

AGE-AT-FORMATION AND DURATION OF LINEAR ENAMEL HYPOPLASIA
AND ACCENTUATED (PATHOLOGICAL) STRIAE IN ANCIENT, MODERN, AND
FORENSIC POPULATIONS AS AN INDICATOR OF DIFFERENTIAL
DEVELOPMENTAL STRESS

by

Lauren Georgiana Koutlias, B.A.

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Committee Members:

Michelle Hamilton, Chair

Nicholas Herrmann

Kate Spradley

Fred Valdez

Lori Wright

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DEDICATION

For

Annalise

Trudy

Pete

Sara

Laila

Kamila

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This thesis is the culmination of two years of mentorship and guidance by Dr. Michelle Hamilton. Without her support and feedback on thesis proposals, grant proposals, thesis drafts, statements of purpose, ideas, thoughts, interesting tidbits, criticisms, vents, and many other forms of academic discourse, I would not have been able to complete this thesis or even be where I am today. I feel much more informed and knowledgeable about the field of biological anthropology and much more assured of my research trajectory because I had Dr. Hamilton as an advisor.

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LIST OF ABBREVIATIONS

Abbreviation	Description
AS	Accentuated striae
DOHaD	Developmental Origins of Health and Disease
GEB	Grady Early Building
GEFARL	Grady Early Forensic Anthropology Research Lab
LEH	Linear enamel hypoplasia
PfBAP	Programme for Belize Archaeological Project
OpID	Operation Identification
ORPL	Osteological Research and Processing Lab
SEM	Scanning Electron Microscope
TXSTDSC	Texas State Donated Skeletal Collection

ABSTRACT

Under the premise that individuals can embody their sociocultural environment, this project was undertaken to analyze differences in age-at-formation and duration of linear enamel hypoplasia (LEH) and accentuated striae (AS) in the mandibular canines of pre-Hispanic Maya individuals from Belize, migrants who died crossing the Texas-Mexico border, and modern American individuals from the Texas State Donated Skeletal Collection (TXSTDSC). LEH and AS, as representative of stress events, were measured using a scanning electron microscope and DinoLite™ from the line to the cervico-enamel junction. These measurements were placed into equations for age calculations. Age-at-formation and duration of stress episodes were statistically analyzed using ANOVA and t-tests. Results show that smaller Maya sites experienced fewer and shorter stress episodes compared to larger, more dense sites, female migrants experienced the first stress events at an earlier age than their male counterparts, and male donors from the TXSTDSC experienced first stress episodes at an earlier age than females, although this may be due to sample size. These results are discussed in terms of the life course and bodily context. The ancient Maya cities were very population dense, exposure of female migrants to systemic and structural violence can be a factor in age-at-formation differences, and in general, differences in weaning practices can contribute to the formation of LEH and AS, with weaning practices differing within varying social contexts.

I. INTRODUCTION

Individuals can biologically embody an adverse sociocultural environment. At a populational level, this can result in physiological health inequalities between groups. This thesis is an investigation of the concept of embodied inequality in the timing, severity, and duration of developmental defects in enamel including linear enamel hypoplasia and accentuated striae in the dentition of three populations; pre-Hispanic Maya from Belize, modern migrant individuals found deceased along the Texas-Mexico border, and modern individuals who donated their skeletal remains to Texas State University for forensic research purposes.

Through the lenses of sex, age, and socioeconomic status, this thesis will examine diachronic and geographic expressions of childhood stress. Examining mandibular canines from these three groups will determine the age-at-formation of macroscopic linear enamel hypoplasia (LEH) – stress lines on the surface of the enamel – and histological accentuated striae (AS) – stress lines on the interior of the enamel. Because the permanent adult dentition develops during childhood, teeth are an excellent source of information for assessing childhood stress.

Anthropologists have recently theorized about the applicability of epigenetics and the Developmental Origins of Health and Disease (DOHaD) hypothesis to anthropological topics related to stress and the skeletal manifestation of disease. LEH, a non-specific stress-induced cessation of dental enamel matrix secretion that presents as lines on the tooth, has frequently been used in paleopathological research and is, in theory, an excellent epigenetic proxy for considering DOHaD, since teeth form during childhood and do not remodel over the lifespan. The examination of both LEH and AS in

these populations may provide more understanding regarding the maternal-fetal environment and generational effects on adult health outcomes.

Research Questions

Among the ancient Maya from Belize, I will macroscopically and microscopically analyze mandibular canines for LEH and AS to assess the effects of population density on developmental stress. I will then compare this data from ancient Central America to the LEH and AS data from a modern group of unidentified migrants who died crossing the Texas-Mexico border, some of whom are also potentially from Mayan regions of Honduras, El Salvador, and Guatemala. Finally, I will examine LEH and AS in a collection of modern American individuals who have donated themselves to the willed body anatomical program at the Forensic Anthropology Center at Texas State.

Population-specific and comparative research questions addressed by this thesis include:

1. Among the Maya sample, is there an inequality in stresses experienced between larger, more populated urban centers of the La Milpa area and those of the smaller, less populated “hinterland” sites? Are there sex and age differences?
2. Among the modern samples, is there an inequality in stressors experienced between unidentified migrants found deceased along the Texas-Mexico border and the American nationals who donated their body for research? Are there sex and age differences?
3. At what age was the first stress event occurring in all populations? Are there sex differences?

4. Does the duration of stress events change between different sites of the archaeological Maya, and between the modern unidentified migrants and the modern donated individuals? Are there sex and age differences?

A synopsis of the populations from which the dental samples were drawn now follows.

Pre-Hispanic Maya of Belize

The pre-Hispanic Maya dental samples are from multiple sites in northwest Belize excavated by the Programme for Belize Archaeological Project (PfbAP), under the direction of Dr. Fred Valdez of the University of Texas at Austin. Known for highly stratified societies, large metropolitan cities, and interconnected hinterlands, analyzing the dentition of these individuals who come from three administrative/ceremonial centers and four commoner sites will help elucidate the effect of population density and urbanism on the stress experience.

Three specific sites that hold administrative or ceremonial significance were investigated. La Milpa, the largest site considered in this thesis, was occupied from the Late Preclassic period (400 BCE – 250 CE) until it was abandoned in the Terminal Classic period (800 CE – 900CE) and was an administrative center which harbored leaders and nobility as well as laborers (Zaro & Houk, 2012). Dos Hombres, a site almost as large as La Milpa, was occupied during the same time periods and may have been a sort of “colony” of agricultural resources for La Milpa (Robichaux & Houk, 2005). The Dos Barbaras site is a smaller ceremonial center and was mostly utilized during the Late Classic (600 CE – 900 CE) with a small Late Preclassic component (400 BCE – 250 CE)

and yielded many artifacts associated with scribal activities and warfare, indicating its ceremonial significance (Hyde, 2012; Lewis, 2005).

Four sites associated with ancient Maya commoners, including commoners of high sociopolitical standing in the community, were also studied. Medicinal Trail is a hinterland community with a complex commoner sociopolitical organization and likely relied on agricultural production for La Milpa (Hyde, 2011). Guijarral is a smaller site dating to the Late and Terminal Classic with an abundance of artifacts associated with land-clearing and agricultural use and water management site modifications (Hughbanks, 2005; Hyde, 2012). El Intruso also dates to the Late and Terminal Classic and is a small site that may have been a local authority over the surrounding area because of its lack of agricultural tools (Hyde, 2012; Lewis, 1995).

These sites were chosen to investigate the complex effect of population density, urbanism, and site specializations on the bodies of the ancient Maya inhabitants of northwest Belize. Previous studies have shown that the ancient Maya embodied cultural ideas of gender and socioeconomic status which represent in the teeth (Cucina & Tiesler, 2003; Somerville, Fauvelle, & Froehle, 2013) and population density has been shown to affect disease loads (Armelagos & Cohen, 1984). If individuals from larger metropolitan centers seemed to experience greater food insecurity and variability and commoner sites tended to have easier access to food resources and were more resilient through time (Somerville et al., 2013), then this should also be evident in the PfBAP burial population of ancient Maya from the La Milpa region (map shown in Figure 1.1; (Scarborough & Valdez, 2003)).

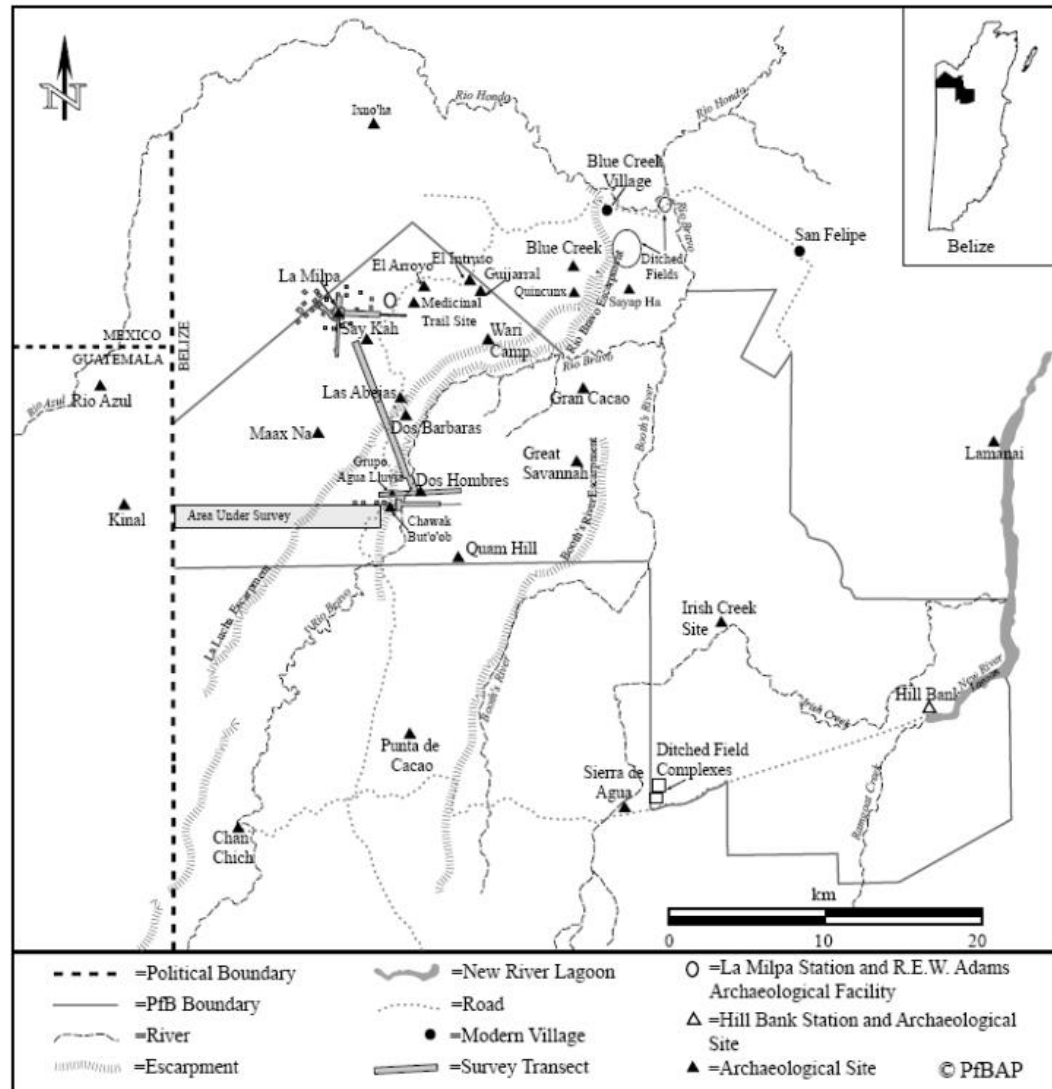


Figure 1.1. Map showing the location of Dos Barbaras, Dos Hombres, Guijarral, El Intruso, and Medicinal Trail in relation to La Milpa. From Hyde (2003) and Scarborough & Valdez (2003).

Operation Identification

The second population examined in this project are unidentified migrants who died while attempting to cross the Texas-Mexico border. These individuals are overseen by Operation Identification (OpID) at Texas State University, an initiative directed by Dr. Kate Spradley that seeks to establish positive identity and to repatriate remains back to their families, and they are presumably of Hispanic ancestry. The term “Hispanic” poses

a challenge in forensic identification since it can be applied to populations from many countries, including Central and South America, and one of the core research programs pursued by OpID involves the narrowing of the forensic ancestry window (Spradley, 2014; Spradley, Jantz, Robinson, & Peccerelli, 2008). While the OpID individuals are presumed to be Hispanic, specifically they may come from a variety of regions including Mexico, Guatemala, Honduras, El Salvador and a number of other countries south of the Texas border. In 2017 alone, more non-Mexican than Mexican nationals were apprehended by U.S. border patrol along the entire length of the border and overall, the percentage of non-Mexican nationals is much larger along Texas border crossings compared to border crossings at other locations along the U.S.-Mexico border (Isacson & Meyer, 2013; Spradley, 2014).

The reasons for migration vary. In Central American countries, reasons may include extreme poverty and the complex factors that feed into it: little to no employment, food scarcity, and violent crime (Isacson & Meyer, 2013). Mexican migrants somewhat differ in their expressed reasons, with many touting more well-paying employment or the presence of family members already living in the United States (Arenas, Goldman, Pebley, & Teruel, 2015). Regardless, some of those who decide to undertake the journey ultimately die while attempting to navigate the inhospitably arid landscape of south Texas.

An investigation of dental stress profiles might help to elucidate the stress experience of these migrating populations, demonstrating that they were experiencing hardship and stress during the most developmentally important time in their lives – childhood. From a humanitarian perspective, this may be of social and forensic relevance

since those experiencing more sociopolitical conflict and violence (i.e., non-Mexican nationals) may reflect differential stress profiles than those who may not have been exposed to the same kind or degree of social upheaval (i.e., Mexican nationals), potentially providing another marker of identity to use when generating the biological profile.

Texas State Donated Skeletal Collection

The third population is the Texas State Donated Skeletal Collection (TXSTDSC) of mostly American nationals housed at the Forensic Anthropology Center at Texas State and comprised of mostly white individuals with a few individuals of Hispanic, Black, and Native American ancestry. Some individuals in the collection donated themselves during their lifetime, and others were donated by their legal next-of-kin. Either donation scenario allowed for the gathering of some contextual medical and lifestyle information - including childhood socioeconomic status - on donation forms. Investigating developmental dental pathologies in the donated individuals allows for a comparative approach looking at modern childhood stress in the United States and how it differs with modern migrants from south of the US border and among the ancient Maya.

By considering these three populations individually and comparatively using a biocultural approach, the stress profiles become more meaningful and explanatory, and a better understanding of the structural and systemic nature of biological-wellbeing may result (Gravlee, 2009).

An introduction to the developmental defects in enamel examined for this thesis will now be presented.

Linear Enamel Hypoplasia (LEH) and Accentuated Striae (AS)

LEH is a malformation in the dental enamel that manifests as a decrease in enamel thickness, usually in the form of linear grooves but is also observed as pits and plane forms in smaller frequencies (Armélagos, Goodman, Harper, & Blakey, 2009; Goodman, Armélagos, & Rose, 1980; Goodman, Martinez, & Chavez, 1991; Goodman, Pelto, Allen, & Chavez, 1992; Goodman & Rose, 1990; Hillson, 2014). Because of the lack of pit and furrow types in the samples analyzed for this thesis, only the linear form will be discussed here. The consensus is that LEH occurs as the teeth are forming during childhood in response to some sort of physiological perturbation (Goodman & Armélagos, 1985b; Goodman et al., 1980; Hutchinson & Larsen, 1988; Neiburger, 1990). Because teeth form during childhood, this makes LEH an excellent proxy for understanding developmental stress and its effect on the life course in ancient and modern populations (Armélagos et al., 2009; Goodman et al., 1980). LEH forms due to a decrease or cessation in ameloblastic activity (the cells that secrete dental enamel matrix) because the body is reallocating energy required for bodily development and function to other more important aspects of development, such as the brain (Hillson, 2014). However, LEH is still characterized as a non-specific stress indicator with several likely etiologies including malnutrition, infectious disease, genetic predisposition, trauma, and perhaps even psychological stress (Goodman et al., 1980; Goodman et al., 1992; Goodman & Rose, 1991; Neiburger, 1990; Nikiforuk & Fraser, 1979). Infant weaning as a possibly etiology for the first occurrence of LEH in the permanent dentition has merit, since the weaning period is associated with increased risk of infection and nutrient-sparse (in comparison to breastmilk) weaning food. This will be discussed more in Chapter 2.

Accentuated striae (AS) (also known as Wilson bands and pathological striae) are an enamel microdefect that can only be observed histologically (Wilson & Shroff, 1970). Some studies have shown them to co-occur with LEH, although this is not conclusive (Fitzgerald & Rose, 2008; Goodman & Rose, 1990; Guatelli-Steinberg & Huffman, 2012; Wright, 1990). While LEH can manifest after an acute stress episode such as a fever, it is usually associated with longer duration, chronic illnesses (Goodman & Rose, 1990; Hillson, 2014). Therefore, it has also been relatively accepted that AS are more a reflection of acute stress since they occur between or on incremental striae of Retzius (Guatelli-Steinberg & Huffman, 2012; Hillson, 2014) and in fact, AS may be present even when an individual has no LEH at all. For this reason, it is important to also consider AS in addition to LEH, since AS may capture acute stress episodes not reflected by LEH.

Accentuated striae are darker, thicker versions of normal striae of Retzius, which are incremental lines of enamel matrix secretion, and the accentuated striae can fall on the striae of Retzius or in between two striae of Retzius (Guatelli-Steinberg & Huffman, 2012) (Figure 1.2). If a darker thicker line is observed, there are a few defining criteria for identification of the line as an accentuated stria: 1. They should be 75% of the enamel thickness, 2. They should be observed in buccal and lingual enamel, and 3. Prism formation should be abnormal in that they are observed in a sharp trough or ridge formation (Hillson, 2014). The latter requires a magnification that is beyond the capabilities of the GEFARL microscope and was therefore not assessed.

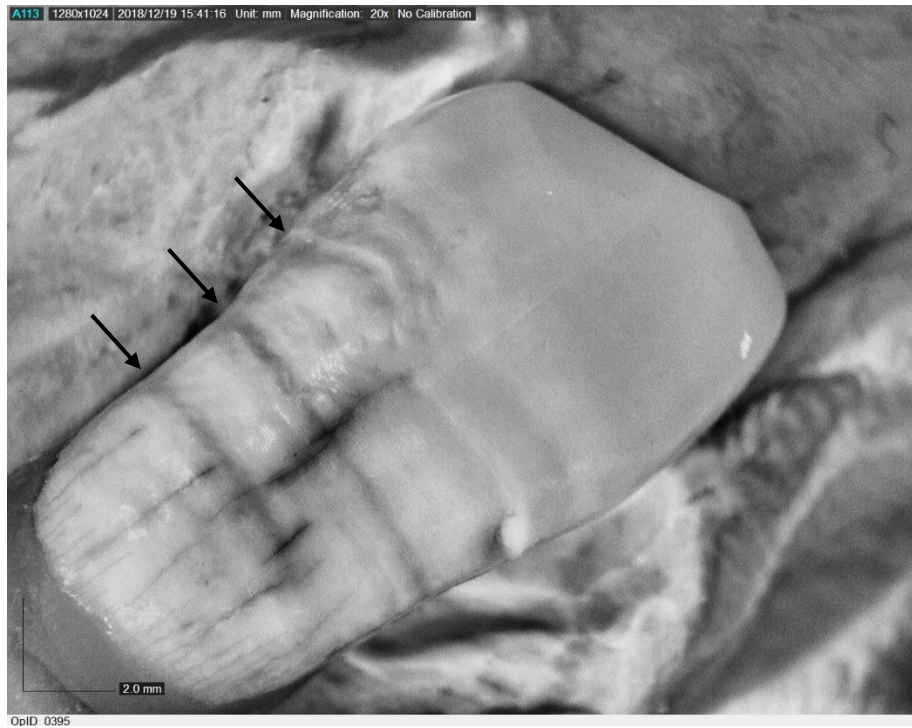


Figure 1.2 Mandibular canine from an individual curated by OpID (0395). Multiple macroscopic linear hypoplastic events are observable (arrows).

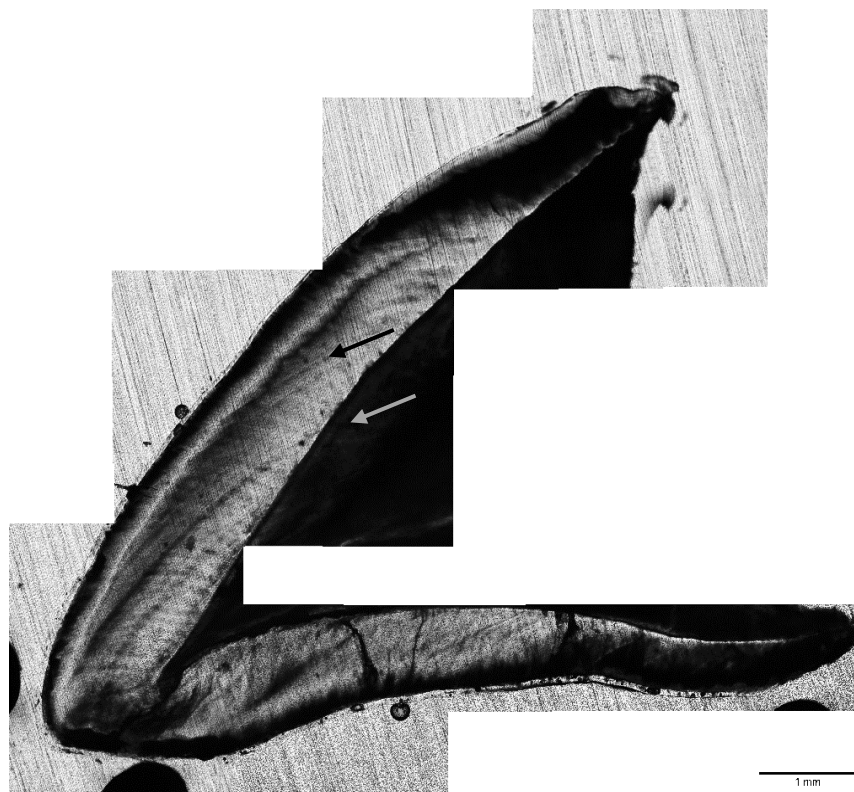


Figure 1.3 Mandibular canine from La Milpa (RB25-V63-A-5) showing clear accentuated striae in the histological thin section (arrows).

Some research teams interested in accentuated striae have looked at primate osteological remains and associated specific incidences of accentuated lines with stressful events in the logbooks of the primatologists studying them. Schwartz, Reid, Dean, and Zihlman (2006) studied fourteen teeth from a single gorilla and correlated specific stress episodes in the histological thin sections with events such as veterinary visits and days during which the juvenile gorilla was bullied by an alpha male. The implication is that psychological stressors may also have an impact on the formation of dental enamel matrix, but this is inconclusive since the sample size was a single individual. Smith and Boesch (2015) analyzed the accentuated striae in the dentition of three wild chimpanzees and found that there were higher rates of accentuated striae in all three individuals during periods of high rainfall, which would have prevented access to movement and food resources. Again, this study is inhibited from having any explanatory power for the etiology of accentuated striae because of the small sample size. However, these two studies show potential for the use of primate dental analyses and their observers detailed note-taking in elucidating the etiology of stress-induced non-specific dental pathologies.

Destructive analysis is not always feasible or allowable in modern or bioarchaeological human skeletal remains. Non-destructive observation of perikymata can therefore be a useful approach to analyzing micro-stress events that are usually only observed through histologically-sectioned teeth demonstrating accentuated striae, since perikymata are incremental microstructural “waves” or lines that sit on the buccal enamel surface (Temple, 2016). Etiologically, perikymata are the surface expressions of the striae of Retzius and therefore have a periodicity of around 6 to 8 days. When the space between two perikymata are greater than the surrounding perikymata, this is typically

identifiable as a micro-stress event (lasting around 6 to 8 days). Objective measurement of perikymata has been proposed as the best method for identifying linear stress episodes, as macroscopic observation of LEH is largely based upon the ability of the researcher to observe them visually or by touch (Cares Henriquez & Oxenham, 2017; Hassett, 2014; Temple, 2016). Observation of only large, macroscopic LEH leaves the researcher with a dearth of micro-stress episodes that the individual undoubtedly experienced. Perikymata analysis can therefore allow a more nuanced consideration of micro- and macro-stress events within a biocultural framework.

Individuals with freshly erupted permanent teeth are usually the best candidates for the identification of continuous perikymata, with archaeological and older modern individuals being afflicted with discontinuous or even completely absent lines for various reasons which include issues of taphonomy and preservation, or even tooth brushing in modern populations. However, methods have been devised which allow for the analysis of micro-LEH and micro-stress events even with the absence of continuous perikymata (Cares Henriquez & Oxenham, 2017) which account for the micro-deviations of normal enamel surface curvature. This newer method requires a modified engineer's measuring microscope, which was not available to the Forensic Anthropology Center at Texas State at the time of this writing.

Goodman and Armelagos (1985a, 1985b) propose that studies of dental enamel defects should focus primarily on the anterior teeth, since those teeth are more likely than posterior teeth to present with defects (Wright, 1997a). The authors also found that the mandibular canine and maxillary incisor are more likely to be affected by defects because of the direction of amelogenesis, tooth crown geometry, and a genetic susceptibility

(Wright 1997; Goodman and Armelagos 1985b). Condon and Rose (1992) proposed that teeth have different rates of amelogenesis. Teeth with slower rates of amelogenesis would be more susceptible to enamel defects (*ibid.*). For these reasons, and in keeping with standard research protocols, only mandibular canines were sampled and observed for incidence of AS and LEH, as these are the most likely teeth to form and preserve defects.

Stress and Disease Markers in Skeletal Material

The term “stress” is often not well defined in skeletal research (Hillson, 2014; Temple & Goodman, 2014). In this thesis, stress is defined as a physiological disruption to homeostasis in response to an adverse biocultural event in the environment of an individual or population (Goodman, Brooke, Swedlund, & Armelagos, 1988; Hillson, 2014; Temple & Goodman, 2014).

The identification of “stress” and “health,” especially in paleopathology, began with a focus on pathologies that left observable marks on bone along with corresponding attempts to identify etiology (Goodman & Martin, 2002). Early focus emphasized skeletal lesions such as porotic hyperostosis, cribra orbitalia, periostitis, osteomyelitis, and LEH, among others. However, these endeavors were focused mostly on description of lesions and analyses of prevalence and frequency between and among sites as proxies for the disease processes in living populations (Goodman & Martin, 2002; Bethany L Turner & Klaus, 2016). This was the issue critiqued by Wood and colleagues’ ‘Osteological Paradox’ since skeletal series are not completely representative of the living population nor are all skeletal assemblages representative of a snapshot of one period of time (and are therefore not death cohorts) (Wood et al., 1992).

The stress events represented by incidence of LEH and AS will be interpreted as such. The opposite, that is, the absence of LEH or accentuated striae, will not be interpreted as evidence of “health” or “healthy” individuals, as that would tread on the premise of selective mortality and hidden heterogeneity in frailty (Temple & Goodman, 2014; Wood et al., 1992). However, the presence of the stress event at the age at which it occurred will be seen as a stress episode potentially affecting other individuals in the population, and it will be assumed to be systemically caused by multiple and complex etiologies (Goodman & Martin, 2002; Temple & Goodman, 2014).

Age-at-formation and duration of LEH and AS will be measured and calculated from sampled teeth using standard methodologies. Dental defect analysis will allow for a more holistic consideration of differential childhood stress experiences among the three populations. The age at which stress is experienced is important for adult morbidity and longevity. Early childhood stress experiences can be more detrimental to adult life than stress experienced during later childhood (Armstrong et al., 2009; Gluckman, Buklijas, & Hanson, 2016; Rebecca L. Gowland, 2015). In addition, duration of a stress event can be related to the ability of the biocultural buffering system to work against longer-term adverse environments (i.e. drought), while shorter duration events might reflect an individual’s increased ability to resist stress episodes (Goodman & Martin, 2002). The age-at-formation method of analyzing LEH and AS has more explanatory power than reporting prevalence and frequency data alone. LEH age-at-formation and the duration of stress episodes can therefore measure different aspects of the stress experience.

Thesis Overview

The second chapter, Theoretical Background and Literature Review, will be a synthesis of relevant background literature as well as theoretical perspectives that may help with understanding how individuals are affected by their sociocultural environment in potentially harmful ways. It will begin with an in-depth look at biocultural theory, the Developmental Origins of Health and Disease (DOHaD), Life Course Theory, and how biological anthropologists have used these theories to say something about human health.

The third chapter, Materials and Methods, will identify the demographic make-up of the study populations and detail the methods by which data collection was conducted and statistical analysis was carried out. Specifically, all aspects of sampling and measurement will be reviewed here. Chapter four will be a presentation of the results and statistical analyses divided by groups, with inter- and intra-population differences highlighted. Chapter five will be a discussion of results and consideration of the data within the theoretical frameworks synthesized in Chapter 2. In the concluding chapter, a summary of the most meaningful findings will be presented, and a strong consideration of future directions for research will be discussed.

II. THEORETICAL BACKGROUND AND LITERATURE REVIEW

Biological anthropologists have been attempting to identify stress levels in ancient and modern populations since, arguably, the founding of the field. In recent years especially, this identification of stress levels has moved from basic reporting of pathological and developmental conditions to a consideration of those conditions as biocultural responses to a changing and hostile environment, with various populations possessing unique stress profiles (Goodman and Martin, 2002). Therefore, researchers have employed various theories such as the Developmental Origins of Health and Disease (DOHaD) hypothesis, Life Course Theory, and embodiment as theoretical orientations that ultimately may yield a more holistic and explanatory view of complex structural and systemic levels of stress on ancient and modern bodies. (Zuckerman & Armelagos, 2011b).

Biocultural Approach

In the previous chapter, stress was identified as a physiological disruption in homeostasis in response to an adverse biocultural event in the environment of an individual or population. By biocultural, I mean some sort of bony response to a stressful episode that occurred because of hostile social or cultural issues (Zuckerman & Martin, 2016). The biocultural approach is usually adopted by investigating a sociocultural issue, for example inequality or socioeconomic status (by gender, ancestry, age, class etc.), and its (possible) corresponding bony manifestation (LEH, bone infections, trauma, etc.) (Goodman et al., 1988; Zuckerman & Armelagos, 2011b; Zuckerman & Martin, 2016). The biocultural approach can allow for more interpretation of the complex and integrative

causes of particular skeletal responses. However, this feature has been deemed by some to be the drawback of applying this approach. If one skeletal lesion can be caused by multiple and intertwined variables and much of the contextual knowledge of the population or individual is lost (i.e. in bioarchaeological remains), much of what can be said about the “culture” in “biocultural” is lost and any attempts at explaining observations are inaccurate (Zuckerman & Armelagos, 2011a; Zuckerman & Martin, 2016).

Regardless, it is important to report observations of skeletal lesions potentially tied to sociocultural inequalities in skeletal remains, and biological anthropologists do this in a number of ways, primarily through the theoretical lenses of DOHaD, life course theory, and embodiment. These three concepts form a framework for interpretations of early life adversity on adult morbidity and mortality.

Developmental Origins of Health and Disease (DOHaD)

The DOHaD hypothesis is a conceptual framework that states that poor or stressful environmental circumstances during the prenatal and postnatal developmental periods can have an effect on the rest of the life course, especially on adult morbidity and mortality (Gluckman et al., 2016; Rebecca L Gowland, 2015). This concept is firmly rooted in the field of epigenetics, a subfield of genetics in which studies show that the environment can have lasting effects on the phenotypic expression of DNA, sometimes adversely, and that these changes can be passed down from parents to offspring (Burdge & Lillycrop, 2010; Rebecca L Gowland, 2015).

A precursor to DOHaD, the Barker hypothesis posited that stressful early fetal and infant environments increased the likelihood that adults would present with

cardiovascular diseases (Barker & Osmond, 1986). Armelagos et al. (2009) attempted to apply this concept to bioarchaeological skeletal material. They synthesized and reinterpreted previous LEH data to show support for the Barker hypothesis and DOHaD. Subsequently, more recent studies have also found correlations with age-at-death and presence of linear enamel hypoplasia (Amoroso, Garcia, & Cardoso, 2014; Watts, 2015; Yaussy & DeWitte, 2018).

The effects of early life adversity, including malnutrition, disease, and physical and psychological trauma, is linked to later adult morbidity and mortality and the maternal-fetal environment plays a role in this fetal and postnatal programming (Burdge & Lillycrop, 2010; Gluckman et al., 2016; Rebecca L Gowland, 2015; Vinkers et al., 2015). A famous example is the Dutch hunger-winter of 1944-1945 during which rations of food were drastically reduced for the entire season. Studies of adults who were *in utero* during this brief period have shown epigenetic changes associated with mental and metabolic disorders (Rebecca L Gowland, 2015; Roseboom et al., 2001). The environmental and social experiences of the pregnant mother have an effect on the susceptible, developing fetus and therefore on the rest of the life course. Since the developmental period is a period of high plasticity and proneness to epigenetic alterations, psychosocial, traumatic, and nutritional stressors on the mother affect the longevity and morbidity of the child. While this focus on adverse generational health has been explicitly placed at the feet of the mother (deemed by some to be harmful rhetoric, see (Richardson et al., 2014), new experimental studies on laboratory mice have now shown that sperm also carry and pass on harmful epigenetic alterations, proving that these

epigenetic mechanisms are not yet well understood (Dickson et al., 2018; Donkin & Barrès, 2018).

Life Course Theory and the Life History Approach

Life Course Theory and the Life History Approach are similar to DOHaD in that they acknowledge that the complexities of human lives are rooted in childhood development. The Life History Approach is a theoretical framework from biology that attempts to explain the differences in development and attainment of specific life stages and life hallmarks such as infancy, puberty, adolescence, adulthood, senescence, menarche, menopause, and death, in addition to developmental rates (Hill, 1993) while Life Course Theory, coming from the perspective of childhood development and behavior, emphasizes how all of these life stages are interconnected, influenced by each other and by changing sociocultural contexts which can create differential developmental trajectories (Elder, 1998). In disciplines outside of anthropology, life course theory is applied by conducting longitudinal studies of living subjects (Elder, Johnson, & Crosnoe, 2003).

In biological anthropology and bioarchaeology, life course approaches have been readily adopted. Life course theory can be applied by looking at adult skeletal morphology – such as age at death, sex, trauma, pathological conditions – along with skeletal features of childhood development – such as LEH, AS, vertebral neural canal diameters, Harris lines, porotic hyperostosis, stature and others (Agarwal, 2016). There is an understanding in the field that differences in environment and behavior can affect developmental plasticity which in turn can cause different bone trajectories and histories – or adult skeletal plasticity (Agarwal & Beauchesne, 2011).

Specifically, biological anthropologists consider the life course when observing changes in the human skeleton at the individual and population levels to explain skeletal differences, and how various events during the lifespan can affect the present morphology (Agarwal, 2016). For the purposes of this thesis, this especially includes a consideration of the effects of developmental stress and plasticity on the rest of the life course (Agarwal & Beauchesne, 2011) as seen through age-at-formation of LEH, AS, and duration of stress episodes, and less of a consideration of biological life history hallmark attainment except through a discussion of weaning (see below). Watts (2011, 2015) does this especially well by observing how vertebral neural canal diameters, formed during early developmental periods, correspond with other features of childhood stress and adult morphology in Medieval London skeletal populations, acknowledging the multiplicative nature of skeletal morphology.

Embodiment

DOHaD and Life Course Approaches have something in common, primarily the concept that early life insults can affect developmental plasticity in childhood that alters bone morphology trajectories in adulthood. DOHaD builds on the idea of a genetic basis for these adverse trajectories; epigenetic alterations result if insults transpire during important developmental windows in early childhood which can then affect adult phenotypes (Gluckman et al., 2016). Life course theory acknowledges that the context of these epigenetic alterations can vary in a variety of complex ways due to sociocultural practices, geographic or climatic environments, individual agency and behavior, or likely, a combination of all of these (Elder et al., 2003). The root of both of these conceptual frameworks is the idea that individuals can embody adverse environments. Embodiment

is the concept of the human body and mind as a subject of, and shaped by, the cultural environment (Csordas, 1990). In bioarchaeology, this means that skeletal biologists should acknowledge the “produc[tion]” of skeletons shaped by the sociocultural environment, and this can help to make skeletal data socially relevant by illustrating the effects of inequality on the human body (Zuckerman, Kamnikar, & Mathena, 2014). Gravlee (2009) explicitly uses the embodiment concept to discuss racial inequalities in biological well-being, such as rates of certain diseases between social races. An entire group of people, ostracized for generations by social race, can embody that inequality in group rates of morbidity and mortality. In this way, DOHaD and life course theory become explanations for embodiment which all fall under the umbrella of the biocultural approach.

Weaning

The weaning period is a transition from breastmilk (or formula) to a diet of solid foods and has gained interest among biological anthropologists as feminist anthropology has grown to pay more attention to culturally prescribed ideas of weaning and how it is linked to issues such as fertility and fecundity, infant morbidity and mortality, and sociocultural and environmental influences (Katzenberg, Herring & Saunders, 1996). The weaning period is well situated within DOHaD and Life Course theory, as it is arguably both an important life course *and* life history event that can affect adult morbidity and mortality if associated with adverse environmental circumstances.

The modern clinical literature on breastfeeding emphasizes the undeniable immunity that breastmilk can provide to the developing immune system of an infant (Parigi, Eldh, & Larssen et al, 2015; Ladomenou, Kafatos, & Galanankis, 2007; Rao &

Rajpathak, 1992). Breastfeeding can have long-lasting positive effects on the immune system of the developing infant that outlast infancy and can be beneficial to immune response and capacity in later life (Hanson, 1998; Hanson et al., 2003; Zamora-Kapoor et al., 2017).

However, in some instances, weaning can be a stressful event. The inability to breastfeed, the decision not to breastfeed, rapid weaning, and nutrient-sparse weaning food may have detrimental effects to infant (and later adult) health. Ogbuanu et al. (2009) conducted a study in the southeastern United States where they interviewed women concerning their breastfeeding practices. Women gave varying responses, including the lack of hospital staff teaching proper breastfeeding practices, household responsibilities, and employment. The authors also found that black women were more likely to have not been taught how to breastfeed and therefore twice as likely not to have breastfed their children. Using biocultural reasoning, the systemic nature of racism and structural violence to black mothers and their children creates a more adverse environment for the growth and development of their babies. The children of the black women in this study were more likely to have immunological issues related to the lack of hospital staff offering breastfeeding information which may have an effect on the life course – including longevity and morbidity. Ahluwalia, Morrow, and Hsia (2005) conducted breastfeeding interviews with women and found that mothers from the lower socioeconomic status cohort were more likely not to breastfeed or to stop breastfeeding early (also found in Davis, Li, Adams-Huet, and Sandon (2018)). Reasons women cited for not breastfeeding included an inadequate milk supply and the infant having difficulties feeding. Considering this in an ancient context, the infants would have had an

increased mortality risk because of the lack of artificial milk substitutes such as modern formula or, if they survived, increased morbidity and longevity.

Weaning is usually a culturally prescribed endeavor and investigations of age-related dental defects or stable isotope studies of teeth can corroborate the modal age of weaning in archaeological populations, sometimes with historical archival evidence (Herring, Saunders, & Katzenberg, 1998; Katzenberg, Herring, & Saunders, 1996; Wright & Schwarcz, 1998). Following the idea of biological/pathological uniformitarianism (Buikstra, Cook, & Bolhofner, 2017), modern issues that prevent or halt breastfeeding or involve the weaning process likely affected women and children in the ancient world. Wright and Schwarcz (1998) collected stable isotope data from the first molars, premolars, and third molars of adults to reconstruct weaning practices at Kaminaljuyu in Guatemala and found that children were consuming maize products before 2 years of age but were still drinking breastmilk by 4 to 6 years old. Stable isotope ratios also varied by skeletal individual. The authors asserted that this may be due to individual preferences of the child and/or mother, or perhaps an underlying trend by socioeconomic class, or it may reflect dietary shifts by cultural time period at Kaminaljuyu, Guatemala. Regardless, the data show that general trends in breastfeeding and weaning practices are observable in the ancient world. What may add to this argument would be an analysis for age-at-formation of LEH in these individuals that were sampled for stable isotopes to better understand if these culturally determined weaning practices were affecting children adversely. This method was adopted by Corruccini, Handler, and Jacobi (1985) on a Caribbean slave population. Most LEH was

occurring between 3 and 4 years of age which is the historically documented high-risk post-weaning period of 2 to 3 years old.

However, the initiation and cessation of the weaning process, while holding the possibility to be stressful, affects individual children in different ways depending on the selective frailty of an individual and the hidden heterogeneity of a population. Weaning as a process is heterogeneous and without the explicit documentation of how it occurred, it is impossible to assess from skeletal remains. For example, the average age of the introduction of solid foods (i.e. the initiation of the weaning process), in Americans is around 5 months of age (Scott, Binns, Graham, & Oddy, 2009), while the recommended age for solid food introduction is after six months of age – and exclusive breastfeeding until then (Shafer & Hawkins, 2017). In modern populations, breastfeeding may not be initiated at all, instead opting to formula feed, and in one study, 52% of Americans who breastfed supplemented with formula (Scariati, Grummer-Strawn, & Fein, 1997). This showcases the heterogeneity in this important life historical and life course event, which must be considered in a study observing incidences of stress during early childhood development.

LEH and Accentuated Striae in the Maya World

As LEH is a common prehistoric dental pathology and requires simple observations and no destructive analysis, many LEH studies have been conducted on Maya skeletal populations. Two studies have looked at a Classic Maya population at Xcambo in the Yucatan (Cucina, 2011; Méndez Collí, Sierra Sosa, Tiesler, & Cucina, 2009). Both of these studies found that all children, regardless of age, sex, or social status, were experiencing stressful events that caused LEH, which is odd considering

inhabitants of Xcambo seemed to have the potential for cultural buffering from stress, with access to many (and balanced) food resources as a port city. Cucina (2011) and Méndez Collí et al. (2009) therefore assert that researchers should be cautious when considering the etiology of LEH as malnutrition and disease in the past, since the Xcambo population likely was not experiencing either of those. Wright (1997b) looked at LEH in a Classic Maya lowland population from the Pasión River region in Guatemala. In her study, Early, Late, and Terminal Classic individuals were separated and statistically analyzed. No differences were found between the three periods with regards to anterior teeth, showing stability in stress load through time. However, posterior teeth (premolars and molars) experienced more stress episodes during the Terminal Classic (Wright, 1997b). Because tooth crown geometry of posterior teeth makes it more difficult for the formation of defects (Goodman & Armelagos, 1985b), this indicates that more severe stresses were affecting children during the Terminal Classic, or the period of the ancient Maya collapse.

Only a handful of studies have been performed utilizing dental histological methods for observing accentuated striae. Wright (1990) sampled 23 individuals from Lamanai, Belize to assess differences between pre-contact and post-contact individuals. Wright concluded that there was an increase in epidemiological stressors in the historic period for the Maya at Lamanai. In addition, she observed that LEH and accentuated striae co-occurred only 25%-50% of the time, and therefore likely had different etiologies. As a result, I also anticipate observing a low rate of co-occurrence between LEH and accentuated striae in sampled canines of PfBAP individuals if this is the case. Another dental histological analysis of accentuated striae and LEH in Maya individuals

was performed by Danforth (1997). She examined individuals from Barton Ramie, Seibal, and Tikal in Guatemala in order to evaluate variances between the three sites in relation to population density, with Tikal being the densest and Barton Ramie the least dense. Danforth concluded that Maya areas with high population densities had the highest rate of accentuated striae and LEH. However, Danforth also identified striae of Retzius -- a normal developmental occurrence in the enamel (Guatelli-Steinberg & Huffman, 2012; Hillson, 2014) as a pathology. As a result, while her striae of Retzius data will not be incorporated into this project, Danforth's accentuated striae and LEH data can be used to shed light on what the PfBAP data might show. While a general report of demographic information of the ancient Maya curated by the PfBAP was conducted (Drake, 2016) no paleopathological data has been analyzed.

Biocultural Stress Profiles of Unidentified Migrants

In a forensic context, LEH and AS have the potential to elucidate the life history of migrants fleeing their country of origin. Frequency and duration of LEH and AS can aid in the forensic identification of individuals as inhabitants of specific regions, since sometimes frequency and/or duration of LEH and AS can be specific to groups of people; for instance, between Mexican nationals versus individuals coming from Central America (Beatrice and Solis 2016). This "stress profile" can be an important component along with the biological profile to help establish identity (Birkby, Fenton, & Anderson, 2008).

Additionally, illustrating how unidentified migrants undergo multiple bouts of stress during the most important developmental time of the life course can be important from a legal and policy perspective. At the population level, LEH and AS can inform on

the life history of unidentified migrants beginning with structural violence experienced during childhood and why their motivation for leaving is important and valid from a humanitarian perspective.

Under the assumption that individuals from Central America have endured more traumatic stress events since childhood than those from Mexican populations, LEH and AS should be more prevalent in people coming from Central American countries. If this is so, using LEH and AS to address the question of stress and migrant population profiles may provide additional information that can contribute to forensic identification by narrowing the window of the population profile. Results may show higher incidences of LEH in the unidentified migrant remains (especially from Central America) because these individuals will embody the structural violence and sociopolitical pressure that affected them in their home countries not only in the skeleton but in the dentition (Beatrice & Soler, 2016; Martínez, Reineke, Rubio-Goldsmith, & Parks, 2014). Lastly, a component of this thesis research involves examination of LEH and AS in archaeological Maya populations, and comparing the modern migrant dental stress patterns to the ancient Maya data may be of diachronic and individualizing value, since the Maya of the past are genetic ancestors for many modern individuals migrating from Central America and crossing the US-Mexico border.

Previous efforts at identification efforts using skeletal indicators of stress have been somewhat successful. Beatrice and Soler (2016) used porotic hyperostosis, cribra orbitalia, and all forms of enamel hypoplasias in unidentified migrant remains in Arizona and compared that to skeletal lesions in modern American donated collections. They found that unidentified migrants were up to 7 times more likely to be affected by one of

these skeletal lesions and therefore a “foreign national” should be easily distinguished from an American national if these lesions were present. Birkby et al. (2008) also cite the presence of decreased dental health and unique dental cosmetic restorations in unidentified migrants at the Pima County Office of the Medical Examiner compared to American whites in the same area.

A Biocultural Synthesis

Recording dental developmental defects can help to elucidate the childhood stress experience of various populations. When using a comparative approach to consider the biocultural context of the growth of modern American teeth, the teeth of unidentified migrants fleeing sociopolitical unrest, and the teeth of the ancient Maya of Belize, a better understanding of structural violence and the embodiment of stress may emerge. This can also hold implications for the anthropological study of migrant and refugee issues, the archaeological study of health and disease in complex societies, and the forensic use of developmental pathologies for identification purposes.

III. MATERIALS AND METHODS

Data Collection Locations

The scope of this project includes the sampling of mandibular canines from three populations: skeletal remains of ancient Maya curated by the Programme for Belize Archaeological Project (PfBAP) in northwest Belize, donated individuals from the Texas State Donated Skeletal Collection (TXSTDSC) housed at the Grady Early Forensic Anthropology Research Laboratory, and unidentified migrant individuals curated by Operation Identification (OpID) at Texas State University.

PfBAP

Excavation and preliminary analyses took place at the PfBAP, and dentition was sampled from sites that included Medicinal Trail, La Milpa, Dos Hombres, Dos Barbaras, Barba Group, Guijarral, and El Intruso (Gateway). The mandibular canines were collected at the field lab and exported to the United States under existing PfBAP protocols and export permits. The second round of analysis took place at the Grady Early Forensic Anthropology Research Laboratory (GEFARL) at Texas State University, where required histological equipment is housed.

All individuals excavated on the Rio Bravo Conservation and Management Area are housed in the PfBAP camp laboratory at La Milpa Station. In addition, during most field seasons, other burials are found during ongoing archaeological excavations and require the specialized excavation methods of trained bioarchaeologists. I performed these excavations with other osteologists using the standard archaeological tool kit and bioarchaeological excavation techniques (i.e. setting up 1m x 1m units, mapping, etc.).

Therefore, already excavated remains as well as those excavated during the 2018 season were considered for inclusion in my project, with a final overall sample size of 31 individuals selected (Table 3.2).

Table 3.1. Individuals sampled from the Programme for Belize Archaeological Project burial collection.								
<i>Site</i>	RB	Op	SubOp	Lot	B#	Sex	Age	Range
<i>Medicinal Trail</i>	62	7	-	-	2	I	Child	10-14
<i>Medicinal Trail</i>	62	12	BO	2		I	Adult	-
<i>Medicinal Trail</i>	62	12	A	19	2	I	Adult	-
<i>Medicinal Trail</i>	62	7	HP	2	7A	I	Middle Adult	-
<i>Medicinal Trail</i>	62	7	FG	11	3	F	Young Adult	20-30
<i>Medicinal Trail</i>	62	7	HP	1	-	I	Young - Middle Adult	-
<i>Medicinal Trail</i>	62	7	GS	1	4	F	Middle Adult	30-50
<i>El Intruso</i>	11	6	X	8	15	M	Young Adult	25-35
<i>Barba Group</i>	S2	1	-	-	1	M	Young Adult	20-35
<i>Guijarral</i>	18	45	G	S	2	M	Young Adult	25-35
<i>La Milpa</i>	25	V	42	-	2	M	Young Adult	20-35
<i>La Milpa</i>	25	V66	A	15	3	M	Young Adult	20-30
<i>La Milpa</i>	25	V68	A	9	-	M	Young Adult	25-35
<i>La Milpa</i>	25	V66	A	15	1	I	Young - Middle Adult	25-40
<i>La Milpa</i>	25	V63	A	5	3	I	Early Child	3-5
<i>Dos Barbaras</i>	4	3	XX	14	-	I	Young Adult	20-30
<i>Dos Barbaras</i>	4	3	BP	1	-	I	Adult	-
<i>Dos Barbaras</i>	4	3	AQ	13	-	I	Adult	-
<i>Dos Barbaras</i>	4	3	AW	9	2	I	Adult	-
<i>Dos Barbaras</i>	4	3	AU	6	-	I	Adult	-
<i>Dos Barbaras</i>	4	3	DJ	1	-	M	Young Adult	25-40
<i>Dos Barbaras</i>	4	3	BD	6	-	M	Middle Adult	35-50
<i>Dos Barbaras</i>	4	3	WWZZ	16	-	F	Young Adult	20-34
<i>Dos Barbaras</i>	4	3	AI	17	10	F	Young Adult	-
<i>Dos Hombres</i>	2	8	38	8	6	M	Young Adult	26-34
<i>Dos Hombres</i>	2	19	1	--	-	M	Young Adult	20-35
<i>Dos Hombres</i>	2	8	21	5	-	I	Middle Adult	30-50
<i>Dos Hombres</i>	2	8	36	6	5b	M	Young Adult	20-30
<i>Dos Hombres</i>	2	28	O	13	3	I	Early Child	3 to 5
<i>Dos Hombres</i>	2	29	V	10	3	F	Middle Adult	35-50
<i>Dos Hombres</i>	2	29	C	12	2	F	Adult	Adult

First, I assessed individual dentition for presence or absence of mandibular canines, the most susceptible tooth to external stressors (Hillson 2016; Danforth 1997). Second, the canines were examined for patterns of cultural modification (e.g., filing, inclusions, drilling). If the tooth was determined to be culturally modified, it was not included in the analysis. Third, I determined if the canine was too heavily worn, since wear can obliterate LEH or AS. Fourth, teeth were photographed before being packed for export, and photos were given to the director to preserve as much information as possible in case of damage during export. Fifth, selected canines were placed in single-use plastic zip-lock bags with a slip of paper with contextual information (provenience) on it well as my name, contact information, date taken, and all burial information. This information was left with the skeletal remains on notecards so future researchers know that a mandibular canine was taken. Sixth, all selected canines in individual bags were packed in sturdy plastic food containers to be exported to the United States.

After the field component of my research at the PfBAP field lab concluded, all selected teeth arrived at GEFARL. Before teeth were embedded for histological thin-sectioning, DinoLite™ images were taken to take more precise measurements of surface features such as LEH. Subsequently, all teeth were taken to the Materials Science Engineering and Commercialization (MSEC) department to the Analysis Research Service Center (ARSC) at Texas State University, where a scanning electron microscope (SEM) is housed. High resolution scanning electron and backscatter electron images were taken of the teeth for observation of perikymata. After SEM images were taken, teeth returned to GEFARL for histological preparation.

Histological thin-sectioning of the mandibular canines then proceeded through a sequence of the following nine steps: First, in order to preserve a proxy of the tooth for future research needs, I created molds and plaster casts of all teeth using Dragon Skin™ mold-making and casting materials and these casts will be returned to the PfBAP to be re-associated with the burial they came from. Second, standard measurements were taken of the teeth to document as much data as possible before thin-sectioning. Third, teeth were embedded in resin to prevent splintering during sectioning and placed in a vacuum to rid the resin of bubbles. Fourth, resin-embedded teeth were sectioned using an Isomet™ thin-sectioning saw. Three slices per tooth were taken at 1mm each. An effort was made to obtain at least one slice through direct midline of the tooth and this slice was prioritized for analysis. Fourth, the teeth were ground and polished to within 80 and 100 µm thick in order to view AS. Fifth, the thin section was secured to a slide using crystal bond mounting medium and examined under a polarized light microscope.

All slides had high-resolution images taken using an Olympus™ microscope camera in the Cellsens™ program at a 10x magnification. Using these images, AS were counted and measured. AS was associated with a developmental age according to standards presented by Cares Henriquez and Oxenham (2018) (discussed below.) All LEH and AS data gathered was handwritten on forms created by me and in a Microsoft Access database. After all data was gathered, statistical tests were then run.

PfBAP Sample Composition and Limitations

The independent variable in this study is the archaeological site from which I drew the samples, since these are an analog for high and low population density. For other research questions, the independent variables were age and sex, since I was

examining how age and/or sex affected the prevalence and severity of dental microdefects.

The dependent variables in my research questions were prevalence and severity of enamel microdefects. The number of AS and LEH will depend on the archaeological site from which the individuals were excavated, whether they were male or female, and/or how old they were at death. A confounding variable that I was forced to take into consideration is preservation. Maya skeletal preservation is especially poor due to the placement of the deceased individuals among the construction fill of homes, public buildings, and plazas (Danforth 1997), and the complex root systems of trees in the dry-rainforest climate often damage or destroy osteological material. In addition, many individuals did not have teeth preserved and some individuals with teeth did not have mandibular canines.

There are a few limitations of my use of PfbAP individuals. First, I only have access to the PfbAP individuals and so can only consider conclusions as specific to the region from which they were excavated (northwest Belize). Second, the Maya migrated between regions frequently and may have lived in multiple locales (e.g., as a child, they lived in a sparse household community and moved to a large ceremonial center in adulthood). Third, I only sampled canines that were present and complete, free from wear or cultural modifications which limited my sample size and sample representation. Fourth, there is a sample bias; larger sites are represented as a larger proportion of my sample than smaller sites such as Medicinal Trail and Barba Group.

Operation Identification

No OpID dental samples were thin-sectioned. All non-destructive mandibular canine sampling took place at the Osteological Research and Processing Laboratory (ORPL) which is an arm of the Forensic Anthropology Center at Texas State (FACTS). Unidentified individuals curated by OpID at Texas State University go through maceration of remains and standard biological profile analyses, the results of which are uploaded to the National Missing and Unidentified Persons Database (NamUs) for an increased likelihood at identification. All individuals curated by OpID were excavated at various cemetery sites in south Texas thanks to the work of director Dr. Kate Spradley and Texas State graduate students, and are assumed to be migrants/refugees from Mexico, El Salvador, Guatemala, Nicaragua, Honduras, and other countries in Central America.

After exclusion criteria were applied, 30 individuals (15 males; 15 females) (see Table 3.3) were picked with suitable mandibular canines. Individuals were excluded from analysis if they were currently awaiting DNA sequencing, if they were awaiting completed biological profile analyses, if they had been identified and were awaiting repatriation, or if their canines were too firmly held in the mandibular body to preclude observation. Age and stature data were compiled using information from the OpID database. This information was generated by OpID personnel, including director Dr. Kate Spradley and graduate students. Age ranges of individuals included in this project ranged from 15 to 69 years of age at the time of death.

No destructive analysis was conducted on teeth sampled from OpID. All teeth were examined under a DinoLite™, where surface features such as LEH could be

observed and measured. Images with embedded measurements were taken. Following documentation of surface LEH, OpID teeth were taken to the MSEC ARSC for SEM analysis of microdefects and perikymata.

Table 3.2 Individuals sampled from Operation Identification.

<i>ID</i>	<i>Sex</i>	<i>Age</i>
365	F	17 -30
368	M	27 -69
377	F	30-50
378	F	34-67
391	M	25-57
395	M	1725
397	M	32-50
404	F	-
406	F	23-30
409	M	23-36
410	M	31-63
411	F	-
416	F	26-50
421	M	20-29
446	F	25-42
448	F	17-26
464	F	21-32
471	M	23-46
473	F	28-54
475	F	15-22
485	F	16-26
487	F	15-20
492	M	30-57
500	M	28-49
503	M	20-45
504	M	20-40
505	F	35-69
510	M	27-45
607	M	29-51
611	M	15-25
<i>Total M</i>	15	
<i>Total F</i>	15	

Texas State Donated Skeletal Collection

The TXSTDSC is composed of donated individuals housed at Texas State University who donated themselves or were donated by their legal next-of-kin under the Texas Anatomical gift Act. All individuals housed in the collection are accompanied by self-reported medical and demographic information, and are therefore useful for studying pathologies with documented context.

Like OpID, no destructive analysis was undertaken on the TXSTDSC samples. Individuals were assessed for presence of mandibular canines. If too much wear was present or the tooth was damaged, it was not included in this analysis. The resulting final TXSTDSC sample size was 29 individuals: 3 Hispanic females, 3 Hispanic males, 13 White females, and 10 White males (see Table 3.3). All mandibular canines were photographed using a DinoLite™ and measurements were taken of LEH. Teeth were then taken to the MSEC ARSC for SEM imagery and perikymata/microdefect measurement.

Table 3.3 Individuals sampled from the Texas State Donated Skeletal Collection.

<i>ID</i>	<i>Sex</i>	<i>Ancestry</i>	<i>Age at Death</i>
<i>D40-2012</i>	F	H	67
<i>D01-2014</i>	F	H	72
<i>D28-2014</i>	F	H	68
<i>D01-2008</i>	M	H	81
<i>D08-2010</i>	M	H	67
<i>D54-2014</i>	M	H	52
<i>D04-2011</i>	F	W	68
<i>D21-2011</i>	F	W	56
<i>D03-2012</i>	F	W	78
<i>D04-2012</i>	F	W	63
<i>D35-2012</i>	F	W	63
<i>D36-2012</i>	F	W	42
<i>D09-2013</i>	F	W	45
<i>D17-2013</i>	F	W	47
<i>D11-2014</i>	F	W	46
<i>D07-2009</i>	F	W	65
<i>D08-2009</i>	F	W	53
<i>D09-2009</i>	F	W	58
<i>D04-2010</i>	F	W	53
<i>D07-2010</i>	M	W	46
<i>D12-2010</i>	M	W	54
<i>D01-2011</i>	M	W	40
<i>D25-2012</i>	M	W	44
<i>D27-2014</i>	M	W	56
<i>D57-2013</i>	M	W	54
<i>D03-2009</i>	M	W	32
<i>D10-2010</i>	M	W	32
<i>D60-2014</i>	M	W	59
<i>D20-2012</i>	M	W	34
<i>TOTAL M</i>	13		
<i>TOTAL F</i>	16		
<i>TOTAL H</i>	6		
<i>TOTAL W</i>	23		

Age-at-Formation Measurements

The age-at-formation of LEH on all teeth was calculated using a newly published exponential regression method (Cares Henriquez & Oxenham, 2018) which accounts for the curvilinear nature of the tooth surface and cuspal enamel formation. Previous iterations of this method, namely the linear regression method, did not account for these issues, and thus underestimated age by months. Using ImageJ™ all macro and micro LEH defects on PfbAP, OpID, and TXSTDSC teeth were marked on the buccal surface and a distance was taken from that mark to the cervico-enamel junction (CEJ). This distance was placed into the equations created using the cross-striation count of a Northern European population and a South African population (Reid & Dean, 2006). While the linear regression method accounts for population specific crown height sizes, it does not account for imbricational and cuspal enamel formation and the curvilinear tooth surface (Cares Henriquez & Oxenham, 2018). OpID, TXSTDSC, and PfbAP individuals are neither of these populations and the ages produced by the two equations were therefore averaged. While the white individuals from the TXDSC could be argued to fit the Northern European equation, they are likely too heterogenous in tooth development as developmental rates of enamel can vary significantly between groups of people (Hillson, 2014).

Table 3.4 Equations for age-at-formation of LEH and AS on mandibular canines. From Cares-Henriquez and Oxenham (2018).

Population	Equation	R ²	S
Northern European	$\text{Age}=6.2e^{-0.141x}$	0.9969	0.11
South African	$\text{Age}=5.2e^{-0.128x}$	0.9976	.08

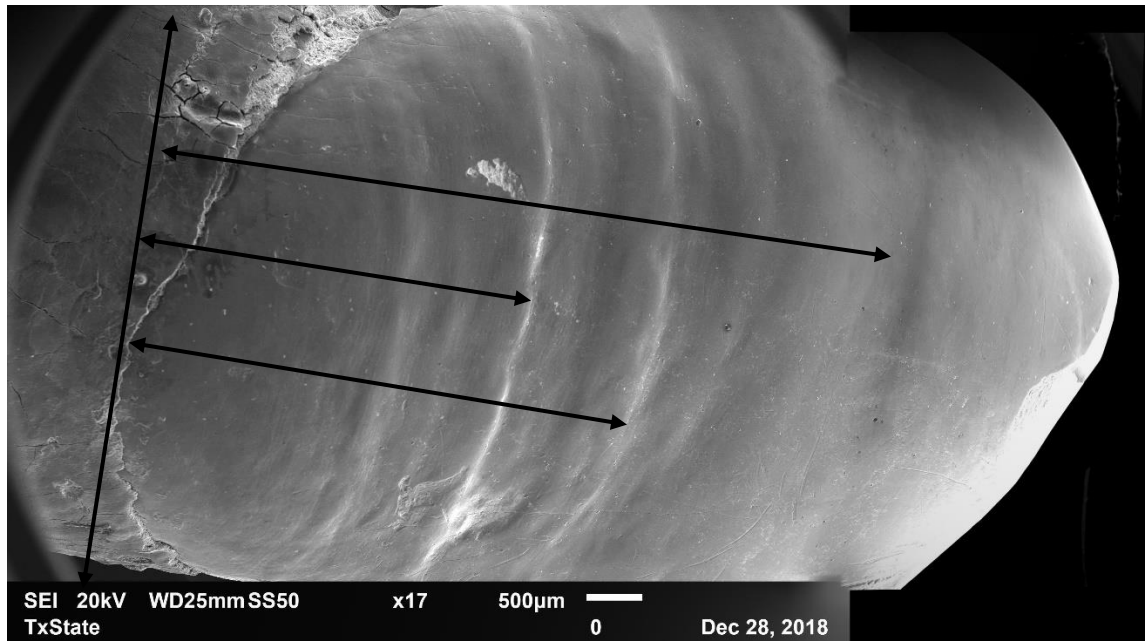


Figure 3.1 Example of measurement taking process for LEH and AS age-at-formation data. The image is of a mandibular left canine, the CEJ on the left-hand side. Arrows/lines show how LEH was measured. An effort was always made to keep lines perpendicular to the tooth midline (the imaginary line going through the midpoint of the CEJ and the very point of the enamel crown). LEH and AS distances were plugged into x of the equations in Table 3.4. (D01-2008 from the TXSTDSC)

Statistical Analysis

The prevalence of LEH and AS are nominal statistical data. I will be counting LEH and AS and scoring whether they are absent or present. This count data can be statistically analyzed using chi-squares, since the samples are equal sizes. To assess if there are statistically significant differences between age-at-formation of LEH and AS, I performed t-tests for equal and unequal variances between the three populations under study depending on Levene's test, and within the population samples to observe differences between sex and age cohorts.

IV. RESULTS

Overall, macro and micro LEH counts were observed in all three population samples. More micro LEH was observed than macro LEH, and macro and micro stress events created a more robust stress profile of the mandibular canine than observation of macroscopic LEH alone – since most observations of macro LEH are on the cervical third of the tooth. Age-at-formation of LEH was statistically tested using the Northern European equation and South African equation provided by Cares Henriquez and Oxenham (2018) and statistically significant results remained the same between both equations. Therefore, all data presented in this chapter was created using the averages of the two ages provided by the two equations rather than presenting the results of both equations. Other results are presented according to study population and the section concludes with a consideration of ancient, modern, and forensic data together.

PfBAP

Out of all PfBAP individuals, 21 out of 31 (68%) had macroscopic LEH on the mandibular canines. This number increased for microscopic LEH: 29 out of 31 (94%). Smaller sites (Medicinal Trail, Guijarral, El Intruso, and Barba Group) experienced fewer incidence of macro or microscopic LEH with 6 out of 10 (60%) possessing macroscopic LEH (longer stress events).

Age-at-formation of LEH was initially analyzed by site or site groupings (higher population versus lower population densities) and descriptive statistics are presented in Table 4a. Medicinal Trail was grouped with Guijarral, El Intruso, and Barba Group as these are the smallest sites. Dos Barbaras, Dos Hombres, and La Milpa were separate

groups. Ultimately, this resulted in 4 groups. 1. Medicinal Trail, Guijarral, El Intruso, and Barba Group, 2. Dos Barbaras, 3. Dos Hombres, and 4. La Milpa. An ANOVA indicated that age-at-formation differences between the four sites/groupings was statistically significant (p-value= .0011). T-tests were calculated for each pair of groupings/sites and differences between all pairs were found to be statistically significant except between Dos Barbaras and Dos Hombres with a p-value of .21. These results are presented in Figure 4.2 and Table 4.1.

Table 4.1 Table showing the p-values for paired groups. The only non-significant p-value was between the age-at-formation results of Dos Hombres and Dos Barbaras.

	MT, GJ, EI, BG	Dos Barbaras	Dos Hombres	La Milpa
<i>MT, GJ, EI, BG</i>	x	.047	.0415	.0104
<i>Dos Barbaras</i>	.047	x	.21	.0001
<i>Dos Hombres</i>	.0415	.21	x	.0028
<i>La Milpa</i>	.0104	.0001	.0028	x

Age-at-formation of the first stress episode differed slightly between males and females (Figure 4d). Levene's test indicated that the variances were equal; a t-test for equal variances resulted in an insignificant result. Age-at-formation of all stress episodes by sex is presented in Figure 4c. Levene's test indicated that the variances were equal and so a t-test for equal variances was conducted. The results were not statistically significant (p-value=.12). However, there is a small difference visible in Figure 4c, Figure 4d, and Table 4a where the mean age-at-formation of females for all stress episodes is slightly higher than that experienced by males.

Duration of stress events was the largest at La Milpa and Dos Hombres, shorter at Dos Barbaras, and the shortest at Medicinal Trail and the other small sites in this group.

However, these results were not significant when run through an ANOVA and t-tests.

Results for duration of stress events by site is presented in Figure 4e.

Table 4.2. PFBAP age-at-formation of stress event descriptive statistics data pooled and by site.

<i>Sample</i>	Mean	Mode	Minimum	Maximum	Skew
<i>Pooled</i>	2.99	2.5	1.3	5.7	0.29
<i>La Milpa</i>	2.34	2	1.3	5.1	1.20
<i>Medicinal Trail *</i>	2.77	1.8	1.311	4.7	0.40
<i>Dos Hombres</i>	3.12	3.44	1.35	5.2	0.04
<i>Dos Barbaras</i>	3.28	4.3	1.4	5.7	0.12

**El Intruso (Gateway), Barba Group, and Guijarral are grouped with Medicinal Trail*

Operation Identification

Out of 30 individuals, 29 (97%) possessed at least one macroscopic stress event and 28 (93%) possessed at least one microscopic stress event. No single individual was free from a childhood stress episode. Descriptive statistics of all OpID individuals (pooled) and calculations by sex are represented in Table 4b and an illustration is presented in Figure 4e.

Age-at-formation of LEH was primarily considered through the lens of sex because of the lack of other contextual information. First age-at-onset differed between males and females (Figure 4g) with females experiencing the first stress event around 2 years old and males around 2.6 years old. Figure 4e illustrates these results in a box-and-whisker plot. Levene's test indicated that the variances were unequal. Subsequently, this result was statistically significant after a t-test for unequal variances yielded a p-value of .001. In addition, there was a much smaller range of age-at-formation than males. No statistically significant differences existed between age-at-death and age-at-formation of the first stress episode.

Duration of stress episodes between males and females was statistically considered. Levene's test indicated equal variances. A t-test for equal variances resulted in these data being statistically insignificant. These results are presented in Figure 4h.

Table 4.3. OpID age-at-formation of stress event descriptive statistics data pooled and by sex.

Sample	Mean	Mode	Min	Max	Skew
<i>Pooled</i>	3.21	2.99	1.74	5.7	0.3
<i>Male</i>	3.86	2.8	1.74	5.7	-0.1
<i>Female</i>	3	2.4	1.7	5.4	0.5

TXSTDSC

Out of 29 individuals analyzed from the TXSTDSC, 10 possessed macroscopic LEH (34%) and 24 possessed microscopic LEH (83%). This count data is represented in Figure 4i. By ancestry, 4 out of 6 (67%) Hispanic individuals had macroscopic LEH present and 6 out of 6 (100%) had microscopic LEH, while 6 out of 23 (26%) White individuals possessed a macroscopic LEH and 18 out of 23 (78%) had microscopic LEH. Descriptive statistics are presented in Table 4c.

Age-at-formation of the first stress episodes were statistically significantly different between males and females from the TXSTDSC, with a p-value of .04 after Levene's indicated that a t-test for unequal variances should be used. Males had a noticeably lower age-at-defect formation for the first insult experience during early childhood and a smaller age-range during which the insults are occurring (Figure 4j). When all stress-episodes are considered, males and females are only slightly different with an average age-at-formation of 3.34 and males 3.13 years (Figure 4k).

Hispanic individuals from the TXSTDSC also experienced the first insult at a younger age than White individuals with a smaller age-range during which stress is experienced (Figure 4l). This difference was found not to be statistically significant after a t-test for unequal variances determined the p-value to be .06. Figure 4m shows the difference between age-at-formation of LEH between Hispanic and White individuals from the TXSTDSC. Again, this is only a slight difference. Levene's test indicated equal variances and a t-test for equal variances found this difference to not be statistically significant (p-value=.16).

Figure 4n shows the age-at-formation of all stress episodes by self-reported childhood socioeconomic status. Any difference observed in Figure 4n was considered statistically insignificant after an ANOVA was performed (p-value=.26). However, individuals self-reporting as middle class tended to have an average age-at-formation of LEH higher than the other groups and almost equal with upper-middle class individuals.

Table 4.4. TXSTDSC age-at-formation of stress event descriptive statistics data pooled by sex.

Sample	Mean	Mode	Min	Max	Skew
<i>Pooled</i>	3.24	4.07	1.54	5.46	0.41
<i>Female</i>	3.34	2.83	1.81	5.46	0.43
<i>Male</i>	3.13	2.98	1.54	4.85	0.21
<i>Hispanic</i>	3.03	2.83	1.52	5.29	0.67
<i>White</i>	3.41	4.01	1.91	5.46	0.25

Overall Comparisons

Overall, modern White individuals from the TXSTDSC had the least linear hypoplastic defects and OpID individuals possessed the most. Out of all sub-populations, individuals from the larger La Milpa site had the earliest first incidences of LEH (Figure 4s) and when comparing females and males from all populations, ancient Maya females

were experiencing the first stress events at the youngest age (Figure 4r). It seems that the first age-at-formation of any stress event among the Maya (excluding La Milpa) is around 2 years old.

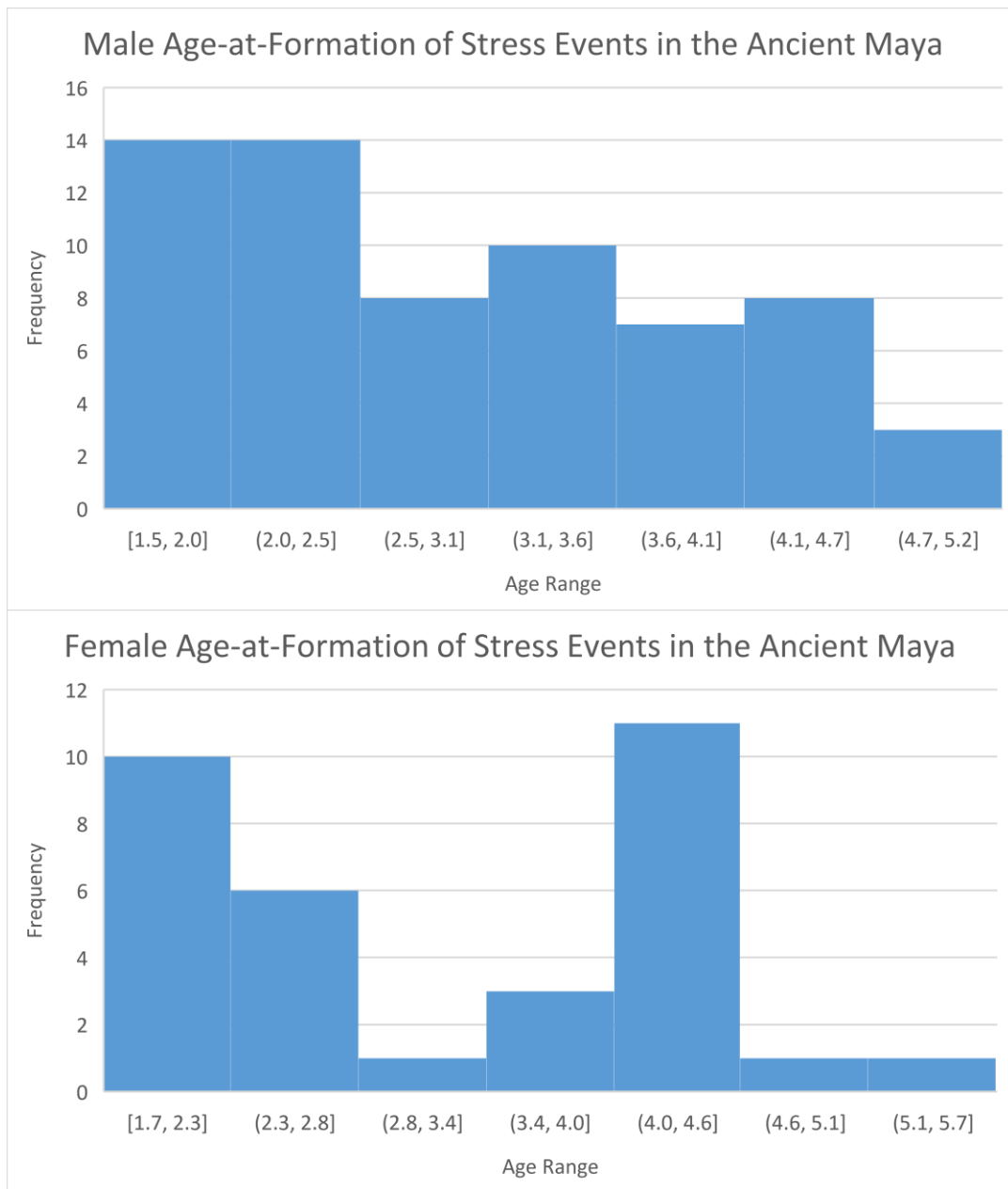


Figure 4.1 Histograms of total number of stress events experienced by male and female individuals of the PfBAP burial collection.

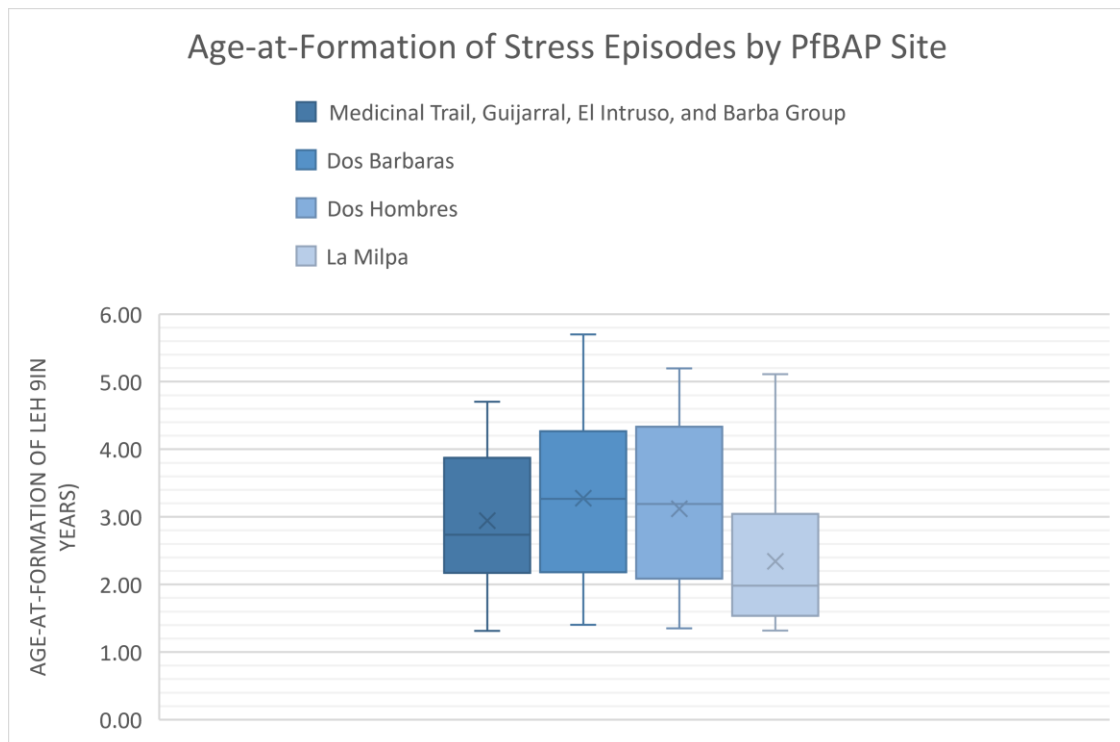


Figure 4.2 Age-at-formation of LEH between small sites (Medicinal Trail, Guijarral, El Intruso, and Barba Group) and medium to larger sites.

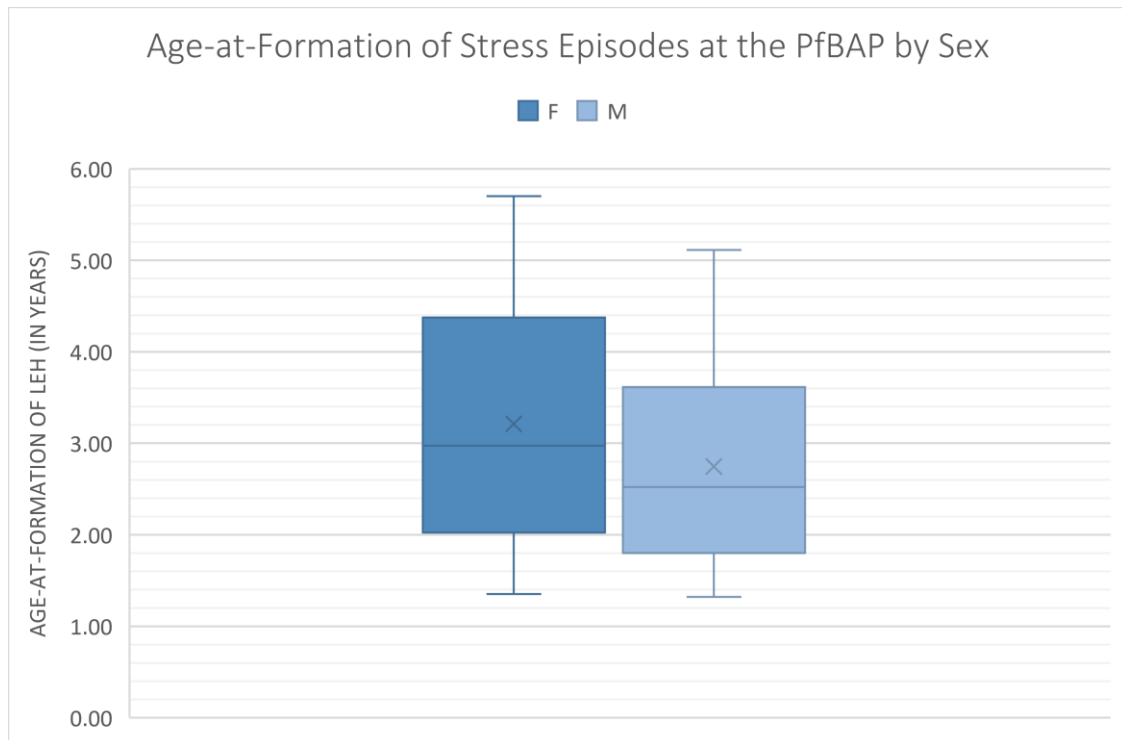


Figure 4.3 Age-at-formation of LEH at the PFBAP considered by sex.

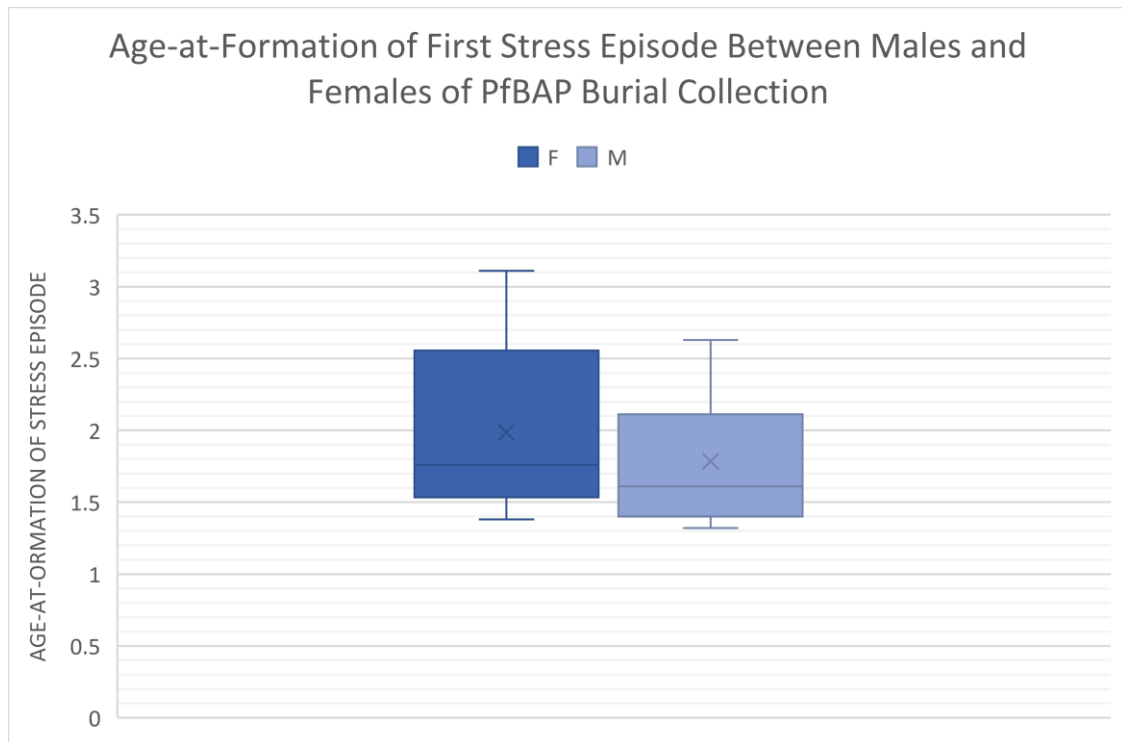


Figure 4.4 Age-at-Formation of the first stress episode between males and females at PfBAP.

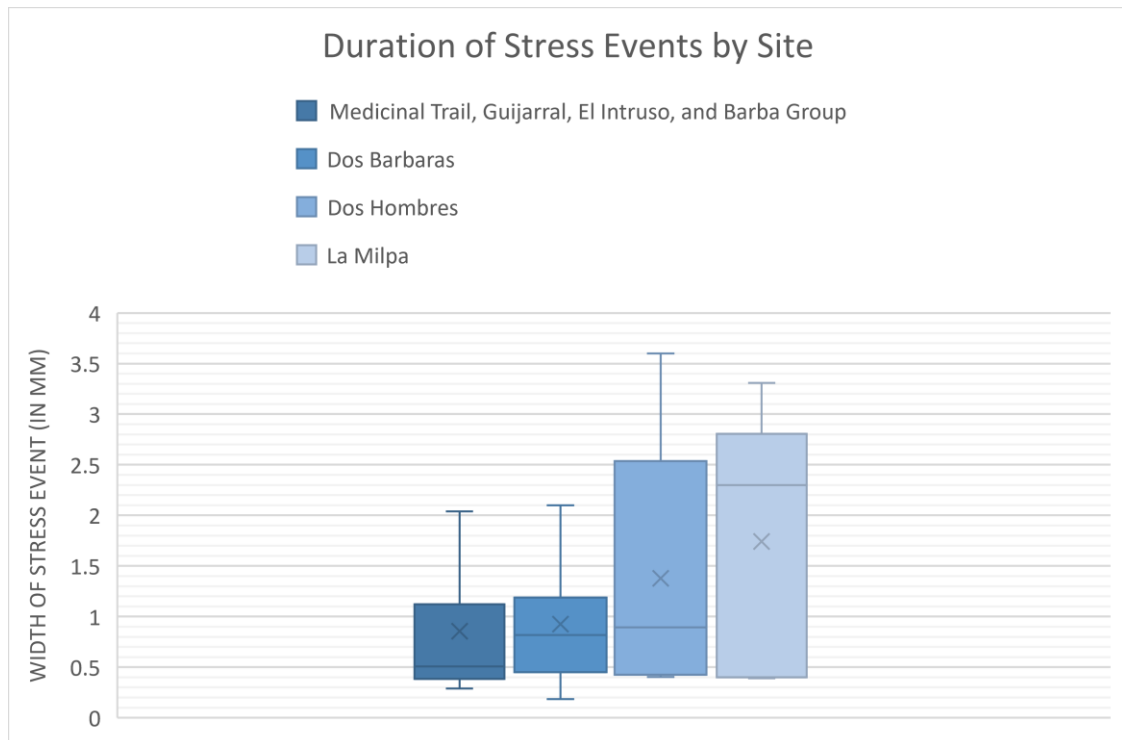


Figure 4.5 Duration of stress episodes at the three medium to larger sites and at smaller sites which are grouped (Medicinal Trail, Guijarral, and Barba Group).

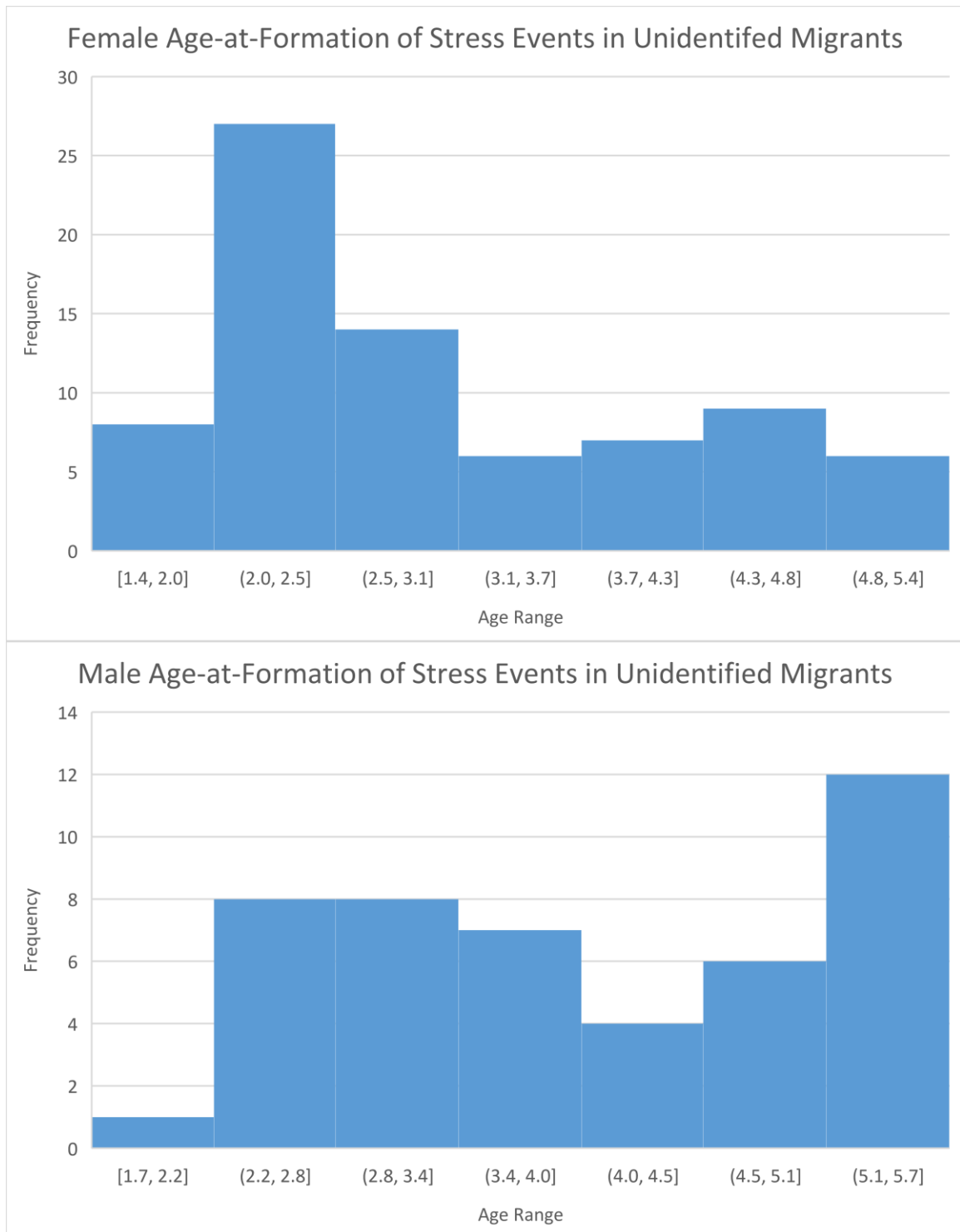


Figure 4.6 Histograms of number of total stress events experienced by males and females of Operation Identification.

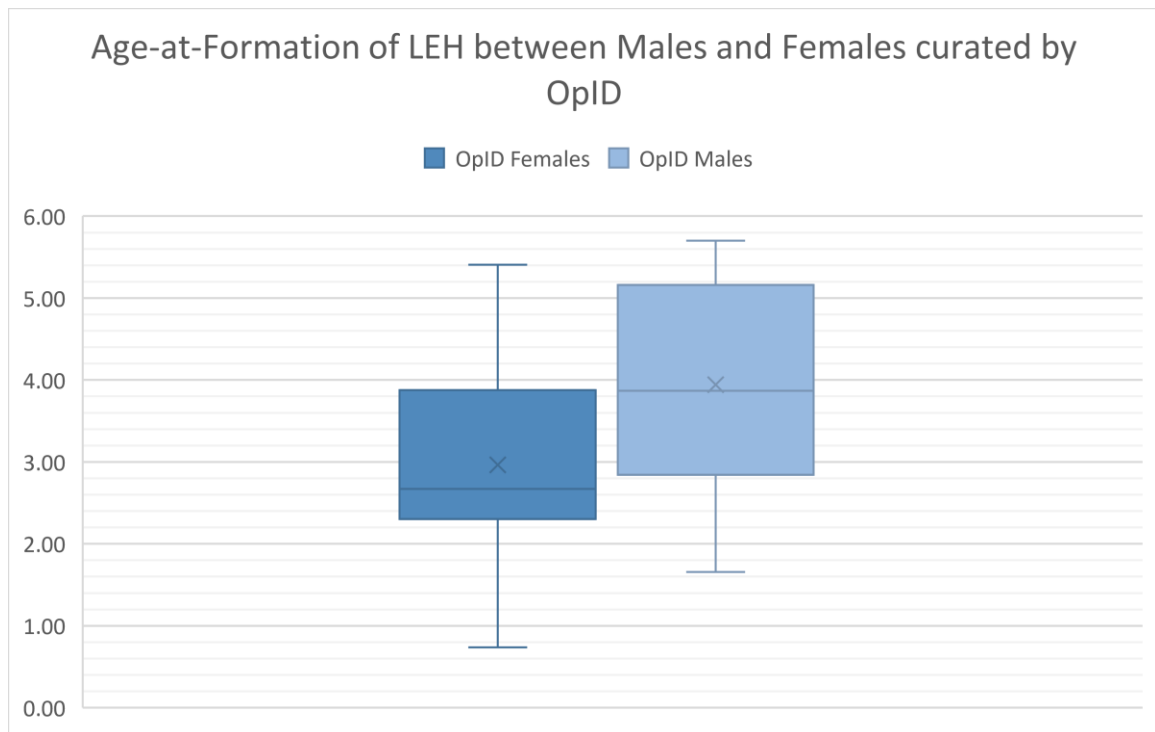


Figure 4.7 Age-at-formation of the first stress episode in males and females curated by Operation Identification at Texas State University.

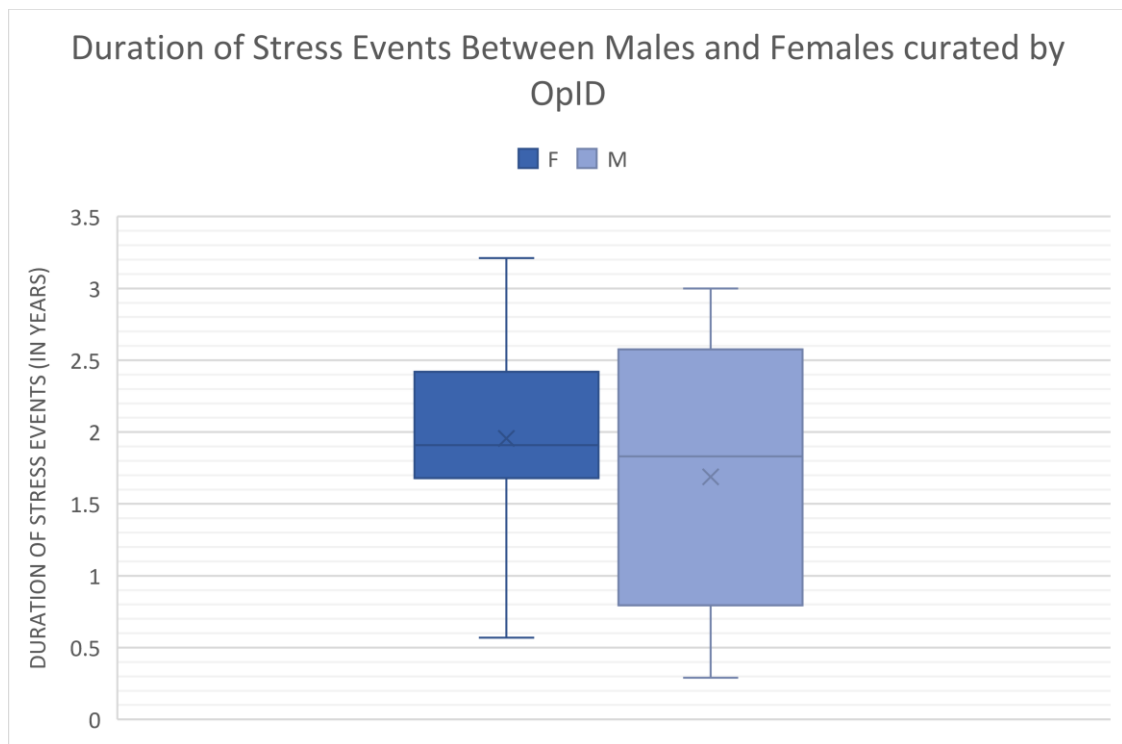


Figure 4.8 Duration of stress events between males and females curated by Operation Identification at Texas State University.

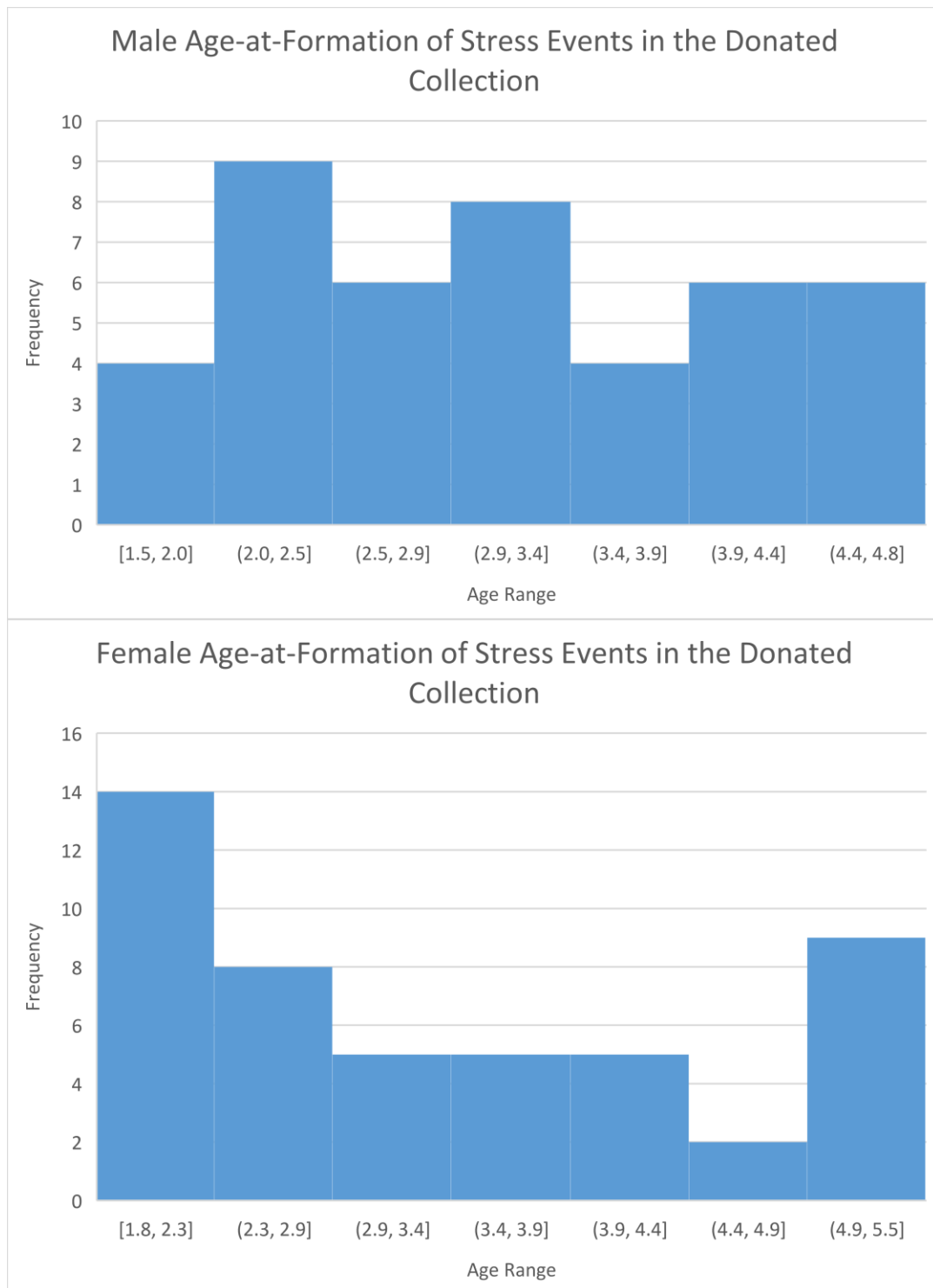


Figure 4.9 Histograms of total number of stress events by sex in the TXSTDSC.

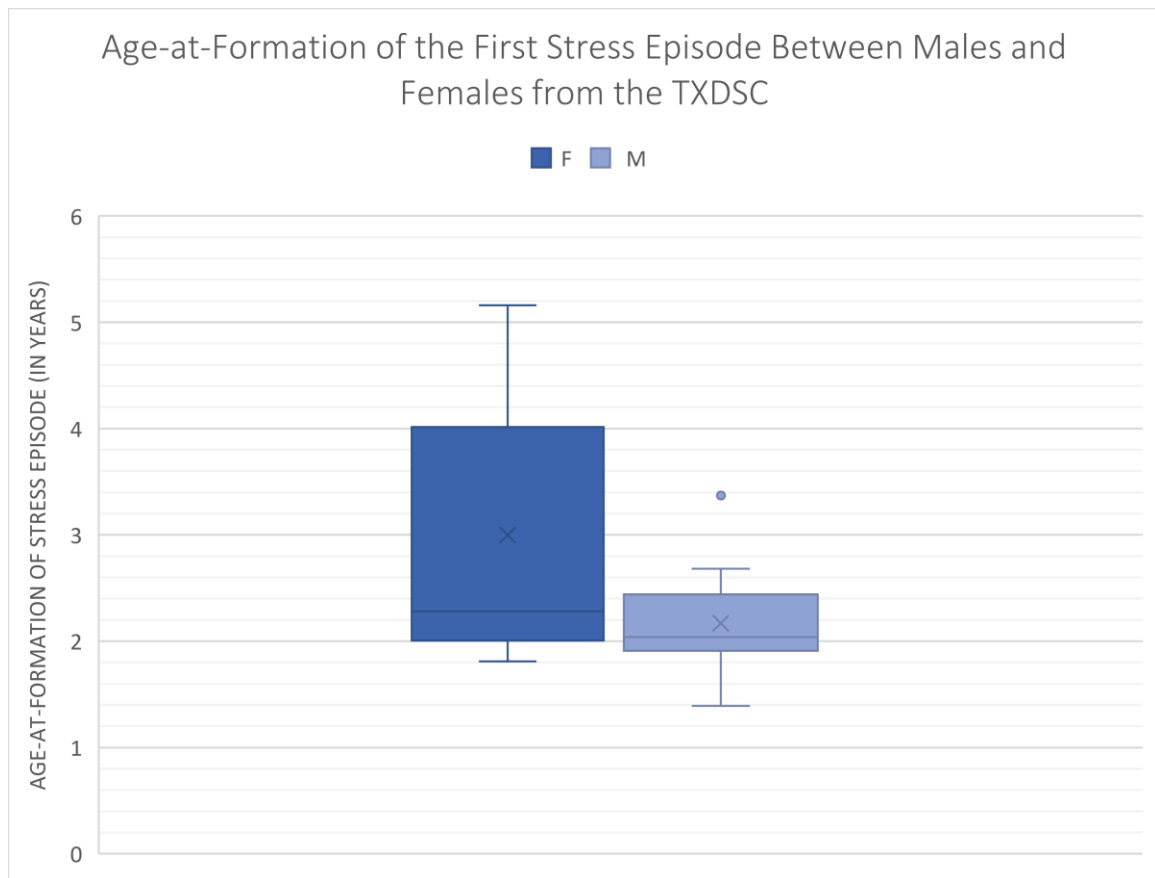


Figure 4.10 Age-at-formation of the first stress episode in males and females from the TXSTDSC.

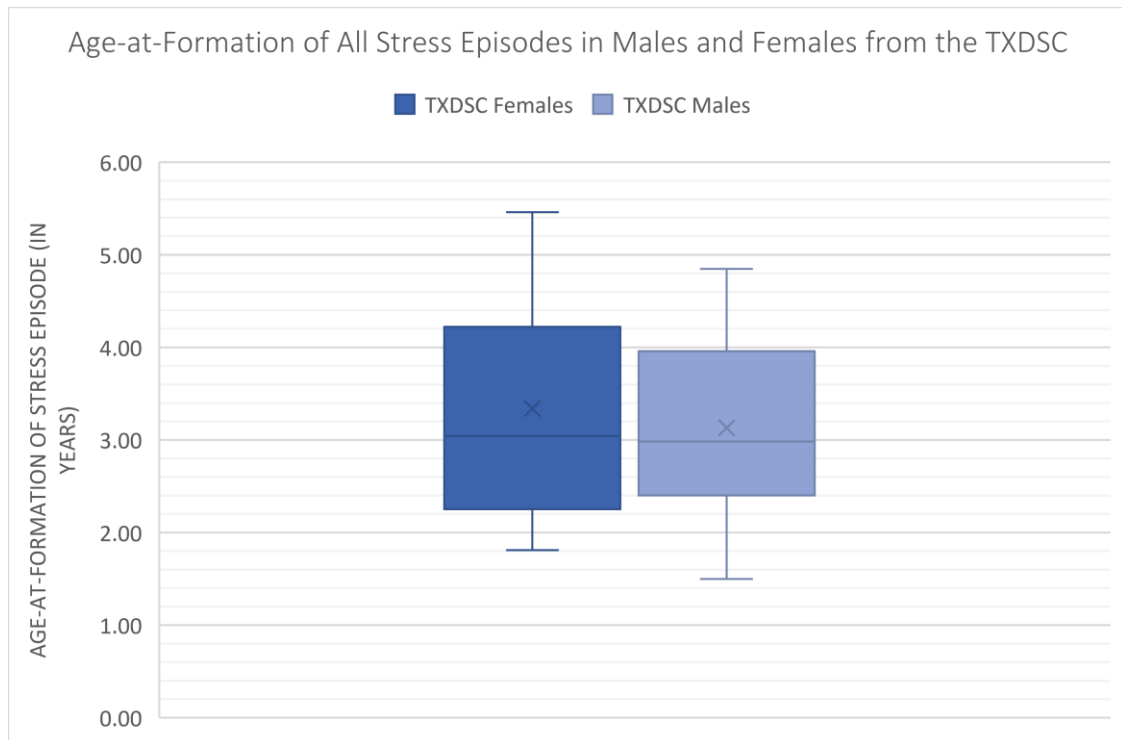


Figure 4.11 Age-at-formation of all stress events in males and females from the TXSTDSC.

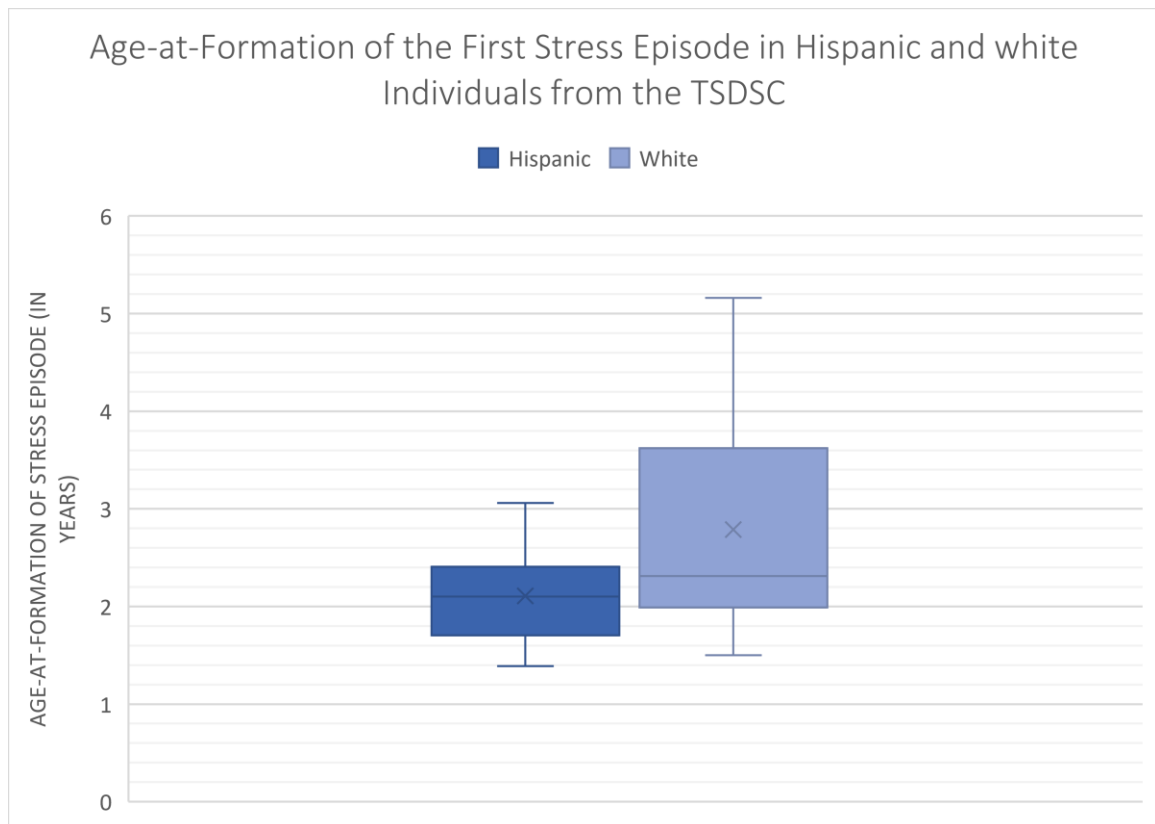


Figure 4.12 Box-and-whisker plot of the age-at-formation of the first stress episode in Hispanic and White individuals in the TXSTDSC.

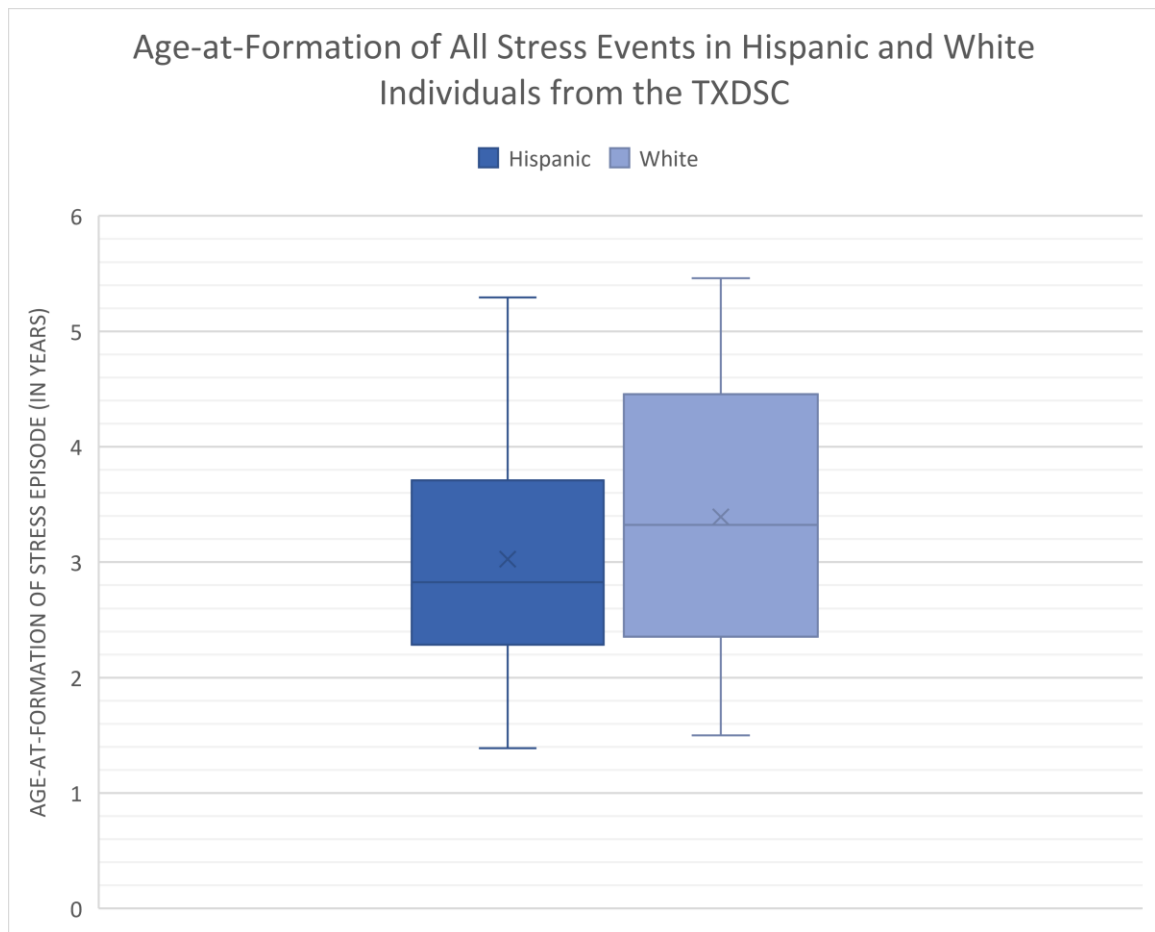


Figure 4.13 Box-and-whisker plot of the age-at-formation of all stress episodes in Hispanic and White individuals from the TXSTDSC.

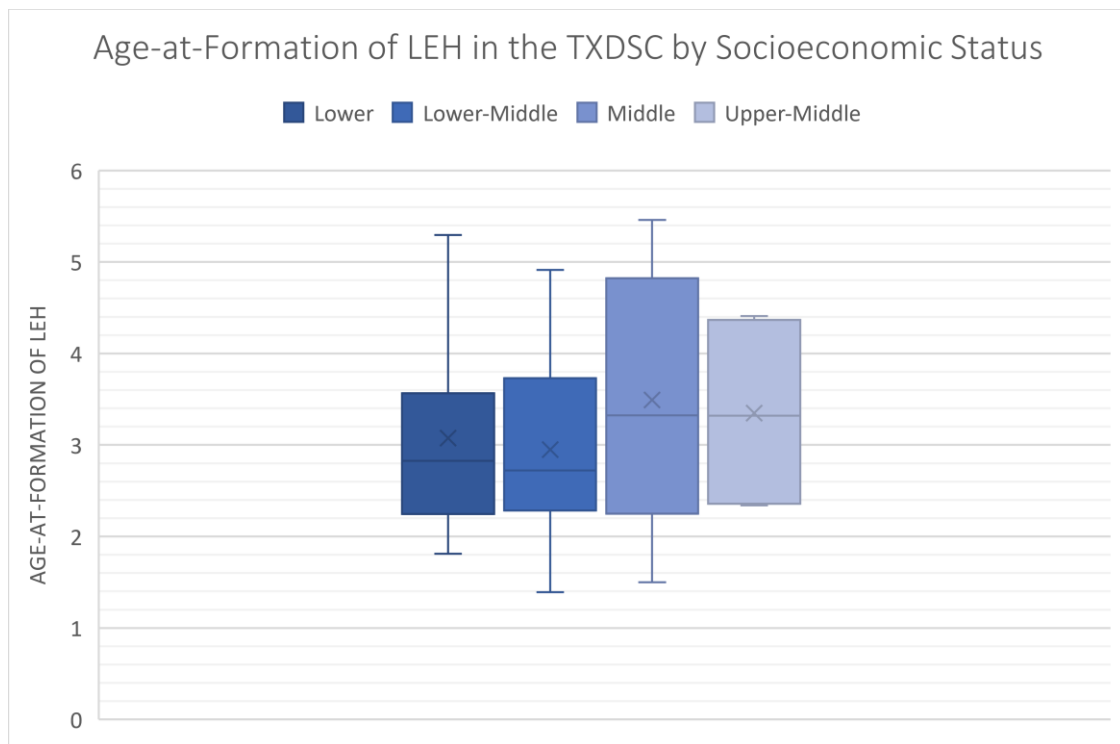


Figure 4.14 Box-and-whisker plot of age-at-formation of all stress episodes between individuals of a certain self-reported socioeconomic status.

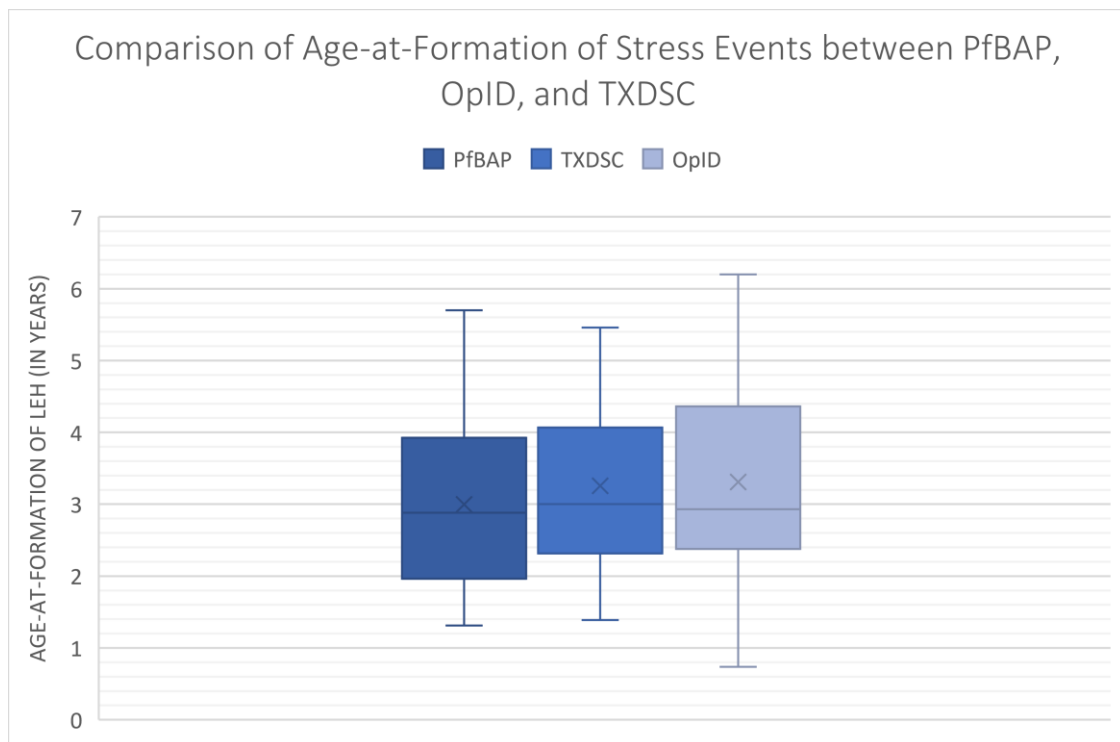


Figure 4.15 Box-and-whisker plot of ages-at-formation of all stress events between PfBAP, OpID, and the TXSTDSC.

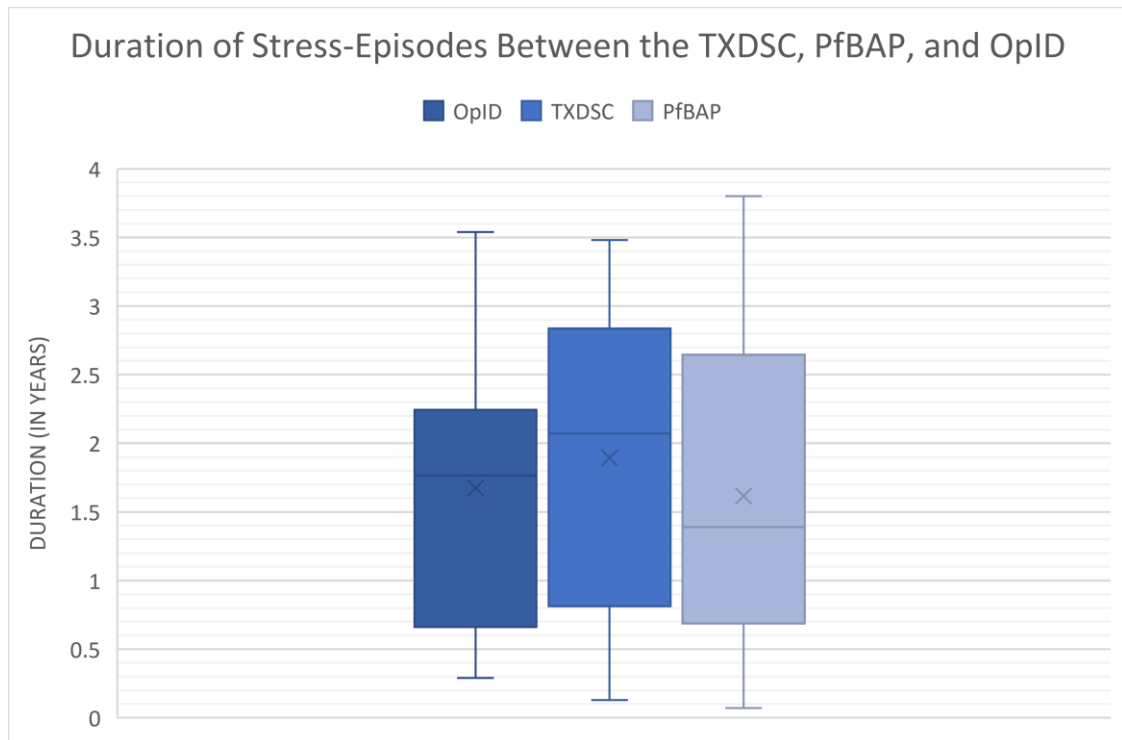


Figure 4.16 Box-and-whisker plot of duration of stress episodes between the TXSTDSC, PfBAP, and OpID.

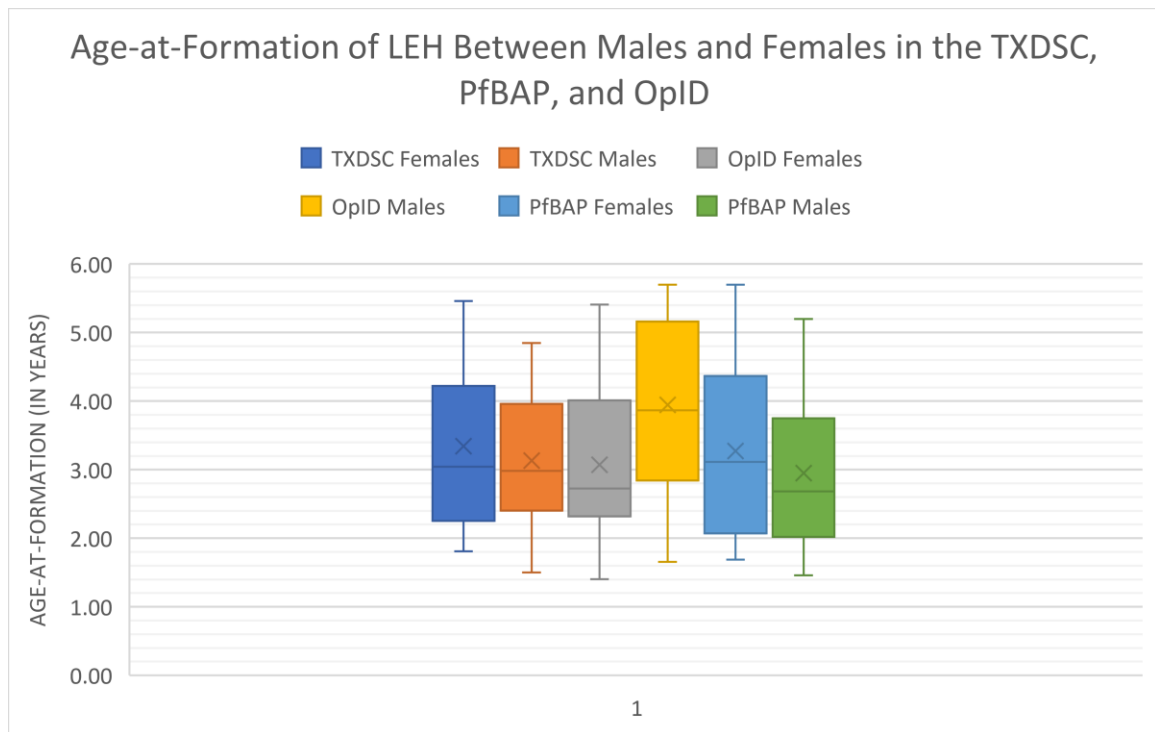


Figure 4.17 Box-and-whisker plots of all ages-at-formation of stress events between males and females of the TXSTDSC, PfBAP, and OpID.

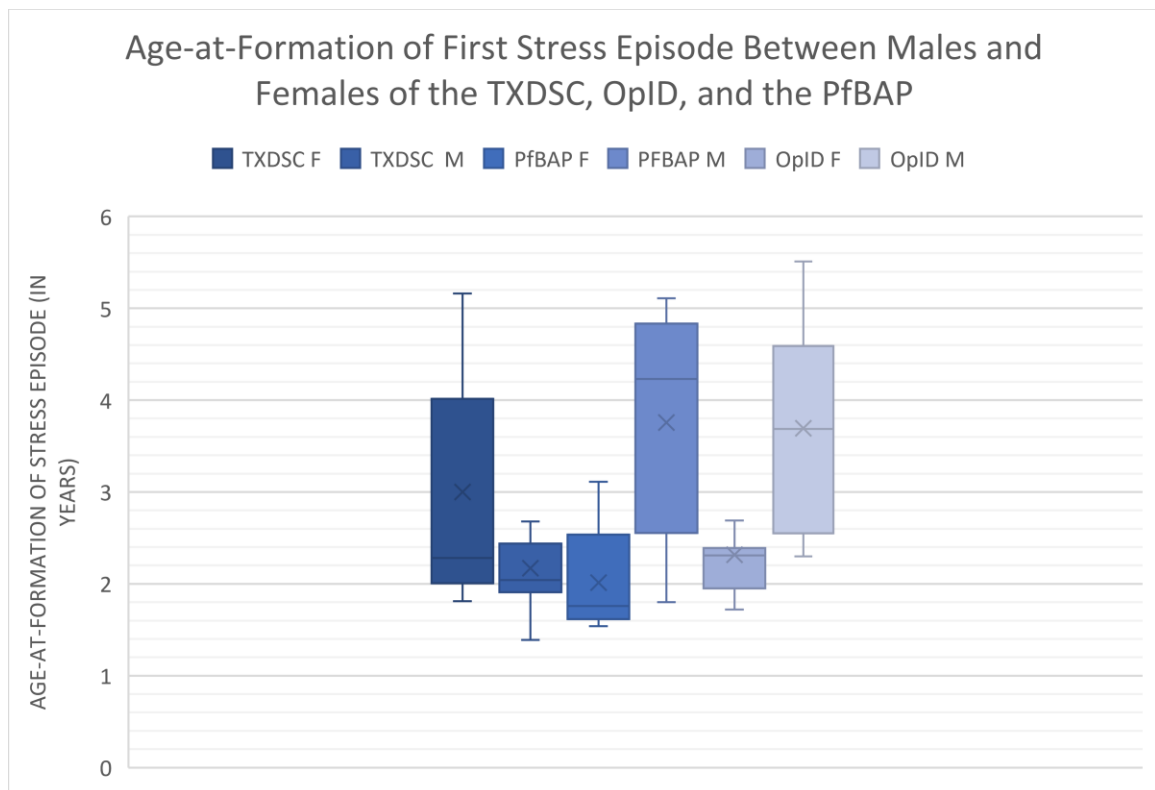


Figure 4.18 Box-and-whisker plot comparing age-at-formation of the first stress episode between males and females of the PfBAP, OpID, and the TXSTDSC.

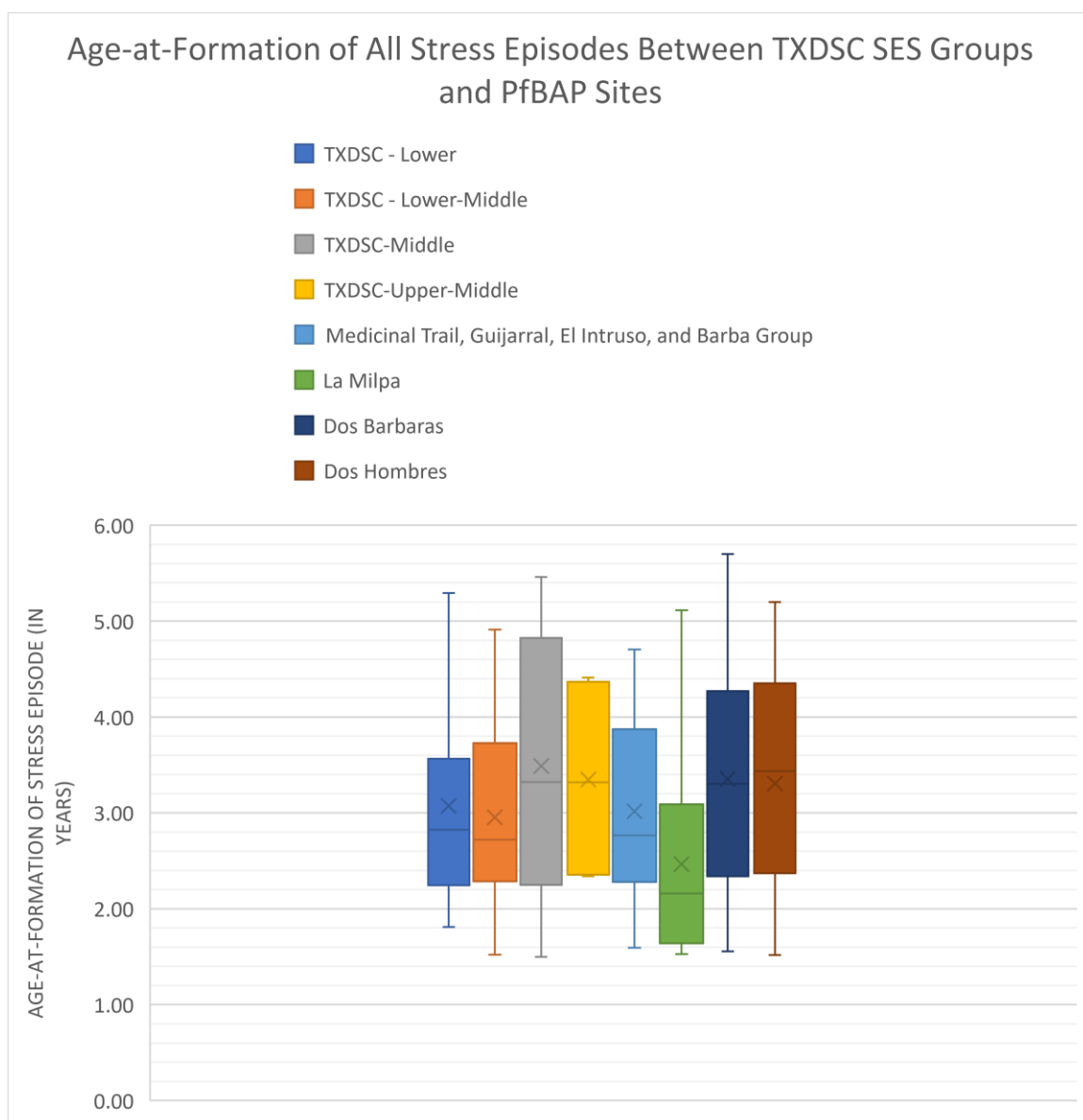


Figure 4.19 Box-and-whisker plots comparing age-at-formation of stress events among SES groups in the TXSTDSC and sites analyzed from the PfBAP.

V. DISCUSSION

The mandibular canine begins forming between 1 to 2 years of age and the enamel crown is finished forming by 7 to 8 years, depending on the population and innate biological variation. Because of the utilization of only mandibular canines in this study, stress events discussed in this chapter occurred only within this age range. One of the most important life course events, weaning, occurs during this time frame and can be potentially harmful to the body of a child if undertaken during adverse environmental events. In theory, when infants begin the process of transitioning from breastmilk to an adult diet of solid food, they are at an increased risk of disease/infection and possibly malnutrition, since many weaning foods are not nutritionally adequate. It is widely accepted that breastfeeding a child affords many benefits, as breastmilk contains the mother's antibodies, lymphocytes, immunoglobins, and resistance to the common bacterium *Staphylococcus aureus* (Hanson, 1998; Hanson et al., 2003; Herring et al., 1998; Katzenberg et al., 1996). I argue that the first stress events observed in all three populations are likely due to the initiation or cessation of the weaning process, whether caused by weaning foods or increased susceptibility to illness. This can lead to increased morbidity and mortality.

However, I also acknowledge the hidden heterogeneity in not only the stress experience (Wood et al. 1991) but also in the weanling experience. Individual children have different needs and trajectories in the initiation and cessation of the weaning process. This process, if stressful, will also affect children differently according to a sliding scale of frailty. In modern populations, including the unidentified migrants and the donated individuals analyzed for this thesis, the possibility that they were formula-fed

is also a very real possibility and must be considered. The possible differential affects of these two methods on life trajectories is unclear.

PfBAP

The ancient Maya that lived in smaller archaeological sites in the PfBAP such as Medicinal Trail, Guijarral, El Intruso, and Barba Group experienced fewer stress events overall *and* experienced shorter duration of stress episodes while those at the larger ceremonial sites of La Milpa and Dos Hombres experienced more perturbations and increased duration of stress events. This data corroborates with other studies that look at population density in the ancient world and its effect on skeletal biology. The best known theory is an examination of the effects of agricultural intensification on population density, diet, and health (Armelagos & Cohen, 1984; Cohen, 2009). Complex agricultural economies create larger populations which can also lead to more complex sociopolitical structures (Cohen, 2009). This confluence of events leads to increases in infectious disease rates and metabolic disorders, particularly affecting the youngest demographic of the population (Armelagos & Cohen, 1984; Armelagos, Goodman, & Jacobs, 1991; Eshed, Gopher, Pinhasi, & HersHKovitz, 2010; Hodges, 1987; Lukacs, 1992; Wright & White, 1996).

Among the ancient Maya, a great deal of evidence has been published that validates this relationship. Based on calculations of agricultural carrying capacity and presence of house sites, population in the ancient Maya lowlands is hypothesized to have been dense, especially at the larger ceremonial centers, and only increased during the Classic period (B. L. Turner, 1976; Wright & White, 1996). The population density at La Milpa was likely typical of other similar sized Maya centers (Scarborough, Becher,

Baker, Harris, & Valdez, 1995). Danforth (1997) analyzed enamel microdefects from three sites of ranging population density and found the most perturbations associated with the largest site with likely the highest population density at Tikal and the least at Barton Ramie in Belize. Population density in general is often cited as one of the causal factors of the ancient Maya collapse (Wright & White, 1996).

It is therefore likely that individuals living at the larger sites of La Milpa and Dos Hombres were experiencing the same sort of physiological stressors as those of other population dense sites and this is reflected in their stress episode prevalence and duration. Not only were they experiencing more stress events, they were also not overcoming the stress episodes as quickly as their neighbors at the less-dense sites. Cultural buffering and resilience was therefore affected by the environment of La Milpa and Dos Hombres. While explanatory power of prevalence is affected by the Osteological Paradox (Wood et al., 1992), the undeniable increase in duration of stress episodes shows that something at La Milpa and Dos Hombres was affecting the ancient Maya living there in ways that were not occurring at the smaller sites examined in this thesis. Somerville et al. (2013) conducted a stable isotope meta-analysis of individuals recovered from metropolitan “elite” sites and hinterland “commoner” sites and found that, in general, commoner diet seemed to be consistent through time, as they likely had easily accessible agricultural resources while elites were more variable through time and at some points may not have been adequately nourished. This was attributed to the reliance of the elite in metropolitan centers on the import of agricultural resources from the hinterlands, which can fluctuate in response to adverse climatic events (*ibid.*). While the terms “elite” and “commoner” are problematic (there were likely laborers/commoners living in the metropolitan

centers), this is also a study of population density, since commoner data came from less dense hinterland sites. This argument may also explain the dental defects found at La Milpa and Dos Hombres, since residents of these sites may have been chronically malnourished starting in childhood because of this dependence on food imported from other locations.

These conclusions could be strengthened with the inclusion of more teeth and an increased sample size (and therefore a wider range of ages) since this study is based on small sample sizes that only included twelve mandibular canines from Dos Hombres and La Milpa and ten canines from the smaller sites of Medicinal Trail, Guijarral, El Intruso, and Barba Group.

Age-at-formation of the first stress episode and all stress episodes was different between sites. Individuals from the La Milpa site experienced the first stress episode at a significantly younger age than those from other sites, including Dos Hombres, while the smaller sites of Medicinal Trail, Barba Group, El Intruso, and Guijarral experienced later first stress episodes. First stress episodes in early childhood can be interpreted as the post-weaning period (Corruccini et al., 1985) which in this case can be either culturally prescribed or biologically mandated by physiology or the environment. In general, the age-at-formation data of stress episodes seemed to coincide with the carbon and oxygen stable isotope data presented by Wright and Schwarcz (1998) where solid foods began to be incorporated into the diets of Maya children before or around 2 years of age. Given that La Milpa was experiencing more stress episodes and greater duration of stress episodes, this earlier age-at-onset of stress events could be due to the general nutritional stress of the population – including the mothers that would be breastfeeding. If La Milpa

children were being weaned earlier than infants in other Maya hinterland communities, malnourishment of the mother could prevent the production of breastmilk altogether which would force early weaning practices (Herring et al., 1998; Katzenberg et al., 1996). Alternatively, cultural norms at La Milpa could have enforced this early weaning period, although this may be unlikely because this data is not emulated at other sites. However, commoner culture and ritual has been shown not to be a reproduction of elite ritual practices (Blackmore, 2011). Just like in recent history (Wolf, 2003), breast milk may have been deemed sub-par to other forms of nutritional supplementation, such as corn gruel, especially among the upper classes, and this may be reflected in the stress experience.

Females were also shown to have experienced slightly later first age-at-formation of stress episodes than males and in general, the range in age-at-formation of all stress events was later. This can potentially elucidate gendered childcare practices among the ancient Maya of northwest Belize. Gendered foodways have been reported among the ancient Maya; White (2005) uses ethnohistoric and archaeological evidence to show that commoner women and men consumed similar foods (and therefore have similar isotopic signatures) while elite women did not consume the same variety of foods as elite males. This finding highlights some form of gender differences in nutritional uptake. It is known that the Maya, like most other groups of people, developed gender roles related to production of commodities and even placed women and men in different spaces (Hendon, 1997). Eerkens and Bartelink (2013) found similar findings to this thesis in a prehistoric site in Central California. Female children seemed to have been weaned later than male children, which the authors argue is a case of preferential treatment of girls initially,

although stable isotope ratios showed that children were weaned onto a gendered diet: with females eating primarily plant foods and males eating primarily meats and fish. While no published stable isotope data exists as of yet for the individuals studied for this thesis, it seems that these circumstances may potentially explain the differential age-at-formation between males and females. Additionally, this may seem like a preferential treatment for female children but male children were weaned earlier because of a culturally determined masculine ideal. Ultimately this may also be due to different physiological nutrient uptake and utilization between the sexes, with females utilizing more iron, for example (Cohen & Bennett, 1993).

Operation Identification

The most important result of the migrant dental stress analysis was that all migrants curated by OpID possessed evidence of a stress event, whether it was macroscopic or microscopic in nature. This means that all migrants experienced some sort of systemic stress during early development. Studies of living Central American migrants show decreased mental health functioning due to depressive and anxiety disorders that cite gang-related violence as the reason for leaving their home country (Keller, Joscelyne, Granski, & Rosenfeld, 2017). Children that have been coming to the U.S. border in recent years who are fleeing life-threatening situations in Central America are also more likely to be afflicted with mental illnesses such as depression, anxiety, and even post-traumatic stress disorder (Sawyer & Márquez, 2017).

Many women and children experience health issues in Central American countries which can manifest as LEH and AS. Galicia, Grajeda, and Lopez de Romana (2016) looked at anemia in women from various Central American countries and found that

among women of reproductive age, 15% of all women in El Salvador, 21% of all women in Guatemala, and 20% of all women in Honduras suffered from anemic conditions, and Galicia and colleagues classify the frequency of anemia as a mild to moderate public health problem. By contrast, the frequency of anemia in developed countries is estimated at around 9% (Abriha, Yesuf, & Wassie, 2014). In addition, Galicia et al. (2016) report the prevalence of growth stunting in Central American countries of children under 5 years old as follows: 50% of Guatemalan children, 30% of Honduran children, and 20% of El Salvadoran children experienced stunted growth. Additionally, Galicia and colleagues classify anemic conditions in children under 5 years old in the following frequencies: 30% of children in Honduras, 48% of children in Guatemala, and 23% of children in El Salvador. These numbers are unusually high and can represent why the unidentified OpID migrants experienced so many long-duration childhood stress episodes.

These factors also come together to create the ideal conditions for early onset of weaning which predominates in women of low socioeconomic status (Ogbuanu et al., 2009). The 30% of malnourished Honduran children is considered by Palacios, Augustinus, Urquía, Saseta, and Berruezo (2017) as the result of early cessation of breastfeeding with only 30% of Honduran children breastfed until 6 months old and 36% of mothers not breastfeeding at all. In Guatemala, El Salvador, Honduras, and Mexico, the likelihood of breastfeeding is determined by complex factors, with ultimately the majority of women in these countries attempting breastfeeding for a few days to a few months and by 6 months, infants are drinking some other substance (Schaefer et al.,

2015). Reasons included social pressure not to breastfeed, Cesarean section, failure of breastmilk production, and “latching” issues.

These high instances of malnutrition, stunting of growth and anemic conditions in children and women of reproductive age (Galicia et al., 2016) and the undeniable sociopolitical and domestic violence that they may face in Central America (Keller et al., 2017) potentially illustrate the reasons behind the high prevalence of stress events in the unidentified migrants curated by OpID. If women are experiencing problems with breastfeeding because of personal illness or personal decisions, the health of the child may be compromised. Undergoing the weaning process in a hostile environment filled with violence and sociopolitical unrest can lead to stress formation in the teeth of children who ultimately grow up to make the trek through Mexico and die while attempting to find refuge in the United States. Ultimately, life course theory holds that bodies are made up of context. Migrant bodies experience these stressors due to a convergence of all factors as they embody the sociopolitical and violent environment around them and while a statistical analysis of DOHaD cannot be explicitly tested, all migrants facing intense bodily stressors are likely to face a host of health problems in adulthood.

Among the OpID migrants, age-at-formation of the first stress episode in females was at a significantly younger age than males but the duration of stress episodes did not differ significantly. Women in Latin America, especially El Salvador, experience high levels of violence, including mental, physical, and sexual abuse and murder, and this is tied to the sociocultural and political environment (e.g., machismo culture) (Essayag, 2017; Gibbons & Luna, 2015; Walsh & Menjívar, 2016). Alvarado-Esquivel, Sifuentes-Alvarez, and Salas-Martinez (2016) have even reported high rates of depression in

pregnant and post-partum mothers who were unhappy with the female sex of the infant in a poor community from the state of Durango, Mexico. Similarly, Pérez-Escamilla et al. (1995) reported that in Brazil, Honduras, and Mexico, mothers were more likely to exclusively breastfeed male infants versus female infants, although they were likely to stop breastfeeding male infants sooner. If women can also embody machismo and marianisma (the desire for women to be passive and pure), then the children they care for are also likely to be affected. This may be a potential explanation for the earlier age-at-formation of stress events in female migrants, because female infants began the weaning process earlier or were not breastfed at all. In this way, migrants can embody their childhood sociocultural context and that context can be observed in the hard tissues of the adult body.

TXSTDSC

Individuals who donated to the TXSTDSC possessed less LEH overall which can be attributed to better healthcare in a modern, developed country. However this may also just mean that individuals are experiencing the same amount of stressors as in the ancient and developing worlds but healthcare prevents the bones from being modified by the agent causing the disease or illness (Wood et al., 1992). The six Hispanic donors in the collection that were analyzed accounted for more of the stress episodes than the 26 White donors.

Males had significantly lower age-at-formation of first stress episodes than females and a smaller age range during which the insults are occurring. When all stress episodes are considered, there was no statistical significance between males and females. The literature on gendered breastfeeding practices in the United States points to an

equality between the sexes except from mothers that identify as Hispanic. Hispanic mothers in the United States were more likely to think that male infants needed nutritional supplementation with formula or cereal and would therefore stop breastfeeding earlier (Shafer & Hawkins, 2017). These TXSTDSC results therefore must be severely affected by sample size. The gathering of more age-at-formation data from teeth other than mandibular canines will undoubtedly ameliorate this statistic. (this paragraph feels out of place – find a better place to put it)

Hispanic individuals experienced the first stress episode younger than White individuals and also had a smaller range during which the first insults were occurring. The small range may be due to sample size of people who identify themselves as Hispanic (n=6). However, most (n=4) of these donors were born in Mexico and moved to the United States so they likely embody Mexican child-rearing practices. In suburban Mexico City, only 2% of mothers of children less than 5 years old breastfeed until 4 months old (Guerrero et al., 1999). This likely falls back on the fact that individuals of lower socioeconomic status tend to wean children younger, or not breastfeed altogether, which can create individuals who experience more developmental stress and life course effects. As stated previously, infant morbidity increases significantly, especially from diarrhea and ear infections, in infants who are not exclusively breastfed for the first 4 to 6 months. Early childhood morbidity can lead to later adult morbidity and decreased longevity.

No statistically significant differences occurred between those who self-reported a childhood socioeconomic status (SES) in categories of lower, lower-middle, middle, and upper-middle. However, middle and upper-middle SES possessed average ages-at-

formation noticeably higher than lower and lower-middle SES individuals. While not so in other countries, lower socioeconomic status in the United States is associated with earlier weaning or never breastfeeding (Rassin et al., 1984).

VI. CONCLUSION

Using the theoretical frameworks of DOHaD, Life Course Theory, and Embodiment, the age-at-formation of LEH and AS in the ancient Maya, unidentified migrants, and modern donated individuals was brought into biocultural context. Considerations of bodies as created by the sociopolitical environment aids in the contextual understanding of the formation of developmental stress episodes.

The ancient Maya of the larger population centers of La Milpa and Dos Hombres embodied problems that reflected increased population density, while those who lived at smaller ceremonial and agricultural centers tended to experience fewer and shorter bouts of stress. In addition, male individuals were experiencing the first stress episode earlier than females, likely representing a gendered norm of child-rearing masculine children. All unidentified migrants from OpID were affected by stress episodes, possibly illustrating the structural violence and unrest present in their home countries. Female migrants may also embody the sociocultural treatment of women prevalent in Latin America since their age-at-formation of LEH was significantly lower. These results were brought into context with the analysis of modern American donors of the TXSTDSC. There were not many instances of stress experienced in these individuals, likely due to the presence of more accessible healthcare systems and fewer sociocultural stressors in the United States. However, Hispanic individuals that were born in Mexico still embodied Mexican child-rearing practices.

The results of this pilot test can undoubtedly be strengthened by the inclusion of more data – especially from other teeth in the dentition. Specifically, more ages and larger age-at-formation of stress episode profiles can be considered. In addition, issues with observing perikymata on the surface of the teeth prevented observation of even smaller stress episodes. No teeth possessed continuous perikymata profiles, however, new method developed by Cares Henriquez and Oxenham (2017) allows for the observation of microscopic LEH in the absence of visible or continuous perikymata. This method should also be utilized in the future. Lastly, other features of childhood developmental stress, such as vertebral neural canal diameters and stature should be utilized to understand the impacts of stress as children age, and indicators of adult stress such as infectious disease can further reflect how childhood stress is embodied in the adult skeleton.

APPENDIX SECTION

APPENDIX I: PFBAP DATA

Medicinal Trail (RB62)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
7			2	I	Child	1	8.1	1.9	1	0.4	4.2	3.3
						2	8.4	1.8				
12	BO	2		I		1	9.5	1.6	0			
12	A	19	2	I		1	8.6	1.8	1	0.8	1.6	4.6
									2	0.6	2.7	4.0
									3	2.0	5.4	2.7
									4	0.3	7.2	2.2
7	HP	2	7A	I	MA	1	7.2	2.2	1	3.0	3.8	3.4
						2	8.2	1.9				
						3	10.8	1.3				
						4	10.9	1.3				
7	FG	11	3	F	YA	1	6.4	2.4	1	0.5	1.4	4.7
						2	8.7	1.8	2	1.4	2.9	3.9
						3			3	0.4	6.2	2.5
7	HP	1			YA	1	5.4	2.8	0			
						2	2.9	3.9				
						3	4.4	3.1				
						4	6.5	2.4				
7	GS	1	4	F	MA	1	4.5	3.1	0			
El Intruso (RB11)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
6	X	8	15	M	YA	1	3.7	3.4	1	1.038	3.81	3.4
						2	5	2.9	2	0.318	2.76	3.9
						3	6.8	2.3	3	0.352	1.88	4.4
						4	8.7	1.8				
Barba Group (RBS2)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
1			1	M	YA	1	5.7	2.6	0.0			

Guijarral (RB18)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
45	G	S	2	M	YA	0			1.0	0.5	6.0	2.5
									2.0	0.4	6.8	2.3
La Milpa (RB25)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
V	42		2	M	YA	1	8.6	1.8	0.0			
						2	9.8	1.5				
						3	10.6	1.4				
						4	10.9	1.3				
V66	A	15	3	M	YA	1	0.8	5.1	1.0	0.4	2.0	4.3
						2	4.1	3.3	2.0	0.4	2.7	4.0
						3	4.5	3.1				
						4	5.8	2.6				
V68	A	9		M	YA	1	5.5	2.7	1.0	3.3	3.5	3.6
						2	6.7	2.3				
						3	7.7	2.0				
						4	7.9	2.0				
						5	9.2	1.6				
						6	9.5	1.6				
						7	9.7	1.5				
						8	9.9	1.5				
						9	10.1	1.5				
						10	10.4	1.4				
V66	A	15	1		YA	1	7.2	2.2	1.0	2.3	4.7	3.0
						2	8.5	1.8				
						3	10.9	1.3				
V63	A	5	3		Early Child	1	3.7	3.5	0.0			
						2	6.4	2.4				
						3	6.6	2.4				
						4	8.1	1.9				
						5	8.6	1.8				
Dos Barbaras (RB4)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
3	XX	14			YA	1	7.2	2.2	1.0	1.9	2.5	4.1
						2	7.5	2.1				

						3	8.2	1.9				
						4	8.3	1.9				
3	BP	1				1	1.0	5.0	0.0			
						2	1.5	4.7				
						3	1.5	4.6				
						4	1.8	4.4				
						5	1.9	4.4				
						6	3.6	3.5				
						7	3.7	3.5				
						8	3.8	3.4				
						9	3.8	3.4				
						10	3.9	3.4				
						11	3.9	3.3				
						12	4.0	3.3				
						13	4.1	3.3				
						14	4.1	3.3				
						15	4.2	3.2				
						16	4.3	3.2				
						17	4.3	3.2				
						18	4.3	3.2				
						19	4.4	3.1				
						20	4.5	3.1				
3	AQ	13				1	0.6	5.3	1.0	0.5	0.9	5.0
						2	7.0	2.2	2.0	0.4	1.8	4.5
						3	8.7	1.8	3.0	0.8	3.5	3.6
						4	9.2	1.7				
						5	9.6	1.6				
						6	10.0	1.5				
3	AI	17		F	YA	1	2.2	4.3	0.0			
						2	5.2	2.8				
						3	5.5	2.7				
						4	6.1	2.5				
						5	7.6	2.1				
						6	7.9	2.0				
3	AU	6				1	0.3	5.4	1.0	0.7	2.1	4.3
						2	4.3	3.2	2.0	0.8	3.1	3.7
						3	7.8	2.0				
						4	7.9	2.0				
3	DJ	1		M	YA	1	1.8	4.5	1.0	2.1	2.7	4.0

						2	2.1	4.3				
						3	3.6	3.5				
						4	6.2	2.5				
						5	8.5	1.8				
						6	10.2	1.4				
						7	10.4	1.4				
3	BD	6		M	MA	1	1.6	4.6	1.0	0.4	1.9	4.4
						2	7.5	2.1	2.0	1.0	3.2	3.7
						3	7.8	2.0	3.0	0.2	4.2	3.2
						4	8.2	1.9	4.0	0.8	5.1	2.9
						5	8.2	1.9	5.0	0.4	6.1	2.5
3	WWZZ	16		F	YA	0		5.7	0.0			
3	AW	9	2			1	0.9	5.0	1.0	0.5	1.0	5.0
						2	2.1	4.3	2.0	0.7	1.6	4.6
						3	2.7	3.9	3.0	0.5	2.3	4.2
						4	3.3	3.7	4.0	0.8	3.2	3.7
						5	5.1	2.9	5.0	0.9	5.3	2.8
									6.0	0.9	7.0	2.2
Dos Hombres (RB2)												
Op	SubOp	Lot	B#	Sex	Age	# AS	Dist.	Age	# LEH	Width	Dist.	Age
8	38	8	6	M	YA	1	6.4	2.4	1.0	0.9	1.8	4.4
						2	10.4	1.4	2.0	0.9	3.4	3.6
						3	10.1	1.5				
19	1			M	YA	1	0.8	5.1	1.0	1.4	3.1	3.8
						2	5.2	2.8				
						3	5.6	2.7				
						4	6.1	2.5				
						5	7.1	2.2				
						6	8.0	2.0				
8	21	5			MA	1	0.7	5.2	1.0	3.6	3.8	3.4
						2	5.8	2.6				
						3	7.5	2.1				
8	36	6	5b	M	YA	1	2.2	4.2	1.0	2.9	2.9	3.9
						2	3.4	3.6	2.0		4.3	3.2
						3	6.3	2.4				
						4	9.8	1.5				
						5	10.2	1.4				
8	40	3	3	F	MA	1	6.9	2.3	1.0	0.4	1.0	5.0

						2	7.5	2.1				
						3	7.7	2.0				
						4	8.5	1.8				
						5	8.5	1.8				
						6	8.6	1.8				
						7	9.0	1.7				
28	O	13	3		Early Child	1	2.2	4.2	0.0			
						2	6.0	2.5				
						3	6.2	2.5				
						4	3.8	3.4				
						5	4.1	3.3				
29	C	12	2	F	YA	1	1.7	4.5	1.0	0.4	2.8	3.9
						2	1.8	4.5	2.0	0.5	2.0	4.4
						3	1.8	4.4				
						4	1.9	4.4				
						5	1.9	4.4				
						6	2.0	4.4				
						7	2.0	4.3				
						8	2.0	4.3				
						9	2.0	4.3				
						10	3.3	3.7				
						11	6.0	2.5				
						12	10.7	1.4				
						13	10.5	1.4				

APPENDIX II: OPID DATA

ID	Sex	Min. Age	Av. Age	Max. Age	# LEH	Min. Age3	Max Age	# Micro	Min. Age2	Max. Age2
365	F	17	23.5	30	1	2.73		4	2.31	4.73
368	M	27	48	69	1	2.85		4	3.56	5.39
377	F	30	40	50	2	0.90	1.54	3	1.86	5.4
378	F	34	50.5	67	2	0.90	2.47	7	2.32	5.41
391	M	25	41	57	1	2.67		0		
395	M	17	21	25	3	1.62	3.18	5	2.91	5.2
397	M	32	41	50	1	2.71		0		
404	F				2	0.90	1.4	4	1.87	5.08
406	F	23	26.5	30	1	2.95		2	2.39	2.99
409	M	23	29.5	36	1	1.66		2	3.81	4.87
410	M	31	47	63	0			5	2.3	5.16
411	F				2	2.32	3.04	5	1.95	2.52
416	F	26	38	50	4	1.90	4	4	2.38	4.48
421	M	20	24.5	29	2	2.82	3.16	5	3.92	5.34
446	F	25	33.5	42	1	3.16		4	2.23	4.56
448	F	17	21.5	26	1	2.73		2	2.69	4.37
464	F	21	26.5	32	1	3.25		3	3.29	5.02
471	M	23	34.5	46	1			1	4.05	
473	F	28	41	54	1			5	1.96	3.76
475	F	15	18.5	22	2	0.74	2.31	9	2.15	4.06
485	F	16	21	26	1			2	1.72	2.27
487	F	15	17.5	20	1			5	3.29	5.24
492	M	30	43.5	57	1			0		
500	M	28	38.5	49	1	2.80		5	2.35	5.35
503	M	20	32.5	45	1			3	5.17	5.46
504	M	20	30	40	1			2	3.53	5.44
505	F	35	52	69	1	2.87		6	2.36	4.21
510	M	27	36	45	1			2	4.77	5.3
607	M	29	40	51	1			1	2.43	
611	M	15	20	25	1			1	5.51	

APPENDIX III: TXSTDSC DATA

ID	Sex	Ancestry	Age at Death	# LEH	Min. Age	Max Age	# Micro	Min. Age	Max. Age
D40-2012	F	H	67	2	2.83	3.58	7	1.81	5.29
D01-2014	F	H	72	2	2.1	2.83	2	3.06	5.13
D28-2014	F	H	68	0			5	2.19	3.31
D01-2008	M	H	81	5	2.28	3.96	5	2.04	4.72
D08-2010	M	H	67	0			2	2.16	2.33
D54-2014	M	H	52	1	2.66		5	1.39	4.07
D04-2011	F	W	68	0			1	3.96	
D21-2011	F	W	56	1	2.25		1	5.16	
D03-2012	F	W	78	1	4.07		0		
D04-2012	F	W	63	0			0		
D35-2012	F	W	63	0			2	4.07	4.97
D36-2012	F	W	42	1	3.79		3	2.04	3.55
D09-2013	F	W	45	0			6	1.92	4.96
D17-2013	F	W	47	0			3	2.02	2.57
D11-2014	F	W	46	0			0		
D07-2009	F	W	65	0			1	3.51	
D08-2009	F	W	53	0			4	1.99	5.46
D09-2009	F	W	58	0			3	2.28	4.91
D04-2010	F	W	53	1	1.91		2	4.97	5.1
D07-2010	M	W	46	0			4	2.04	4.81
D12-2010	M	W	54	1	4.07		4	2.44	2.98
D01-2011	M	W	40	1	2.68		1	3.37	
D25-2012	M	W	44	0			4	2.34	4.41
D27-2014	M	W	56	0			2	1.99	4.47
D57-2013	M	W	54	0			4	1.91	3.6
D03-2009	M	W	32	0			4	1.5	4.78
D10-2010	M	W	32	0			4	2.68	4.85
D60-2014	M	W	59	0			0		
D20-2012	M	W	34	0			0		

APPENDIX II: PFBAP HISTOLOGICAL IMAGES



Figure 1. RB2-8-38-8



Figure 2. RB2-28-O-13



Figure 3. RB2-8-40-3



Figure 4. RB2-Burial2

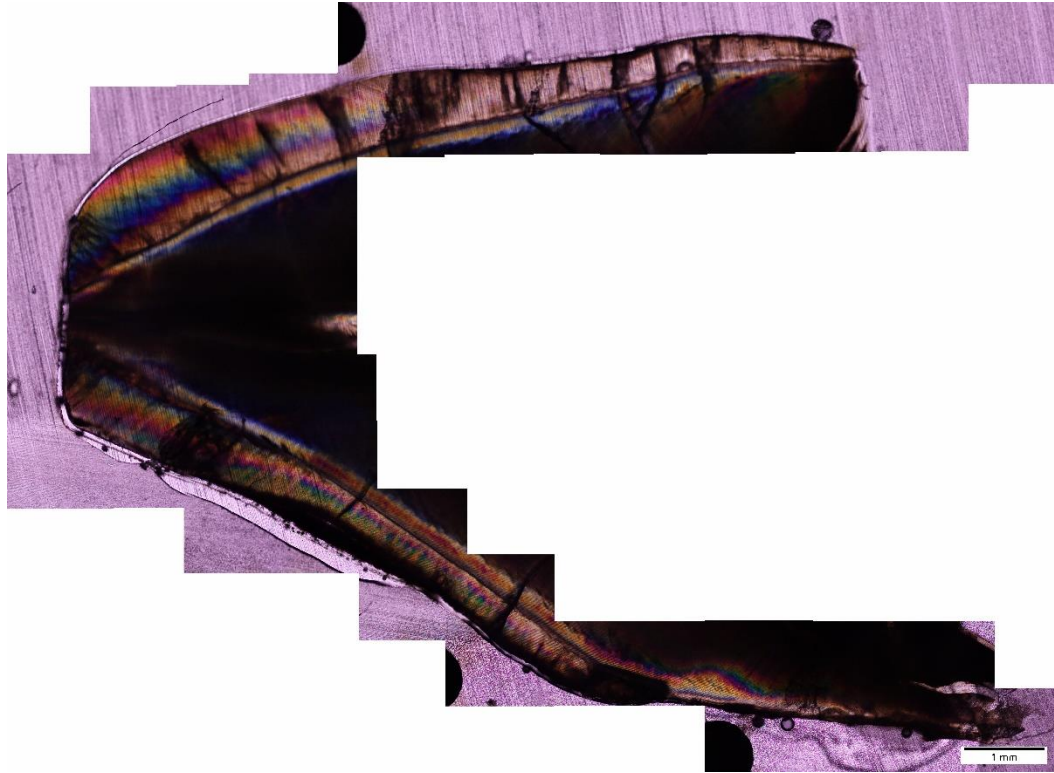


Figure 5. RB2-8-36-6



Figure 6. RB2-19-1

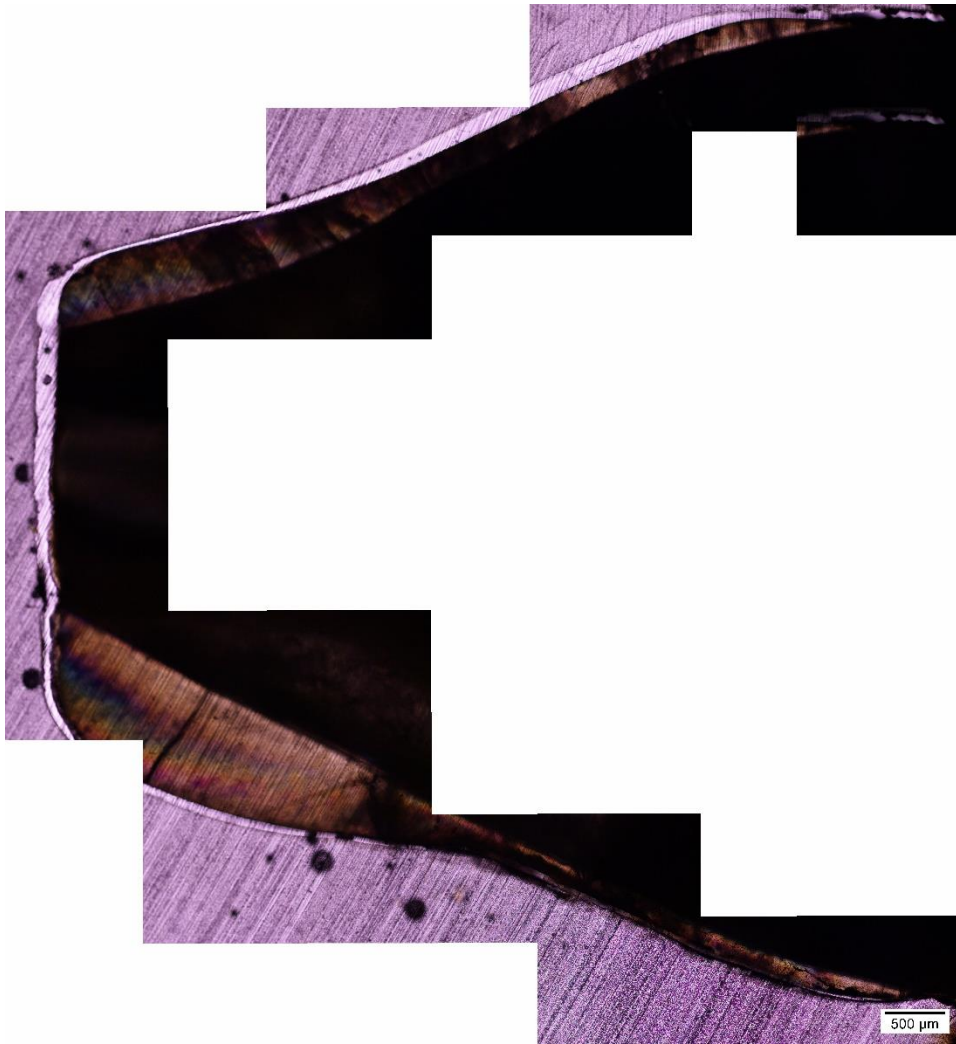


Figure 7. RB2-8-21-5



Figure 8. RB4-3-XX-14



Figure 9. RB4-3-BP-1



Figure 10. RB4-3-AQ-13



Figure 11. RB4-3-AU-6



Figure 12. RB4-3-AW-9



Figure 13. RB4-3-WWZZ-13



Figure 14. RB4-3-BD-6

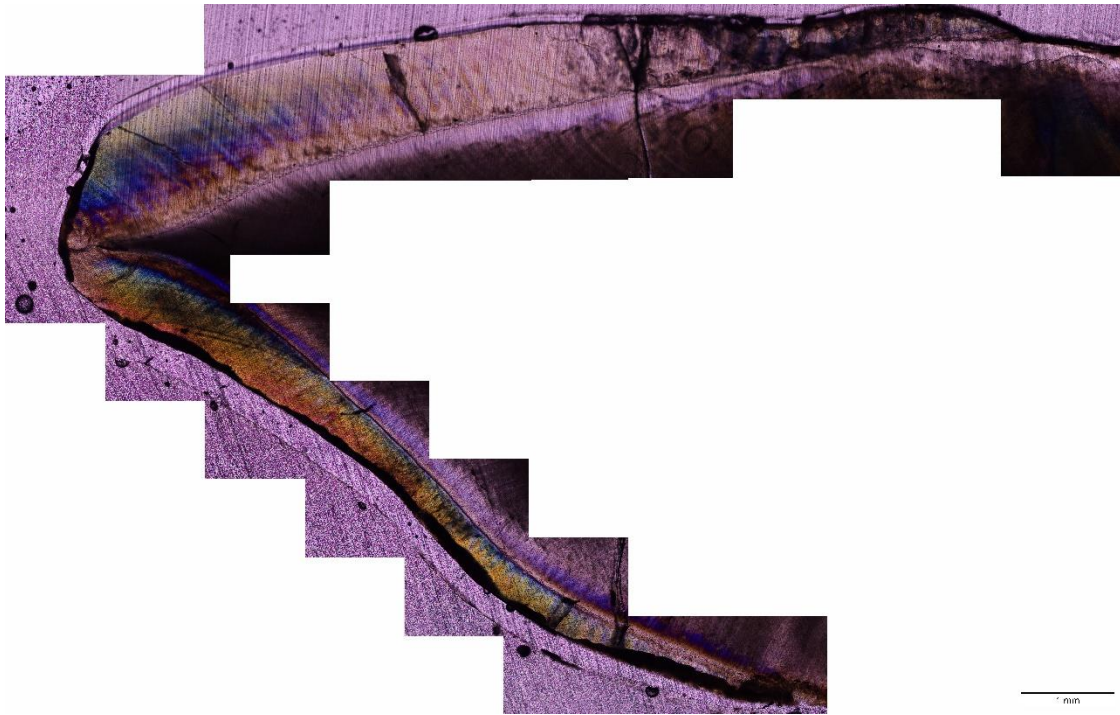


Figure 15. RB4-3-DJ-1



Figure 16. RB4-3-AI-14



Figure 17. RB11-6-X



Figure 18. RB18-45-G-5



Figure 19. RB25-V63-A-5



Figure 20. RB25-V68-A-15(B.1)



Figure 21. RB25-V68-A-9



Figure 22. RB62-7-GS-1



Figure 23. RB25-V66-A-15(B.3)



Figure 24. RB62-7-HP-1



Figure 25. RB25- V42

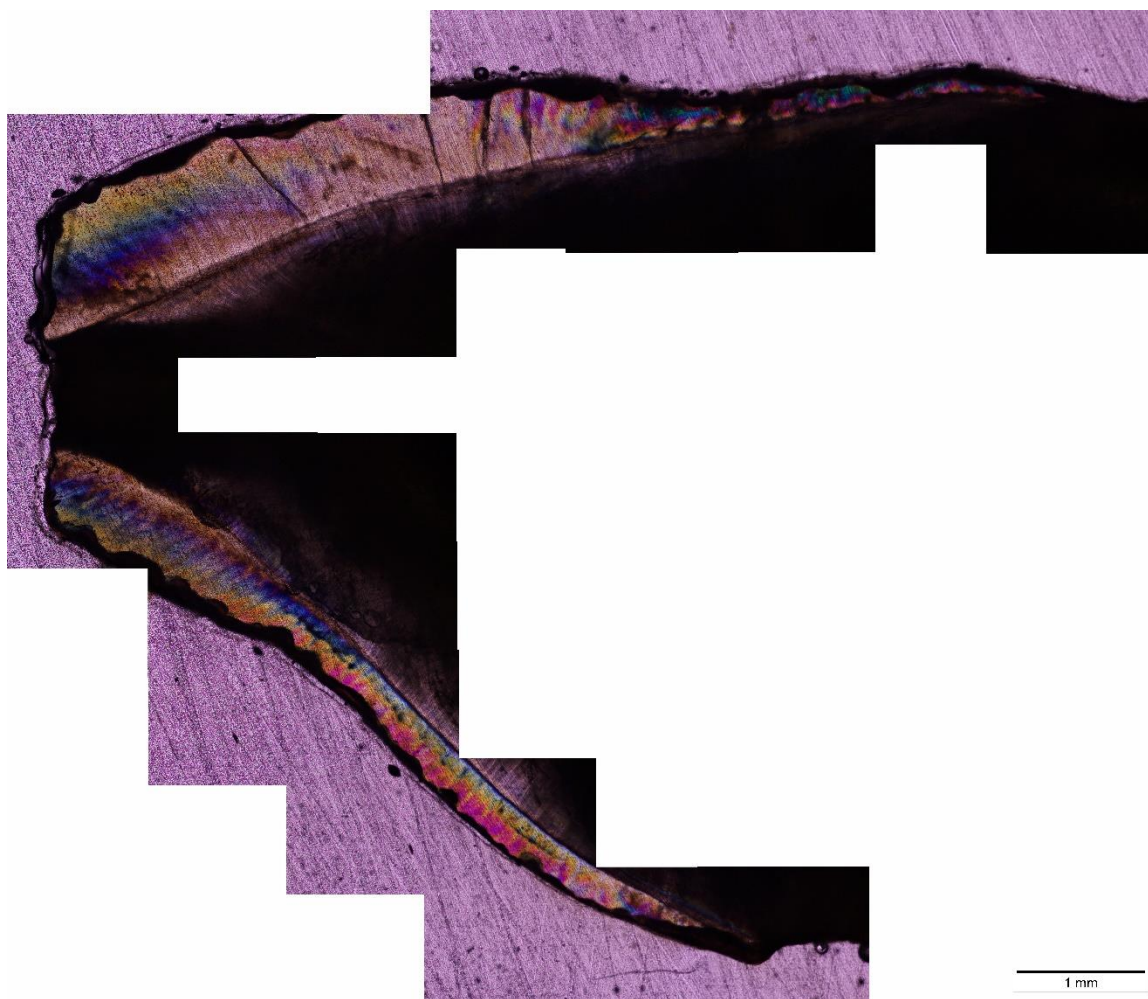


Figure 26. RB62-7-FG-11



Figure 27. RB62-7-HP-2



Figure 28. RB62-12-BO-2



Figure 29. RB62-12-A-19



Figure 30. RB62-7(B.2)



Figure 21. RBS2-1

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