# INVESTIGATING BIOLOGICAL DISTANCE AND SKELETAL STRESS IN A LATE ANTIQUE AND EARLY MEDIEVAL TUSCAN TOWN

By

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## **ABSTRACT**

The Late Antique and Early Medieval eras in the Mediterranean (5th-10th centuries AD) were an extremely turbulent time. In the centuries following the fall of the Western Roman Empire (c. 476 AD), Italy experienced the Gothic War, the plague of Justinian, the Lombard invasion, the rise of the Carolingian dynasty, and the spread of Christianity. The nature of this transitional period has garnered considerable debate in archaeological and historical literature, resulting in some scholars advocating for a "Dark Age" of isolation, disease, and cultural stagnation, while others have suggested these eras were marked by adaptation, interconnectivity, and resilience. Notably, there has been relatively little focus on the ways in which bioarchaeology can contribute to this conversation. At present, no studies have used a diachronic, intracemetery analysis with paleopathological and biological distance (biodistance) approaches to understand the effects of this time period on the residents of Tuscany, Italy.

In order to address these scholarly gaps, this dissertation focuses on the site of *Rusellae* (Tuscany, Italy) and its cemetery, which was in use between the 6th and 12th centuries AD. The present study focuses on a sample of 160 adults excavated in a collaboration between the Art and Archaeology Museum of the Maremma and the Archaeological Superintendence of Tuscany between 1987 and 1991. The burials comprise individuals from two archaeologically-defined phases: Phase I (6th-7th centuries AD) and Phase II (8th-12th centuries AD). The primary foci of this study are: an examination of biological distance within *Rusellae* and among *Rusellae* and contemporary sites using craniometrics; a craniometric population affinity analysis within *Rusellae*; an exploration of craniometric variation over time; and the frequency of skeletal stress indicators (*cribra orbitalia*, porotic hyperostosis, periosteal reaction, and linear enamel hypoplasias) between cemetery phases and population affinities.

The results showed that, despite exhibiting an overall greater affinity with contemporary European samples than African references, *Rusellae* was a biologically heterogeneous site comprising several craniofacially distinct groups. Further, the presence of several individuals consistent with African affinity was preliminarily attested at the site. Notably, when compared with individuals showing greater European affinity, the individuals with a greater affinity to African reference samples showed no significant difference in frequencies of the skeletal stress markers analyzed, with the exception of linear enamel hypoplasia of the maxillary central incisor. In addition, there were no statistically significant differences in either skeletal stress indicators or cranial measurements between the earlier and later phases of the cemetery.

Broadly, these results suggest genetic continuity between *Rusellae* and other European populations, but not to the exclusion of some contribution from the African continent. These findings are consistent with continued circum-Mediterranean trade and migration, including exchange between Tuscany and North Africa, during this period. Additionally, the lack of diachronic change in skeletal stress indicators and craniometrics suggests a degree of biological, ecological, environmental, and psychosocial stability throughout Late Antiquity and the Early Middle Ages at *Rusellae*. Further, the findings indicate a generally similar experience between affinities, at least with respect to the stress indicators analyzed, possibly due to a social structure that prioritized religious cohesion over ancestral divisions. Thus, the totality of the evidence supports a view of the transitional period between the 6th and 12th centuries, not as a "Dark Age" characterized by collapse, but as an era of continuity, connectivity, and resilience for *Rusellae*.

This dissertation is dedicated to my family. I couldn't have done it without you.

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# TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION
CHAPTER TWO: BIOARCHAEOLOGICAL APPROACHES TO SKELETAL STRESS AND BIOLOGICAL DISTANCE
CHAPTER THREE: ARCHAEOLOGY AND HISTORY OF LATE ANTIQUE AND EARLY MEDIEVAL ITALY
CHAPTER FOUR: RESEARCH QUESTIONS AND EXPECTATIONS79
CHAPTER FIVE: MATERIALS AND METHODS87
CHAPTER SIX: RESULTS
CHAPTER SEVEN: DISCUSSION AND CONCLUSION
REFERENCES

### **CHAPTER ONE: INTRODUCTION**

The Late Antique and Early Medieval eras (5<sup>th</sup>-10<sup>th</sup> centuries AD) are tumultuous periods in Mediterranean history. These periods are characterized by human mobility, sociopolitical shifts, changing settlement structures, and multicultural interactions. At the beginning of Late Antiquity, which roughly coincides with the fall of Rome in AD 476, the Italian peninsula fell into an amalgamation of kingdoms under the rule of various Germanic peoples (S. Mitchell, 2007). The following centuries saw the Gothic War, the plague of Justinian, the Lombard invasion of Italy, the rise of the Carolingian dynasty, and the spread of Christianity, all of which have been well-examined in a historical and archaeological context and all of which inevitably impacted the individuals who lived through these events (Brogiolo, Marazzi, & Giostra, 2017; Wickham, 1981). Italy was situated at the core of the Western Roman Empire and was a diverse center of contact and trade within the broader Mediterranean during the Classical period; this position as a nucleus, however, made Italy all the more vulnerable to the effects of the end of the Western Roman Empire.

This transformative time in Italy has garnered considerable debate as to its nature: was it a "Dark Age" wherein trade ceased, disease was rampant, and cultural progress halted? Or, perhaps, was this period marked by cultural continuity, reconfiguration of settlement structures, and the maintenance of trade networks? Further, what was the nature and extent of mobility and migration during this period, and what were its biological and social impacts? Though these debates are ongoing, archaeological and historical studies have increasingly revealed that this period was resilient, marked by adaptations in subsistence, settlement, trade, and political systems, rather than an outright collapse (Riccomi, 2021).

There has been comparatively little focus on the ways in which bioarchaeology can contribute to these debates. A small number of studies have analyzed skeletal indicators of stress between the Classical and post-Classical periods, showing that a disastrous transition was not felt ubiquitously throughout Italy. Instead, studies have shown regional variation in health outcomes during this time (Belcastro et al., 2007; Giannecchini & Moggi-Cecchi, 2008; Manzi, Salvadei, Vienna, & Passarello, 1999; Riccomi, 2021; Viva, 2020). Additionally, ancient DNA and stable isotope analyses of skeletal remains have been used to understand the extent of mobility in Italy after the fall of Rome, generally concluding that Late Antiquity and the Early Middle Ages represent a period of coexistence between genetically diverse groups, including Lombards, Romans, Central Europeans, and even North Africans (Amorim et al., 2018; Antonio et al., 2019; Coia et al., 2023; Francisci et al., 2020; Tian et al., 2024; Viva, Lubritto, Cantini, & Fabbri, 2022).

Few of these studies focus on Tuscany and still fewer use a diachronic, intra-cemetery approach to skeletal stress analyses. No studies to date have undertaken a biological distance analysis at any scale to explore biodiversity, and none have integrated craniometric and stress data to examine how skeletal stress might vary by population affinity. Consequently, perspectives surrounding health and migration in Late Antiquity and the Early Middle Ages, especially in Tuscany, are largely drawn from historical and archaeological data and lack a biological component. This dissertation aims to fill these gaps by contributing novel data towards a better understanding of the human experience of the social, economic, and political shifts of Late Antique and Early Medieval Italy through biological distance and skeletal stress analyses at the site of *Rusellae*, in southern Tuscany, Italy.

Rusellae is an ancient city that was occupied from the Etruscan times through the Middle Ages. Rusellae was founded during the 9<sup>th</sup> century BC and an enormous fortification wall was constructed between the 8<sup>th</sup> and 7<sup>th</sup> centuries BC (Sebastiani, 2017). The Etruscan occupants of Rusellae fought the Romans from approximately 302-294 BC, when the city was finally besieged and captured for Rome (Grant, 1980). Rusellae seems to have briefly recovered until the Etruscans were finally displaced and a Roman colony was established at Rusellae in the early first century BC (Barker & Rasmussen, 1998). In the 1<sup>st</sup> century AD, construction began on a forum, amphitheater, temple, and domus (Campana, 2018; Sebastiani, Chirico, Colombini, & Cygielman, 2015).

Between the 4<sup>th</sup> and 5<sup>th</sup> centuries AD, coincident with the fall of Rome, much of Tuscany experienced a substantial decline in the number of sites and occupants. *Rusellae* was not immune to this regional experience, and portions of the site were abandoned, including the forum and most public buildings (Sebastiani, 2017). Despite this general decline, *Rusellae* remained occupied, even while many contemporary sites were abandoned; as one of the few remaining inhabited cities in the area, *Rusellae* was chosen as the center of a diocese in the late 5<sup>th</sup> century AD (Celuzza, Cencetti, & Pacciani, 2002; Sebastiani, 2017). Soon thereafter, a church and cemetery complex were constructed on the remains of a Hadrianic-era bathhouse. Additional construction of a new perimeter wall and fortification of the amphitheater around this time provide further evidence for the resilience of *Rusellae* (Celuzza et al., 2002; Sebastiani, 2017). Importantly, it was during this period of resurgence when the Lombards conquered *Rusellae* in approximately AD 593 (Sebastiani et al., 2015). However, construction and craftsmanship maintained their importance at *Rusellae*; this is attested through continued refurbishment of the

church in the late 8<sup>th</sup> and early 9<sup>th</sup> centuries AD as well as the construction of a bell tower in the late 11<sup>th</sup> century AD (Celuzza et al., 2002; Sebastiani, 2017).

The cemetery at *Rusellae* was excavated in a collaboration between the Art and Archaeology Museum of the Maremma and the Archaeological Superintendence of Tuscany between 1987 and 1991. Approximately 170 tombs have been excavated, containing the remains of roughly 200 individuals (Celuzza et al., 2023). Excavations revealed that the cemetery was used between the 6<sup>th</sup> and 12<sup>th</sup> centuries and comprised two general archaeological phases: Phase I (6<sup>th</sup>-7<sup>th</sup> centuries AD) and Phase II (8<sup>th</sup>-12<sup>th</sup> centuries AD). The layout of the cemetery ranges from neatly arranged tombs in Phase I to earthen inhumations nearing the abandonment of the cemetery. A few graves from Phase I (6<sup>th</sup> and 7<sup>th</sup> centuries) contain grave goods, including personal adornments and bone combs. One grave contains Lombard-style funerary objects, including a knife and a belt dating to the 7<sup>th</sup> century; these grave inclusions and this date are consistent with the Lombard occupation of *Rusellae* (Celuzza et al., 2002). The following phase does not contain grave goods and the burials are arranged more opportunistically with reuse of graves occurring frequently. The latest recorded use of the cemetery was in the 12<sup>th</sup> century AD, when the bishopric was transferred to nearby Grosseto in 1138 AD (Celuzza et al., 2002).

Rusellae is an especially important site for understanding the transition between Classical and Post-Classical Italy for several reasons. Rusellae was located in an advantageous position near the Tyrrhenian Sea and adjacent to the Via Aurelia, which connected the city to Rome, Pisa, and the greater Mediterranean region through networks of trade and exchange (Sebastiani et al., 2015). Its location on a hilltop was also likely a key to its survival amongst many other declining sites. As the Tuscan settlement structure shifted from the Roman-era villa system to the Medieval structure, fortified hilltop sites were favored, and Rusellae rose to prominence in the

Tuscan landscape (Francovich, 2007). There is archaeological and historical evidence of contact with the Lombard people from Central Europe whose cultural influence is widely attested in the literature but whose influence on biological diversity in Italy is only just beginning to be understood (Amorim et al., 2018). In addition, the construction and refurbishment of the church and cemetery made *Rusellae* a religious and civic center in the territory. This would have attracted people from throughout the region and Christianity likely formed the basis for social cohesion in the city. Studying *Rusellae* has the potential to contribute to a better understanding of regional population history and health outcomes. Specifically, skeletal analysis can contribute information about environmental and ecological influences, shifting settlement structures, the spread of Christianity, human mobility, and a changing sociopolitical landscape between the Classical and post-Classical periods.

However, limited bioarchaeological analysis of the site has previously been undertaken, with the exception of a 2023 publication by the research team from Michigan State University and our Italian collaborators. This chapter applied a paleopathology approach to the Rusellae sample in order to examine the effects of malaria on the site; results indicated that malarial infection was present at Rusellae potentially in up to 66% of the individuals with similar frequencies across phases, sexes, and age cohorts. Notably, many of the lesions showed signs of healing, and their presence in older individuals indicates a degree of endemicity in the sample, wherein malarial infection was not acutely fatal (Celuzza et al., 2023). While the malaria study provided important insights into past health at *Rusellae*, it also highlighted how much was left to be explored. In fact, it was in the process of analyzing these remains with a focus on malaria that this dissertation came into being.

While recording data on pathologies and the biological profile, one skull stood out from the rest (Figure 1.1). When viewed through a forensic anthropology lens, this skull exhibited features consistent with African affinity, such as the shape and width of the nasal aperture and interorbital region, facial prognathism, and a post-bregmatic depression. The possible presence of an African individual at *Rusellae* was exciting to both our research team and our Italian colleagues, and it led us to wonder about the extent of craniometric variation at the site and its implications for understanding population dynamics in the past.



Figure 1.1. Rusellae 82-1103, anterior and lateral views. Photos by Dr. Todd Fenton.

To explore this and related questions, this dissertation uses a biocultural approach to situate biodistance and skeletal stress analyses within the broader historical framework of *Rusellae*. The biocultural approach accounts for the complex and reciprocal relationship between biology and culture, and is the foundational theory of bioarchaeological studies (Zuckerman & Martin, 2016). Craniometric data and non-specific skeletal stress indicators can provide insight

into the population history and health outcomes of Medieval Tuscans, as well as the ways in which they adapted to social, political, economic, and environmental changes during a period of major transition. The craniometric data is used as a proxy within a biodistance framework to understand the degree of genetic variation within Rusellae, as well as how Rusellae relates to geographically and temporally proximate samples. These data will contribute to better understanding how Rusellae's nodal position within the Mediterranean trade network might have contributed to biological diversity at the site and how the Lombard invasion of Italy may have influenced craniometric variation at *Rusellae*. Additionally, within a biocultural framework, skeletal stress indicators including *cribra orbitalia*, porotic hyperostosis, periosteal reaction, and linear enamel hypoplasia can provide insight into the biological impact of Germanic subsistence strategies, the influence of collapsing Roman settlement system and consequent coalescence of people in a fortified settlement, and how the spread of Christianity may have influenced the system of social stratification. Therefore, by examining craniofacial variation and skeletal stress at Rusellae, these questions derived from the gaps in current historical and archaeological knowledge can be addressed through direct skeletal observation of individuals who lived during this time.

#### **Research Goals**

This study aims to integrate several lines of evidence, including craniometric data, non-specific skeletal stress indicators, and the historical and regional context of *Rusellae* to provide a more in-depth view of life in Late Antique and Early Medieval Tuscany. The specific research goals are as follows:

 To understand how craniofacial variation at *Rusellae* resembles or differs from that of geographically and temporally proximate reference samples;

- To understand which variables drive craniometric variation within *Rusellae*, and whether there is heterogeneity or homogeneity in overall craniometric variation at the site;
- To ascertain whether some individuals at *Rusellae* show a closer craniometric affinity to African reference samples as compared to European reference samples;
- To understand whether differences in affinity correlate with different frequencies of skeletal stress indicators;
- To examine whether craniofacial variation changes over time at *Rusellae*;
- And to explore diachronic changes in the frequency of skeletal stress indicators.

# **An Outline of the Present Study**

In order to achieve these goals, the dissertation is organized into seven chapters. The present chapter, **Chapter One: Introduction**, begins with a brief discussion of the relevant historical context of Late Antiquity and Early Medieval Europe. Then, the current archaeological and historical debates regarding this time frame are discussed, as well as several studies that begin to address these questions. The study site, *Rusellae*, is introduced and its relevance to the major debates and capacity to address gaps in the literature using a biocultural approach are discussed. Finally, the research goals are outlined.

Chapter Two: Bioarchaeological Approaches to Skeletal Stress and Biological Distance, provides insight into the utility of bioarchaeology and the biocultural approach for addressing broad questions of identity, ethnicity, and health. It then describes how skeletal stress indicators provide a framework for examining patterns of physiological disruptions and detail the four specific indicators used in this study: linear enamel hypoplasias, *cribra orbitalia*, porotic hyperostosis, and periosteal reactions. Next, the chapter provides an overview of biological distance analyses, including current practices, different avenues of research, statistical analyses,

and methodological advancements and critiques. It concludes with a discussion of population affinity and its relevance to the present study.

Chapter Three: Archaeology and History of Late Antique and Early Medieval Italy begins with a brief outline of the major historical events in the Mediterranean between AD 400 and AD 1200 that are relevant to this dissertation. Then, the chapter introduces *Rusellae* and its cemetery. Next, *Rusellae* is situated in the context of the Tuscan countryside in order to better understand its position in the regional system. Subsequently, the chapter provides an overview of the research agendas in modern Tuscan archaeology, followed by a discussion of the major archaeological debates surrounding this time period. The chapter closes with a survey of Mediterranean bioarchaeology, including studies on migration and skeletal stress.

Chapter Four: Research Questions and Expectations outlines the four research questions guiding this dissertation. Each question is followed by a hypothesis and a justification for the expected outcome using historical and archaeological evidence.

Chapter Five: Materials and Methods presents an overview of the skeletal samples included in the study, including the *Rusellae* cemetery sample and the six reference groups included in the biodistance analyses. It then discusses the limitations of bioarchaeological samples. Next, this chapter details the data collection methods employed, including those for sex estimation, age estimation, cranial measurements, and skeletal stress indicators. Finally, this chapter outlines the analytical methods and variables used for each research question.

Chapter Six: Results presents the results of the study, organized by research question.

Finally, in Chapter Seven: Discussion and Conclusion, each research question and its key

findings are reiterated before providing an in-depth explanation for the findings. The discussion

also provides possible alternative explanations for the results, if necessary. It also discusses the

limitations of each research question in turn. Additionally, this chapter presents the broader implications of the research and the potential impacts of the study. The discussion includes avenues of future research based on the findings and limitations of the present study. The chapter concludes with remarks that connect the overarching findings to the archaeological and historical debates that inspired this research.

# CHAPTER TWO: BIOARCHAEOLOGICAL APPROACHES TO SKELETAL STRESS AND BIOLOGICAL DISTANCE

Bioarchaeology is the study of skeletal remains within their corresponding historical and archaeological context with the goal of interpreting past lifeways and gaining a broader understanding of the human condition throughout time and space (Larsen, 2015). Beginning as a field primarily focused on osteological description and typology, modern bioarchaeology now benefits from a multidisciplinary approach, incorporating theory and methods not only from osteology and archaeology, but also from biology, chemistry, epidemiology, and population genetics (Baker & Agarwal, 2017; Buikstra, 2006; Buikstra et al., 2022; Zuckerman & Martin, 2016). Further, advancements in statistical methods have allowed for more accurate interpretations of small samples, better handling of missing data, and more rapid assessment of complex datasets (Buikstra et al., 2022). These theoretical and methodological foundations allow for a wide variety of research programs within bioarchaeology, including studies of health, intersectional identities, migration, marginalization, and population history, among many others (Baker & Agarwal, 2017; Buikstra et al., 2022; Larsen, 2002).

A common thread of bioarchaeological inquiry, no matter the topic, is the biocultural approach, which accounts for the complex and reciprocal relationship between biology and culture with respect to a given human phenomenon (Zuckerman & Martin, 2016). Notably, the cultural and historical facets of biological anthropology were not always at the forefront of early scholarship in the field; it was not until the 1950s and Washburn's "new physical anthropology" that a paradigm shift occurred towards synthetic, population-level research with a basis in theory and hypothesis testing (Zuckerman & Martin, 2016). Modern biocultural scholarship has diversified its focus to include questions of social and economic status, identity, embodiment, gender, and structural violence, among many other avenues of research. However, these higher-

order questions are only answerable by contextualizing the skeletal data with the corresponding archaeological and historical information. It is this context that the biocultural approach provides. Bioarchaeology, then, is the application of the biocultural approach to questions of past populations.

In this dissertation specifically, the biocultural approach provides an interpretive framework for biological distance (biodistance) and skeletal stress analyses. These analyses will be used to look at changes in skeletal stressors throughout time as well as population history and population affinity at *Rusellae*. Bioarchaeology and the biocultural approach are relevant to this study because these topics are not purely biological in nature. Instead, examining shifting skeletal stress over time involves an understanding of how ancient social structures and environment influence the experience of nutrition and disease. Further, exploring population history involves social constructs such as identity and ethnicity through the ways in which concepts of self, otherness, and group membership influence mate choice and migration. In order to situate this dissertation within relevant research, this chapter will first outline the overarching biocultural concepts of identity, ethnicity, and health. Then, I will discuss the study of skeletal indicators of stress and their specific application to this research. Finally, I will provide an overview of biological distance analyses, including their application to ancestry estimation in forensic anthropology.

# **Identity: Ethnicity and Health**

In an effort to critically examine the current status of bioarchaeology, a group of international scholars participated in a workshop in early 2020 and established topics of current importance to bioarchaeology, as well as identifying several avenues for growth in the field (Buikstra et al., 2022). One of the theoretical frameworks identified by the workshop participants

as useful for linking skeletal data with cultural factors is identity, or the embodiment of biological attributes such as age, sex, ancestry, and health outcomes and their relationship with social constructs such as social age, gender, ethnicity, and social status (Buikstra et al., 2022; Gowland, 2017; Knudson & Stojanowski, 2008, 2020; Zuckerman, 2020). In bioarchaeology, identity theory refers to the ways in which individuals and groups produce, maintain, and manipulate their identities through aspects of biology, material culture, mortuary customs, and social organization (Buikstra et al., 2022). The general consensus, within bioarchaeology at least, is that identity is flexible, but not infinitely changeable. Identity is seen as being constructed both by the self and by others. In this way, identity is both situational and relational (Hu, 2013). Gowland elegantly summarizes how identity manifests skeletally, noting that life is an embodied experience; interactions with the local environment, diet, migration, activity, experiences of gender, and social structure are all undertaken with the physical body, which thereby records these experience in the skeletal and dental tissue (2017). An emphasis is also placed on understanding the intersections and interactions between various aspects of identity (Buikstra et al., 2022; Knudson & Stojanowski, 2020; Torres-Rouff & Knudson, 2017). An additional consideration of research involving ancient identities is cultural relativism. That is, scholars should avoid extrapolating modern Western beliefs and social constructs to past populations (Beck & Quinn, 2022; Buikstra et al., 2022; Quinn & Beck, 2016). Two specific aspects of identity will be further explored in this dissertation: ethnicity and health status. A brief discussion of each follows.

# **Ethnicity**

Ethnicity, as it is currently conceptualized, is a social construct that involves the complicated interplay between geographic origin, ancestry, skin color, religion, language and

traditions (Gowland, 2017). Contrary to the culture-historical approach, which defined discrete ethnic groups by their material culture, the modern biocultural approach considers the relationship between biological factors like genetic ancestry and cultural elements such as grave goods, burial patterns, or body modification (Zakrzewski, 2011). Ethnicity is simultaneously distinct from and overlapping with shared genetic ancestry; geographic origin or ancestry is a component of ethnic identity, but the two do not have a direct correlation. Despite the somewhat nebulous nature of ethnicity as a construct, the lived experience of ethnicity has biological implications due to the social relationship between ethnic identity and access to resources, social roles in society, and proximity (or lack thereof) to power (Gowland, 2017; Gowland & Thompson, 2013; Zuckerman, 2020). Therefore, although ethnicity is a cultural construct, it has undeniable biological consequences that can be studied in the skeleton (Gowland, 2017; Sofaer, 2006). Further, migrants and their descendants whose ethnicity differs from local individuals may manifest their journeys and subsequent adaptations skeletally, potentially allowing for identification of differing ethnic cohorts in ancient contexts (Gregoricka, 2021).

In bioarchaeology, ethnicity is studied through a combination of biological and material factors; genetic ancestry ascertained through ancient DNA, stable isotopes, and biodistance analyses are all used to explore population affinity and relatedness in bioarchaeological settings (Nikita, 2024). Comparisons of these biological data with archaeological variables such as material culture and mortuary practices or other biological variables such as sex, age, or health status therefore reflect cultural or social ethnic affiliations that may or may not align with biological population affinity. Integrating the two lines of evidence allows bioarchaeologists to understand the degree to which biological relatedness or genetic ancestry influences cultural practices. Ethnicity has been effectively studied through the comparison of ancient DNA with

funerary customs in 6th-century Hungary and Northern Italy (Amorim et al., 2018); with an intersectional approach to understand the experiences of enslaved African women in colonial Peru (Maass, 2023); and by evaluating craniometric variation in a medieval Islamic cemetery in Spain (Zakrzewski, 2011).

Studying ethnicity in a modern context is fraught with challenges, and making inferences about ethnicity in the past is no different. Ethnicity is situated firmly in a particular time and place; ethnic identity changes throughout time, which complicates the endeavor of studying ethnicity in the past. Care should be taken not to extrapolate modern constructs of ethnicity to past societies (Buikstra et al., 2022). Notably, ethnicity is but one component of identity, and the lived experience of any ethnic identity is inextricably linked to other aspects of identity, including social status, gender, or social age. Further, understanding ethnicity in ancient samples is predicated upon the use of strong methods for estimating population affinity or geographic origin in the past; these methods (e.g. ancient DNA, stable isotopes, biodistance), although often used effectively, come with their own set of issues and assumptions. In addition, material markers of ethnic identity may have been mimicked by those with less power in order to socially connect with or show allegiance to groups of greater power, which may skew interpretations of the construction of ancient ethnicity if biological or biogeochemical factors are not also taken into account (Gregoricka, 2021).

This dissertation uses biodistance analyses to contribute to the conversation of ethnicity in Late Antique and Early Medieval Italy. Specifically, cranial measurements are used to examine broad relationships between the study site and contemporary reference samples as well as craniometric variation within the study site. This variation will then be explored through the lens of ethnicity; that is, if heterogeneity is identified in cranial measurements in the *Rusellae* 

sample and if that heterogeneity shows any geographic patterning, there may exist similarly variable ethnicities at *Rusellae*. However, as ethnicity is not directly correlated with population affinity, this variation will be further explored by understanding skeletal stress at *Rusellae*; the skeletal stress markers utilized (e.g. *cribra orbitalia*, porotic hyperostosis, periostitis, and linear enamel hypoplasias) can provide information about diet, health, disease, climate and activity throughout the life course. By comparing patterned craniometric variation with cultural variables (and their skeletal correlates) such as these, preliminary conclusions surrounding ethnicity at *Rusellae* may be drawn.

# Health

Now we turn to a discussion of another aspect of identity examined in this dissertation: health. In bioarchaeology, health refers to an individual or population's experience of illness or wellness within the context of their specific culture, which can be explored, in part, by analyzing skeletal lesions that represent instances of "stress" or disease during life (Larsen, 2018; Reitsema & McIlvaine, 2014). Notably, these stress markers do not provide an overall "health index," as they do not directly correlate with all aspects of health such as mental and social well-being; instead, they point to periods during which physiological homeostasis was disrupted. Therefore, health and stress are related, but not interchangeable (Pilloud & Schwitalla, 2020; Reitsema & McIlvaine, 2014; Temple & Goodman, 2014). Health is inextricably linked to identity because access to resources and care, and therefore wellness, is historically patterned by intersectional identities and marginalization based on age, gender, ethnicity, or social status (DeWitte & Kowaleski, 2017; Gowland, 2017; Larsen, 2018; Zuckerman, 2020). Health differences that fall along lines of identity, such as sex or ancestry, might consequently be indications of larger social structures and systemic identity-based discrimination in both modern and ancient societies (de la

Cova, 2011, 2012; Harrod, Thompson, & Martin, 2012; Zuckerman, 2020). Therefore, exploring ancient experiences of health can also provide insight into these other aspects of identity, such as gender, age, or ethnicity.

Health is studied in bioarchaeology, in part, through examining skeletal indications of stress, disrupted growth, infection or disease, trauma, or degenerative changes (Reitsema & McIlvaine, 2014). Most of these features can be identified macroscopically by a trained investigator, although differential diagnosis of pathological conditions can often be challenging (Ortner, 2012). Recent methodological improvements have allowed for greater specificity when understanding ancient health, such as isotopic analyses for exploring ancient diet, nutrition, and metabolic stress, as well as analyzing pathogen DNA for more accurate diagnoses of illnesses in the past (Klaus, 2014; Larsen, 2018). A biocultural approach to health further links these skeletal markers to cultural dimensions such as subsistence, labor, and social status (Larsen, 2018). Within the biocultural approach, a political-economic perspective emphasizes power differentials and their corollaries such as class and access to resources and medical care within different cultural contexts (Zuckerman & Armelagos, 2011). In addition, the intersection of multiple forms of identity-based discrimination have been shown to influence health (de la Cova, 2011; Harrod et al., 2012). For example, different types of labor can lead to occupational markers in the skeleton or greater degenerative changes than expected for an individual of a particular age (Harrod et al., 2012). Dietary differences linked to different subsistence strategies or climate change may lead to varying degrees of malnutrition, which can produce stunting or linear enamel hypoplasias if these nutritional insults occur during childhood or adolescence (Temple & Goodman, 2014). Social stratification may lead to unequal resource distribution and unsanitary living conditions, potentially causing malnutrition, illness, or evidence of nutrient deficiency in

marginalized groups (de la Cova, 2011; Harrod et al., 2012). Psychosocial stress associated with marginalization has also been shown to have negative health consequences, and poor health in Roman Britain has been hypothesized to result from the increasingly hierarchical social structure imposed on its residents (Gowland, 2017).

Similarly to the study of ethnicity, the study of ancient health comes with a variety of challenges and ethical considerations. First, methodological and theoretical issues leave room for uncertainty when interpreting past disease; the Osteological Paradox has shown that skeletal indicators of stress are not necessarily a direct indicator of illness or wellness in a skeletal sample (Buikstra et al., 2022; Wood, Milner, Harpending, & Weiss, 1992). Similarly, "poor" health is not an experience exclusive to the marginalized; high status in the Roman Empire has been linked to increased caries and rickets due to a sweeter diet and more time spent indoors (Gowland, 2017). Issues with differential diagnosis in paleopathology are caused by the fact that many illnesses either leave similar lesions to one another or no lesions at all; therefore, a skeleton that appears free of lesions may or may not be a "healthy" individual (Wood et al., 1992). In addition, negative health outcomes can be associated with intergenerational trauma as a result of epigenetic factors (Gowland, 2017; Klaus, 2014). Importantly, health is experienced through the lens of a given time and place, so care must be taken to avoid extending modern assumptions about health and disability to past populations (Buikstra et al., 2022). Finally, health is inextricably tied to marginalization, so researchers must be cautious not to retraumatize or stigmatize vulnerable communities.

This dissertation will contribute to the conversation surrounding health at *Rusellae* by examining several non-specific skeletal stress markers: *cribra orbitalia*, porotic hyperostosis, linear enamel hypoplasia, and periosteal reaction. As noted previously, these lesions cannot

provide a full picture of ancient health since they do not provide information about aspects of health such as self-perception or mental wellness. They do, however, provide insight into specific experiences of stress, malnutrition, or infection that are relevant to the interests of this dissertation: that is, they contribute data to understand how physiological insults, malnutrition, and infection may have changed throughout time or varied by population affinity. Within a biocultural framework, the osteological data will be integrated with demographic information, archaeological evidence from *Rusellae* and the broader region of Tuscany, and historical information to illustrate the potential consequences of structural inequity and transitions of power on health in the past.

#### **Skeletal Stress Indicators**

Non-specific stress indicators are the embodied markers of physiological disruption, malnutrition, or infections that are recorded and can be observed in the skeleton (Beatrice, Soler, Reineke, & Martínez, 2021; Pilloud & Schwitalla, 2020). These indicators have been identified and studied in a wide variety of contexts, ranging from modern cases of undocumented migrants (Beatrice et al., 2021) to medieval Italian cemeteries (Belcastro et al., 2007; Viva, 2020) in order to reconstruct past health, social structure, environment, and living conditions. As noted previously, stress indicators cannot and do not give an overall picture of health. Much like sex is a biological component of the socially constructed concept of gender, stress is a biological component of the socially constructed concept of health. The bioarchaeological study of health was discussed in the previous section; this section will outline the model used to interpret skeletal stress, detail challenges with the evaluation of stress markers in bone, and end with an overview of the specific stress markers used in this dissertation.

Goodman and colleagues outlined a stress model in order to interpret stress in skeletal samples; according to their model, the impact of stress on an individual is a product of their physical environment, the cultural system in which they live, and the resistance of the host to the stressor (Goodman, Martin, & Armelagos, 1984). Importantly, this model emphasizes that stress cannot be understood from a purely physiological perspective; the model is inherently biocultural in nature, as it takes into account the effects of sociopolitical processes and psychosocial stress on biology, as well as the influence of cultural elements on mitigating stress (Goodman, Thomas, & Swedlund, 1988). In cases where stress is not sufficiently mitigated, the stress model suggests that the consequent biological stress response can leave a lasting and identifiable impact on an individual's bones and teeth.

Although stress models provide a valuable framework for examining patterns of physiological disruptions, the implementation of these models relies on accurately interpreting stress indicators; due to the complex and multifactorial nature of these indicators, this accurate interpretation has historically been challenging (Pilloud & Schwitalla, 2020). In addition, debates surrounding the relationship between health and stress still feature prominently in anthropological literature (Pilloud & Schwitalla, 2020; Reitsema & McIlvaine, 2014; Temple & Goodman, 2014). Recent publications have called for a better definition of "stress" within the context of bioarchaeology, arguing that the term has lost its meaning and that the complicated or unknown etiologies of some of these indicators leave little to be gleaned from their presence (Edinborough & Rando, 2020; Hillson, 2014). The prevailing idea in this perspective is that little, if anything, is being done to ascertain the true causative agents of these pathologies, and that a blanket diagnosis of "stress" is minimally informative (Edinborough & Rando, 2020).

Similarly, the osteoblastic and/or osteoclastic activity that causes these pathologies may be

initiated by a variety of factors, and it is important to consider that these defects may indicate periods of metabolic disruption throughout an individual's life, instead of simply a snapshot of their frailty at death (Pilloud & Schwitalla, 2020).

Though the concerns raised surrounding stress analysis are legitimate, they do not preclude useful analyses of stress indicators, as several solutions and recommendations have been presented. For example, using multiple indicators—as this dissertation does—is recommended in order to provide the most holistic understanding of an individual's stress response (Agarwal, 2016). Certain indicators are related to stress during specific phases of growth and development; by analyzing a variety of these factors, a picture of an individual's life history emerges, as opposed to a snapshot of stress during a limited period of time (Larsen, 2015). Further solutions to this issue include differentiating between healed and active lesions, as well as framing results in the context of the osteological paradox (Pilloud & Schwitalla, 2020). Finally, utilizing a life course approach and incorporating embodiment theory to evaluate the influence of intersecting identities on stress response at the individual level—in addition to population-level analyses—facilitates a view of the skeleton as the product of a lived experience (Agarwal, 2012, 2016; Knudson & Stojanowski, 2008; Sofaer, 2006).

This dissertation focuses on four non-specific skeletal stress indicators: linear enamel hypoplasias, *cribra orbitalia*, porotic hyperostosis, and periosteal reactions. These lesions have been clinically identified in modern populations, and their presence in the past can provide insight into facets of life such as trauma, infection, and disease (Beatrice et al., 2021; O'Donnell, Hill, Anderson, & Edgar, 2022; Vercellotti et al., 2014). A discussion of each indicator follows.

# **Linear Enamel Hypoplasias**

Linear enamel hypoplasias are linear defects of decreased enamel thickness, running roughly parallel to the cemento-enamel junction (Ritzman, Baker, & Schwartz, 2008). They are a signature of arrested enamel development attributed to three causes: genetic anomalies, dental trauma, or illness, disease, or malnutrition that causes systemic metabolic distress (Larsen, 2015). However, it is systemic metabolic stress that causes a vast majority of linear enamel hypoplasias in both bioarchaeological and modern skeletal populations. Clinical studies have identified several causes of metabolic stress that can result in linear enamel hypoplasias, such as disease, health issues during the newborn phase, and dietary deficiencies. Malnutrition and undernutrition are particularly problematic for the development of healthy enamel; research on the dentition of individuals born during famine in the People's Republic of China shows that enamel developed during starvation times was defective in comparison with enamel developed before and after the famine (Zhou & Corruccini, 1998). Given the wide range of potential causes of linear enamel hypoplasias, they are considered a non-specific indicator of stress (Larsen, 2015). Importantly, enamel cannot be remodeled, so linear enamel hypoplasias are permanent records of developmental insults (M. Lewis & Roberts, 1997).

Enamel formation follows a relatively consistent pattern across teeth, although the timing differs by tooth. Cuspal enamel is first laid down in dome-like layers, beginning with the cusp of the tooth and proceeding cervically. When cuspal enamel has completed formation, enamel is then deposited on the walls of the crown in a tile-like pattern; this is called the lateral enamel. When this pattern is disrupted, a linear enamel hypoplasia is formed. However, the hypoplasia is only visible macroscopically in the lateral enamel. Defects in the cuspal enamel can only be viewed via histological sections (Ritzman et al., 2008). Tooth enamel begins development in

utero, first with the deciduous incisors, and completes development once the crowns of the third molars have finished forming, at approximately twelve years old (Larsen, 2015).

Enamel defects do not affect all teeth equally. Instead, they are more commonly observed on the anterior dentition—the incisors and canines—as well as the cervical two thirds of the crown (Larsen, 2015). This pattern is related to enamel development, so linear enamel hypoplasias are considered to reflect developmental stressors, as opposed to those encountered later in life. This is due to the fact that the defects are formed while the tooth enamel is undergoing development, which varies in age by tooth. Therefore, based on the tooth on which the defect is present, as well as its distance from the cemento-enamel junction, it may be possible to roughly ascertain the age at which an individual experienced some sort of stress or insult (Larsen, 2015). Some researchers have argued, however, that traditional macroscopic methods (e.g. measuring the distance between the cemento-enamel junction and the linear enamel hypoplasia), underestimate the age of insult, as they do not take into account cuspal enamel formation time or the varying rates of enamel deposition by tooth and throughout the dentition (Ritzman et al., 2008). Further, these methods falsely assume that the rate of tooth development in a bioarchaeological population is the same as in a modern healthy population, from which these methods were derived (M. Lewis & Roberts, 1997).

# Cribra orbitalia and Porotic Hyperostosis

Two notable porosities are among the most commonly observed in bioarchaeological material: porosity of the outer table of the cranial vault (porotic hyperostosis) and porosities of the superior aspect of the eye orbits (*cribra orbitalia*). Both lesions were initially subsumed under the umbrella of porotic hyperostosis, with J. Lawrence Angel having coined the term in 1966. Prior to 2009, both conditions had long been linked to iron deficiency anemia or a

response to overwhelming pathogen load in the environment (Stuart-Macadam, 1992). The connection seemed a reasonable one; both iron deficiency anemia and these porotic lesions were widespread in the global population. This hypothesis led bioarchaeologists to connect the presence of porotic hyperostosis and/or *cribra orbitalia* to conditions causing iron deficiency, such as diarrheal disease or intestinal parasites (Walker, Bathurst, Richman, Gjerdrum, & Andrushko, 2009).

However, iron deficiency anemia alone is not a sufficient explanation for these commonly observed lesions (Walker et al., 2009). In fact, the marrow expansion characteristic of porotic hyperostosis and *cribra orbitalia* requires such an intense proliferation of red blood cell production that iron deficiency anemia alone cannot cause. As a result, hemolytic and megaloblastic anemias are more likely causes for porotic hyperostosis, while the etiology of *cribra orbitalia* likely differs, possibly linked to sub-periosteal bleeding associated with nutritional deficiencies (Walker et al., 2009). Therefore, it is no longer advisable to associate either of these lesions exclusively with iron deficiency anemia. Instead, these porosities can be more accurately linked to chronic disease and malnutrition.

Walker and colleagues propose a constellation of variables that can lead to porotic hyperostosis and *cribra orbitalia*, including inadequate nutrition, poor sanitation, and infectious disease (2009). On the other hand, porotic hyperostosis and *cribra orbitalia* may also be caused by infection, injury, or a nutrient deficiency such as scurvy. Macroscopically, etiologies like these may produce a similar appearance to anemia-induced porotic hyperostosis, but through an activation of the periosteum instead of enlargement of the diploe. In cases such as these, the characteristic "hair on end" radiographic appearance is not observed. Given the possibility that the same macroscopic appearance can result from a number of different disease processes, it is

recommended that porotic hyperostosis and *cribra orbitalia* be considered non-specific indicators of stress, as opposed to a specific diagnosis (Ortner, 2003). Although some scholars have argued that orbital and vault lesions have the same etiology (Stuart-Macadam, 1987, 1989), it should not be assumed that both lesions are caused by anemic marrow hypertrophy. Instead, it has been argued that *cribra orbitalia* is more likely to be caused by subperiosteal inflammation due to vitamin deficiencies or trauma-related hematomas (Larsen, 2015).

On a biological level, the marrow expansion observed in cases of porotic hyperostosis is a response to the body's attempt to maintain homeostasis with respect to red blood cell count. That is, the body should produce red blood cells at a rate similar to that with which they are destroyed. When hemoglobin levels drop, the body begins producing red blood cells more quickly. Therefore, in conditions of chronic anemia and low hemoglobin, red blood cells are overproduced. In extreme cases, red blood cells are overproduced in the diploe of the cranial vault, causing it to expand while the outer table resorbs. This process produces the characteristic lesion known as porotic hyperostosis. When viewed radiographically, the diploe undertakes a "hair on end" appearance. Notably, the sustained and massive marrow hypertrophy that creates porotic hyperostosis is most consistent with hemolytic or megaloblastic anemias; in contrast, iron deficiency anemia causes a decrease in red blood cell production, due to the lack of hemoglobin in the blood (Walker et al., 2009).

Osteologically, the bones of the cranial vault undergo a multi-stage process that results in visible porosities. First, very fine pitting of the vault occurs in small, circumscribed areas, typically on the parietal bones and on the occipital bones, parallel to the lambdoidal suture. Further, the trabeculae of the diploe begin to orient perpendicular to the inner and outer tables of the vault. Second, the areas of porosity grow larger, and the pits themselves also expand due to a

conglomeration of the initial, smaller pits. The affected regions of the vault also expand to include the occipital squama. During this phase, the vault thickens and the traditional "hair on end" appearance can be identified; this is most readily observed radiographically. The third phase involves a marked thickening of the skull vault, giving way to a sponge-like, porous appearance. Remodeling can occur throughout this process, producing rounded edges and an infilling of the pores (Ortner, 2003).

Porotic hyperostosis and *cribra orbitalia* can be observed in both adults and subadults, and can also undergo healing if and when an anemic or inflammatory episode ends. However, it has been argued that these conditions are most likely to occur in subadults, as the location of red blood cell production shifts from the diploe of the cranial vault and medullary cavities of the long bones in subadults, to the thoracic skeleton in adults. Further, most active lesions are observed in subadults, while healed lesions are more commonly associated with adult individuals (Stuart-Macadam, 1985; Walker et al., 2009).

#### **Periosteal Reaction**

Periosteal reactions are caused by inflammation of the periosteum, or the connective tissue lining surrounding bone. This inflammation stimulates osteoblast activity, causing new bone formation on the cortex of the injured or infected bone. This commonly observed condition can be caused by a variety of factors, including bacterial infection, traumatic injury, or any other disruption of the periosteum. Periosteal lesions appear similar to tree bark, generally with demarcated borders and a plaque-like or raised appearance. Similarly to porotic hyperostosis and *cribra orbitalia*, periosteal lesions can appear healed or unhealed; the unhealed lesion tends to appear "woven" in texture, with loosely organized new bone. On the contrary, healed lesions generally have a more dense, raised, and undulating appearance. Periosteal reactions can appear

localized, or can be widespread and involve several skeletal elements, depending on the severity of the infection or injury. Periostitis is considered a non-specific indicator of health, as its presence cannot be attributed to a particular disease, bacteria, or injury (Larsen, 2015).

The presence and prevalence of periosteal reactions has been used widely in bioarchaeological research. The frequency of periostitis has been compared between males and females in order to understand sex-based differences in the non-specific stress indicator among a wide variety of groups. Further, this type of analysis has been used in conjunction with other variables to attempt to identify status differences between individuals in a sample (Larsen, 2015).

# **Methodological Limitations of Skeletal Stress Analyses**

The last three decades of bioarchaeological inquiry have involved extensive introspection and have been characterized by the need to grapple with important methodological considerations, some seemingly the death knell of paleodemography (Bocquet-Appel & Masset, 1982) and others simply a stern but cautionary note on careful interpretation of evidence (Wood et al., 1992). In 1982, Bocquet-Appel and Masset "bid farewell" to the field of paleodemography by elucidating their concerns about age estimation in demographic samples. The authors brought to light three issues—age mimicry, secular change, and poor correlation of aging methods to skeletal indicators—that necessarily bias the results of a paleodemographic inquiry into a cemetery assemblage (Bocquet-Appel & Masset, 1982).

Publishing an additional critique of bioarchaeology, without the inherent pessimism of Bocquet-Appel and Masset, were Wood and colleagues with their seminal paper entitled "The Osteological Paradox" (1992). The three fundamental conceptual issues with bioarchaeological inference raised by the authors are demographic nonstationarity, selective mortality, and heterogeneity in risk of disease and death. Unlike Bocquet-Appel and Masset, Wood and

colleagues are less eager to call for the abandonment of bioarchaeology and paleodemography. Instead, they urge researchers to continue to press forward, but to take these conceptual issues to heart when making assessments about skeletal samples (1992).

Bioarchaeologists and paleodemographers have attempted to address and relieve the concerns raised by Bocquet-Appel and Masset and Wood and colleagues, with varying degrees of success, in recent years (Wright & Yoder, 2003). Improvements in sex estimation methods, particularly using DNA for sex estimation in subadults, as well as the expansion of ageestimation methods to improve their utility in cases of poor preservation, and transition analysis for more accurate age estimation within a statistical framework, have allowed researchers to create a better demographic profile of the dead. In an effort to elucidate hidden heterogeneity in risk, researchers have used isotopes and biodistance analyses to identify subgroups, such as migrants, within a cemetery sample. Additionally, studies of paleodiet have provided information about childhood nutritional status that can illustrate hidden frailty in particular individuals.

Another way to strengthen bioarchaeological conclusions is to consider several lines of evidence at once, such as comparing adult stature with paleodiet for an indication of nutrition status, as well as evidence of childhood stress such as linear enamel hypoplasias to ascertain whether childhood stress affects adult stature. Finally, the recent use of pathogen DNA to better connect skeletal indicators of disease to the specific disease process has the potential to allow researchers to determine whether an individual was affected by a disease, regardless of whether or not any lesions are present in the skeleton. Therefore, we might begin to understand whether skeletons with extensive healed lesions may, in fact, be "healthier" than those without lesions at all (Wright & Yoder, 2003).

# **Biological Distance Analysis**

Biological distance, in its most basic form, refers to the degree of similarity or dissimilarity between groups or populations (Auerbach, 2023; Hefner, Pilloud, Buikstra, & Vogelsberg, 2016). Biological distance analyses, as they are employed in bioarchaeology and forensic anthropology, have traditionally explored population relatedness based on continuous and discrete traits of the bones and teeth within a multivariate statistical framework (Hefner et al., 2016). Historically, studies using craniometry were undertaken by typologists intent on reifying "racial" differences based on measurements of the skull and evaluating deviations from "normal types" (Hefner et al., 2016). These groups were seen as fixed and associated with distinct origins (Auerbach, 2023). Today, biodistance studies using skeletal and dental measurements and traits focus on characterizing human variation and interpreting that variation within a biocultural context using model-free and model-bound population genetic approaches (Auerbach, 2023). Improvements in biogeochemistry, DNA analysis, and statistical methods in recent decades have expanded the types of analyses anthropologists use to evaluate relatedness in past populations, as well as population affinity in modern forensic samples (Hefner et al., 2016).

This section will first provide a brief discussion of important population genetics concepts. Then, it will outline the goals, underlying assumptions, and levels of analysis for biodistance studies. Next, it will describe current practices in biodistance research, including those using genetic data, biogeochemical methods, statistical methods, cranial and dental metrics and morphology, and the relationships between these avenues of analysis. This section will conclude with a critical discussion of ancestry and population affinity within forensic anthropology and its implications for the present study.

## **Population Genetics**

Several population genetics concepts are useful for this dissertation, as they provide a potential explanation for the observed results. Population genetics refers to the genetic composition of a population and how it changes over time (Relethford, 2012). Broadly, population genetics theory is a form of evolutionary theory that can be applied to questions of human population history, ancient human migrations, and the impact of geography on mate choice. An equilibrium model of evolution, one that assumes no selection, gene flow, genetic drift, or mutation, often forms the basis of population genetics studies. When populations deviate from this equilibrium, researchers can hypothesize that non-random genetic events have occurred. Two of these non-random processes that influence genetic variation—gene flow and assortative mating—are important to this dissertation and will be discussed briefly here.

Gene flow is the exchange of genetic material between populations (Relethford, 2012). Gene flow is often the result of migration, either temporary or long-term. It introduces new alleles to a population and reduces genetic differentiation between populations. In other words, gene flow increases within-population genetic variation and decreases between-population variation. The degree of gene flow in humans is expected to be proportionate to the geographic distance between populations. This is known as the isolation-by-distance model. Nearby populations should exhibit more gene flow than those that are more distant because humans are more likely to choose a mate from nearby than from far away. The result of this model is that geographically proximate groups should be more genetically similar to one another than they are to geographically distant groups (Relethford, 2012).

A second non-random event that affects genetic variation is assortative mating. This is a form of sexual selection wherein individuals choose to mate with others based on similar

phenotypic characteristics (Relethford, 2012). Assortative mating is culturally mediated and involves the social influence of group membership and phenotypic preferences specific to a time and place (Robinson et al., 2017). The selection of a partner phenotypically similar to oneself influences overall genetic variation by producing subgroups with less within-group variation and more between-group variation. In a sample of individuals with different geographic ancestries all living in the same place, assortative mating based on ancestry may produce genetic subsamples within the overall population.

The influence of non-random genetic events such as gene flow or assortative mating will influence the degree of variation at *Rusellae* and can help explain the dynamics that may have led to the observed variation.

#### **Distance Studies**

Distance studies are a form of population genetic analysis that seek to understand biological relatedness between pairs of populations in order to explore their population structure and history (Relethford, 2016). Distance studies use one of two types of data: genetic information, such as nuclear or mitochondrial DNA, or phenotypic data, such as cranial or dental metrics or morphology. Phenotypic distance studies, which are commonly used in bioarchaeology, are also known as biological distance, or "biodistance" studies (Relethford, 2016). In contrast to genetic distance, wherein genetic data is explored directly, biodistance studies use phenotypic data as a proxy for genetic relationships, following the assumption that phenotypic similarity generally aligns with genetic similarity based on theoretical population-genetic models (Relethford, 2016). Importantly, phenotypic expression is a result of both genetic and environmental influences, and is therefore not directly interchangeable with genetic data. However, studies have routinely shown that genetic data and skeletal trait expression are

correlated highly enough to reveal biological relationships using phenotypic data and that much of global human cranial morphology is shaped by selectively neutral evolutionary forces (Herrera, Hanihara, & Godde, 2014; Martínez-Abadías et al., 2009; Relethford, 1994, 2001, 2004; Roseman, 2004; Roseman & Weaver, 2004; von Cramon-Taubadel, 2014). Further, metric and nonmetric traits have reliably shown non-zero heritabilities (Carson, 2006a, 2006b; Martínez-Abadías et al., 2009; Šešelj, Duren, & Sherwood, 2015).

Biodistance studies have been employed at varying levels of analysis, including at the global, regional, and intra-site levels (Hefner et al., 2016; Paul & Stojanowski, 2017). Globallevel biodistance studies have been used to explore peopling of the Americas through dental nonmetric traits (Scott et al., 2023) and microevolutionary processes that shape the human craniomandibular complex (von Cramon-Taubadel, 2016). On a regional scale, studies have examined migration in the Middle Bronze Age Nile Delta (Maaranen et al., 2021), population history in medieval Nubia (Streetman, 2018; Vollner, 2016), and mobility in Roman Phoenicia (Mardini, Badawi, Zaven, Gergian, & Nikita, 2023). Intra-site biodistance analyses include studies of kinship and cemetery structure, post-marital residence, sample aggregate phenotypic variability, temporal microchronology, and age-structured phenotypic variation. These analyses treat a site as if it comprises distinct subgroups, as opposed to assuming that the site is a homogenous unit (Stojanowski & Schillaci, 2006). Intra-site analyses have explored interindividual dental morphology in Neolithic and Iron Age Germany using Mahalanobis distance (Rathmann, Lismann, Francken, & Spatzier, 2023) and post-marital residence and biological kinship in an Alameda, California shellmound (Broehl, 2023).

#### **Current Practices in Biodistance**

Current practices in biodistance research involve several analytical methods and measures of relatedness that merit discussion. These include genetic data, skeletal and dental metric and nonmetric analyses, biogeochemical methods, and advances in statistical methods. Each of these analytical avenues can be used alone or in conjunction with the other methods to illuminate ancient relationships. A discussion of each—as well as their interactions with one another—follows.

Distance Studies in Bioarchaeology: Ancient DNA

In bioarchaeology, the genetic information used in distance studies is ancient DNA (aDNA) derived from skeletal and dental tissue. Its use has revolutionized archaeology by providing information about the origin and evolution of ancient pathogens, and it has helped clarify methods of domestication of crops and animals. Importantly for this dissertation, ancient DNA has also been used to contribute to debates surrounding population structure, migration, and group relatedness (T. A. Brown, 2023). By its nature, genetic data provides the most direct measure of relatedness, as it does not involve parsing out the additional, complicating layer of phenotypic expression used in biodistance studies. Since aDNA was first used to sequence genetic data from 150-year-old faunal remains 1980s (Higuchi, Bowman, Freiberger, Ryder, & Wilson, 1984), molecular anthropologists and geneticists have refined their methods to allow for data extraction from much older specimens, such ancient Egyptian mummies (Hefner et al., 2016; Pääbo, 1985a, 1985b). Further, advances in DNA amplification, including polymerase chain reaction (PCR) and single primer extension (SPEX), have facilitated more accurate detection of postmortem DNA alteration or contamination (Brotherton et al., 2007; Hefner et al., 2016; Mullis & Faloona, 1987), while next-generation DNA sequencing (NGS) has allowed

scientists to make complex inferences about population structure using mass quantities of DNA data (T. A. Brown, 2023).

Several types of DNA can be used in genetic distance studies, including mitochondrial DNA (mtDNA), which traces the maternal lineage, Y-chromosome DNA, which traces the paternal lineage, and autosomal DNA, which is individual-specific. Since nuclear aDNA tends to be more fragmentary, studies of the mitochondrial genome are particularly prevalent; a greater number of copies of mtDNA exist in each cell, so mtDNA is more readily recovered (Smith, Hulsey, (Pack) West, & Cabana, 2016). Notably, in a study of cranial and dental traits, mtDNA, and Y-chromosome DNA, Herrera and colleagues found that mtDNA was more highly correlated with cranial measurements, while Y-chromosome data is more strongly associated with cranial nonmetric traits (Herrera et al., 2014).

Although ancient DNA has been considered the "gold standard" by which all other distance studies are measured, the destructive, expensive, and specialized analyses required preclude many researchers from utilizing this analytical tool. Additionally, DNA preservation is not guaranteed in archaeological samples, and is highly contingent upon the taphonomic environment and curation process. Finally, aDNA can provide information about genetic ancestry, but does not directly measure mobility. Therefore, although it may be useful in migration studies, other distance measures should also be employed to produce a fuller picture of ancient movements (Nikita, 2024).

Biodistance in Bioarchaeology: Skeletal and Dental Metrics and Nonmetrics

In cases where ancient DNA is either unavailable or unable to be used, phenotypic proxies can be employed in place of genetic data in order to evaluate distance. Traditional biodistance studies use metric and morphological (nonmetric) data from the skull and dentition;

these metric or nonmetric traits are the phenotypes used as genetic proxies to explore relatedness. In general, nonmetric traits are discrete traits scored in a categorical way, typically as a binary "present" vs. "absent" or using an ordinal scoring system (Buikstra & Ubelaker, 1994; Pink, Maier, Pilloud, & Hefner, 2016). Dental nonmetric traits, specifically, refer to the shape and form of teeth (Pilloud, Edgar, George, & Scott, 2016). One of the most commonly-used systems for nonmetric dental analysis is the Arizona State University Dental Anthropology System (ASUDAS), which presents standardized plaques and definitions for dental traits that have been consistently defined (Irish, 2015; Pilloud et al., 2016; Turner, Nichol, & Scott, 1991). Further, datasets such as Ossenberg's (2013) cranial nonmetric trait database can be used for global comparisons and Konigsberg's online publication of statistical code for evaluating biodistance have improved the utility of cranial nonmetric data for biodistance research (Pink et al., 2016).

Nonmetric traits are especially useful in fragmentary or incomplete assemblages, as are common in bioarchaeological settings, because small fragments that may not provide useful metric data can still contain scorable nonmetric traits. Further, these traits are relatively simple to identify, the data is straightforward to collect, and there are a variety of statistical methods available for analysis, including Smith's mean measure of divergence (Pink et al., 2016). However, asymmetry in trait expression, inter-trait correlations, and the relationship between traits and biological variables such as age and sex all require consideration prior to undertaking nonmetric analyses (Pink et al., 2016). Cranial and dental nonmetric traits have been used for large-scale studies of biodistance that have identified geographically clustered dental complexes (Hanihara, 1967), regional migration in Hungary and Italy using dental nonmetric traits (Piccirilli et al., 2023), population affinity in the United States and India (Edgar, 2014; Hefner, 2009;

Pillai, Parmar, Babu, & Vyas, 2023), and genetic diversity in Classic period Teotihuacan (Meza-Peñaloza, Zertuche, & Morehart, 2021), among a variety of other research avenues.

Metric methods for biodistance analysis use continuous distance data, which are regularly applied to a multivariate statistical framework in order to explore human diversity (Dudzik & Kolatorowicz, 2016). Dental metric analyses specifically utilize dental dimensions, typically crown length and width, but sometimes involving additional measures such as crown height, diagonal or cervical crown measurements, or measures of enamel thickness (Kieser, 1990; Pilloud & Kenyhercz, 2016). Craniometric analyses rely on standardized landmark and interlandmark distance definitions; many of the craniometric definitions used today originated with Martin (1914), were refined by Howells (1973), and collated for modern use by Fleischman and Crowder (Fleischman & Crowder, 2018). Howells' worldwide reference craniometric dataset is available online (1996), which has arguably formed the foundation for modern craniometric biodistance studies. Generally, cranial and dental metric methods tend to be cost-effective and non-destructive, allowing for biodistance analyses with as little as a pair of calipers (Dudzik & Kolatorowicz, 2016). However, in cases of fragmentary samples or poor preservation, the number and type of possible measurements may be limited. Further, issues of sexual dimorphism, age, inter-trait correlation, and inter-tooth correlations must be incorporated into research design (Pilloud & Kenyhercz, 2016).

# Critiques of Phenotypic Proxies

Despite the observed utility of biodistance studies, there are several modern critiques of phenotypic proxies for genetic data that merit discussion. These include concerns relating to developmental plasticity, including recent work on epigenetic influences, as well as potential

issues with the isolation-by-distance model that underpins much of the bioarchaeological biodistance research to date.

The baseline assumption for biodistance research is that metric and nonmetric human variation mirrors variation in neutrally evolving genetic markers, driven by genetic drift, mutation, and gene flow. As noted previously, studies have routinely shown the heritability of cranial metric and nonmetric traits (Carson, 2006a, 2006b; Martínez-Abadías et al., 2009; Šešelj et al., 2015) and that the global variation in these traits generally conforms to an isolation-bydistance model (Relethford, 1994). However, the environment encountered during development also plays a crucial role in structuring the craniofacial complex; this environmental influence results in the phenomenon of cranial plasticity, or the capacity for phenotypic alterations due to the impacts of the environment and non-neutral selective forces. The fetal environment and the role of intergenerational inheritance of matrilineal physiological and psychosocial stress has also been reported as influencing the adult craniofacial complex through epigenetic effects, which provides an additional framework for understanding how environment influences phenotype (Kuzawa, 2005; Mulligan, 2016; F. Ross, Pentecost, & Moll, 2023; Thayer & Non, 2015). Epigenetics refers to modifications to the genome that do not alter DNA sequence, but still impact gene expression (Mulligan, 2016). Research with non-human mammals has shown epigenetic effects on craniofacial morphogenesis (Hallgrímsson, Lieberman, Liu, Ford-Hutchinson, & Jirik, 2007). Craniofacial variation results from variation in brain growth as well as developmental constraints in basicranial and facial dimensions; these constraints result from epigenetic processes, thereby providing evidence for an epigenetic component of craniofacial plasticity (Hallgrímsson et al., 2007). Recent data has also shown that epigenetic regulation of a gene responsible for craniofacial morphogenesis may cause the globular skull shape of

anatomically modern humans, as compared with archaic hominins (Di Pietro et al., 2021). Further, epigenetic changes have been linked to phenotypic differences between human and chimpanzee craniofacial structure (Prescott et al., 2015; Selleri & Rijli, 2023). Overall, epigenetic modification has been consistently shown to influence human craniofacial structure (Selleri & Rijli, 2023).

Since Boas argued for the impact of the environment on changing cranial form in the descendants of immigrants (1912), the importance of cranial plasticity on human variation has garnered debate (Gravlee, Bernard, & Leonard, 2003a, 2003b; Sparks & Jantz, 2002, 2003) with the general conclusion that plasticity has a small, but significant, effect on cranial variation that mostly exists as "noise" when exploring population-level research questions (Relethford, 2004; Winburn, Yim, & Stock, 2022). Further, recent studies have shown that most developmental plasticity in the human skull is related to the functionality of the masticatory apparatus (Katz, Grote, & Weaver, 2016; von Cramon-Taubadel, 2009; Winburn et al., 2022), which retains its utility for elucidating population history despite the environmentally-induced variability of this region, as the effects of mastication are significantly smaller than the influence of neutral evolutionary processes (Collard & Wood, 2007; Katz et al., 2016; von Cramon-Taubadel, 2009). Therefore, cranial plasticity does not inhibit the use of craniometric methods for population history analyses (Winburn et al., 2022).

An additional concern for biodistance research is the utility of the isolation-by-distance (IBD) model, which is routinely cited when justifying the use of craniometrics as a phenotypic proxy for genetic information. Relethford's IBD model states that geographically proximate populations should be more genetically similar than geographically distant populations, due to the limitation of geographic distance on gene flow. Relethford showed that this model holds true

on a global scale and that craniometric traits mirror genetic information derived from red blood cell polymorphisms and microsatellite DNA markers (2004). However, critiques of and alternatives to the IBD model have been presented in recent literature (Border et al., 2024; Fletcher Jr. et al., 2022; Petkova, Novembre, & Stephens, 2016). IBD models assume a homogenous physical landscape in which distance is the only deterrent to mate selection. However, in a non-homogenous landscape (which is more applicable to the real world), movement of people, and thus gene flow, is limited by physical barriers. This model, called isolation by resistance, integrates physical landscape with genetic models to predict genetic differentiation (Petkova et al., 2016). Similarly, new statistical methods such as spatial absorbing Markov chains have been recommended to account for impediments to migration, directional migration, and landscape-induced mortality during migration, all of which influence gene flow (Fletcher Jr. et al., 2022). In addition, social and behavioral dynamics such as non-random assortative mating also influence genetic differentiation and deviate from expectations of an isolation-by-distance model (Border et al., 2024).

Another potential issue with the isolation-by-distance model is how scale of analysis influences the model. As previously described, biodistance can be explored on scales ranging from intra-cemetery to global. Therefore, an important consideration in biodistance research is how to handle analyses on a small scale and with a small number of interlandmark distances. When attempting to use craniometrics as a proxy for genetics at small geographic and temporal scales, it is important to use the most complete data set possible. In cases where few individuals and landmarks are used, the resolution of the analysis decreases significantly and craniometrics may correlate weakly with genetic data (Smith et al., 2016). Due to the fact that biodistance studies using craniometric data will always involve fewer variables than genetic studies, as well

as the fact that morphological differentiation of human crania is constrained by trait correlations, there will always be less differentiation between groups in a craniometric analysis than expected in a genetic analysis (Strauss & Hubbe, 2010).

#### Advances in Statistical Methods

Methodological advancements for biodistance analysis have come in the form of improved statistical classification methods and techniques. Machine learning (ML) models are computationally-intensive classification methods that use an iterative approach with few assumptions that can circumvent issues with traditional approaches, such as linear discriminant function analysis (LDFA) (Ousley, 2016). LDFA approaches require normal distributions and are prone to overfitting, whereas machine learning models "learn" based on a training set of data with no a priori assumption of group membership and iteratively fine-tune themselves until they establish the best criteria for group separation. Machine learning methods have the advantage of combining various data types and they produce high classification accuracies. They also incorporate resampling and simulation in order to handle issues of small sample size that regularly occur in skeletal samples (Ousley, 2016). The utility of several machine learning models have been demonstrated for biodistance applications, including neural networks, random forests, and support vector machines (Hefner & Ousley, 2014; Ousley, 2016). Although LDFA is broadly useful for craniometric classification (Ousley, 2016), ML methods have shown greater accuracies for cranial morphoscopic data (Hefner & Ousley, 2014), mixed craniometric and morphoscopic data (Hefner, Spradley, & Anderson, 2014), and dental morphology data (Edgar & Ousley, 2013). In addition to machine learning models, probabilistic clustering algorithms and finite mixture analysis have shown promise for assigning group affinity probability and assessing overlap within samples, instead of assuming discrete group membership (Algee-Hewitt, 2016; Algee-Hewitt, Hughes, & Anderson, 2018; Algee-Hewitt, 2017a, 2017b; Winburn et al., 2022).

An additional craniometric analytical method that has gained popularity with biodistance researchers is geometric morphometric (GM) analysis. Geometric morphometric methods utilize three-dimensional landmark data to analyze shape variation across a study sample (Mitteroecker & Schaefer, 2022). Traditionally, a Generalized Procrustes Analysis (GPA) is used, which uses superimposition in order to standardize position, orientation, and scale to facilitate comparison across a dataset and produce a newly derived set of shape variables (Mitteroecker & Schaefer, 2022; Rohlf & Slice, 1990; Urbanová & Ross, 2016). GM methods have been used to explore population history and ancestral origin since shape variables tend to be correlated with population affinity, whereas size variables are more highly correlated with sex (Dudzik, 2019; Urbanová & Ross, 2016). GM models have been shown to illustrate more phenotypic variation than is present in traditional craniometric data using interlandmark distances (Dudzik, 2019; McKeown & Jantz, 2005; Spradley & Jantz, 2016). In fact, non-traditional landmarks and GM approaches have exhibited more discriminating power than the traditional metric approach (Spradley & Jantz, 2016). Although GM methods offer a more sophisticated analysis of cranial data than traditional interlandmark distance analyses, the sheer volume of data, landmarks, and semi-landmarks used in GM approaches may be challenging to connect to developmental and evolutionary processes, even with advancements in multivariate statistics and machine learning models (Mitteroecker & Schaefer, 2022).

Measures of Mobility: A Biogeochemical Approach

In addition to the aforementioned distance approaches, biogeochemical methods such as stable isotope analysis have also contributed to the exploration of origins and mobility in skeletal samples. Although not a direct measure of distance, per se, stable isotopes can be integrated with genetic and biodistance methods to refine estimates of geographic origin and study past migrations (Nikita, 2024). Stable isotope analysis, very simply, refers to the study of the chemical composition of bones and teeth with respect to certain elements that can provide information about diet, demography, and life history (Katzenberg, 2008). Strontium and oxygen isotopes, specifically, provide information about origin and residence throughout life, as they are tied to local drinking water and geology, which are then incorporated into skeletal and dental tissue through eating and drinking (Bentley, 2006; Nikita, 2024; Pederzani & Britton, 2019). Analysis of dental enamel, which is formed during childhood, provides information about an individual's early years of residence (Montgomery, 2010), whereas isotope ratios in bone can illustrate mobility patterns in the later years of an individual's life (Pederzani & Britton, 2019). Therefore, comparing tissues within the same individual can therefore illustrate a person's movement (or lack thereof) during life. Further, comparison of an individual's childhood isotopic signature to that of their burial location allows for a broad assignment of "local" vs. "non-local" (Pederzani & Britton, 2019). Although isotope analysis is excellent at providing direct data about ancient mobility, the analyses are destructive, can be costly, and requires specialized laboratories (Nikita, 2024). In addition, isotopic signatures are not unique to a specific region; instead, the same isotopic signature can be observed in various regions (Nikita, Mardini, Mardini, & Degryse, 2022; Prowse, 2016). Further, the resolution of isotope analyses are somewhat limited. While they are useful for comparing childhood and adult residence, they may not be as useful for understanding short-term or short-distance migrations (Nikita, 2024). Finally, human behavior can influence isotopic signatures; for example, drinking non-local water that was brought in via aqueducts can influence oxygen isotope values (Bruun, 2010).

Integrating (Bio)distance Methods

Studies that integrate several—or all—of the aforementioned methods have proliferated within bioarchaeology, displaying the utility of theoretically-grounded, hypothesis-driven, and interdisciplinary endeavors. For example, Stark and colleagues employed strontium and oxygen isotopes as well as nonmetric traits to explore mobility at the imperial Roman sites of Isola Sacra and Velia, revealing a diverse Roman population with evidence of migration, especially by females and children (Stark, 2016; Stark et al., 2020; Stark et al., 2021). Additional work at the Roman site of Vagnari using isotopic and DNA analyses found a small but significant contingent of non-local individuals, as well as some individuals with genetic affinity with sub-Saharan Africa and Asia (Emery, 2018; Emery, Duggan, et al., 2018; Emery, Stark, et al., 2018; Prowse, 2016; Prowse, Barta, von Hunnius, & Small, 2010). More recently, isotopes, dental morphology, and ancient DNA were integrated and used to identify an African-born migrant in the catacombs of Saints Peter and Marcellinus in Rome. Two individuals in the assemblage were identified as distinctive based on dental morphology; one was identified as having African origin through subsequent isotopic and genetic analyses, reiterating the idea that long-distance migration was a component of Imperial Roman life (Salesse et al., 2021). Additionally, an analysis of craniometric data and strontium and oxygen isotopes in Roman York, England established the likely presence of non-local individuals and an overall ancestrally diverse population (Leach, Eckardt, Chenery, Müldner, & Lewis, 2010; Leach, Lewis, Chenery, Müldner, & Eckardt, 2009). Similar distance studies incorporating macromorphoscopic traits, stable isotopes, and ancient DNA have identified several possible circum-Mediterranean migrants from Africa-and possibly Asia-in Roman-era and Medieval London (Redfern et al., 2016; Redfern & Hefner, 2019). In addition, stress variables and mortality were explored with relation to ancestry; higher hazards of death from the plague were identified in individuals with African affinity (Redfern, DeWitte, Hefner, & Kim, 2023).

As has hopefully been established, modern biodistance measures can be used to explore patterns of variation and relationships within and between groups. The present study uses biological distance as an analytical tool to explore craniofacial variation within *Rusellae* and between *Rusellae* and relevant global reference samples in order to contribute to the conversation surrounding the population history and demographic structure of the site throughout its occupation. The results of these analyses will be integrated with known archaeological and historical data, as is recommended in order to develop a more nuanced understanding of mobility (Buikstra et al., 2022; Gregoricka, 2021), in order to better understand population structure and population history in Late Antique and Early Medieval Tuscany.

# **Methodological Limitations of Biodistance Research**

As previously discussed, biodistance analyses use a phenotypic proxy—in this case, cranial measurements—to illuminate underlying genetic relationships. Therefore, these analyses rely on using appropriate reference samples and biologically meaningful craniometric variables. Although the actual underlying relationships within and between groups do not change based on which reference groups are included or excluded from the analysis, perceptions and interpretations of these relationships can vary due to methodological choices (Key, 1982). Therefore, it is crucial to include the most temporally and geographically relevant reference samples in biodistance analyses; any deviations from the hypothetical "most accurate" reference sample will necessarily introduce error. This issue, coupled with the forced classification of methods like Linear Discriminant Function Analysis, will cause instances of misclassification in the study.

Another important consideration in biodistance research is how to handle analyses on a small scale and with a small number of interlandmark distances. The overall completeness of the sample will be the primary driver of which data points are included in the analysis. However, whenever it is possible, preference should be given to collecting data from regions of the skull with higher heritability (Dudzik & Kolatorowicz, 2016). When fewer individuals and landmarks are used, the resolution of the analysis decreases significantly to the point where cranial morphology corresponds weakly with genetic data from the same sample and may no longer be informative of biological distance (Smith et al., 2016). Due to the fact that biodistance studies using craniometric data will always involve fewer variables than genetic studies, as well as the fact that morphological differentiation of human crania is constrained by trait correlations, there will always be less differentiation between groups in a craniometric analysis than expected in a genetic analysis (Strauss & Hubbe, 2010).

# **Ancestry & Population Affinity**

Although this dissertation is bioarchaeological in nature, it employs classification methods commonly used in forensic anthropology to estimate the ancestral origin of several unknown individuals from the *Rusellae* cemetery using measurements of the cranium. Therefore, this dissertation requires a brief discussion of biodistance methods as they are applied to forensic ancestry estimation. Following guidelines outlined by the Scientific Working Group for Forensic Anthropology (SWGANTH), "ancestry" refers to the ancestral origin or geographic region associated with a particular population (2013). As it is used in modern forensic anthropology, ancestry estimation refers to the classification of an unknown individual as most similar to a reference group or likely region of geographic origin using classification statistics (Dunn, Spiros, Kamnikar, Plemons, & Hefner, 2020) and ultimately results in the prediction of a probable social

race based on cranial metrics and/or morphology (Stull et al., 2021). Social race refers to the culturally-constructed and peer-perceived category derived from an individual's phenotype (Dunn et al., 2020). Accurate ancestry estimation relies on the non-zero correlation between social race, skeletal morphology, and geographic origin that exists in the United States as a byproduct of assortative mating and institutionalized racism (Dunn et al., 2020; Ousley, Jantz, & Freid, 2009). These methods also rely on the heritability of the craniofacial complex and its use as a proxy for genetic data (Dunn et al., 2020). Fundamentally, ancestry estimation methods utilize a biodistance approach to inform forensic models, moving from a population-wide scale to an individual one (Dunn et al., 2020).

Before diving further into ancestry estimation, it is important to make some terminological clarifications. Historically, the term "race" was used to describe an individual's classification into one of three broad groups: African, Asian, or European (A. H. Ross & Williams, 2021). In light of incontrovertible evidence against the existence of biological races, the term "ancestry" has largely subsumed the term "race" in the literature over the past several decades (Pilloud et al., 2021; Tallman, Parr, & Winburn, 2021). However, many argue this has been a change in name only, with methods for ancestry estimation remaining largely the same as those used to assess "race" (Albanese & Saunders, 2006; Pilloud et al., 2021; Tallman et al., 2021). More recently, the term "population affinity" has been adopted by some scholars, who argue that contemporary methods are ultimately testing the similarity (or affinity) of an unknown individual to a known reference sample using biodistance methods (Spradley & Jantz, 2021). I argue that this dissertation utilizes a population affinity approach within a biodistance framework, and it is this term that I have adopted for use when referring to the present study. However, this section primarily uses the term "ancestry," as it is this term (and the associated

methods) that have been the center of most of the debates and discussions within forensic anthropology over the past two decades.

Ancestry can be estimated using one or several of the following types of data: cranial measurements, cranial morphoscopic traits, dental measurements, and dental nonmetric traits (Dunn et al., 2020). The most commonly-used analytical methods in craniometric ancestry estimation are linear discriminant function analysis (LDFA) for classification and Mahalanobis distance for a measure of similarity. LDFA was first introduced in this context by Giles and Elliot for discrimination between American Black, American White, and Native American groups (1962). However, the relatively low accuracies reported by the authors led to a search for improved methods of classification, which culminated in the development of FORDISC, a software that facilitates sex, stature, and ancestry estimation based on statistical models and a robust reference database (Dunn et al., 2020; Jantz & Ousley, 2005). Additional software exists for ancestry estimation using macromorphoscopic cranial traits (Hefner, Ousley, & Richardson, 2024), metric and morphological analysis of the mandible (Berg & Kenyhercz, 2017), and geometric morphometric methods 3D-ID (Slice & Ross, 2009).

The history of ancestry estimation in forensic anthropology has garnered shame from nearly all modern practitioners due to the overtly racist misapplications of the science for centuries (Stull et al., 2021). Typological studies using anthropometrics and morphology have a long and infamous history in biological anthropology; they have been used to document differences between "racial types" and were associated with non-biological traits, including intelligence, and then injected into a hierarchical structure devolving from the apical position of the White male (Dunn et al., 2020; Spradley & Weisensee, 2017). This unpalatable history has led to critical discussions within forensic anthropology regarding the ethics of estimating

ancestry, and for good reason (Bethard & DiGangi, 2020; DiGangi & Bethard, 2021; Stull et al., 2021). Biological anthropology's foundation in colonialism and eugenics, historical reification of typological and racist thinking, and perpetuation of scientific racism and pseudoscience all warrant considerable reflection and amelioration, where possible (Stull et al., 2021).

In light of these considerations, a number of reflexive questions have arisen as scholars have grappled with the complex nature of ancestry estimation and its implications. These debates surround the state of ancestry estimation in modern practice, the terminology that should be used in reference to patterned human variation, the representativeness and applicability of modern reference samples, the influence of genetic data on ancestry estimation and, importantly, whether forensic anthropologists should estimate ancestry at all (Edgar & Pilloud, 2021). On this last question, the field remains divided; some scholars believe that human biological variation is structured and that phenotypic traits are correlated with social categories, including ancestry, making that structure useful and detectable (Edgar & Pilloud, 2021; Stull et al., 2021). Others have argued that patterned variation does not align with social races, that these social categories do not have a biological meaning, that the traits used in ancestry estimation are poorly understood and typological, that ancestry research has been co-opted by White Nationalists for nefarious purposes, and that ancestry estimation in casework is harmful and may hinder personal identification due to institutionalized racism within the American criminal justice system (Adams & Pilloud, 2021; Albanese & Saunders, 2006; Bethard & DiGangi, 2020; DiGangi & Bethard, 2021; Edgar & Pilloud, 2021). In extreme cases, some have called for immediate cessation of ancestry estimation within forensic anthropology, citing its use as unethical and harmful (Bethard & DiGangi, 2020; DiGangi & Bethard, 2021).

Despite the ongoing discussion, contemporary scholars on both sides of this debate overwhelmingly reject the typological approach to ancestry and the existence of discrete, biological races (Dunn et al., 2020). Therefore, ancestry estimation remains one of the four pillars of the biological profile (Edgar & Pilloud, 2021). As such, a variety of justifications for ancestry estimation and solutions for the above-cited problems have been proposed. First, proponents of ancestry estimation have pointed to methodological accuracy as providing more than enough justification for its use (Sauer, 1992). Accuracy rates have consistently been used to justify the continued practice of ancestry estimation (Ousley et al., 2009; Sauer, 1992) and studies have shown relatively low error rates for current methods (Hughes, Juarez, & Yim, 2021; Thomas, Parks, & Richard, 2017; Winburn & Algee-Hewitt, 2021). Notably, however, these low error rates may be the product of bias, as only identified cases are included in the studies; this possible artificial inflation of accuracy rates deserves further study (Parsons, 2021). Additionally, recent studies have addressed Bethard and DiGangi's (2020) criticism of morphoscopic traits, beginning to unpack the relationships between morphoscopic traits, genes, and climate. The results suggest that morphoscopic traits can serve as genetic proxies in biodistance analyses and, by extension, forensic anthropology (Plemons, 2022). Additional recommendations for improving ancestry estimation include: contextualizing results using evolutionary theory and the concepts of selection, genetic drift, and gene flow (Edgar & Pilloud, 2021; A. H. Ross & Pilloud, 2021; Winburn et al., 2022); clarifying terminology and defining terms when they are used to avoid ambiguity (Pilloud et al., 2021; Tallman et al., 2021); avoid using terms that imply "racial purity" or reify racial typology or hierarchy (e.g. "admixture" suggests mixing of pure "types") (Adams & Pilloud, 2021); engage in meaningful conversation with stakeholders within the

medicolegal system (Stull et al., 2021); and improve and diversify reference samples to better capture extant variation (Edgar & Ousley, 2021; Go, Yukyi, & Chu, 2021).

Admittedly, it is unsettling to conclude that even the most prolific scholars in the field don't have an answer for many of the issues that arise with respect to ancestry estimation, and even more unsettling to realize that the field may be wrestling with these issues in perpetuity, particularly as American society continues to reckon with the undeniable and insidious implications of institutionalized racism in our country. Ultimately, forensic anthropologists should ensure their analyses are rooted in cultural context and should continuously reflect on the implications of their work, especially when it is uncomfortable (Edgar & Pilloud, 2021).

This chapter has presented the broader theoretical and methodological underpinnings of the dissertation. The next chapter will provide the requisite regional and historical context for this dissertation and forms the basis for the specific research questions guiding this study.

# CHAPTER THREE: ARCHAEOLOGY AND HISTORY OF LATE ANTIQUE AND EARLY MEDIEVAL ITALY

The Late Antique and Early Medieval periods in Italy, commonly considered to last from the 5th through the 10th centuries AD, are characterized by social change, migration, and cultural and religious transitions. This period of upheaval has been widely studied in archaeology and history, leading to a number of richly debated questions in these fields. Notably, it has taken until relatively recently for bioarchaeology, or the biocultural analysis of human skeletons, to be included in these debates. Therefore, the aim of this dissertation in general, and this chapter in particular, is to provide the historical and archaeological context of *Rusellae* and the surrounding region, to characterize the existing archaeological and historical debates, and to show how skeletal analysis and bioarchaeology can and has contributed to these larger research questions.

#### **Historical Context**

It is impossible to catalogue the entirety of the historical events that are relevant to this dissertation. Doing so would extend more than a millennium prior to Late Antiquity, from the origins of the Etruscan civilization, through the formation of the Roman Empire, to its expansion, eventual end, and beyond. A vast majority of the goings-on throughout the Mediterranean are important and impact *Rusellae* but are outside the scope of this project. However, certain consequential periods warrant discussion, as they provide context for the research questions asked in this dissertation. These questions investigate how craniometric variation at *Rusellae* was related to that of contemporary sites, what degree of craniometric variation was present at *Rusellae* and what that might tell us about possible Late Antique and Early Medieval migration, and how the people of *Rusellae* experienced life at the site from approximately the 5th century AD through its abandonment in the 12th century AD. It is important to understand the major migration events occurring in Tuscany throughout the study

period, as well as major sociopolitical shifts that may have impacted health, diet, and disease, as this information provides vital context for the results of this dissertation. The historical context is broken into two sections that roughly correspond to the Late Antique and Early Medieval eras: the first section will discuss the period between AD 400 and AD 800, and the second section will outline the important events from AD 800 through AD 1200.

## Late Antiquity (circa AD 400-800)

The Roman empire was already in decline in the west prior to its collapse. Climate change, endemic and epidemic disease, decreasing military strength, and economic change all weakened the empire from the inside (Holmes, 1988). The Alemanni, Franks, and Goths began raiding portions of the Roman territory during the mid-fourth century AD, capitalizing on the internal destabilization of Rome. The Visigoths crossed the Danube into the Empire and settled in Roman territory in the late fourth century AD. In AD 410, Alaric, the king of the Visigoths, sacked Rome. Following the death of Alaric, the Visigoths withdrew to Gaul, but this invasion would not be the last to fall upon Rome. Odoacer, a commander of Germanic mercenaries, deposed Romulus Augustulus in AD 476, thereby ending the western Roman empire (Holmes, 1988). The increasingly frequent incursions of barbarian forces destabilized Rome to the degree that it could no longer maintain its footing in the west, falling instead into an amalgamation of kingdoms under the rule of various Germanic peoples.

After the fall of Rome, Germanic kingdoms began to emerge in the West, replacing the former western provinces of the Roman Empire. These included the Visigoths in southwest Gaul and Spain, the Burgundians in the upper Rhone valley, the Merovingian Franks in northern and Central Gaul, and the eastern Goths in Pannonia (S. Mitchell, 2007). At the same time, the Huns were destabilized due to the death of Attila, leaving a space for the Bavarians to move into the

upper Danube and the Avars to move into Hungary (Holmes, 1988). These kingdoms became independent but primarily maintained the Roman legal and political systems with a mixed Germanic and Roman culture. The Germanic tribes did not force the Roman inhabitants out of these kingdoms; instead, it is hypothesized that the populations mixed (S. Mitchell, 2007).

In Italy, the Ostrogoths were first ruled by Odoacer beginning in AD 476, and then his successor Theodoric beginning in AD 493. Theodoric maintained Roman customs and politics, and brought nearly 100,000 Goths into Italy, who settled in the Po valley in the north, as well as near Ravenna and in Naples and Sicily (S. Mitchell, 2007). Following the death of Theodoric in AD 526, a struggle ensued for power. Justinian seized on this instability, eventually retaking the Italian peninsula in the Gothic War between 535-554 AD but decimating it in the process. The siege of urban and rural areas put pressure on precious resources, interrupting trade and communication and causing widespread famine. The plague of Justinian in AD 542 also triggered enormous demographic decline. The destabilization caused by the Gothic War left Italy weak and ripe for subsequent invasions (Brogiolo et al., 2017).

Following the Gothic War, the Lombards launched a catastrophic invasion of Italy, moving from Pannonia and occupying northern Italy by AD 569. Eventually, the Lombards controlled most of Italy, with the exception of the Duchy of Rome and a few southern Byzantine ports (Wickham, 1981). By the year AD 605, the Lombard kingdom in Italy consisted of three components: northern Italy and Tuscany, and the southern duchies of Spoleto and Benevento, with the capital at Pavia (Wickham, 2006). The Lombard invasion was especially successful because Byzantium was at war with Persia in the east as well as struggling with a Muslim onslaught, leaving few forces to defend Italy. In AD 643 the Lombards issued a code of law under Rothari, and the Lombard kingdom was recognized by the empire in AD 680. The reign of

the Lombards in Italy lasted until AD 774, when they were defeated by the Frankish king Charlemagne at the behest of the Catholic church, ushering in a Frankish rule that would last for another century (Holmes, 1988). However, the Lombards remained in control of the Duchy of Benevento until AD 1077 (Brogiolo et al., 2017).

### Early Medieval Italy (circa AD 800-1200)

Following the deposition of the Lombards in AD 774, Italy fell under the rule of Charlemagne and the Carolingian line. Charlemagne ascended to the role of King of the Franks in AD 768 after his father's death and became the sole ruler of the Franks after the death of his brother in AD 771. Charlemagne's alliance with the papacy and the Catholic church motivated his movement against the Lombards in AD 774, thereby initiating Frankish rule in the northern Italian peninsula and making Charlemagne King of the Lombards. Charlemagne was crowned Holy Roman Emperor in AD 800 by Pope Leo III and, thereby, the king of Italy. His rule was characterized by the reunion and consolidation of much of Western and Central Europe for the first time since the fall of the Roman Empire in the West (Wickham, 2016). Carolingian kings would rule after Charlemagne, but infighting within the dynasty and the subsequent division of territory produced weakness in the Carolingian line (Holmes, 1988). Substantive Carolingian rule ended in Italy in AD 887 when Charles the Fat was deposed (Wickham, 2016). By this time, the former Roman Empire comprised three main political segments: Islamic Spain and North Africa, the Byzantine Empire, and the kingdoms of the West (Holmes, 1988).

The next notable ruler in Italy was Otto I of Germany, who was crowned king in AD 961 and emperor in AD 962, marking the beginning of a stable period during which the Holy Roman Emperor and the King of Italy were typically the same (Wickham, 2016). In the 10<sup>th</sup> century AD, however, upheaval began again in Italy with raiding Muslims from North Africa (Holmes, 1988).

Stability wavered further when divisions between the papacy and the Holy Roman Empire were ignited in the late 11<sup>th</sup> century AD, resulting soon thereafter in the formation of Republican city-states such as Florence, Pisa, Lucca, and Siena, which were independent of the Holy Roman Emperor. Also, in the 11<sup>th</sup> century came the Norman conquest of southern Italy and Sicily, including previously Byzantine, Arab, and Lombard territories. In 1130 AD, these territories became known as the Kingdom of Sicily (Wickham, 2016).

#### Rusellae

Situated on a hilltop overlooking the ancient Lake Prilus in Tuscany, Italy, the site of *Rusellae* was undoubtedly subject to the complex sociopolitical developments of the Late Antique and Early Medieval periods (Figure 3.1). The occupation of *Rusellae* can be dated from the Etruscan era through the Middle Ages; the Etruscans founded the city during the 9<sup>th</sup> century BC and was then besieged and captured by the Romans in 294 BC, finally becoming a Roman colony in the 1<sup>st</sup> century BC (Grant, 1980). In the 1<sup>st</sup> century AD, the city underwent a revival with the construction of the forum, amphitheater, and sacred areas, while domus are recorded both in the center of the city and on the Northern hill (Campana, 2018; Sebastiani et al., 2015).

As with other Tuscan towns at the time, *Rusellae* experienced a substantial decline in the 4<sup>th</sup> and 5<sup>th</sup> centuries AD, including abandoning the forum and most public buildings (Sebastiani, 2017). The transition of the Domus of the Mosaics from an opulent residence to a workshop and its eventual abandonment attests to the overall transformation of *Rusellae* during this period (Michelucci, 1985). However, since *Rusellae* was one of the few inhabited cities during the decline of the 4<sup>th</sup>-5<sup>th</sup> century AD, it was chosen as the center of a diocese in the late 5<sup>th</sup> century AD (Celuzza et al., 2002; Sebastiani, 2017). *Rusellae*'s survival was partly due to its

advantageous position near the Tyrrhenian Sea and its location adjacent to the Via Aurelia vetus, which connected the city to Rome (Sebastiani et al., 2015).



Figure 3.1. Map of Italy highlighting the location of *Rusellae* (red bubble).

After *Rusellae* was chosen as the seat of the diocese, the church/cemetery complex was rebuilt in the late 5<sup>th</sup> and early 6<sup>th</sup> centuries AD, and an overall resurgence of the site began in the 6<sup>th</sup> century, including the construction of a new perimeter wall and the fortification of the amphitheater (Celuzza et al., 2002; Sebastiani, 2017). During this period, craftsmanship grew in importance, becoming a primary resource of the area. Additionally, the Lombards took *Rusellae* in approximately AD 593 (Sebastiani et al., 2015). The cathedral appears to have undergone a period of refurbishment and decoration during the late 8<sup>th</sup>-early 9<sup>th</sup> century AD, and a bell tower was constructed in the late 11<sup>th</sup> century, just before the movement of the bishopric to Grosseto (Celuzza et al., 2002; Sebastiani, 2017).

The widespread appearance of North African pottery, African red slip (ARS) wares, on the north hill of *Rusellae* suggest a circum-Mediterranean trade relationship between *Rusellae* and Tunisia during the 2<sup>nd</sup>-late 6<sup>th</sup> centuries AD. Although ARS wares were consistently used throughout this period, their use fluctuated in response to the Gothic Wars, the demographic decline in Tuscany, and the Vandal conquest of Carthage during the 4<sup>th</sup> and 5<sup>th</sup> centuries AD. The Lombard conquest of *Rusellae* in approximately 593 AD effectively severed trade ties between *Rusellae* and North Africa, at least for the following century (Vaccaro, 2011).

Although excavations have been ongoing at *Rusellae* for over half a century, the cemetery itself was excavated in coordination with the Art and Archaeology Museum of the Maremma and in collaboration with the Archaeological Superintendence of Tuscany between 1987 and 1991. Presently, approximately 170 tombs have been unearthed, but the complete extent of the cemetery is as yet unknown. Importantly, the cemetery at *Rusellae* contains some of the earliest excavated burials in the region, making its contribution to the anthropological and historical understanding of Late Antique Tuscany quite substantial. The cemetery at *Rusellae* is located on

the slopes of the northern hill, near the Hadrianic-era bathhouse. The baths, abandoned in the fourth century AD, were repurposed as a church complex between the sixth and seventh centuries AD. The cemetery was subsequently used between the 6<sup>th</sup> and 12<sup>th</sup> centuries AD (Celuzza et al., 2023).

#### **Phase I Burials**

The earliest burials are characterized by a more organized layout and are dated to the sixth and seventh centuries AD based on the personal ornaments contained therein, including earrings, bracelets, necklaces, and combs. The graves tend to be constructed from reused travertine from Roman-age paving stones; they are organized in terraces and neatly arranged in a northwest orientation. One grave in particular contains Lombard-style funerary objects, including a sax and a belt dating to the 7<sup>th</sup> century AD, which is consistent with the Lombard occupation of *Rusellae* during this period (Celuzza et al., 2002). It has been suggested that these early burials represent an elite group who chose burial near the church for its "privileged" location (Vaccaro, 2011). Additionally, a Lombard inhumation cemetery was discovered southwest of *Rusellae*, producing about twenty-five burials with datable grave goods and stone coffins; scholars have suggested this represents a cemetery for a local middle-class family during the Lombard period (Campana, 2023).

#### **Phase II Burials**

The second phase of burials ranges from the 8th century AD until the abandonment of the site in the 12th century AD. Aside from dating to a later period, these burials also differ substantially from the Phase I burials in several ways. Unlike the earlier graves, the Phase II burials do not include personal adornments and are less organized than the Phase I burials. Not only are the Phase II burials greater in number, they are arranged more opportunistically,

constructed using thin slabs in whatever space and orientation was available. In some cases, graves were reused, with the earlier remains pushed to one end of the grave to make room for the new interment.

In the latest stage of Phase II, nearing the abandonment of the site, a number of commingled and earthen burials are associated with the construction of a tower, tentatively dated to the 11th century AD. The construction of this tower had interrupted earlier burials, which were disinterred, moved to an area near the church, and buried in the ground. These interrupted burials generally cannot be segregated into individuals and appear instead as a mass secondary burial. A bronze pin and shoe buckle associated with the commingled remains suggests that at least some of the disturbed burials were from earlier stages of Phase II. Several primary burials can also be dated to this latest phase of interment. Finally, the cemetery was no longer used in the 12<sup>th</sup> century when the bishopric was transferred to nearby Grosseto in AD 1138 (Celuzza et al., 2002).

## Rusellae in Context: The Tuscan Countryside

Life at *Rusellae* can only be fully understood or studied by situating it in the context of the surrounding region, as the distinctions between *Rusellae* and contemporary sites may help explain some of the demographic shifts and health outcomes seen in this dissertation. In Tuscany, the height of rural settlement occurred between the 2<sup>nd</sup> century BC and the 1<sup>st</sup> century AD, with a substantial number of villas and surrounding settlements concentrated along the coast and near rivers. During the 2<sup>nd</sup>-3<sup>rd</sup> centuries AD, settlement density markedly declined, with an even more substantial decrease in the number of rural sites between the early 4<sup>th</sup> and mid-5<sup>th</sup> centuries. However, the presence of ARS ware during this period suggests that a circum-Mediterranean economy was in operation at this time, despite the decline in the number of rural sites. In the late

5<sup>th</sup>-mid 6<sup>th</sup> centuries, there is evidence for the continuity of some villages, the reuse of Roman settlements, some dispersed settlements, and even cave dwellings (Vaccaro, 2008).

During this period, craft production overcame agriculture as the primary economic driver of the region; the site of Spolverino functioned as a glass and bone workshop, and metalworking is also attested archaeologically in the area (Sebastiani, 2016). Notably, the coastal area of the *ager Rusellanus* represented an interconnected settlement network in the region of *Rusellae*, articulated by the *via Aurelia vetus*, the *via Aemilia Scauri*, the Ombrone river, and the Tyrrhenian Sea. These routes, both on land and over water, connected several settlement areas, including Alberese, Bagni di *Rusellae*, and Castiglione della Pescaia, which were likely supply centers for *Rusellae*. Further, this network connected the hinterland to the coast and, consequently, the broader Mediterranean. Tyrrhenian ports accepted imports of common goods from regions throughout the Mediterranean, including North Africa (Chirico & Sebastiani, 2023). However, the Gothic Wars likely decimated many of these settlements and their interconnected routes by the mid-6<sup>th</sup> century AD (Vaccaro, 2008).

In fact, the 6<sup>th</sup> century AD can be considered a turning point for Tuscany, representing a transition between Roman urban structure and the Early Medieval layout of the landscape. The archaeological sites of Scoglietto, Spolverino, and Umbro Flumen in Alberese, Italy, conform to these trends, declining between roughly the 3<sup>rd</sup> and 5<sup>th</sup> centuries AD and having been abandoned by the first half of the 6<sup>th</sup> century AD. These sites were never reoccupied, suggesting that many Early Medieval settlements were probably developed *de novo* in rural Tuscany (Sebastiani, 2017). *Rusellae*, however, maintained its occupation throughout this otherwise tumultuous period in the region.

The 6<sup>th</sup> and 7<sup>th</sup> centuries also mark a decline in building materials and craft production sites. During this time, many residences were built of perishable materials, including wood and thatched roofs, as opposed to brick and stone (Ward-Perkins, 2005). Additionally, sites of craft production such as Spolverino were deserted after the 6<sup>th</sup> century AD (Sebastiani, 2016).

The collapse of the Roman settlement system between the 4<sup>th</sup> and 7<sup>th</sup> centuries AD in rural Tuscany was characterized by overall demographic decline and the concentration of people in fortified, nucleated hilltop villages (Francovich, 2007). By the 7<sup>th</sup> and 8<sup>th</sup> centuries AD, lowland settlements had decreased in number, and these hilltop villages were almost ubiquitous (Francovich, 2008). A stabilization in the number of sites occurred until the 8<sup>th</sup> century AD, followed by a slight increase during the 11<sup>th</sup> and 12<sup>th</sup> centuries (Bertoldi, Castiglia, & Castrorao Barba, 2019). At this time, hilltop castles were constructed through the process of *incastellamento* (Vaccaro, 2008). The 8th century AD also appears to represent a turning point in the social structure of many sites in Tuscany, showing evidence of a transition from a more egalitarian society to social differentiation under an increasingly powerful Carolingian aristocracy. This shift is attested in the archaeological and historical literature through prominent dwellings inhabited by a lord, workshops, storage areas, and defensive fortifications (Barbiera & Dalla-Zuanna, 2009).

Overall, the demographic decline in Tuscany of the 5<sup>th</sup> century AD and the abandonment of many lowland village sites in the 6<sup>th</sup> century in favor of fortified hilltop villages, such as *Rusellae*, by the 7th and 8th centuries AD is clear. This shift in settlement practice is a response to the drastic decline of the Mediterranean economy, the interaction with invading Germanic groups, and the destruction caused by the Gothic Wars and the plague of Justinian. Tuscany in general, and *Rusellae* in particular, are deeply interwoven with the economy and society of

western Europe, and the outcomes attested in the history and archaeology of the region show that the impacts made by these large transitions clearly reverberated on a small scale.

### Case Study: Cosa

Although the present dissertation is focused on *Rusellae*, an understanding of contemporary sites can provide important historical and regional context. One key comparative case is the hilltop village, Cosa, which overlaps temporally in occupation with *Rusellae* and is located approximately 65 km to its south. Notably, Cosa has provided some of the only other evidence of African presence in Late Antique and Early Medieval Tuscany (Fentress, 2003); as this knowledge formed part of the impetus for the present study, a brief discussion of Cosa's history and cemeteries is warranted.

Cosa was established as a Roman colony in 273 BC on land that had been parceled out from the territory of the defeated Etruscan city of Vulci. Many of the original inhabitants of this colony were drawn from Rome proper; the colony was laid out systematically mimicking the organization of Rome, and probably consisted of about 9,000 people in the city of Cosa and the surrounding farms (Barker & Rasmussen, 1998). The collapse of the original colony required a new injection of colonists in 197 BC; these colonists built most of the domestic and public works in the town, but the settlement again collapsed in approximately 70 BC. In fact, Cosa was settled no fewer than six times, each resulting in decline or collapse (Fentress, 2003). However, a more recent reevaluation of the excavation data, particularly the numismatics, suggests minimal continuity of the site during Late Antiquity. Two phases of its settlement—in the fifth-sixth and the tenth-fourteenth centuries AD—contain burials that overlap with the cemetery of *Rusellae* and deserve special consideration here (De Giorgi, Hobart, Ludke, & Scott, 2023; Fentress, 2003).

The fortified settlement of the sixth century was small and civilian in nature, consisting of a bread oven, a cemetery, a few houses, a granary, stables, a barn, and a church. The skeletons of seven individuals, two adults and five children, contemporary with this occupation suggest relatively poor health. The presence of ARS wares, north African inscriptions, and eastern Mediterranean amphorae is consistent with long-distance trade despite the humble size and ephemeral nature of the settlement. This settlement appears to have been abandoned at the end of the 6<sup>th</sup> century, beginning a period of relative stagnation at the site until the tenth century (Fentress, 2003).

The Early Medieval cemetery (primarily 11<sup>th</sup> century AD) appears to have been used in a number of phases, vacillating between organized burial and random interments with almost no grave goods. Anthropologically, the individuals in this cemetery appeared to have undergone some sort of anemic response, potentially indicating the presence of malaria at the site.

Importantly, the tentative presence of sub-Saharan African individuals was reported at this cemetery based on morphological features of the skeletons (Fentress, 2003).

## **Archaeology of Tuscany**

It should be clear by now that the rural landscape of Tuscany provides a rich opportunity for understanding the transition from Roman to Early Medieval eras. However, much of this landscape has historically been understudied because traditional archaeological survey methods, including field walking, are insufficient to detect "negative features" such as ditches and pits that might provide evidence for rural settlements. The regions of time and space that have been deemed "less visible" archaeologically can be considered "emptyscapes" (Campana, 2017). It has not been until relatively recently that consideration has been given to these "emptyscapes,"

thereby allowing a more complete picture of a time and place that has long been considered archaeologically invisible (Campana, 2018).

Specifically, the use of geophysical methods and a transition from a site-based to a landscape-based research perspective has allowed for more focus on environment, communication and trade, agriculture, and settlement patterns with the ultimate goal of illuminating the "archaeological continuum" with evidence from all time and space in a defined area (Campana, 2017). This method has recently been applied to rural Tuscany, revealing a shift from dispersed lowland villages to nucleated hilltop settlements; this evolution is now seen as characteristic of the transition between the Roman and Early Medieval periods in central Italy (Hodges & Whitehouse, 1983).

A similar framework is used by the nEU-Med Project, a large, interdisciplinary research endeavor aiming to understand how a sample region of southern Tuscany experienced the upheaval of the seventh through twelfth centuries AD through archaeological, historical, and environmental investigations (Dallai & Marasco, 2023). This project, organized by the University of Siena, addresses historical questions relating to the Mediterranean economy between the 7th and 12th centuries AD, which Wickham (2023) has argued deserve special regard. Remarkably, the project focuses on the shift in settlement patterns, landscapes, farming, exploitation of resources, and the political structure of the period. The project is focused in the northern Maremma region, including the coast of the Tyrrhenian Sea, which is considered a representative sample of much of the Western Mediterranean. This region is also helpful for study as many of its Early Medieval structures, settlements, and production sites have been surveyed, excavated, and well-documented. The cemetery at Vetricella has also provided skeletal and bioarchaeological data to the nEU-Med project (Bianchi & Hodges, 2018).

Another important Tuscan archaeological Project is the Alberese Archaeological Project, which studies settlement patterns and trade networks between the mid-Republican period and the Late Antique period in Tuscany. This project, established in 2009, focused primarily on three sites: the temple and sanctuary of *Diana Umbronensis* at Scoglietto, the manufacturing district of Spolverino, and the settlement of *Umbro flumen*. Excavation of these sites provided evidence for a significant transition in settlement patterns in the mid-6th century AD, including the abandonment of several once-bustling cultural and economic centers (Sebastiani, 2017).

The Alberese region is also studied within the Impero (Interconnected Mobility of People and Economy Along the River Ombrone) Archaeological Project, organized by the University at Buffalo. Launched in 2017, the Impero Project focuses on economic and settlement trends in southern Tuscany between roughly the 2nd and 9th centuries AD. More specifically, the Impero Project aims to understand how the Ombrone River connects the micro-level Tuscan settlements and landscapes to the macro-level Mediterranean economy. This goal is achieved, in part, through the excavation of a Roman to Late Antique villa and a medieval castle, as well as field surveys of the surrounding region. Importantly, the Impero Project seeks to understand the relationship between the coast, the hinterland, and the urban center of *Rusellae* (Sebastiani, 2017).

# **Major Archaeological Debates**

The end of the Western Roman Empire, traditionally marked by the deposition of Romulus Augustulus in AD 476, ushered in a complex period characterized by population mobility, shifting economies, and changing settlement structures. Between the 5<sup>th</sup> and 10<sup>th</sup> centuries AD, this transitional period has been studied extensively by historians and archaeologists alike. This research has led to a number of notable debates within archaeological

and historical scholarship. Importantly, these debates, in part, inspired the research questions guiding this dissertation, and the goal of the present research is to use a biocultural approach to skeletal data to contribute to the ongoing historical and archaeological conversations.

# The Nature of Late Antiquity

The nature of the "fall" of the Roman Empire has been the subject of intense debate, with two primary schools of thought dominating the literature: the "Late Antiquity" cultural continuity model and the "decline and fall" model. These models provide a lens through which to view historical events, archaeological finds, and material culture.

For many historians of Europe, the end of the Roman Empire was considered cataclysmic. When Rome fell in AD 476, it was thought that civilization had ended in the West and Europe had plunged into a "Dark Age" from which it would not recover until at least the 10<sup>th</sup> century. Further, the fall of Rome was seen to mark the end of Classical Antiquity and herald the arrival of the Medieval period. Although many scholars had debated the veracity of this viewpoint for at least a century, most notably Pirenne, it was not until Brown's "The World of Late Antiquity" was published in 1971 that scholarly perspectives on the fall of the empire shifted. Brown popularized the term "Late Antiquity," which refers to the period between the rise of Diocletian in the 3<sup>rd</sup> century AD and the rise of Islam in the east in the 8<sup>th</sup> century AD. The term "Late Antiquity" is meant to imply a continuity with Classical Antique thought and culture. Brown primarily used the rise of the Abrahamic religions (Christianity, Islam, and Judaism) during this period as evidence that civilization did not, in fact, end after AD 476. Instead, the rise of monasticism, the development of major world religions, and the proliferation of art suggested a continuation of civilization and culture (P. Brown, 1971). Brown's viewpoint is a product of cultural relativism, which rejects the notion of "civilizations" as more or less complex. Instead,

cultural relativism posits that different cultures exist equally, irrespective of their complexity.

Thus, in Brown's perspective, the Roman civilization did not end; instead, the Roman culture continued until it was eclipsed by the rise of Islam in the east.

Whereas Brown envisions cultural and religious continuity until the 8<sup>th</sup> century AD, Horden and Purcell argue that there is no natural "break" at which point classical antiquity ends and Medieval culture begins. They use the notions of connectivity and redistribution as a proxy for the economic health of the Mediterranean. Additionally, they view the Mediterranean as a complex aggregation of variable microregions. Using evidence from ship logs, material culture, and piracy, Horden and Purcell argue that the circum-Mediterranean trade was ongoing during the so-called "Dark Ages." They also propose that, while this connectivity and redistribution may have slowed down and become more regional in nature, it never entirely ceased. This suggests that the Mediterranean economy never fully collapsed, as is implied by the "decline and fall" model (Horden & Purcell, 2000).

A third perspective, quite distinct from Brown and Horden and Purcell, is that of Ward-Perkins. Much like his predecessor, Gibbon, Ward-Perkins ascribes to the idea of the decline and fall of the Roman empire. His work is a response to the notion, espoused primarily by Brown, that the end of the Roman empire was a period of "transformation and accommodation." Using economic history, Ward-Perkins describes a "demise of comfort." This refers to a decline in the quality of goods and building materials, including a decreased number and sophistication of ceramics, limited coinage in the 5th and 6<sup>th</sup> centuries AD, and a decrease in graffiti, which Ward-Perkins equates to a decline in literacy (Ward-Perkins, 2005). For Ward-Perkins, the fall of the Roman empire in AD 476 marks the beginning of Medieval Europe, with no intermittent "Late Antiquity."

Importantly, Tuscany in general, and *Rusellae* in particular, can be examined through these lenses in order to understand which of these three models, if any, best fits the archaeological data. In general, Tuscany follows the expectations set forth by Ward-Perkins in terms of substantial economic decline, decreased sophistication of material culture, and lesser quality of building materials. However, I argue that the "decline of comfort" described by Ward-Perkins did not occur in AD 476 in Tuscany. Instead, it seems that *Rusellae* experienced some continuity with classical antique culture until the Lombard invasion in AD 569. This continuity was facilitated by the Ostrogoths, who took great pains to maintain Roman culture and political structure. They appointed Roman civil servants to political office and oversaw the construction of Roman-style castles, palaces, theaters, baths, and fortifications. This strategy aimed to gain legitimacy by reproducing "Roman-ness" through monumental construction (Brogiolo, 1999; Brogiolo et al., 2017; Revell, 2008). However, the Gothic Wars and Lombard invasion of the 6<sup>th</sup> century AD would spell the end of cultural continuity and the acceleration of economic decline.

The substantial demographic constriction in Tuscany following the Lombard invasion and the disappearance of ARS wares from the archaeological context in the 6<sup>th</sup> century AD suggests that Ward-Perkins' interpretation has some merit. Additionally, the reuse of Roman sites and the occupation of cave sites during the 5<sup>th</sup> and 6<sup>th</sup> centuries, after the fall of Rome and during the Gothic Wars, implies a decrease in construction that is to be expected based on Ward-Perkins' theory of "the disappearance of comfort" (2005). However, the continual occupation of *Rusellae*, even while most other sites in the area disappeared, suggests at least some level of continuity throughout this era that falls most in line with Horden and Purcell's notion. As a coastal, fortified, hilltop site, *Rusellae* likely acted as a node in the more regional trade networks proposed by Horden and Purcell, leading to at least a low level of sustained craft production and

trade. Additionally, Brown might argue that the centering of Christianity at *Rusellae* is a significant factor for its resilience, citing the importance of Christianity in determining which sites remained viable during Late Antiquity.

Although archaeological evidence can and does contribute to an understanding of how *Rusellae* might have experienced the period between the late 5th and 10th centuries AD, there is a gap in the literature regarding skeletal analysis. The human skeleton can record evidence of stress and disease that might have impacted the inhabitants of *Rusellae*, which can thereby facilitate an understanding of the Late Antique environment there. For example, high levels of non-specific stress indicators may indicate a degree of malnutrition, dietary deficiency, or infection within the population; this finding could corroborate the "decline and fall" model and suggest changes in traditional subsistence strategies or interruptions in trade that forced a transition to reliance on different sources of food or income. On the other hand, low levels of skeletal stress in the population could suggest an adherence to the continuity model, wherein *Rusellae* maintained its local and regional socioeconomic connections, and overall health at the site was not significantly impacted by the transition between Imperial and medieval times. This dissertation, therefore, aims to add to the ongoing conversation about the nature of Late Antiquity through the use of skeletal analysis.

#### The Lombards: Invasion or Assimilation?

A second debate involves the nature of migration and the impact of shifts in power between the Lombard tribes and local Romans during Late Antiquity. This period undoubtedly saw substantial movement of people, particularly of central European groups, into the Italian peninsula. However, it is not always clear how the influx of migrants impacted the autochthonous Romans culturally, whether these migrants or their descendants existed in great

enough numbers or assimilated with locals enough to influence genetic variation in their new homes, and how the transition of power might have affected overall health and stress in the existing population. *Rusellae* provides an opportunity to explore this debate through an analysis of craniometric—and, by proxy, genetic—variation and health outcomes during and after the Lombard period at a site of known Lombard occupation.

When the Lombards began to enter northern Italy in AD 568, they encountered a politically, socially, and geographically fragmented region. The two decades of the Gothic War (AD 535-554) had destroyed the Italian peninsula, causing famine, plague, and massive demographic decline. Fiscal resources were limited, and economic relationships with other parts of the empire were severed, leaving the Byzantines unable to defend the Italian peninsula from the invaded Lombards (Brogiolo et al., 2017).

Historical texts do not provide a complete or necessarily accurate account of what happened to the Romans when the Lombards moved through Italy. It is assumed that the Lombard invasion was violent and that the Romans were either enslaved or forced out of their homes. On the contrary, archaeological evidence suggests an assimilation of the groups and a much less radical Lombard invasion than originally hypothesized (Wickham, 1981), resulting in local communities with substantial cultural communication and crossover between the two groups. For example, the Lombards used Latin and copied the design of Roman gravestones and sarcophagi (S. Mitchell, 2007). The Lombards also made an effort to maintain existing fortifications and infrastructure but were not always able to prevent the decline of these structures over time (Brogiolo et al., 2017). This is a product of the breakdown of the taxation system during the Lombard era, which led to a less wealthy government than the preceding Byzantines. The lack of wealth also likely caused the decline in imported wares during this

period, particularly African red slip ceramics, as the Lombard government could no longer afford to import these goods (Wickham, 1994).

Despite the archaeological evidence of assimilation by a small group of Lombard elites, there is some suggestion that the effect of the Lombard invasion differed based on geography; the frontiers of battle were probably devastated, while the remaining imperial territories in Italy were moderately affected by disruptive political change. Finally, the new Lombard regions probably experienced such a rapid invasion that the lives of Roman peasants were not drastically altered (Holmes, 1988). Therefore, *Rusellae*'s location in Tuscany may be more consistent with a less devastating and disruptive invasion than what was experienced in the towns and cities along the northern Italian "frontier" between the Romans and the Lombards. Importantly, this dissertation aims to contribute to the conversation surrounding the nature of the Lombard "invasion" by analyzing evidence of skeletal stress at *Rusellae* during the Lombard period.

A secondary debate surrounding the Lombards involves their demographic makeup and to what degree their presence influenced the genetic variation within the *in situ* Roman population. The concept of who, exactly, constituted the "Lombard" people is somewhat abstract (Brogiolo, 2000). The inhabitants of Lombard Italy are generally referred to as Lombards; however, the great majority of people in Italy during this period would have been Roman, with ethnic Lombards possibly comprising no more than 8% of the population (Wickham, 1981). The Lombards were probably biologically heterogeneous, primarily united by a common culture. However, the people the Lombards encountered as they moved throughout Italy were also heterogeneous; although Roman from a cultural perspective, these people were Byzantines, Eurasians, Middle Easterners, North Africans, and Western Europeans who had either come from

or migrated to the Italian peninsula in a religious, trade, or military capacity (Brogiolo et al., 2017).

In sum, the hypothesis presented by historians is that the Lombard people were genetically heterogeneous and probably made up only a tiny percentage of the overall population of Italy during their occupation. However, there is renewed archaeological interest in further exploring the demographics and genetic ancestry of the Lombard people, particularly at central European and northern Italian sites such as Szolad and Collegno (Alt et al., 2014; Amorim et al., 2018; Tian et al., 2024). *Rusellae* offers an opportunity to contribute to the scholarly conversation by exploring craniometric variation in a time and place of known Lombard occupation and by contextualizing that variation within a broader Mediterranean context. Therefore, this dissertation will offer further data to better understand who the Lombard people were and how their presence influenced the occupants of *Rusellae*.

## Mediterranean Bioarchaeology

The Mediterranean, particularly in the classical period, has been studied extensively with respect to history and archaeology. However, the utility of skeletal analysis for answering archaeological questions has only sometimes been obvious to historians of the Mediterranean. It has only been in recent decades that interdisciplinary and collaborative conversations between archaeologists, historians, and biological anthropologists have given rise to well-contextualized and interesting bioarchaeological research questions in the Late Antique and Early Medieval periods. Anthropologists have the unique power to provide skeletal data, which historians can set within the appropriate historical framework, thereby producing nuanced studies on topics such as paleopathology, health, diet, disease, stress, and mobility, among others. Further, newlydeveloped and rapidly-improving methods such as stable isotope analysis and ancient DNA

techniques can provide robust information to the existing historical narrative. Additionally, integrating anthropological, archaeological, and historical research programs has shifted focus from site-specific case studies to landscape and population-based approaches (Sperduti, Bonditi, Craig, Prowse, & Garnsey, 2018).

# Migration

One of the most prevalent research topics within Mediterranean bioarchaeology is migration. Specifically, skeletal data have been used to contribute to conversations surrounding the demographic composition and movement of people from the Imperial era through the Middle Ages using stable isotopes, ancient DNA, and craniometric analyses.

## Roman-Era Migration

Overall, the genetic composition of Europe during the Imperial period showed a shift in ancestry toward the eastern Mediterranean but maintained a heterogeneity in ancestral composition and included individuals of North African ancestry (Antonio et al., 2019). The presence of non-local individuals in Roman-era cemeteries has been attested using strontium, oxygen, and carbon isotopes in the Imperial Roman cemeteries Casal Bertone and Castellaccio Europarco (Killgrove & Montgomery, 2016) and Isola Sacra (Prowse et al., 2007). However, each of these cemeteries is located in a well-connected area surrounding Rome, where migrants are expected. Interestingly, a more rural, southern Italian necropolis at Vagnari (1st-4th centuries AD) showed through oxygen and strontium isotopes that only 7% of the individuals at the site were migrants. Even at this site, however, researchers found isotopic and mtDNA evidence of North African migrants and non-local subadults at the cemetery (Emery, Stark, et al., 2018).

In a study of cranial morphological variation during the Roman era, Hens and Ross used geometric morphometric analyses in three cemeteries dating from the 1st-3rd centuries AD

(2017). Two of the cemeteries (Isola Sacra and Velia) were significantly distinct from one another with respect to cranial morphology, while the third (Castel Malnome) was intermediate between the two. The authors suggest that Castel Malnome may be indistinguishable from the other two sites because it exhibited significant heterogeneity in cranial morphology. This variation, they argued, was because Castel Malnome represents a population of freed slaves with a wide variety of geographic origins (Hens & Ross, 2017).

Evidence of migration has also been identified more broadly throughout Europe using the skeleton; Shaw and colleagues found evidence of individuals of North African origin in Imperialera London through lead and strontium isotope analysis of tooth enamel (Shaw, Montgomery, Redfern, Gowland, & Evans, 2016). Using strontium and oxygen isotopes in tandem with cranial measurements, Leach and colleagues found evidence of African migrants and their descendants in Roman York, England (2009). One young female (4th century AD) was buried in a stone coffin with rich grave goods. Her cranial measurements indicate an affinity with African reference groups and her oxygen isotope content suggests a childhood outside of York, signifying longdistance migration and subsequent acculturation during the Roman era (Leach et al., 2010). In an additional study of Roman London, a combination of macromorphoscopic analysis and oxygen isotopes also showed the likely presence of individuals of African ancestry within Roman Britain (Redfern et al., 2016). Further, one late Roman cemetery (AD 330-400) from Bavaria comprised approximately 30% non-local individuals, many of whom had migrated as children (Schweissing & Grupe, 2003). Based on these results, long-distance migration was a significant component of Roman life, and the skeletons can provide essential data about how people moved throughout the Roman Empire.

Late Antique and Early Medieval Periods

Skeletal analysis has also contributed to a better understanding of the demographic makeup of the Lombard people and their interactions with the autochthonous inhabitants they encountered. In a study of two 6<sup>th</sup>-8<sup>th</sup> century AD Lombard cemeteries, one in Hungary (Szólád) and one in Northern Italy (Collegno), ancient genomic researchers found that each site consisted of at least two separate genetic groups—one with northern/central European ancestry and another with southern European ancestry. The two groups each exhibited distinct burial practices, potentially suggesting the coexistence of Lombard migrants and indigenous Romans within the cemeteries. However, the authors are cautious to note that neither of these genetic groups is homogenous. However, the degree of variation is significant enough to identify groups with differing genetic ancestries in two sites of known Lombard contact (Amorim et al., 2018). Additional isotopic analyses at Szólád indicate a wide heterogeneity in the geographic origin of individuals interred there; approximately 31% of the sample was non-local according to strontium isotope ratios. These results confirm the notion that migration was common during the Lombard period (Alt et al., 2014). Similarly, more recent isotopic and genomic research at Collegno concluded that the site comprised individuals with diverse genetic ancestries and isotopic origins, suggesting a historical model of integration of new members of the community (Tian et al., 2024; Viva et al., 2022).

Contemporary sites throughout Italy have yielded similar results. For example, paleogenomic research in South Tyrol revealed a complex genetic history of individuals in the cemetery of Malles Burgusio Santo Stefano (4th-7th centuries AD), with the primary genetic ancestry deriving from southern Europe, but with additional contributions from southwestern, western, and northern Europe. This heterogeneous ancestry data points to hybridization of both

culture and genetics in northern Italy during the Late Antique and Early Medieval periods (Coia et al., 2023). Additionally, strontium isotope analysis at the site of Povegliano Veronese (Verona, Italy, 6th-8th century AD) has identified at least nine individuals whose geochemical signatures match a region in Hungary, hypothesized to be the origin of the Lombard people. In contrast, the remaining individuals in the cemetery appeared to be of local origin (Francisci et al., 2020). Similarly to other studies, this evidence points to an integration of non-local Lombard peoples with autochthonous groups in Italy. The research described here shows that anthropological scholarship throughout the last decade has moved towards using the human skeleton to identify trends in migration, demographics, and ancestry in Italy throughout Late Antiquity and the Early Middle Ages.

## **Skeletal Health Studies**

Another significant research program within Mediterranean bioarchaeology uses skeletal stress markers to study health outcomes over time and throughout the landscape. As noted previously, a particularly popular archaeological and historical debate involves the nature of Late Antiquity—was there a "decline and fall" of the Roman Empire, leading to a "Dark Age," or was there cultural continuity resulting in a long Late Antiquity? Historical evidence suggests that disease and stress were widespread during the sixth century AD as a result of the Gothic Wars, the plague of Justinian, leprosy, famine, malaria, and economic decline during this time (McCormick, 2001). Additionally, the population of Italy declined by half between the 3<sup>rd</sup> and 6<sup>th</sup> centuries AD (Belcastro et al., 2007). Therefore, one might expect to observe skeletal manifestations of poor health during this transitionary period.

However, when studying skeletal data, it becomes clear that a disastrous transition was not felt ubiquitously throughout Italy. Instead, various studies have shown differing health

outcomes during this time. For example, a study of stature as a proxy for health showed an increase in stature between the Roman and Medieval periods, indicating a generally positive health trend over time (Giannecchini & Moggi-Cecchi, 2008). An additional study comparing the frequency of linear enamel hypoplasias, *cribra orbitalia*, and periostitis in Molise, Italy, shows that poor health was actually consistent from the Roman through the Early Medieval period (Belcastro et al., 2007). An analysis of carbon and nitrogen dietary isotopes at the site of Cosa in Tuscany also found no evidence of a substantial shift in diet between the Early Medieval and Medieval occupations (Scorrano et al., 2014).

On the contrary, other studies have shown a decline in health using the frequency of dental lesions in Roman and Early Medieval cemeteries in Latium (Manzi et al., 1999). In a study attempting to ascertain the nature of the transition between Late Antique and Early Medieval Croatia, a comparison of *cribra orbitalia*, linear enamel hypoplasia, non-specific periostitis, and trauma between various sites showed a continuity in health outcomes in continental Croatia, but a worsening of health in Adriatic Croatia (Slaus, 2008).

With respect to the Middle Ages, skeletal evidence from the site of Vetricella (10th-11th century AD; Scarlino, Grosseto, Tuscany, Italy) suggests high levels of childhood stress (between the ages of approximately 2.5 and 4 years) in the form of linear enamel hypoplasia in nearly 90% of the studied sample. Further, 76.9% of the study sample with at least one orbit present exhibited *cribra orbitalia*, while 52.6% of the sample with at least one parietal bone present exhibited parietal hyperostosis; the incidence of both these lesions remains high even when age is considered, but the incidence of both lesions is significantly higher in females than males in the study sample (Viva, 2020). These results suggest an environment in which health outcomes varied by sex, and the frequency of non-specific stress indicators is more similar to the Roman

era than the Late Antique period in this part of Tuscany. Interestingly, the high prevalence of porotic hyperostosis and *cribra orbitalia* was attributed, through isotope studies, to congenital anemia, as opposed to nutritional deficiency anemia (Viva et al., 2021).

This seemingly conflicting research actually shows that the transition from the Late Roman to Early Medieval periods was experienced differently in different regions of the Mediterranean. Bioarchaeology has given us a substantially more nuanced perspective on what some historians and archaeologists call a "Dark Age." By using the human skeleton, bioarchaeologists have the power to explore individual experiences and aggregate their data to make site- and region-wide comparisons.

This chapter has provided an overview of the historical and archaeological background of *Rusellae* and the surrounding Tuscan countryside. The next chapter will use this data to contextualize the four research questions guiding this study as well as their respective hypotheses.

# CHAPTER FOUR: RESEARCH QUESTIONS AND EXPECTATIONS

The present research focuses on understanding the lived experience of individuals interred in the cemetery at *Rusellae*, Tuscany, Italy during the Late Antique and Early Medieval periods (5<sup>th</sup>-12<sup>th</sup> centuries AD). These eras represent a time of intense social, political, religious, and economic change throughout Europe, characterized by population mobility and cultural transitions. This study aims to understand the relationship between craniofacial variation, physiological stress, and biocultural factors throughout these widely debated and well-studied periods. These data can provide insight into how individuals navigated differing identities, political climates, and health statuses during a time of great change in Italy.

Bioarchaeology is a more recent avenue of research in Italy, which can contribute greatly to the numerous archaeological and historical debates surrounding the Late Antique and Early Medieval periods. Due to historical underrepresentation of skeletal analyses in Italian bioarchaeology, there are a number of gaps in understanding of ancient life that can be addressed by direct skeletal observation, particularly with regard to lived experiences. By studying skeletons in separate phases of a cemetery, anthropologists can also gain an understanding of how health and burial practices changed over time.

The present study focuses specifically on the site of *Rusellae*, which serves as an example of a Late Antique and Early Medieval town in Tuscany. Although *Rusellae* followed the general trajectory of many Italian towns during this time period (Sebastiani, 2017), it was also chosen as the center of a diocese, suggesting it held a place of importance in the landscape (Celuzza et al., 2002). Although the results of this dissertation will be informative for understanding *Rusellae*'s place in history, they may not be generalizable to all contemporary sites. However, this research

is valuable to inform an understanding of ancient life in a particular place and over a period of time that remains under debate by historians and archaeologists alike.

This research builds on questions such as: (1) What is the nature of Mediterranean migration, and what are the range of homelands represented by migrants throughout the former Roman Empire? (Horden & Purcell, 2000; Killgrove & Montgomery, 2016; Leach et al., 2010; Leach et al., 2009; McCormick, 2001; Scheidel, 2004, 2005); (2) Is there evidence for African migrants in Medieval Europe? (Redfern et al., 2016; Shaw et al., 2016); and (3) How did health outcomes change during major shifts of power during and after the Roman Imperial Era? (Belcastro et al., 2007; Giannecchini & Moggi-Cecchi, 2008; Manzi et al., 1999). These research questions have been explored in recent literature using craniometric, macromophoscopic, stable isotope, ancient DNA, and pathological analyses. I propose to use similar analyses to understand craniofacial variation, health, and identity during the Late Antique and Early Medieval periods in Italy.

The following research questions represent an initial step in understanding the daily lives of people living at *Rusellae*. Each research question will be presented, followed by the hypothesized results and a justification for these expectations. The research questions guiding this dissertation are as follows:

**Research Question 1:** How does craniofacial variation at *Rusellae* resemble or differ from that of geographically and temporally proximate reference samples, and what might these relationships suggest about population continuity, migration, or gene flow? Further, what variables drive craniometric variation within *Rusellae*, and does there appear to be heterogeneity or homogeneity in overall craniometric variation at the site?

**Hypothesis 1:** I expect that *Rusellae* will more closely resemble medieval European craniometric reference samples and differ most from African reference samples, suggesting greater genetic continuity with the European continent. I also expect to find evidence of significant craniofacial variation within *Rusellae*, suggesting the potential presence of multiple craniofacially distinct groups at the site.

First, and most obviously, as *Rusellae* is in Europe, I expect to find more similarity between *Rusellae* and other European groups due simply to geographic proximity. Despite the fact that human migration was a substantial component of Roman and Late Antique life, and even knowing that contact with Africa regularly occurred, the largest proportion of that migration was likely within the European continent. Therefore, gene flow is primarily expected between European groups, with a lesser (but non-zero) degree of gene flow with African groups. Further, central European groups such as those studied here are subject to similar environmental influences as one another, especially when compared with African groups. These environmental factors, such as temperature and humidity, are known to impact cranial traits and craniometrics (Plemons, 2022; Relethford, 2004, 2010). Therefore, it is expected that samples with similar environmental pressures should exhibit a more similar phenotypic trait expression.

Although I hypothesize that *Rusellae* as a whole will cluster most closely with other European groups, indicating greater genetic similarity with Europeans as compared with African samples, I also expect to find substantial craniofacial variation within *Rusellae*. Like much of the former Western Roman Empire, *Rusellae* likely would have been composed of a number of different population groups. First, *Rusellae* occupies a coastal location, serving as a node in a trade network connecting the sea and inland sites as part of the "Ombrone system," a commercial network in the Ombrone valley (Bertoldi et al., 2019). Further, *Rusellae*'s location adjacent to

the Via Aurelia vetus connected the city to Rome (Sebastiani et al., 2015). As a node on the coast, *Rusellae* would have attracted traders, migrants, and members of the army from the rest of the Empire as they traveled throughout Italy. Inevitably, some would have settled in *Rusellae*, produced offspring, and died, thus leaving their remains in the cemetery. In fact, merchants often moved throughout the empire as they sold their wares, as well as members of the Roman army, who sometimes settled in the lands they conquered. Often, these migrants were assimilated into the Roman world, which was relatively open to foreigners (Horden & Purcell, 2000). This migration would have presented the opportunity for gene flow between native Romans and migrant groups, thereby introducing more craniofacial variation into the skeletal sample. Further, the presence of individuals of diverse origins, or their descendants, also increases expected craniofacial variation within the sample.

In addition, *Rusellae* was occupied for an extended period of time, having initially been founded by the Etruscans in the 9th century BC (Sebastiani, 2017) and not having been abandoned until the 12th century AD (Celuzza et al., 2002). During this approximately 2,000-year occupation, there is both ample time for and ample evidence that several groups of people migrated to *Rusellae* and made it their home. Most notably, *Rusellae*'s history of having been conquered by the Romans and then the Lombards corroborate this hypothesis. *Rusellae* was established as a Roman colony in the early first century BC (Barker & Rasmussen, 1998), at which point the Etruscans were displaced and a cultural shift occurred at *Rusellae*. Much later, the Lombards moved into *Rusellae* in approximately AD 593 (Sebastiani et al., 2015), initiating a further change in both the population and culture of the site. When the temporally aggregated cemetery assemblage is viewed at once, as is the case here, it is expected that the shifting demographics of *Rusellae* throughout its occupation will manifest as observed craniofacial

variation. Overall, *Rusellae* is expected to exhibit a degree of variation that indicates the presence of a number of groups with distinct craniofacial features throughout the duration of its occupation.

**Research Question 2:** Do any individuals at *Rusellae* show a closer affinity to African reference samples than European reference samples (Phases I and II)?

**Hypothesis 2:** I expect to find that some individuals at *Rusellae* show a closer affinity to African reference samples than European reference samples.

As previously discussed, Rusellae was a cosmopolitan city that was deeply integrated in the trade networks of Late Antique and Early Medieval Tuscany, which were in turn connected to the Mediterranean economy as a whole. Evidence of North African migration throughout the Roman Empire has been identified broadly throughout Europe; Shaw and colleagues found evidence of individuals of North African origin in Imperial-era London through lead and strontium isotope analysis of tooth enamel (Shaw et al., 2016). Using strontium and oxygen isotopes in tandem with cranial measurements, Leach and colleagues found evidence of African migrants and their descendants in Roman York, England (2009). One young female (4th century AD) was buried in a stone coffin with rich grave goods. Her cranial measurements indicate an affinity with African reference groups and her oxygen isotope content suggests a childhood outside of York, signifying long-distance migration and subsequent acculturation during the Roman era (Leach et al., 2010). In an additional study of Roman London, a combination of macromorphoscopic analysis and oxygen isotopes also showed the likely presence of individuals of African ancestry within Roman Britain (Redfern et al., 2016). Based on these results, it is clear that long-distance migration, particularly between Africa and Europe, was a significant component of Roman life.

Further, Cosa, a site contemporary and near to *Rusellae*, may provide evidence for African migration into Italy. The tentative presence of sub-Saharan African individuals was reported at this cemetery based on morphological features of the crania. Additionally, the presence of African Red Slip (ARS) wares, north African inscriptions, and eastern Mediterranean amphorae is consistent with long-distance trade despite the humble size and ephemeral nature of the settlement at Cosa (Fentress, 2003).

Based on evidence from other sites similar to and contemporary with *Rusellae*, as well as substantial textual and archaeological evidence of long-distance trade involving Africa and Italy, there is reason to believe that *Rusellae* might have been the home of individuals who show greater craniofacial affinity to African reference samples as compared with European reference samples.

**Research Question 3:** Is there evidence of differential life experience, evaluated using skeletal stress indicators, between individuals with African affinity as compared with individuals with European affinity in Phase II?

**Hypothesis 3:** I expect to find similar frequencies of stress indicators in individuals with African affinity when compared to individuals with European affinity in Phase II.

The relevant literature suggests that acculturation was a critical part of maintaining cohesion in the Roman and post-Roman world. Archaeological and historical evidence suggests that the Ostrogoths, who reigned in Italy during the early 6th century AD, took great care to maintain and imitate many features of the Roman empire, including government and architecture in an effort to establish their legitimacy (Brogiolo et al., 2017). The Lombards, who ruled Italy in the late 6th century AD and early 7th century AD, likely also assimilated with the native people, invading so rapidly that the lives of the Roman peasants they encountered were not drastically

changed (Holmes, 1988). Therefore, I anticipate that individuals who lived together and were buried in the same cemetery likely experienced similar physiological stressors in life.

**Research Question 4:** Do detectable craniofacial differences exist between Phase I (6th-7th centuries AD) and Phase II (8th-12th centuries AD) burials at *Rusellae*, suggesting either shifting population demographics or relative population continuity? Further, do differences exist in the frequency of *cribra orbitalia*, porotic hyperostosis, periosteal reaction, and linear enamel hypoplasia between phases, suggesting changing environmental stressors, diet, disease, or other cultural factors over time?

**Hypothesis 4:** I do not expect to find significant craniofacial and health differences between individuals interred during Phase I and Phase II at *Rusellae*.

I expect to find minimal craniofacial and stress indicator differences between Phase I and Phase II individuals, representing general population and health stability over time at *Rusellae*. On a regional scale, the 6th-7th centuries AD (Phase I) represent a turning point in Tuscany, between Roman urban structure and the Early Medieval layout of the landscape. During this period, many contemporary sites and workshops were abandoned and never reoccupied (Sebastiani, 2017). *Rusellae*, however, maintained its occupation during what was otherwise a period of regional upheaval, indicating a degree of population continuity and socioeconomic stability within the site. In fact, when many lowland and rural sites were being abandoned, people concentrated in fortified, nucleated hilltop villages such as *Rusellae* (Francovich, 2008). Therefore, I expect *Rusellae* to represent a relatively diverse population comprising people from throughout the region, as well as potential central European migrants—such as the Lombards—or their descendants during Phase I.

During Phase II, from roughly the 8th-12th centuries AD, *Rusellae* maintained its importance as a craft production site, and its occupants continued construction and improvements on the church and cemetery complex, even constructing a tower near the abandonment of the site (Celuzza et al., 2002; Sebastiani, 2017; Sebastiani et al., 2015). This suggests a degree of socioeconomic stability at *Rusellae*, despite a transition in power from Lombard rule to Carolingian aristocratic rule during Phase II.

Further, changes in burial practices, from earlier rock-lined tombs with grave goods to earthen inhumations without decoration, are consistent with a shift in religious practice from pre-Christian to Christian burial traditions. Although some grave goods from Phase I suggest the presence of a high-status, non-local Lombard individual, the overwhelming majority of graves are consistent with the expected tradition for Romans throughout time. Since the distinction in burial type is consistent with expectations for change over time and not with an invading group or massive cultural shift, I expect the inhabitants to represent a diverse, but relatively stable, population.

Given that *Rusellae* was a fortified hilltop site throughout Phases I and II with continued construction and increasing craft production, especially during and after a Tuscan demographic decline, I expect that its inhabitants experienced some degree of overall population continuity and were also buffered from some of the diet and environmental stressors that may have affected smaller, isolated, lowland settlements. Therefore, I do not expect to find significant differences in craniometrics or stress indicators between Phase I and Phase II individuals.

Now that the research questions have been presented, the next chapter will discuss in detail the materials and methods used to answer them.

#### CHAPTER FIVE: MATERIALS AND METHODS

This chapter introduces the analytical and reference skeletal samples that are used in this dissertation. Additionally, this chapter describes each of the different types of data, data collection protocols, and analytical methods utilized in this study. The first section details the *Rusellae* skeletal sample, for which novel craniometric and skeletal stress data were collected for this dissertation. This section also discusses features of the *Rusellae* skeletal sample and the cemetery from which it was derived, including demographic information, burial chronology, and general layout of the cemetery. Further, the first section describes the comparative reference samples used in this study, including Howells' samples of African and European crania and samples from Mis Island in modern-day Sudan. The second section in this chapter describes the types of data and data collection methods used in this dissertation, including sex and age indicators, craniometric data, and skeletal health data. Lastly, this chapter details the analytical methods used to answer the previously outlined research questions.

## The Skeletal Samples

The materials for the present research include the skeletal sample from *Rusellae*,

Tuscany, Italy, as well as three European and three African craniometric reference samples.

## Rusellae Sample

The overall *Rusellae* skeletal sample represents all individuals excavated from the *Rusellae* cemetery for which age and sex data could be ascertained. A total of 202 individuals excavated from *Rusellae* were sufficiently complete to permit estimation of age and sex. The assemblage consists of 42 subadults (20.8%) and 160 adults (79.2%). Subadults were further divided into age cohorts as follows: 16 infants (7.9%), 17 children (8.4%), and 9 adolescents (4.5%). Adults were subdivided into the following age cohorts: 42 young adults (20.8%), 71

middle adults (35.1%), 18 old adults (8.9%), and 29 adults over 20 years (14.4%). Adult individuals were further subdivided into males and females. Of the 160 adults in the sample, 72 are female (45%), 81 are male (50.6%), and 7 are of indeterminate sex (4.4%). Table 5.1 outlines the demographic breakdown of the cemetery.

Table 5.1. Demographic breakdown of *Rusellae* sample

	Male	Female	Indeterminate	Total
Infant (0-3 years)	0	0	16	16
Child (3-12 years)	0	0	17	17
Adolescent (12-20 years)	0	0	9	9
Young Adult (20-35 years)	19	23	0	42
Middle Adult (35-50 years)	43	24	4	71
Old Adult (>50 years)	8	10	0	18
Adult (>20 years)	11	15	3	29
Total	81	72	49	202

The cemetery at *Rusellae* was in use between the 6<sup>th</sup> and 12<sup>th</sup> centuries AD and comprises two general phases of use (Celuzza et al., 2023). Phase determinations were initially made by the archaeologists who excavated the site, with additional data added recently through radiocarbon dating of several bone samples (Table 5.2).

The earlier burials, designated Phase I, date to the sixth and seventh centuries AD based on grave inclusions. The Phase I graves are generally orderly, laid out in a northwest orientation, and tend to be constructed from stone. These graves roughly correspond to the "Lombard Period," or the time during which Germanic tribes moved from Central Europe into portions of Italy, including *Rusellae*.

The later burials, designated Phase II, date from the 8th century AD until the abandonment of the site in the 12th century AD. These burials are less organized than the Phase I burials and include several instances of secondary burials and reuse of graves. Further, the Phase

II burials are generally lacking in grave goods, which is consistent with the Christian burial tradition of the time (Celuzza et al., 2002).

Phase data was available for 138 individuals; 27 individuals were dated to Phase I (19.6%) and 111 individuals were dated to Phase II (80.4%).

Table 5.2. Skeletal samples and their associated radiocarbon dates

Skeleton	Dates (AD)	Phase (Radiocarbon and Archaeology)
1174	993-1175	Phase 2
1185	990-1171	Phase 2
1216	820-992	Phase 2
1219	1020-1165	Phase 2
1220	976-1163	Phase 2
3514	1022-1175	Phase 2
1263	1122-1273	Phase 2
1081	1033-1216	Phase 2
1091	978-1159	Phase 2
1094	1155-1279	Phase 2
5103	578-679	Phase 1
5102	771-992	Phase 1
5523	661-777	Phase 1
2006	890-1048	Phase 2
2009	771-977	Phase 2
1103	1038-1169	Phase 2

## **Howells' Datasets**

Several reference samples from W. W. Howells' craniometric dataset (1973, 1989) are also included in this study in order to situate the *Rusellae* sample within a broader global context. W.W. Howells collected craniometric data for a variety of samples across the globe in order to analyze the degree of craniofacial variation among and between groups around the world (1973). The four subsamples of the Howells dataset used in this dissertation are the Berg, Zalavar, and Norse samples from Europe and the Dogon sample from Africa (Howells, 1973). The specific makeup of each skeletal sample and its relevance to the present study are outlined below and in Table 5.3. The geographic locations of each sample are illustrated in Figure 5.1.

Africa: Dogon (West Africa)

The Dogon sample comprises 48 males and 53 females from the burial caves of the Dogon tribe in Mali and dating to AD 1055-1750 (Howells, 1973; Willett, 1971). The sample was collected by Professor Marcel Griaule in 1934 and comprises an earlier and later phase which are pooled into one sample due to relatively little difference in cranial form between the groups. Howells estimated sex for these individuals using cranial morphology during the process of measurement (1973).

The Dogon sample was selected for use as a reference due to its origin in northwest Africa and its temporal overlap with portions of the *Rusellae* cemetery. Although Mali is not specifically a hypothesized origin of individuals at *Rusellae*, Howells notes that this sample is a generally good representation of a West African series (1973), and the sample provides a good temporal comparison; therefore, the Dogon sample is an effective reference group for this dissertation.

Europe: Medieval Norse (Northern Europe)

The Medieval Norse sample is a collection of 54 male and 54 female crania from several medieval parish graveyards in the vicinity of Oslo, Norway. According to Howells, these people were relatively isolated and the sample likely includes primarily local individuals, representing a relatively homogenous sample (1973, 1989). In fact, 85% of the sample derives from one cemetery near St. Nicolaus Church (Howells, 1989). Howells does not specify the dates of this sample beyond classifying it as "Medieval." Sex estimation was based on cranial and postcranial skeletal traits, when present (Howells, 1989).

This sample was selected for use as a reference in this dissertation due to both its time period and its location. Despite the lack of specific dates, the Medieval era is generally

considered to span between AD 500 and AD 1500, which is concurrent with the cemetery at *Rusellae*. Further, Norse incursions are historically documented in Tuscany in the late 9th century (Haywood, 2015), providing evidence for at least some degree of contact between Medieval Norway and Medieval Italy. Due to the overlapping time frame and a possible historical connection between the reference region and the study region, the Norse sample provides an effective comparative group.

Europe: Zalavar (Central Europe)

The Zalavar sample comprises 53 males and 45 females and comes from the cemeteries at Zalavar in western Hungary. The remains date from the 9th to 11th centuries AD and represent a heterogeneous population, comprising Avars, Romanized and newly arrived Germanic and Slavic individuals, and a few of the earliest Magyars. Howells estimated sex for this sample using traits of the postcranial skeleton (Howells, 1973, 1989).

The Zalavar sample was selected for comparison in this dissertation due to its time frame and ancestral makeup. In fact, the aforementioned ethnic groups are among the Germanic tribes that are historically attested to have been present in Italy during the time period under investigation in this dissertation. Therefore, the Zalavar reference group is the most similar sample to the somewhat nebulous "Lombard" people who are known to have occupied *Rusellae* for at least a century, beginning in the late 6th century (Sebastiani et al., 2015), making this a valuable reference sample.

Europe: Berg (Central Europe)

The Berg sample is made up of 56 males and 53 females from a small mountain village in Carinthia, Austria. The remains were recovered from a charnel house in an isolated village and contain almost all the crania from the village's population over the course of five generations.

Therefore, the dates for this sample are challenging to reconstruct, although they are likely to range from AD 1700 to AD 1900. Sex estimation in this sample was particularly challenging, according to Howells, and sex was solely determined based on visual traits of the skulls (Howells, 1973).

The Berg sample was selected for comparison to *Rusellae* primarily due to its geographic origin. Although the Berg sample is later in time than the *Rusellae* cemetery, it represents the nearest geographic reference sample to *Rusellae*. In fact, the modern-day region of Carinthia borders the northeast Italian region, Friuli-Venezia Giulia. Notably, Carinthia would have been part of the Roman Empire and its occupants would have been considered "Romans" until the region fell to the Ostrogoths in the late 5th century (Arnold, Bjornlie, & Sessa, 2016). Therefore, the sociopolitical trajectory of this region mimics that of *Rusellae*, making the Berg sample an interesting comparative group in this study.

## **Nubian Datasets**

In order to develop a more temporally and geographically representative reference sample, two medieval Nubian datasets are also used for comparative purposes. Craniometric data collected by Dr. Jennifer Vollner for individuals from Mis Island cemeteries 3-J-10 and 3-J-11, located in present-day Sudan, are used to bolster the reference sample with North African individuals corresponding to the Late Antique and Early Medieval periods (Figure 5.1, Table 5.3) (Vollner, 2016). The samples contain adult individuals with no apparent cranial pathology or cranial modification.

Mis Island is located upstream from the fourth cataract of the Nile River in present-day Sudan. The individuals from Mis Island were interred in two cemeteries from the Christian period, 3-J-10 and 3-J-11. Cemetery 3-J-10 was in use from AD 1100-1500 and contained 13

males and 11 females with sufficiently complete crania for analysis, totaling 24 individuals. Cemetery 3-J-11 was in use from AD 300-1400 and was much larger than 3-J-10, containing 33 males and 42 females with sufficiently complete crania, totaling 75 individuals. Sex was estimated for this sample using pelvic morphology (Vollner, 2016).

The two Mis Island cemetery samples were selected for comparison in this dissertation due to their temporal and geographic parameters. Their location in northeast Africa provides a generally good representation of the possible origin of African migrants to Tuscany. Further, the time periods of the two cemeteries together entirely overlap with the cemetery at *Rusellae*, creating a good temporal comparative sample. Notably, the two cemeteries will be treated separately in this dissertation due to their distinct time frames, with Cemetery 3-J-11 having a longer and earlier span of usage compared to Cemetery 3-J-10.

Table 5.3. Craniometric reference samples

Group	Continent	Region	Time Period	Male	Female
Berg	Europe	Carinthia, Austria	Unknown; likely AD 1700-1900	56	53
Norse	Europe	Oslo, Norway	Medieval	54	54
Zalavar	Europe	Zalavar, Hungary	AD 800-1000	53	45
Dogon	Africa	Mali, West Africa	AD 1055-1750	47	52
Mis Island 3-J-10	Africa	Nubia, Northeast Africa	AD 1100-1500	13	11
Mis Island 3-J-11	Africa	Nubia, Northeast Africa	AD 300-1400	33	42

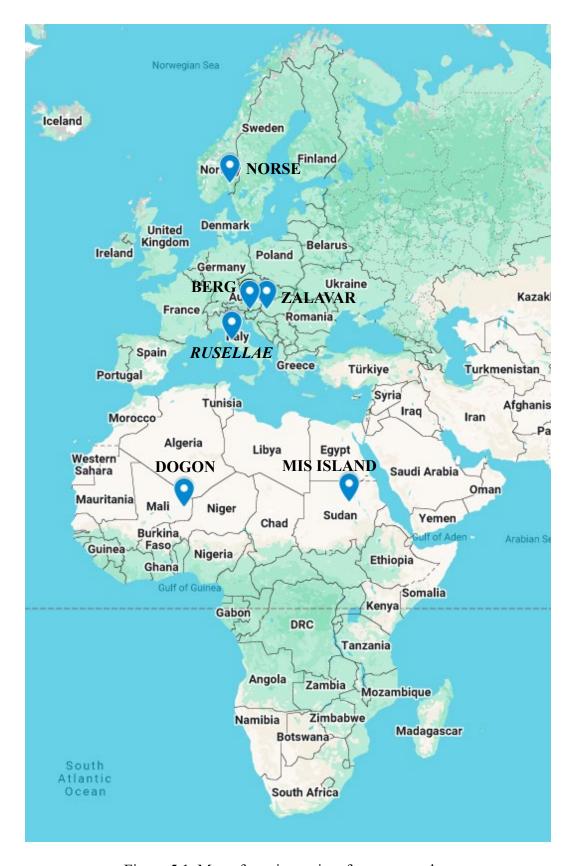


Figure 5.1. Map of craniometric reference samples.

## **Sample Limitations**

Bioarchaeological skeletal samples necessarily differ from modern skeletal samples in a number of ways that need to be addressed. The cemetery sample is a result of decisions made by the living, including variables such as how the corpses are disposed (inhumation vs. cremation), whether the burials are temporary or permanent, or where in the cemetery people can be buried (Weiss-Krejci, 2011). For example, in Medieval Italy females tend to be underrepresented in cemeteries compared to the Roman period and Late Antiquity (Barbiera, Castiglione, & Dalla-Zuanna, 2017). This may be the result of different burial practices for females, such as inhumation in a separate part of the cemetery or burial directly in the ground instead of in a rock-lined tomb. Similarly, in the present study, individuals from Phase I are particularly sparse. This is due in part to the fact that many of the Phase I graves were re-used during Phase II, and the remains originally interred were commingled and reburied, meaning that fewer Phase I "individuals" were preserved (Celuzza et al., 2002).

Following deposition of the remains, taphonomic processes can either preserve or destroy the quality of the osseous material. Factors such as soil acidity, burial environment, exhumation and reburial, and construction can all influence the preservation of the remains, which in turn affects the depth of analysis that is possible. As a result of these taphonomic processes, skeletal samples are regularly fragmentary. Skeletal trauma or disease near the time of death may also make the skeleton more fragile, resulting in poor preservation. Curation or handling during analysis further increases the possibility of postmortem damage. This fragmentation can substantially restrict the number and type of cranial landmarks available for study. This directly impacts the granularity with which phenotypic, and subsequently genotypic, variation can be understood.

Sample bias is also introduced during excavation of the remains, where the research interests, time, money, or political constraints of the archaeologists dictates what gets excavated, what gets recorded, and how it is curated. This can lead to incomplete cemetery recoveries or a focus on monumental burials instead of cemeteries containing common folks. Although examining elite burials is important and interesting, they are often not representative of the true lived experience for most people in a particular context. Further, the interests of Italian archaeologists and classicists have not always involved the human skeleton (Gowland, 2017; Killgrove, 2005). In some of the most egregious cases, grave goods were carefully excavated, while human remains were subsequently disposed of without further analysis. Fortunately, this practice is no longer legal. Excavations rarely included skeletal analysis beyond reporting body position within a grave. When excavated, these skeletons were often subject to poor curation with limited associated contextual information, making future research challenging (Killgrove, 2005). Working with skeletal collections that were excavated decades earlier is challenging due to the loss of taphonomic and archaeological context (Nikita, 2024).

When it comes to a statistical analysis of the remains, a sampling strategy is typically used to ensure that the sample under study is representative of the site or population at large. It is this representativeness that allows for larger conclusions to be drawn from a smaller sample group. When working with skeletal samples, researchers do not always have the luxury of randomly selecting specimens from a larger population. Skeletal samples are often relatively small, requiring the use of the complete, excavated assemblage as opposed to a statistically-derived study sample. The results of such investigations are informative, but they may be limited in their scope and generalizability.

Another avenue through which bias is introduced in the *Rusellae* sample is temporal aggregation, which refers to the grouping of individuals who lived over a broad time scale and considering them to be one population. Individuals who lived several hundred years apart (such as those in the earlier vs. later part of Phase II) would have likely led quite different lives. In this study, however, they are considered to represent one time period. This approach subsequently masks some of the variation inherent within each phase by lumping together several generations into one group.

Each of the aforementioned issues with bioarchaeological samples contributes to a trend of small sample sizes in bioarchaeological research. Although limited samples should not deter research, there are considerations or concessions that need to be made. In some cases, when data are too sparse to allow for a statistical analysis, an osteobiography approach can be used to examine the lived experience of a single individual (Hosek & Robb, 2019; Robb, 2024). Specific statistical methods for small samples, such as the Fisher's exact test and data imputation methods, can be used to maximize the sample and apply statistical rigor (Buikstra et al., 2022). Overall, sample limitations must be acknowledged, and results presented with the appropriate caveats.

#### **Data Collection Methods**

Several types of data were collected or generously provided by colleagues for the present research: skeletal indicators of sex, age, and stress, and cranial measurements. All possible data points were collected for each skeleton, depending on the level of completeness and preservation in each case. The data collection procedures for each type of data are described below.

#### **Sex Estimation**

For this dissertation, sex was estimated by morphological analyses of the pelvis and skull, as well as measurements of the postcranium. The three primary pelvic features used in sex estimation are the ventral arc, subpubic concavity, and the medial aspect of the ischiopubic ramus, which can be used to estimate sex with approximately 95% accuracy (Phenice, 1969). These three traits, in addition to the morphology of the greater sciatic notch and preauricular sulcus, were scored using the system outlined in Buikstra and Ubelaker (1994). Generally, the presence of a ventral arc, subpubic concavity, a narrow ischiopubic ramus, and a wide greater sciatic notch are consistent with female, while the lack of a ventral arc, no subpubic concavity, a broad ischiopubic ramus, and a narrow greater sciatic notch are consistent with male.

Similarly to the pelvis, the skull also exhibits sexual dimorphism in several features, five of which are traditionally used in sex estimation: the mental eminence, orbital margin, glabellar area, nuchal area, and mastoid process. These traits were also scored following Buikstra and Ubelaker (1994), with a more gracile mental eminence, glabella, nuchal area, mastoid process, and sharp orbital margins scored as consistent with female. Conversely, a robust mental eminence, glabella, nuchal area, mastoid process, and blunt orbital margins are consistent with male.

Finally, when present, the humeral and femoral heads were measured using spreading calipers and the measurements were recorded in millimeters. These measurements were compared with Stewart's sectioning points for sex estimation (1979). For the humeral head, a vertical diameter less than 43 mm indicates female, 43-44 mm indicates probable female, 45 mm is indeterminate, 46-47 mm indicates probable male, and greater than 47 mm indicates male. For the femoral head, a maximum diameter less than 42.5 mm indicates female, 42.5-43.5 mm

indicates probable female, 43.5-46.5 is indeterminate, 46.5-47.5 mm indicates probable male, and greater than 47.5 mm indicates male (Stewart, 1979).

When present and observable, morphological features of the pelvis were prioritized in sex estimation. However, all the aforementioned features were used in combination to assess sex overall, using the categories of undetermined, female, probable female, ambiguous, probable male, and male. For the purposes of this dissertation, the categories of female and probable female are lumped together as female, probable male and male are lumped together as male, and individuals with undetermined and ambiguous sex are not included in the sex-specific analyses of this study.

# **Age Estimation**

Adult age is estimated using predictable degenerative changes in the skeleton that can be correlated to chronological age. The primary methods of adult age estimation in this dissertation include the fusion of the medial clavicle and the morphologies of the pubic symphysis, auricular surface, and sternal end of the rib.

The fusion of the medial clavicle epiphyses, when observable, is used as an indicator of adult versus subadult age. That is, when the medial clavicle epiphysis is fused, the individual is considered to have reached skeletal maturity, which allows for the use of the following sex estimation methods. This epiphysis generally fuses between the ages of 18 and 32 years (Buikstra & Ubelaker, 1994).

Features of the face of the pubis bone were utilized for age estimation in the adult skeleton. With time, the pubic symphysis transitions from a sturdy, billowing surface with little delineation of the face, to a flat, finely grained face with a complete rim, and finally to a depressed, porous face with lipping and other irregularities (Suchey & Katz, 1998). The Suchey-

Brooks method, which outlines six phases of changes in pubic symphysis morphology, employs descriptions and casts to aid practitioners in its use. Males and females were analyzed separately, as they undergo age-related changes at different rates and exhibit some different features.

Similarly to the pubic symphysis, the morphology of the auricular surface of the ilium was also used to estimate adult age. Over time, the auricular surface undergoes a transition from a youthful, billowy surface to a poorly-organized, irregular surface with marked apical activity. Features evaluated for aging include the transverse organization, surface texture, microporosity, macroporosity, and apical changes of the auricular surface. For the present research, these features are taken in combination and scored using a phase method to estimate age (Lovejoy, Meindl, Pryzbeck, & Mensforth, 1985). As with the pubic symphysis, males and females were analyzed separately as the age-related changes vary slightly by sex.

The sternal rib end also undergoes predictable changes with age, transitioning from a relatively flat and billowy surface to a thin-walled surface with irregular projections. Ribs were sorted into phases from 0-8, each corresponding to an age range with an associated 95% confidence interval, based on traits such as scalloping of the margins, the shape and depth of the pit at the sternal end, and the presence of any irregular claw-like projections (Iscan, Loth, & Wright, 1984, 1985). Males and females were scored separately in this method as well, as they undergo these changes along a slightly different time scale.

After using all possible methods to estimate age, a consolidated age estimate was produced using the data gleaned from each method. The pubic symphysis data was weighed most heavily, when present, and the age range was adjusted as necessary using data from the auricular surface and sternal rib ends. After the age range was established, each individual was placed into an age category as follows: young adult (20-35 years), middle adult (35-50 years), old adult (50+

years). If age indicators are limited due to lack of skeletal completeness or poor preservation, a broader age category of subadult (< 20 years) or adult (> 20 years) was assigned.

#### **Craniometrics**

Coordinate data and interlandmark distances were recorded for each cranium using a Microscribe 3D digitizer and the program 3Skull (Ousley, 2014). Cranial landmarks are defined points that are theoretically homologous across all individuals, allowing for a comparison of landmark locations across several samples. This study utilized the 108 landmarks prompted in the 3Skull software package and defined in the Harris County Standard Operating Procedure (Fleischman & Crowder, 2018). All possible landmark data were recorded for each cranium, even if the cranium was incomplete.

Coordinate data were collected using a Microscribe 3D digitizer, which records x, y, and z coordinates for each landmark. Before a cranium is digitized, several instrumentally-determined landmarks are measured with sliding or spreading calipers and marked with a pencil, including ectoconchion, upper orbital border, cheek height superior point, cheek height inferior point, zygion, eurion, frontotemporale, maximum frontal point, glabella, and ectomolare.

Additionally, several points are also marked for ease of identification during the digitizing process, including sphenion, krotaphion, stephanion, asterion, opisthion, and basion. After locating and marking these landmarks, the cranium is set on three clay pedestals and secured to prevent any movement during the digitizing process. The 3Skull software prompts the analyst to collect each landmark, at which point the Microscribe's stylus is placed on the landmark and a foot pedal is depressed to collect the coordinate data. If a landmark is not present due to pathology or poor preservation, a note is recorded in 3Skull and data for that landmark is not recorded.

Finally, these coordinates are imported through the 3Skull software program into a Microsoft Excel spreadsheet. 3Skull also calculates interlandmark distances, or the distance between two landmarks in space, from these coordinates; these data are recorded in millimeters and exported to a separate Microsoft Excel spreadsheet.

#### **Skeletal Stress Indicators**

Linear Enamel Hypoplasia

All present teeth were observed for evidence of linear enamel hypoplasias. When necessary, directed lighting was used to facilitate observation of enamel defects. These traits were scored according to Buikstra and Ubelaker (1994). If present, the type of defect was scored as either linear horizontal grooves, linear vertical grooves, linear horizontal pits, a nonlinear array of pits, or single pits. The location of each lesion was then measured in millimeters from the cemento-enamel junction using digital calipers. The defects were recorded by tooth for each tooth present.

#### Skeletal Porosities

Porosities of the cranium, including porotic hyperostosis and *cribra orbitalia*, were identified and scored following the standardized scoring system outlined in Buikstra and Ubelaker (1994). When observed, these porosities were scored in terms of expression and activity. The expression of the observed lesions was characterized as either barely discernible/very indistinct porosity, true porosity/porosity only, coalescing pores, or coalescing pores and expansive changes. Activity was scored as either active, healed, or a mixed reaction of the two. Specifically, active lesions exhibit sharp lesion margins and a sieve-like appearance, while healed lesions exhibit a more smooth texture with evidence of infilling of the pores (Mensforth, Lovejoy, Lallo, & Armelagos, 1978).

### Periosteal Reactions

The long bones were observed for evidence of periosteal reactions and data were recorded regarding the presence, location, extent, and vascularization of the lesions, as outlined in Cook (1976) and Weston (2008). The reactions were also characterized as focal vs. diffuse and woven vs. sclerotic vs. mixed. The extent of the reaction was scored as either normal, isolated on <1/3 of the surface, isolated on 1/3-2/3 of the surface, isolated on > 2/3 of the surface, or > 2/3 of the surface with elevation > 2-3 mm. The vascularization was scored as normal, multiple small striae, multiple small foramina, multiple large striae, multiple large foramina, or mixed abnormal vascularization. Further, focal reactions exhibited clearly defined boundaries, while diffuse reactions occupied a larger area with less clear boundaries. Finally, woven bone appears fibrous and unconsolidated, while sclerotic bone appears as woven bone that has been filled in, exhibiting a more dense texture (Cook, 1976; Weston, 2008).

### **Analytical Methods**

## **Research Question 1**

The first research question seeks to understand the craniometric variation at *Rusellae* on two levels: between *Rusellae* and geographically and temporally relevant craniometric reference samples and within *Rusellae* as a whole. Each of these levels of variation will be explored using specific statistical methods, as outlined below. All analyses for this research question are performed in R (v. 4.4.1), a freely-available open-source programming language used primarily for statistical computing and graphics (R Core Team, 2024).

Twenty-one craniometric variables were selected for analysis. These variables were selected because a) they correlate well with genetic data (Relethford, 1994); b) they capture the morphology of the midface and the cranial vault; c) they are commonly used in forensic

anthropology population affinity estimation as well as biodistance analyses; d) they have standardized definitions and measurement protocols (Fleischman & Crowder, 2018); and e) they maximize the sample size in the present study as they are better preserved than other variables. The twenty-one variables under study are listed in Table 5.4. Measurements and abbreviations are adapted from Fleischman and Crowder (2018).

Table 5.4. Craniometric variables used in Research Question 1

Abbreviation	Measurement
GOL	Maximum cranial length
BNL	Basion-nasion length
BBH	Basion-bregma height
XCB	Maximum cranial breadth
ZYB	Bizygomatic breadth
AUB	Biauricular breadth
ASB	Biasterionic breadth
BPL	Basion-prosthion length
NPH	Nasion-prosthion height
NLH	Nasal height
NLB	Nasal breadth
MDH	Mastoid height
OBH	Orbital height
OBB	Orbital breadth
DKB	Interorbital breadth
ZMB	Bimaxillary breadth
EKB	Biorbital breadth
FRC	Frontal chord
PAC	Parietal chord
OCC	Occipital chord
FOL	Foramen magnum length

Prior to analysis, all datasets included in both the reference and study sample must be complete. That is, there can be no missing measurements in any of the individuals for any of the 21 variables under analysis. Although the Howells reference data is complete, the *Rusellae* study sample and the Mis Island 3-J-10 and Mis Island 3-J-11 cemeteries include individuals with an incomplete set of observations. This fragmentation is common in bioarchaeological samples as a result of poor preservation or postmortem damage caused by taphonomic processes or during

excavation, curation, or analysis. Therefore, data imputation will be used to complete these datasets. Kenyhercz and Passalacqua (2016) showed that accurate classifications can still be achieved when up to 50% of data is missing. Consequently, data is imputed for individuals missing ten or fewer data points out of the 21 total craniometric variables used in the study.

Missing data for the *Rusellae* and Mis Island samples are imputed using the Multivariate Imputation by Chained Equations (MICE) method using the 'mice' package in R (Van Buuren & Groothuis-Oudshoorn, 2011). The MICE method is effective for imputing incomplete multivariate data simultaneously on a variable-by-variable basis (Van Buuren & Groothuis-Oudshoorn, 2011). Each dataset is imputed separately by population (e.g. each Mis Island cemetery and the *Rusellae* sample were treated separately); this conservative strategy is recommended by Kenyhercz and Passalacqua (2016) to minimize the difference between imputed and actual values that may occur by pooling datasets prior to imputation. The specific method of imputation is 'predictive mean matching', which evaluates the range of realistic values for each variable and fills in missing data while preserving the distribution of the original dataset (Van Buuren & Groothuis-Oudshoorn, 2011). This analysis employed 50 iterations to produce five imputed datasets. The first imputed dataset was selected for further analysis for all three imputed datasets.

Following imputation, all seven groups used in this analysis are combined: Berg, Zalavar, Norse, Dogon, Mis Island 3-J-10, Mis Island 3-J-11, and *Rusellae*. Then, the data are standardized by scaling and centering. Scaling and centering sets the mean of each variable to zero and the standard deviation to one, which prevents variables with large magnitudes (in this case, larger size) and variables with large ranges (as in the range between male and female

values) from biasing the results (Kabacoff, 2022). This approach allows males and females to be pooled in order to maximize sample size.

In order to compare the overall craniometric variation at Rusellae to temporally and geographically relevant reference samples, a Mahalanobis distance (MD) statistic is employed, followed by a Principal Components Analysis (PCA) to visualize intergroup distances in multivariate space. The Mahalanobis distance can be employed in both the original variable and the Principal Components (PC) spaces; here, the MD is applied to the original scaled data. The MD is a distance between a point and a distribution; one of the most commonly-used distance measures in modern research, MD outperforms simple Euclidean distance as it is a model-free approach that takes into account the variance within the dataset and the correlations between variables, such as those between interlandmark distances of the cranium (De Maesschalck, Jouan-Rimbaud, & Massart, 2000; Relethford, 2016). Mahalanobis distance using cranial measurements correlates with genetic distances in studies using matched datasets (Relethford, 2004). The present analysis measures similarity and dissimilarity between the groups in order to understand the degree of relatedness among the samples. For this analysis, the Mahalanobis distance between all group centroids is calculated. Permutation testing is used to test for significant differences. Then, a PCA is performed on the complete dataset and group centroids are plotted against PC1 and PC2 to visualize group relatedness in multivariate space.

In order to understand within-group variation at *Rusellae*, a Principal Components Analysis (PCA) is employed on the *Rusellae* craniometric data alone. Like the Mahalanobis Distance analysis, PCA requires a complete dataset; therefore, the imputed *Rusellae* dataset produced using the MICE method is analyzed here. The data are again scaled and centered to allow for pooling of sexes.

PCA is an analytical method that reduces dimensionality in multivariate data to maximize the variance within the data. PCA takes a large number of correlated variables (in this case, interlandmark distances) and transforms them into a smaller and more manageable set of uncorrelated variables, known as principal components (PCs). The output of this analysis is a series of PCs, ranked in order of how much variation they capture from the original dataset. Typically, the first few PCs explain a large proportion of data variation, which allows for dimensionality reduction while maintaining as much variance in the dataset as possible (Kabacoff, 2022). Further, PCA does not require a grouping variable; therefore, all individuals at *Rusellae* can be evaluated against one another without any a priori assumptions about group membership (sex, age, ancestry, phase, etc.).

The PCA is performed in R version 4.4.1 using the prcomp function in base R (R Core Team, 2024). The PCA is performed on the scaled dataset and the principal components are extracted. The PCs with an eigenvalue greater than 1.0 are retained, per the Kaiser-Harris criterion; eigenvalues greater than 1.0 suggest the principal component explains more variance than a single variable alone (Kabacoff, 2022). The results of this analysis quantify combinations of craniometric variables most significantly driving the craniometric variation within the cemetery at *Rusellae*.

In order to further understand the within-cemetery craniometric variation, a hierarchical cluster analysis is performed on the retained PCs with eigenvalues greater than 1.0. The resulting dendrogram is visualized and the tree is cut at the number of clusters established from the dendrogram. Finally, a scatterplot is produced of individuals in the cemetery based on the first two PCs to visualize the clusters in multivariate space.

## **Research Question 2**

The second research question aims to understand whether individuals of African ancestry might have been present at *Rusellae* throughout the occupation of the cemetery. This question will be addressed using linear discriminant analysis (LDA). LDA is a form of classification statistics that relies on building a model using a known reference sample, followed by classifying unknowns into one of the reference groups (Zhao, Zhang, Yang, Zhou, & Xu, 2024). All analyses for this research question are performed in R (v. 4.4.1) (R Core Team, 2024).

The same 21 craniometric variables from Research Question 1 are also used in this analysis. As with MD and PCA, LDA requires complete datasets; therefore, the *Rusellae* and Mis Island datasets were again completed using the 'mice' package. Following data imputation, the two Mis Island cemetery reference datasets are added to the four Howells datasets (Berg, Norse, Zalavar, and Dogon) to produce the overall reference sample used in this analysis. Then, each dataset (the combined reference sample and the *Rusellae* sample) is scaled and centered in order to minimize the effects of size and to allow for pooling of sexes.

After the reference and study datasets are clean, a linear discriminant analysis (LDA) is performed. LDA is a form of discriminant function analysis that maximizes between-group variance and minimizes within-group variance by producing the linear combination of variables, in this case cranial measurements, that best separates each group (Zhao et al., 2024). The LDA is performed using the MASS package in R (Venables & Ripley, 2002), where the model is built to classify observations into one of the six reference groups based on the 21 craniometric variables in the dataset. Effectively, the reference groups act as a training sample used to generate the model. The model applies equal prior probabilities to each of the six groups in the reference dataset. The overall correct classification rate of the model is calculated, as well as the correct

classification rate for each group. Then, the model is used to make predictions about group membership for the individuals in the unknown dataset; here, the *Rusellae* individuals act as the holdout or testing set for the model. Therefore, the model is cross-validated using separate testing and training datasets.

### **Research Question 3**

The third research question seeks to understand whether skeletal stress differed between individuals of estimated African and European ancestry. In order to evaluate this question, the frequency of each non-specific stress indicator is calculated by dividing the number of instances of each indicator by the total number of observable instances in the assemblage. Both affinity groups are divided by sex and differences in frequency are compared between males and females using a Fisher's exact test. If no significant difference is found between males and females within each affinity group, the sexes are pooled and frequencies are compared between African and European affinity, again using a Fisher's exact test.

A Fisher's exact test assesses the independence of categorical variables, especially in cases where presence or absence counts for a given variable are less than five (Kim, 2017). This statistical method is used to understand whether there is a relationship between any health/stress indicators (porotic hyperostosis, periosteal reaction, *cribra orbitalia*, and LEH) and estimated affinity or whether the frequency of a stress indicator is independent of affinity.

#### **Research Ouestion 4**

In order to examine craniofacial variation over time at *Rusellae*, individuals from Phase I are compared with individuals from Phase II with respect to cranial measurements. This is achieved through a multivariate analysis of variance (MANOVA) performed on a standardized craniometric dataset grouped by phase. MANOVAs test for the significance of group differences

over multiple variables at once (Kabacoff, 2022). All analyses for this research question are performed in R (v. 4.4.1) (R Core Team, 2024).

Since phase data is only available for a small subset of individuals with crania present, the sample size for this research question is quite limited. To mitigate some issues associated with statistical analysis of small samples, the number of craniometric variables used in this analysis is also limited, with the aim of preserving all four individuals from Phase I in the dataset. Therefore, craniometric variables with data for at least three of the four Phase I individuals were retained, leaving 12 variables (Table 5.5). To impute missing data following the recommendation of Kenyhercz and Passalacqua (2016), case deletion was performed for all individuals not having data for at least 6 of the 12 variables. Missing data are imputed using the MICE method for each phase separately in order to maintain any variation that exists between phases in the data. The resulting dataset comprises 12 craniometric variables for four individuals from Phase I and 20 individuals from Phase II.

Table 5.5. Craniometric variables used in Research Question 4

Abbreviation	Measurement
WFB	Minimum frontal breadth
NPH	Nasion-prosthion height
NLH	Nasal height
JUB	Jugal breadth
NLB	Nasal breadth
OBH	Orbital height
OBB	Orbital breadth
DKB	Interorbital breadth
ZMB	Bimaxillary breadth
FMB	Bifrontal breadth
UFBR	Upper facial breadth
UFHT	Upper facial height

Following imputation, the complete Phase I and Phase II datasets are combined and scaled and centered to minimize the effect of size and to allow for pooling of sexes. Then, the

two phases are compared using a MANOVA to determine if there are significant differences in craniometric variables over time. Following the MANOVA, univariate one-way analyses of variance (ANOVAs) are performed on each variable to determine whether and where significant differences exist between phases on any of the 12 variables included in the analysis.

The final research question also seeks to understand whether skeletal stress indicators were more frequent in the earlier phase (Phase I) or later phase (Phase II) of the cemetery. All individuals with phase data who had at least one scorable stress indicator are included in the analysis. In order to evaluate this question, the frequency of each non-specific stress indicator is calculated by dividing the number of instances of each indicator by the total number of observable instances in the assemblage. Each phase is divided by sex and differences in frequency are compared between males and females using a Fisher's exact test. If no significant difference is found between males and females within each phase, the sexes are pooled and frequencies are compared between Phase I and Phase II, again using a Fisher's exact test.

This chapter provided the specific skeletal samples and methods used to answer the research questions. The next chapter will present the results of these analyses.

### **CHAPTER SIX: RESULTS**

First, I will report summary data for the craniometric datasets as well as illustrations of missingness and the effects of imputation on the datasets. This chapter will then present results organized by each research question, as outlined in **Chapter Four: Research Questions and Expectations**.

## **Summary Data**

Summary data for the seven craniometric datasets are presented in Tables 6.1 through 6.7. These show details of the data prior to imputation. Missingness is illustrated in Figures 6.1 through 6.3. Following imputation, a series of graphics were generated to ensure no unrealistic values were imputed. Figure 6.4 provides an example of the pre- and post-imputation distribution of GOL in the *Rusellae* sample.

Table 6.1. Descriptive statistics, Rusellae

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	24	184	7.56	172	198
BNL	22	98.8	5.59	84	111
BBH	22	128	5.97	117	136
XCB	23	139	6.29	127	152
ZYB	13	128	6.35	112	137
AUB	23	121	7.78	103	134
ASB	21	113	5.46	102	123
BPL	19	95.3	4.36	85	104
NPH	25	63.8	3.44	58	72
NLH	27	50.1	3.05	45	57
NLB	27	25.1	1.67	22	28
MDH	23	29.3	2.85	24	37
OBH	28	32.6	2.09	28	36
OBB	28	38.8	1.85	34	41
DKB	28	22.5	2.16	18	27
ZMB	23	92.2	5.28	79	104
EKB	25	96.4	3.52	90	102
FRC	28	109	6.22	97	123
PAC	27	115	5.88	107	134
OCC	24	95.9	5.29	84	110
FOL	21	37.2	2.79	33	43
WFB	29	94.9	4.28	87	102
JUB	25	112	4.61	104	119
FMB	29	97.3	3.72	91	105
UFBR	29	103.1	3.83	96	110
UFHT	26	66.8	3.69	61	78

Table 6.2. Descriptive statistics, Mis Island 3-J-10

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	23	181.2	4.85	172	191
BNL	23	98.3	4.15	90	105
BBH	23	129.2	5.25	119	141
XCB	23	126.7	4.51	116	135
ZYB	19	122.3	4.78	114	131
AUB	23	112.3	4.31	103	120
ASB	23	104.8	5.00	96	116
BPL	22	98.0	6.12	86	109
NPH	23	65.0	4.02	56	75
NLH	23	47.3	2.74	41	52
NLB	24	25.4	1.41	23	29
MDH	23	27.3	4.08	18	36
OBH	23	33.0	1.85	29	37
OBB	23	37.2	1.93	32	41
DKB	24	21.8	1.49	19	25
ZMB	23	91.3	4.12	85	101
EKB	22	93.4	2.77	87	99
FRC	24	108.5	4.62	101	117
PAC	24	117.3	5.66	105	130
OCC	23	92.5	4.34	84	102
FOL	23	36.3	3.10	30	44

Table 6.3. Descriptive statistics, Mis Island 3-J-11

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	74	182.1	6.17	171	195
BNL	74	99.3	4.66	89	114
BBH	74	128.7	4.71	117	138
XCB	74	126.9	4.80	113	138
ZYB	60	120.9	5.42	111	133
AUB	73	112	4.17	100	121
ASB	74	104.6	4.24	93	119
BPL	71	98.1	4.95	88	112
NPH	72	65.1	4.67	54	77
NLH	72	47.1	3.43	40	56
NLB	74	26.6	1.90	22	32
MDH	72	26.6	3.09	20	34
OBH	72	32.5	2.05	28	37
OBB	72	37.4	1.68	33	41
DKB	74	23.1	1.96	19	30
ZMB	71	93.0	4.66	83	107
EKB	69	94.2	3.12	86	102
FRC	75	109	3.98	101	120
PAC	75	115.6	5.06	102	137
OCC	75	93.3	4.59	83	104
FOL	75	35.5	2.57	30	43

Table 6.4. Descriptive statistics, Berg

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	109	175.6	8.45	155	198
BNL	109	95.8	5.03	84	110
BBH	109	127.4	5.34	115	141
XCB	109	144.1	6.22	131	161
ZYB	109	131.1	6.37	117	148
AUB	109	124	6.1	110	140
ASB	109	111	5.34	98	127
BPL	109	91.9	5.72	81	108
NPH	109	65.8	4.63	52	79
NLH	109	50	3.41	42	58
NLB	109	25.2	1.86	22	31
MDH	109	27	3	18	36
OBH	109	33.3	1.89	28	39
OBB	109	39.3	1.59	36	44
DKB	109	22.5	2.35	18	32
ZMB	109	91.5	4.47	80	104
EKB	109	97	3.41	89	105
FRC	109	108.7	4.60	96	122
PAC	109	107.7	5.70	95	120
OCC	109	92.8	5.21	79	105
FOL	109	37.6	3.10	31	50

Table 6.5. Descriptive statistics, Dogon

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	99	173.6	6.94	157	194
BNL	99	96.6	4.02	86	107
BBH	99	130	4.92	118	152
XCB	99	134.6	5.17	121	148
ZYB	99	125.1	5.69	112	138
AUB	99	112.2	4.64	98	123
ASB	99	101.8	4.61	89	115
BPL	99	97.9	4.70	84	108
NPH	99	63.1	4.17	52	72
NLH	99	46.9	2.65	41	54
NLB	99	28.0	1.68	25	32
MDH	99	27.0	3.22	20	35
OBH	99	33.2	1.85	29	37
OBB	99	38.8	1.79	35	43
DKB	99	22.8	2.30	17	28
ZMB	99	94.6	4.38	84	105
EKB	99	97.1	3.81	88	107
FRC	99	107.8	4.71	98	118
PAC	99	109.8	6.08	97	128
OCC	99	94.2	5.07	79	106
FOL	99	34.1	2.62	29	42

Table 6.6. Descriptive statistics, Norse

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	108	184.2	6.55	170	201
BNL	108	99.5	4.22	88	112
BBH	108	128.9	5.45	117	141
XCB	108	139.1	5.26	127	152
ZYB	108	129.5	6.31	118	142
AUB	108	121.2	5.25	108	134
ASB	108	109.3	4.63	96	123
BPL	108	95.5	4.97	86	114
NPH	108	66.5	4.38	57	76
NLH	108	50.6	2.98	44	59
NLB	108	24.8	1.80	19	30
MDH	108	27.8	3.48	19	37
OBH	108	33.5	2.15	27	38
OBB	108	39.8	1.62	36	44
DKB	108	21.4	2.40	16	27
ZMB	108	92.1	5.15	79	109
EKB	108	96.8	3.72	88	106
FRC	108	110.6	4.99	99	123
PAC	108	112	5.79	99	128
OCC	108	96.3	4.41	86	107
FOL	108	35.7	2.51	30	43

Table 6.7. Descriptive statistics, Zalavar

Measurement	n	Mean (mm)	SD	Min (mm)	Max (mm)
GOL	98	181.1	7.24	165	196
BNL	98	99.1	4.74	90	109
BBH	98	132.1	5.91	120	144
XCB	98	139.3	4.74	127	149
ZYB	98	129.5	5.32	114	139
AUB	98	121.4	4.77	110	133
ASB	98	109.5	4.68	97	122
BPL	98	94.8	6.07	83	110
NPH	98	66.0	4.71	54	79
NLH	98	50.1	3.09	42	57
NLB	98	25.0	1.60	22	30
MDH	98	28	3.22	21	35
OBH	98	32.4	1.89	28	36
OBB	98	39.4	1.57	34	43
DKB	98	21.1	2.39	16	27
ZMB	98	92.6	4.46	82	105
EKB	98	96.6	3.22	88	105
FRC	98	110.3	4.85	98	120
PAC	98	113.2	5.60	100	127
OCC	98	95.3	4.76	85	108
FOL	98	36.3	2.64	30	43

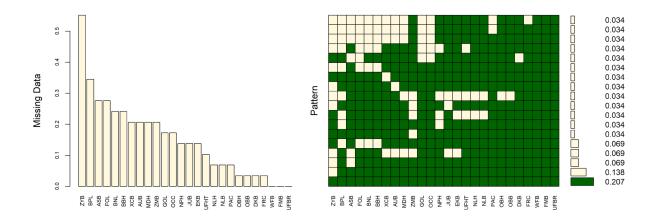


Figure 6.1. Missing data by variable, Rusellae.

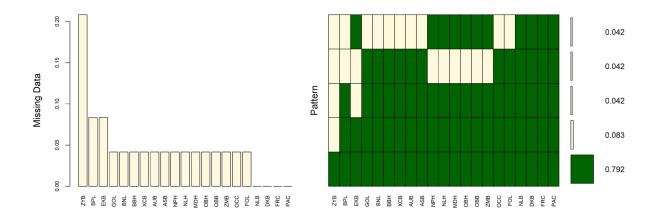


Figure 6.2. Missing data by variable, Mis Island 3-J-10.

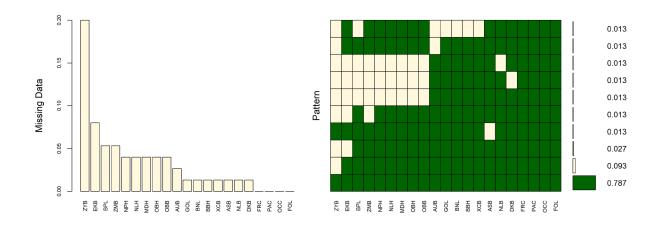


Figure 6.3. Missing data by variable, Mis Island 3-J-11.

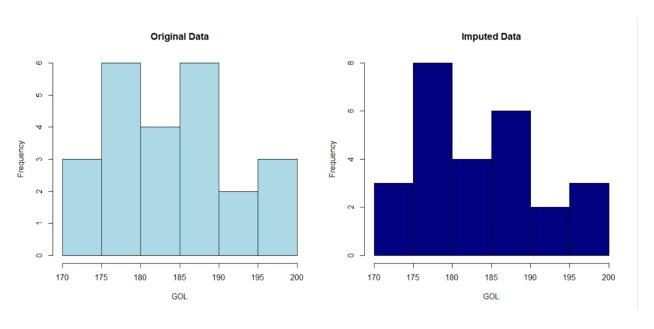


Figure 6.4. Pre- and post-imputation distribution of GOL, Rusellae.

## **Research Question 1**

A subset of sufficiently complete crania from *Rusellae* (n=26) were evaluated using Mahalanobis Distance (MD) and Principal Components Analysis (PCA) to explore craniofacial variation between *Rusellae* and global reference samples, as well as within the *Rusellae* sample itself. Of the 26 individuals from *Rusellae*, 13 are female (50%) and 13 are male (50%). Phase data is available for 22 of the individuals; 2 are from Phase I (9.1%) and 20 are from Phase II

(90.9%). The demographic breakdown of the six reference samples (Berg, Zalavar, Norse, Dogon, Mis Island 3-J-10, and Mis Island 3-J-11) is outlined in Chapter Five: Materials and Methods.

In order to understand how *Rusellae* compares with geographically and temporally relevant reference samples, a Mahalanobis Distance test is performed. This test allows for understanding of intergroup similarity and dissimilarity in multivariate space between the Berg, Zalavar, Norse, Dogon, Mis Island 3-J-10, Mis Island 3-J-11, and *Rusellae* groups using the 21 craniometric variables described in Chapter Five: Materials and Methods. The resulting Mahalanobis distances are depicted in Table 6.8. A permutation test for significance found significant differences at the  $\alpha$ =0.05 level for all pairs of groups except between the two Mis Island cemeteries (p=0.113).

Table 6.8. Mahalanobis distances between *Rusellae* and the six reference samples

	Berg	Dogon	Mis Island 3-J-10	Mis Island 3-J-11	Norse	Rusellae	Zalavar
Berg	0	5.89	7.88	7.22	3.49	5.04	2.99
Dogon	5.89	0	7.13	5.95	5.67	8.30	5.25
Mis Island 3-J-10	7.88	7.13	0	1.58	5.72	6.00	5.25
Mis Island 3-J-11	7.22	5.95	1.58	0	4.70	6.64	4.75
Norse	3.49	5.67	5.72	4.70	0	4.26	1.48
Rusellae	5.04	8.30	6.00	6.64	4.26	0	4.14
Zalavar	2.99	5.25	5.25	4.75	1.48	4.14	0

A Principal Components Analysis on the complete Howells, Nubian, and *Rusellae* dataset can visualize the major contributing variables to the craniometric variation between the seven groups (Figure 6.5). Generally, the Dogon and Mis Island cemeteries cluster most closely and are separated from the cluster that contains the Norse, Zalavar, and *Rusellae* samples based on the first two PCs. The Berg sample is closer to the European centroids than the African centroids,

but is generally distant from all groups. Notably, the 95% confidence ellipse for *Rusellae* is larger than that of the other groups, and is the only one that encompasses the centroids of all groups, suggesting *Rusellae* exhibits more craniometric variation than the other samples included in the analysis.

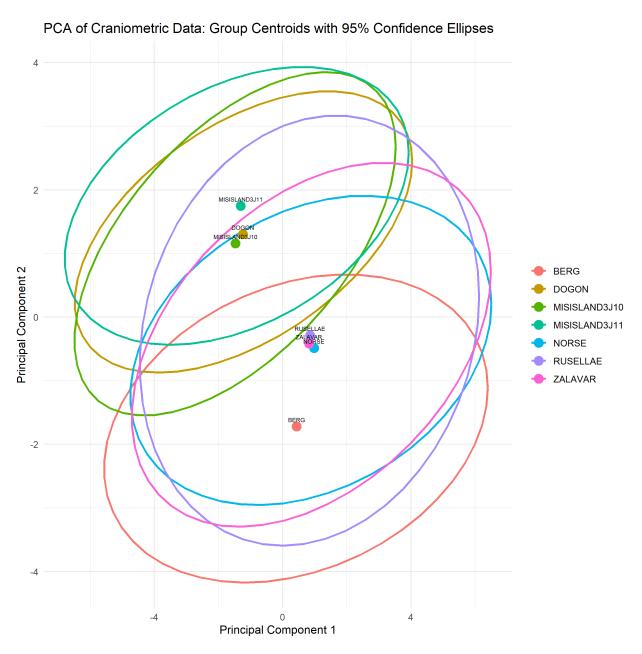


Figure 6.5. Scatterplot of Principal Components 1 and 2 with associated 95% confidence ellipses.

In order to evaluate within-cemetery craniometric variation at *Rusellae*, a Principal Components Analysis was performed using the same 21 craniometric variables used in the MD. The PCA resulted in seven PCs with eigenvalues greater than one (Table 6.9); these seven PCs each represent more variation than a single craniometric variable alone. Together, they explain 78% of the craniofacial variation within the cemetery. A Scree plot of all PCs shows the percentage of variance explained by each PC, with a red dashed line separating those PCs with eigenvalues greater than one from the remaining PCs (Figure 6.6).

Table 6.9. Variable loadings for first seven PCs

Measurement	PC1	PC2	PC3	PC4	PC5	PC6	PC7
GOL	-0.30989	-0.19032	0.166309	0.05782	-0.00026	0.218944	0.059634
BNL	-0.10075	-0.41491	0.241611	-0.16011	0.237165	0.082573	-0.03839
ВВН	-0.19478	-0.17129	0.097424	0.088396	0.470154	-0.31719	0.029472
XCB	-0.23126	0.341286	0.120018	-0.17997	0.085946	-0.14171	0.137811
ZYB	-0.24729	0.214291	0.306868	0.120946	-0.21028	-0.08604	0.215344
AUB	-0.15659	0.247669	0.389715	-0.21223	-0.08353	0.029023	-0.30747
ASB	-0.21717	0.215467	-0.00281	-0.09293	0.382177	0.110742	0.308816
BPL	0.027653	-0.45243	0.041557	-0.16661	-0.18641	-0.04152	0.10423
NPH	-0.30611	-0.01822	-0.14961	0.013714	-0.23966	0.210768	-0.23547
NLH	-0.31013	-0.03473	-0.10931	0.142414	-0.21957	0.092342	-0.2553
NLB	-0.09139	-0.25151	-0.05112	0.229791	-0.34542	-0.55552	0.017364
MDH	-0.21179	-0.0062	0.179922	0.363026	0.112131	0.023016	-0.1302
OBH	-0.09735	0.269546	-0.22442	-0.1193	0.039423	0.016722	-0.51568
OBB	-0.19232	-0.06643	-0.35411	-0.46318	-0.10448	0.120076	0.222219
DKB	-0.19519	-0.13264	-0.20851	-0.01524	0.400852	-0.31681	-0.34125
ZMB	-0.21447	-0.04607	0.23645	-0.27727	-0.07592	-0.15694	-0.07987
EKB	-0.24173	0.049198	-0.25822	-0.33528	-0.1365	-0.36785	0.122389
FRC	-0.24031	-0.03549	-0.38664	0.367566	0.036197	0.167469	0.053638
PAC	-0.2937	0.035017	-0.16196	0.152358	0.069146	0.131685	0.333506
OCC	-0.16202	-0.34578	0.105232	-0.18314	0.012577	0.342968	-0.11537
FOL	-0.25776	0.036735	0.210613	0.139805	-0.2049	-0.04705	0.097779

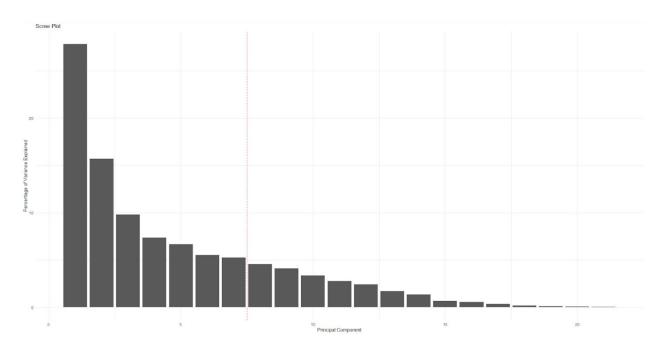


Figure 6.6. Scree Plot of Rusellae Principal Components.

The first two Principal Components explain the majority of the variation within the sample. The craniometric variables with the highest absolute loading on PC1 are maximum cranial length (-0.31), nasal height (-0.31), nasion-prosthion height (-0.31), and the parietal chord (-0.29). Overall, these measurements contribute to the height and length of the cranium in the sagittal plane. The variables that contribute most to PC2 are basion-nasion length (-0.41), basion-prosthion length (-0.45), occipital chord (-0.35), and maximum cranial breadth (0.34); these variables primarily relate to cranial base length and cranial vault breadth. The loadings of the length variables on PC2 are all negative while the loading on the breadth variable is positive; this may represent a general shape component wherein cranial length and breadth are inversely related. Figure 6.7 is a scatterplot showing all 21 craniometric variables and their loadings on PC1 and PC2.

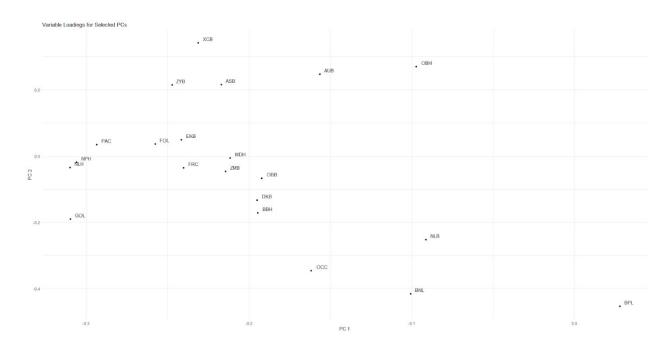


Figure 6.7. Scatterplot of interlandmark distances on PCs 1 and 2.

The remaining PCs explain smaller proportions of overall variance. The variables with the highest positive loading on PC3 are bizygomatic breadth (0.31) and biauricular breadth (0.39), while the variables with the highest negative loading are frontal chord (-0.39) and orbital breadth (-0.35). In general, PC3 may represent a component of facial breadth. PC4 is heavily weighted by orbital breadth (-0.46), with moderate contributions from biorbital breadth (-0.34), bimaxillary breadth (-0.28), mastoid height (0.36), and frontal chord (0.37). PC4 may be capturing the relative dimensions of the eye orbits to the rest of the face. PC5 has the highest contributions from basion-bregma height (0.47), interorbital breadth (0.40), and biasterionic breadth (0.38); this may generally relate to a cranial base shape component. PC6 is weighted heavily by nasal breadth (-0.56), with moderate contributions from basion-bregma height (-0.32), interorbital breadth (-0.32), biorbital breadth (-0.37), and the occipital chord (0.34), representing a relationship between cranial base shape and midfacial structure. Finally, PC7 is primarily driven by orbital height (-0.52), with moderate contributions from biauricular breadth (-0.31),

biasterionic breadth (0.31), interorbital breadth (-0.34), and parietal chord (0.34); this PC appears to represent a shape variable connecting midfacial structure with cranial breadth.

The *Rusellae* sample is plotted with each individual's scores for PC1 and PC2 to visualize craniometric variation throughout the cemetery (Figure 6.8). In general, PC 1 separates males on the left from females on the right, suggesting a size component to PC1.

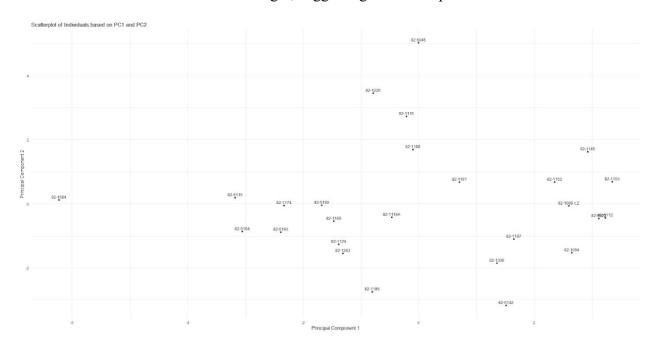


Figure 6.8. Rusellae individuals plotted against PCs 1 and 2.

In order to establish whether the variation present in the cemetery produces statistically distinct clusters of individuals, a hierarchical cluster analysis is performed on the seven retained PCs with eigenvalues greater than 1.0. The resulting dendrogram is shown in Figure 6.9.

Although there is some overlap, the major cluster on the left primarily comprises females, while the major cluster on the right is mostly composed of males.

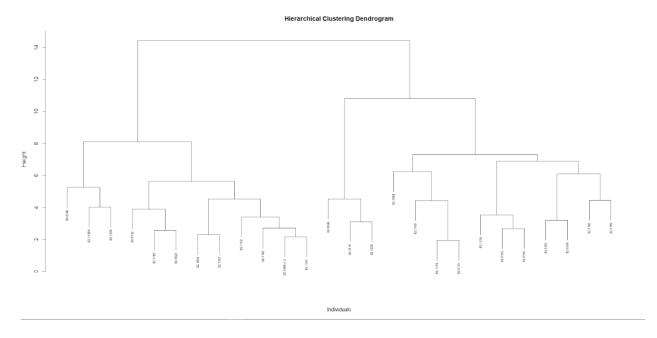


Figure 6.9. Hierarchical clustering dendrogram.

The hierarchical clustering dendrogram shows three general clusters, so the dendrogram is cut at k=3. The *Rusellae* individuals are plotted again based on their PC1 and PC2 scores, but this time color coded by cluster (Figure 6.10).

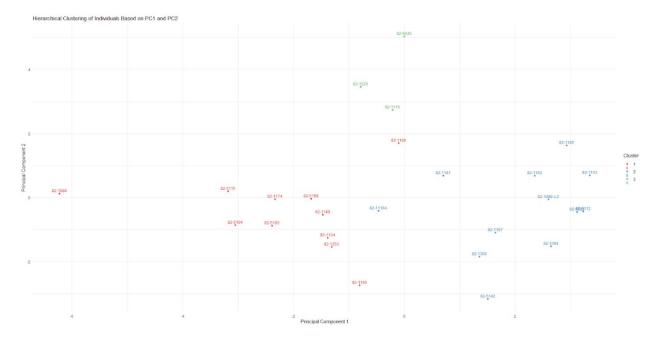


Figure 6.10. Rusellae individuals plotted against PC 1 and 2, color coded by cluster.

## **Research Question 2**

The same subset of crania as in Research Question 1 (n=26) were tested using the linear discriminant analysis (LDA) to explore population affinity throughout the cemetery. The model produced from the LDA using six reference datasets (Berg, Norse, Zalavar, Dogon, Mis Island 3-J-10, and Mis Island 3-J-11) had an overall correct classification rate of 77.2%. The correct classification rates for each reference group are outlined in Table 6.10.

Table 6.10. Correct classification rates for groups used to build LDA model

Group	Correct Classification
Berg	81.7%
Norse	69.4%
Zalavar	67.3%
Dogon	94.9%
Mis Island 3-J-10	75.0%
Mis Island 3-J-11	72.0%

Of the 26 sufficiently complete individuals analyzed, 11 individuals (42.3%) showed closer affinity to one of the African groups (Dogon, Mis Island 3-J-10, and Mis Island 3-J-11), while 15 individuals (57.7%) showed closer affinity to one of the European groups (Berg, Norse, and Zalavar) (Table 6.11).

Table 6.11. Affinity estimates for Rusellae individuals

Skeleton ID	Sex	Phase	Group	<b>Posterior Probability</b>	Continent
82-1084	Male	Unknown	Norse	0.5095	Europe
82-1088 c.2	Female	Unknown	Mis Island 3-J-11	0.8165	Africa
82-1094	Male	Phase II	Mis Island 3-J-11	0.6712	Africa
82-1103	Female	Phase II	Dogon	0.9933	Africa
82-1116	Male	Unknown	Berg	0.9338	Europe
82-1118A	Female	Phase II	Zalavar	0.6541	Europe
82-1124	Male	Phase II	Dogon	0.7760	Africa
82-1152	Male	Phase II	Zalavar	0.5540	Europe
82-1174	Male	Phase II	Zalavar	0.7089	Europe
82-1181	Female	Phase II	Norse	0.5058	Europe
82-1185	Female	Phase II	Norse	0.4219	Europe
82-1186	Male	Phase II	Norse	0.8069	Europe
82-1187	Female	Phase II	Norse	0.4956	Europe
82-1188	Male	Phase II	Norse	0.6938	Europe
82-1189	Male	Phase II	Berg	0.4301	Europe
82-1220	Female	Phase II	Berg	0.9605	Europe
82-1263	Female	Phase II	Dogon	0.3990	Africa
82-1308	Female	Unknown	Dogon	0.9963	Africa
82-5045	Female	Phase I	Berg	0.9964	Europe
82-5104	Male	Phase II	Norse	0.8689	Europe
82-5112	Female	Phase II	Mis Island 3-J-10	0.6058	Africa
82-5115	Male	Phase II	Zalavar	0.3817	Europe
82-5142	Female	Phase II	Mis Island 3-J-11	0.9479	Africa
82-5193	Male	Phase II	Mis Island 3-J-10	0.7878	Africa
82-5199	Male	Phase II	Dogon	0.9984	Africa
82-5523	Female	Phase I	Dogon	0.7223	Africa

The values for the unknowns in the first two linear discriminant functions are plotted to visualize their relationship with the centroids of the six reference groups (Figure 6.11). The first linear discriminant axis separates the European and African groups. The second axis roughly separates the Berg and Dogon samples from the Norse, Zalavar, and Mis Island reference groups.

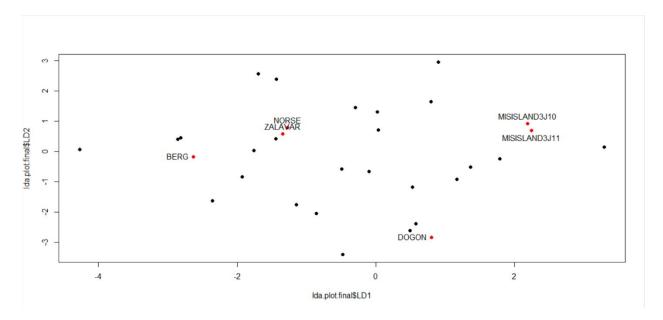


Figure 6.11. *Rusellae* unknowns (black dots) and reference group centroids (red dots) plotted against the first two linear discriminant functions.

### **Research Ouestion 3**

In this section, I will refer to individuals of African affinity and individuals of European affinity for the sake of brevity. Please note, this shorthand should be taken to mean individuals showing closer statistical affinity to the African or European reference samples used in the craniometric analysis.

### Cribra orbitalia

Of the total sample of 160 adults, 20 (12.5%) had an estimated affinity and at least one scorable eye orbit in order to evaluate presence or absence of *cribra orbitalia*. Of these, eight (40%) show greater African affinity and 12 (60%) show greater European affinity. Of the

individuals with African affinity, four are male (50%) and four are female (50%). Seven of the individuals with European affinity are male (58.3%), while five (41.7%) are female (Table 6.12).

Table 6.12. Individuals with data for affinity and cribra orbitalia

	Male	Female	Total
African Affinity	4	4	8
<b>European Affinity</b>	7	5	12
Total	11	9	20

In individuals with a closer affinity to the African reference samples, *cribra orbitalia* is present in zero out of four males (0%) and one of four females (25%) (Table 6.13). A Fisher's exact test showed no significant differences in the frequency of *cribra orbitalia* between males and females with African affinity (p=1.00).

Table 6.13. Counts for *cribra orbitalia* by sex and African affinity

	Present	Absent	Total
African Affinity Male	0	4	4
African Affinity Female	1	3	4
Total	1	7	8

In individuals with European affinity, *cribra orbitalia* is present in one out of seven males (14.3%) and zero out of 5 females (0%) (Table 6.14). A Fisher's exact test showed no significant difference in the frequency of *cribra orbitalia* between males and females with European affinity (p=1.00). Consequently, males and females were pooled for the overall analysis comparing frequency of *cribra orbitalia* between individuals of African and European affinity.

Table 6.14. Counts for *cribra orbitalia* by sex and European affinity

	Present	Absent	Total
<b>European Affinity Male</b>	1	6	7
<b>European Affinity Female</b>	0	5	5
Total	1	11	12

In the African affinity pooled sexes sample, *cribra orbitalia* is present in one out of eight individuals (12.5%). In the European affinity pooled sexes sample, *cribra orbitalia* is present in one out of 11 individuals (9.1%) (Table 6.15). A Fisher's exact test showed no significant differences in the frequency of *cribra orbitalia* between individuals with African and European affinity (p=1.00).

Table 6.15. Counts for *cribra orbitalia* by affinity, pooled sexes

	Present	Absent	Total
African Affinity	1	7	8
<b>European Affinity</b>	1	11	12

## **Porotic Hyperostosis**

Of the total sample of 160 adults, 20 (12.5%) had an estimated affinity and at least one parietal bone present in order to evaluate presence or absence of porotic hyperostosis. Of these, eight (40%) show greater African affinity and 12 (60%) show greater European affinity. Of the individuals with African affinity, four are male (50%) and four are female (50%). Seven of the individuals with European affinity are male (58.3%), while five (41.7%) are female (Table 6.16).

Table 6.16 Individuals with data for affinity and porotic hyperostosis

	Male	Female	Total
African Affinity	4	4	8
<b>European Affinity</b>	7	5	12
Total	11	9	20

In individuals with African affinity, porotic hyperostosis is present in two out of four males (50%) and one of four females (25%) (Table 6.17). A Fisher's exact test showed no significant differences in the frequency of porotic hyperostosis between males and females with African affinity (p=1.00).

Table 6.17. Counts for porotic hyperostosis by sex and African affinity

	Present	Absent	Total
African Affinity Male	2	2	4
<b>African Affinity Female</b>	1	3	4
Total	3	5	8

In individuals with European affinity, porotic hyperostosis is present in three out of seven males (42.9%) and zero out of 5 females (0%) (Table 6.18). A Fisher's exact test showed no significant difference in the frequency of porotic hyperostosis between males and females with European affinity (p=0.2045). Consequently, males and females were pooled for the overall analysis comparing frequency of porotic hyperostosis between individuals of African and European affinity.

Table 6.18. Counts for porotic hyperostosis by sex and European affinity

	Present	Absent	Total
<b>European Affinity Male</b>	3	4	7
<b>European Affinity Female</b>	0	5	5
Total	3	9	12

In the African affinity pooled sexes sample, porotic hyperostosis is present in three out of eight individuals (37.5%). In the European affinity pooled sexes sample, porotic hyperostosis is present in three out of 12 individuals (25%) (Table 6.19). A Fisher's exact test showed no significant differences in the frequency of porotic hyperostosis between individuals with African and European affinity (p=0.6424).

Table 6.19. Counts for porotic hyperostosis by affinity, pooled sexes

	Present	Absent	Total
African Affinity	3	5	8
<b>European Affinity</b>	3	9	12

#### **Periosteal Reaction**

Of the total sample of 160 adults, 16 (10%) had an estimated affinity and at least one tibia or femur present in order to evaluate presence or absence of periosteal reaction. Of these, six (37.5%) show greater African affinity and 10 (62.5%) show greater European affinity. Of the individuals with African affinity, three are male (50%) and three are female (50%). Six of the individuals with European affinity are male (60%), while four (40%) are female (Table 6.20).

Table 6.20. Individuals with data for affinity and periosteal reaction

	Male	Female	Total
African Affinity	3	3	6
<b>European Affinity</b>	6	4	10
Total	9	7	16

In individuals with African affinity, periosteal reaction is present in three out of three males (100%) and three out of three females (100%) (Table 6.21). A Fisher's exact test showed no significant differences in the frequency of periosteal reaction between males and females with African affinity (p=1.00).

Table 6.21. Counts for periosteal reaction by sex and African affinity

	Present	Absent	Total
African Affinity Male	3	0	3
African Affinity Female	3	0	3
Total	6	0	6

In individuals with European affinity, periosteal reaction is present in all six males (100%) and all four females (100%) (Table 6.22). A Fisher's exact test showed no significant difference in the frequency of periosteal reaction between males and females with European affinity (p=1.00). Consequently, males and females were pooled for the overall analysis comparing frequency of periosteal reaction between individuals of African and European affinity.

Table 6.22. Counts for periosteal reaction by sex and European affinity

	Present	Absent	Total
<b>European Affinity Male</b>	6	0	6
<b>European Affinity Female</b>	4	0	4
Total	10	0	10

In the African affinity pooled sexes sample, periosteal reaction is present in all six individuals (100%). In the European affinity pooled sexes sample, periosteal reaction is present in all ten individuals (100%) (Table 6.23). A Fisher's exact test showed no significant differences in the frequency of periosteal reaction between individuals with African and European affinity (p=1.00).

Table 6.23. Counts for periosteal reaction by affinity, pooled sexes

	Present	Absent	Total
African Affinity	6	0	6
<b>European Affinity</b>	10	0	10

# **Linear Enamel Hypoplasia: Maxillary Canine**

Of the total sample of 160 adults, 17 (10.6%) had an estimated affinity and at least one scorable maxillary canine in order to evaluate presence or absence of linear enamel hypoplasia. Of these, eight (47.1%) show greater African affinity and nine (52.1%) show greater European affinity. Of the individuals with African affinity, four are male (50%) and four are female (50%). Five of the individuals with European affinity are male (55.6%), while four (44.4%) are female (Table 6.24).

Table 6.24. Individuals with data for affinity and maxillary canine LEH

	Male	Female	Total
African Affinity	4	4	8
<b>European Affinity</b>	5	4	9
Total	9	8	17

In individuals with African affinity, LEH of the maxillary canine is present in three out of four males (75%) and three of four females (75%) (Table 6.25). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary canine between males and females with African affinity (p=1.00).

Table 6.25. Counts for maxillary canine LEH by sex and African affinity

	Present	Absent	Total
African Affinity Male	3	1	4
<b>African Affinity Female</b>	3	1	4
Total	6	2	8

In individuals with European affinity, LEH of the maxillary canine is present in four out of five males (80%) and all four females (100%) (Table 6.26). A Fisher's exact test showed no significant difference in the frequency of LEH of the maxillary canine between males and females with European affinity (p=1.00). Consequently, males and females were pooled for the overall analysis comparing frequency of LEH of the maxillary canine between individuals of African and European affinity.

Table 6.26. Counts for maxillary canine LEH by sex and European affinity

	Present	Absent	Total
European Affinity Male	4	1	5
<b>European Affinity Female</b>	4	0	4
Total	8	1	9

In the African affinity pooled sexes sample, LEH of the maxillary canine is present in six out of eight individuals (75%). In the European affinity pooled sexes sample, LEH of the maxillary canine is present in eight out of nine individuals (88.9%) (Table 6.27). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary canine between individuals with African and European affinity (p=0.5765).

Table 6.27. Counts for maxillary canine LEH by affinity, pooled sexes

	Present	Absent	Total
African Affinity	6	2	8
<b>European Affinity</b>	8	1	9

## Linear Enamel Hypoplasia: Maxillary Lateral Incisor

Of the total sample of 160 adults, 14 (8.8%) had an estimated affinity and at least one scorable maxillary lateral incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, seven (50%) show greater African affinity and seven (50%) show greater European affinity. Of the individuals with African affinity, three are male (42.9%) and four are female (57.1%). Four of the individuals with European affinity are male (57.1%), while three (42.9%) are female (Table 6.28).

Table 6.28. Individuals with data for affinity and maxillary lateral incisor LEH

	Male	Female	Total
African Affinity	3	4	7
<b>European Affinity</b>	4	3	7
Total	7	7	14

In individuals with African affinity, LEH of the maxillary lateral incisor is present in two out of three males (66.7%) and one of four females (25%) (Table 6.29). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary lateral incisor between males and females with African affinity (p=0.4857).

Table 6.29. Counts for maxillary lateral incisor LEH by sex and African affinity

	Present	Absent	Total
African Affinity Male	2	1	3
<b>African Affinity Female</b>	1	3	4
Total	3	4	7

In individuals with European affinity, LEH of the maxillary lateral incisor is present in two out of four males (50%) and all three females (100%) (Table 6.30). A Fisher's exact test

showed no significant difference in the frequency of LEH of the maxillary lateral incisor between males and females with European affinity (p=0.4286). Consequently, males and females were pooled for the overall analysis comparing frequency of LEH of the maxillary lateral incisor between individuals of African and European affinity.

Table 6.30. Counts for maxillary lateral incisor LEH by sex and European affinity

	Present	Absent	Total
European Affinity Male	2	2	4
<b>European Affinity Female</b>	3	0	3
Total	5	2	7

In the African affinity pooled sexes sample, LEH of the maxillary lateral incisor is present in three out of seven individuals (42.9%). In the European affinity pooled sexes sample, LEH of the maxillary lateral incisor is present in five out of seven individuals (72.4%) (Table 6.31). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary lateral incisor between individuals with African and European affinity (p=0.5921).

Table 6.31. Counts for maxillary lateral incisor LEH by affinity, pooled sexes

	Present	Absent	Total
African Affinity	3	4	7
<b>European Affinity</b>	5	2	7

#### **Linear Enamel Hypoplasia: Maxillary Central Incisor**

Of the total sample of 160 adults, 15 (9.4%) had an estimated affinity and at least one scorable maxillary central incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, seven (46.7%) show greater African affinity and eight (53.3%) show greater European affinity. Of the individuals with African affinity, four are male (57.1%) and three are female (42.9%). Five of the individuals with European affinity are male (62.5%), while three (37.5%) are female (Table 6.32).

Table 6.32. Individuals with data for affinity and maxillary central incisor LEH

	Male	Female	Total
African Affinity	4	3	7
<b>European Affinity</b>	5	3	8
Total	9	6	15

In individuals with African affinity, LEH of the maxillary central incisor is present in three out of four males (75%) and all three females (100%) (Table 6.33). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary central incisor between males and females with African affinity (p=1.00).

Table 6.33. Counts for maxillary central incisor LEH by sex and African affinity

	Present	Absent	Total
African Affinity Male	3	1	4
<b>African Affinity Female</b>	3	0	3
Total	6	1	7

In individuals with European affinity, LEH of the maxillary central incisor is present in one out of five males (20%) and one out of three females (33.3%) (Table 6.34). A Fisher's exact test showed no significant difference in the frequency of LEH of the maxillary central incisor between males and females with European affinity (p=1.00). Consequently, males and females were pooled for the overall analysis comparing frequency of LEH of the maxillary central incisor between individuals of African and European affinity.

Table 6.34. Counts for maxillary central incisor LEH by sex and European affinity

	Present	Absent	Total
European Affinity Male	1	4	5
<b>European Affinity Female</b>	1	2	3
Total	2	6	8

In the African affinity pooled sexes sample, LEH of the maxillary central incisor is present in six out of seven individuals (85.7%). In the European affinity pooled sexes sample, LEH of the maxillary central incisor is present in two out of eight individuals (25%) (Table

6.35). A Fisher's exact test showed a significant difference in the frequency of LEH of the maxillary central incisor between individuals with African and European affinity (p=0.04056,  $\alpha$ =0.05), with a greater frequency in LEH of the maxillary central incisor in individuals with African affinity.

Table 6.35. Counts for maxillary central incisor LEH by affinity, pooled sexes

	Present	Absent	Total
African Affinity	6	1	7
<b>European Affinity</b>	2	6	8

# Linear Enamel Hypoplasia: Mandibular Canine

Of the total sample of 160 adults, 17 (10.6%) had an estimated affinity and at least one scorable mandibular canine in order to evaluate presence or absence of linear enamel hypoplasia. Of these, seven (41.2%) show greater African affinity and ten (58.8%) show greater European affinity. Of the individuals with African affinity, three are male (42.9%) and four are female (57.1%). Six of the individuals with European affinity are male (60%), while four (40%) are female (Table 6.36).

Table 6.36. Individuals with data for affinity and mandibular canine LEH

	Male	Female	Total
African Affinity	3	4	7
<b>European Affinity</b>	6	4	10
Total	9	8	17

In individuals with African affinity, LEH of the mandibular canine is present in two out of three males (66.7%) and all four females (100%) (Table 6.37). A Fisher's exact test showed no significant differences in the frequency of LEH of the mandibular canine between males and females with African affinity (p=0.4286).

Table 6.37. Counts for mandibular canine LEH by sex and African affinity

	Present	Absent	Total
African Affinity Male	2	1	3
<b>African Affinity Female</b>	4	0	4
Total	6	1	7

In individuals with European affinity, LEH of the mandibular canine is present in four out of six males (66.7%) and all four females (100%) (Table 6.38). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular canine between males and females with European affinity (p=0.4667). Consequently, males and females were pooled for the overall analysis comparing frequency of LEH of the mandibular canine between individuals of African and European affinity.

Table 6.38. Counts for mandibular canine LEH by sex and European affinity

	Present	Absent	Total
<b>European Affinity Male</b>	4	2	6
<b>European Affinity Female</b>	4	0	4
Total	8	2	10

In the African affinity pooled sexes sample, LEH of the mandibular canine is present in six out of seven individuals (85.7%). In the European affinity pooled sexes sample, LEH of the mandibular canine is present in eight out of ten individuals (80%) (Table 6.39). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular canine between individuals with African and European affinity (p=1.00).

Table 6.39. Counts for mandibular canine LEH by affinity, pooled sexes

	Present	Absent	Total
African Affinity	6	1	7
<b>European Affinity</b>	8	2	10

## Linear Enamel Hypoplasia: Mandibular Lateral Incisor

Of the total sample of 160 adults, 15 (9.4%) had an estimated affinity and at least one scorable mandibular lateral incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, eight (53.3%) show greater African affinity and seven (46.7%) show greater European affinity. Of the individuals with African affinity, four are male (50%) and four are female (50%). Three of the individuals with European affinity are male (42.9%), while four (57.1%) are female (Table 6.40).

Table 6.40. Individuals with data for mandibular lateral incisor LEH

	Male	Female	Total
African Affinity	4	4	8
<b>European Affinity</b>	3	4	7
Total	7	8	15

In individuals with African affinity, LEH of the mandibular lateral incisor is present in two out of four males (50%) and one out of the four females (25%) (Table 6.41). A Fisher's exact test showed no significant differences in the frequency of LEH of the mandibular lateral incisor between males and females with African affinity (p=1.00).

Table 6.41. Counts for mandibular lateral incisor LEH by sex and African affinity

	Present	Absent	Total
African Affinity Male	2	2	4
<b>African Affinity Female</b>	1	3	4
Total	3	5	8

In individuals with European affinity, LEH of the mandibular lateral incisor is present in one out of three males (33.3%) and one out of four females (25%) (Table 6.42). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular lateral incisor between males and females with European affinity (p=1.00). Consequently, males and females

were pooled for the overall analysis comparing frequency of LEH of the mandibular lateral incisor between individuals of African and European affinity.

Table 6.42. Counts for mandibular lateral incisor LEH by sex and European affinity

	Present	Absent	Total
<b>European Affinity Male</b>	1	2	3
<b>European Affinity Female</b>	1	3	4
Total	2	5	7

In the African affinity pooled sexes sample, LEH of the mandibular lateral incisor is present in three out of eight individuals (37.5%). In the European affinity pooled sexes sample, LEH of the mandibular lateral incisor is present in two out of seven individuals (28.8%) (Table 6.43). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular lateral incisor between individuals with African and European affinity (p=1.00).

Table 6.43. Counts for mandibular lateral incisor LEH by affinity, pooled sexes

	Present	Absent	Total
African Affinity	3	5	8
<b>European Affinity</b>	2	5	7

# Linear Enamel Hypoplasia: Mandibular Central Incisor

Of the total sample of 160 adults, 12 (7.5%) had an estimated affinity and at least one scorable mandibular central incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, seven (58.3%) show greater African affinity and five (41.7%) show greater European affinity. Of the individuals with African affinity, three are male (42.9%) and four are female (57.1%). Three of the individuals with European affinity are male (60%), while two (40%) are female (Table 6.44).

Table 6.44. Individuals with data for affinity and mandibular central incisor LEH

	Male	Female	Total
African Affinity	3	4	7
<b>European Affinity</b>	3	2	5
Total	6	6	12

In individuals with African affinity, LEH of the mandibular central incisor is present in one out of three males (33.3%) and one out of the four females (25%) (Table 6.45). A Fisher's exact test showed no significant differences in the frequency of LEH of the mandibular central incisor between males and females with African affinity (p=1.00).

Table 6.45. Counts for mandibular central incisor LEH by sex and African affinity

	Present	Absent	Total
African Affinity Male	1	2	3
<b>African Affinity Female</b>	1	3	4
Total	2	5	7

In individuals with European affinity, LEH of the mandibular central incisor is present in one out of three males (33.3%) and one out of two females (50%) (Table 6.46). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular central incisor between males and females with European affinity (p=1.00). Consequently, males and females were pooled for the overall analysis comparing frequency of LEH of the mandibular central incisor between individuals of African and European affinity.

Table 6.46. Counts for mandibular central incisor LEH by sex and European affinity

	Present	Absent	Total
<b>European Affinity Male</b>	1	2	3
<b>European Affinity Female</b>	1	1	2
Total	2	3	5

In the African affinity pooled sexes sample, LEH of the mandibular central incisor is present in two out of seven individuals (28.6%). In the European affinity pooled sexes sample, LEH of the mandibular central incisor is present in two out of five individuals (40%) (Table

6.47). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular central incisor between individuals with African and European affinity (p=1.00).

Table 6.47. Counts for mandibular central incisor LEH by affinity, pooled sexes

	Present	Absent	Total
African Affinity	2	5	7
<b>European Affinity</b>	2	3	5

#### **Research Question 4**

The results for Research Question 4 comprise two parts. In the first part, differences in the craniometric variables will be compared between Phase I and Phase II. Then, the frequency of each skeletal stress variable (*cribra orbitalia*, porotic hyperostosis, periostitis, linear enamel hypoplasia) will be compared between Phases I and II.

#### **Craniometric Variation**

In order to examine craniofacial variation over time at *Rusellae*, individuals from Phase I are compared with individuals from Phase II with respect to cranial measurements using MANOVA and ANOVA. The MANOVA testing for craniometric differences between Phase I and Phase II did not show significant differences ( $\alpha$ =0.05, p=0.8827). The one-way ANOVAs comparing measurements for each variable by phase also showed no significant differences ( $\alpha$ =0.05) between phases for any of the 12 variables included in the analysis.

#### Cribra orbitalia

Of the total sample of 160 adults, 51 (31.9%) had data for Phase and at least one scorable eye orbit in order to evaluate presence or absence of *cribra orbitalia*. Of these, six (11.8%) date to Phase I and 45 (88.2%) date to Phase II. Of the individuals from Phase I, three are male (50%) and three are female (50%). Twenty-six of the 45 Phase II individuals are male (57.8%), while 19 (42.2%) are female (Table 6.48).

Table 6.48. Individuals with data for Phase and cribra orbitalia

	Male	Female	Total
Phase I	3	3	6
Phase II	26	19	45
Total	29	22	51

In Phase I, *cribra orbitalia* is present in one out of three males (33.3%) and zero of three females (0%) (Table 6.49). A Fisher's exact test showed no significant differences in the frequency of *cribra orbitalia* between males and females in Phase I (p=1.00).

Table 6.49. Counts for cribra orbitalia by sex in Phase I

	Present	Absent	Total
Phase I Male	1	2	3
Phase I Female	0	3	3
Total	1	5	6

In Phase II, *cribra orbitalia* is present in six out of 26 males (23.1%) and one out of 19 females (5.3%) (Table 6.50). A Fisher's exact test showed no significant difference in the frequency of *cribra orbitalia* between males and females in Phase II (p=0.2108). Consequently, males and females were pooled for the overall analysis comparing frequency of *cribra orbitalia* between Phases I and II.

Table 6.50. Counts for cribra orbitalia by sex in Phase II

	Present	Absent	Total
Phase II Male	6	20	26
Phase II Female	1	18	19
Total	7	38	45

In the Phase I pooled sexes sample, *cribra orbitalia* is present in one out of six individuals (16.7%). In the Phase II pooled sexes sample, *cribra orbitalia* is present in seven out of 45 individuals (15.6%) (Table 6.51). A Fisher's exact test showed no significant differences in the frequency of *cribra orbitalia* between Phase I and Phase II (p=1.00).

Table 6.51. Counts for cribra orbitalia by Phase, pooled sexes

	Present	Absent	Total
Phase I	1	5	6
Phase II	7	38	45

## **Porotic Hyperostosis**

Of the total sample of 160 adults, 79 (49.4%) had data for Phase and at least one parietal bone present in order to evaluate presence or absence of porotic hyperostosis. Of these, 12 (15.2%) date to Phase I and 67 (84.8%) date to Phase II. Of the individuals from Phase I, five are male (41.7%), six are female (50.0%), and one is of indeterminate sex (8.3%). Thirty-six of the 67 Phase II individuals are male (53.7%), 30 (44.8%) are female, and one is of indeterminate sex (1.5%) (Table 6.52).

Table 6.52. Individuals with data for Phase and porotic hyperostosis

	Male	Female	Indeterminate	Total
Phase I	5	6	1	12
Phase II	36	30	1	67
Total	41	36	2	79

For the by-sex analysis, the individuals of indeterminate sex are left out. In Phase I, porotic hyperostosis is absent in all five males and all six females. Therefore, no statistical analyses are employed to evaluate significant differences between the sexes in Phase I. In Phase II, porotic hyperostosis is present in 13 out of 36 males (36.1%) and six out of 30 females (20%) (Table 6.53). A Fisher's exact test showed no significant difference in the frequency of *cribra orbitalia* between males and females in Phase II (p=0.1803). Consequently, males and females were pooled for the overall analysis comparing frequency of *cribra orbitalia* between Phases I and II.

Table 6.53. Counts for *cribra orbitalia* by sex in Phase II

	Present	Absent	Total
Phase II Male	13	23	36
Phase II Female	6	24	30
Total	19	47	66

In the Phase I pooled sexes sample, porotic hyperostosis is present in none of the 12 individuals (0%). In the Phase II pooled sexes sample, porotic hyperostosis is present in 19 out of 67 individuals (28.4%) (Table 6.54). A Fisher's exact test showed no significant differences in the frequency of porotic hyperostosis between Phase I and Phase II (p=0.06001). However, due to the complete absence of porotic hyperostosis in Phase I, these results should be interpreted cautiously.

Table 6.54. Counts for porotic hyperostosis by Phase, pooled sexes

	Present	Absent	Total
Phase I	0	12	12
Phase II	19	48	67

#### **Periosteal Reaction**

Of the total sample of 160 adults, 93 (58.1%) had data for Phase and at least one tibia or femur present in order to evaluate presence or absence of periosteal reaction. Of these, 15 (16.1%) date to Phase I and 78 (83.9%) date to Phase II. Of the individuals from Phase I, six are male (40.0%), seven are female (46.7%), and two are of indeterminate sex (13.3%). Forty-five of the 78 Phase II individuals are male (57.7%), 31 (39.7%) are female, and two are of indeterminate sex (2.6%) (Table 6.55).

Table 6.55. Individuals with data for Phase and periosteal reaction

	Male	Female	Indeterminate	Total
Phase I	6	7	2	15
Phase II	45	31	2	78
Total	51	38	4	93

For the by-sex analysis, the individuals of indeterminate sex are left out. In Phase I, periosteal reaction is present in five out of six males (83.3%) and six of seven females (85.7%) (Table 6.56). A Fisher's exact test showed no significant differences in the frequency of periosteal reaction between males and females in Phase I (p=1.00).

Table 6.56. Counts for periosteal reaction by sex in Phase I

	Present	Absent	Total
Phase I Male	5	1	6
Phase I Female	6	1	7
Total	11	2	13

In Phase II, periosteal reaction is present in 41 out of 45 males (91.1%) and 23 out of 31 females (74.2%) (Table 6.57). A Fisher's exact test showed no significant difference in the frequency of periosteal reaction between males and females in Phase II (p=0.05972). Consequently, males and females were pooled for the overall analysis comparing frequency of *cribra orbitalia* between Phases I and II.

Table 6.57. Counts for periosteal reaction by sex in Phase II

	Present	Absent	Total
Phase II Male	41	4	45
Phase II Female	23	8	31
Total	64	12	76

In the Phase I pooled sexes sample, periosteal reaction is present in 12 of the 15 individuals (80%). In the Phase II pooled sexes sample, periosteal reaction is present in 66 out of 78 individuals (84.6%) (Table 6.58). A Fisher's exact test showed no significant differences in the frequency of periosteal reaction between Phase I and Phase II (p=0.7036).

Table 6.58. Counts for periosteal reaction by Phase, pooled sexes

	Present	Absent	Total
Phase I	12	3	15
Phase II	66	12	78

## Linear Enamel Hypoplasia: Maxillary Canines

Of the total sample of 160 adults, 63 (39.4%) had data for Phase and at least one scorable maxillary canine in order to evaluate presence or absence of linear enamel hypoplasia. Of these, nine (14.3%) date to Phase I and 54 (85.7%) date to Phase II. Of the individuals from Phase I, six are male (66.7%) and three are female (33.3%). Twenty-seven of the 54 Phase II individuals are male (50%), while 27 (50%) are female (Table 6.59).

Table 6.59. Individuals with data for Phase and maxillary canine LEH

	Male	Female	Total
Phase I	6	3	9
Phase II	27	27	54
Total	33	30	63

In Phase I, linear enamel hypoplasia of the maxillary canine is present in five out of six males (83.3%) and two of three females (66.6%) (Table 6.60). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary canine between males and females in Phase I (p=1.00).

Table 6.60. Counts for maxillary canine LEH by sex in Phase I

	Present	Absent	Total
Phase I Male	5	1	6
Phase I Female	2	1	3
Total	7	2	9

In Phase II, linear enamel hypoplasia of the maxillary canine is present in 21 out of 27 males (77.8%) and 15 out of 27 females (55.6%) (Table 6.61). A Fisher's exact test showed no significant difference in the frequency of LEH of the maxillary canine between males and females in Phase II (p=0.148). Consequently, males and females were pooled for the overall analysis comparing frequency of LEH of the maxillary canine between Phases I and II.

Table 6.61. Counts for maxillary canine LEH by sex in Phase II

	Present	Absent	Total
Phase II Male	21	6	27
Phase II Female	15	12	27
Total	36	18	54

In the Phase I pooled sexes sample, LEH of the maxillary canine is present in seven out of nine individuals (77.8%). In the Phase II pooled sexes sample, LEH of the maxillary canine is present in 36 out of 54 individuals (66.7%) (Table 6.62). A Fisher's exact test showed no significant differences in the frequency of maxillary canine LEH between Phase I and Phase II (p=0.7064).

Table 6.62. Counts for maxillary canine LEH by Phase, pooled sexes

	Present	Absent	Total
Phase I	7	2	9
Phase II	36	18	54

# Linear Enamel Hypoplasia: Maxillary Lateral Incisors

Of the total sample of 160 adults, 58 (36.3%) had data for Phase and at least one scorable maxillary lateral incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, seven (12.1%) date to Phase I and 51 (87.9%) date to Phase II. Of the individuals from Phase I, six are male (85.7%) and one is female (14.3%). Twenty-five of the 51 Phase II individuals are male (49%), while 24 (47.1%) are female and two are of indeterminate sex (3.9%) (Table 6.63).

Table 6.63. Individuals with data for Phase and maxillary lateral incisor LEH

	Male	Female	Indeterminate	Total
Phase I	6	1	0	7
Phase II	25	24	2	51
Total	31	25	2	58

In Phase I, linear enamel hypoplasia of the maxillary lateral incisor is present in four out of six males (66.7%) and absent in the female (Table 6.64). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary lateral incisor between males and females in Phase I (p=0.4286).

Table 6.64. Counts for maxillary lateral incisor LEH by sex in Phase I

	Present	Absent	Total
Phase I Male	4	2	6
Phase I Female	0	1	1
Total	4	3	7

For the sex-specific analysis, individuals of indeterminate sex are left out. In Phase II, linear enamel hypoplasia of the maxillary lateral incisor is present in six out of 25 males (24%) and eight out of 24 females (33.3%) (Table 6.65). A Fisher's exact test showed no significant difference in the frequency of LEH of the maxillary lateral incisor between males and females in Phase II (p=0.538). Consequently, males, females, and individuals of indeterminate sex were pooled for the overall analysis comparing frequency of LEH of the maxillary lateral incisor between Phases I and II.

Table 6.65. Counts for maxillary lateral incisor LEH by sex in Phase II

	Present	Absent	Total
Phase II Male	6	19	25
Phase II Female	8	16	24
Total	14	35	49

In the Phase I pooled sexes sample, LEH of the maxillary lateral incisor is present in four out of seven individuals (57.1%). In the Phase II pooled sexes sample, LEH of the maxillary lateral incisor is present in 14 out of 51 individuals (27.5%) (Table 6.66). A Fisher's exact test showed no significant differences in the frequency of maxillary lateral incisor LEH between Phase I and Phase II (p=0.1874).

Table 6.66. Counts for maxillary lateral incisor LEH by Phase, pooled sexes

	Present	Absent	Total
Phase I	4	3	7
Phase II	14	37	51

## **Linear Enamel Hypoplasia: Maxillary Central Incisors**

Of the total sample of 160 adults, 57 (35.6%) had data for Phase and at least one scorable maxillary central incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, eight (14%) date to Phase I and 49 (86%) date to Phase II. Of the individuals from Phase I, five are male (62.5%) and three are female (37.5%). Twenty-four of the 49 Phase II individuals are male (49%), while 24 (49%) are female and one is of indeterminate sex (2%) (Table 6.67).

Table 6.67. Individuals with data for Phase and maxillary central incisor LEH

	Male	Female	Indeterminate	Total
Phase I	5	3	0	8
Phase II	24	24	1	49
Total	29	27	1	57

In Phase I, linear enamel hypoplasia of the maxillary central incisor is present in one out of five males (20%) and one of three females (33.3%) (Table 6.68). A Fisher's exact test showed no significant differences in the frequency of LEH of the maxillary central incisor between males and females in Phase I (p=1.00).

Table 6.68. Counts for maxillary central incisor LEH by sex in Phase I

	Present	Absent	Total
Phase I Male	1	4	5
Phase I Female	1	2	3
Total	2	6	8

For the sex-specific analysis, individuals of indeterminate sex are left out. In Phase II, linear enamel hypoplasia of the maxillary central incisor is present in nine out of 24 males (37.5%) and 12 out of 24 females (50%) (Table 6.69). A Fisher's exact test showed no

significant difference in the frequency of LEH of the maxillary central incisor between males and females in Phase II (p=0.5612). Consequently, males, females, and individuals of indeterminate sex were pooled for the overall analysis comparing frequency of LEH of the maxillary central incisor between Phases I and II.

Table 6.69. Counts for maxillary central incisor LEH by sex in Phase II

	Present	Absent	Total
Phase II Male	9	15	24
Phase II Female	12	12	24
Total	21	27	48

In the Phase I pooled sexes sample, LEH of the maxillary central incisor is present in two out of eight individuals (25%). In the Phase II pooled sexes sample, LEH of the maxillary central incisor is present in 21 out of 49 individuals (42.9%) (Table 6.70). A Fisher's exact test showed no significant differences in the frequency of maxillary central incisor LEH between Phase I and Phase II (p=0.4533).

Table 6.70. Counts for maxillary central incisor LEH by Phase, pooled sexes

	Present	Absent	Total
Phase I	2	6	8
Phase II	21	28	49

# **Linear Enamel Hypoplasia: Mandibular Canine**

Of the total sample of 160 adults, 72 (45%) had data for Phase and at least one scorable mandibular canine in order to evaluate presence or absence of linear enamel hypoplasia. Of these, ten (13.9%) date to Phase I and 62 (86.1%) date to Phase II. Of the individuals from Phase I, six are male (60%) and four are female (40%). Thirty-three of the 62 Phase II individuals are male (53.2%), while 28 (45.2%) are female and one is of indeterminate sex (1.6%) (Table 6.71).

Table 6.71. Individuals with data for Phase and mandibular canine LEH

	Male	Female	Indeterminate	Total
Phase I	6	4	0	10
Phase II	33	28	1	62
Total	39	32	1	72

In Phase I, linear enamel hypoplasia of the mandibular canine is present in six out of six males (100%) and two out of four females (50%) (Table 6.72). A Fisher's exact test showed no significant differences in the frequency of LEH of the mandibular canine between males and females in Phase I (p=0.1333).

Table 6.72. Counts for mandibular canine LEH by sex in Phase I

	Present	Absent	Total
Phase I Male	6	0	6
Phase I Female	2	2	4
Total	8	2	10

For the sex-specific analysis, individuals of indeterminate sex are left out. In Phase II, linear enamel hypoplasia of the mandibular canine is present in 23 out of 33 males (69.7%) and 22 out of 28 females (78.6%) (Table 6.73). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular canine between males and females in Phase II (p=0.562). Consequently, males, females, and individuals of indeterminate sex were pooled for the overall analysis comparing frequency of LEH of the maxillary central incisor between Phases I and II.

Table 6.73. Counts for mandibular canine LEH by sex in Phase II

	Present	Absent	Total
Phase II Male	23	10	33
Phase II Female	22	6	28
Total	45	16	61

In the Phase I pooled sexes sample, LEH of the mandibular canine is present in eight out of 10 individuals (80%). In the Phase II pooled sexes sample, LEH of the mandibular canine is

present in 46 out of 62 individuals (74.2%) (Table 6.74). A Fisher's exact test showed no significant differences in the frequency of mandibular canine LEH between Phase I and Phase II (p=1.00).

Table 6.74. Counts for mandibular canine LEH by Phase, pooled sexes

	Present	Absent	Total
Phase I	8	2	10
Phase II	46	16	62

# Linear Enamel Hypoplasia: Mandibular Lateral Incisor

Of the total sample of 160 adults, 62 (38.8%) had data for Phase and at least one scorable mandibular lateral incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, nine (14.5%) date to Phase I and 53 (85.5%) date to Phase II. Of the individuals from Phase I, six are male (66.7%) and three are female (33.3%). Twenty-seven of the 53 Phase II individuals are male (50.9%), while 25 (47.2%) are female and one is of indeterminate sex (1.9%) (Table 6.75).

Table 6.75. Individuals with data for Phase and mandibular lateral incisor LEH

	Male	Female	Indeterminate	Total
Phase I	6	3	0	9
Phase II	27	25	1	53
Total	33	28	1	62

In Phase I, linear enamel hypoplasia of the mandibular lateral incisor is present in zero out of six males and one out of three females (33%) (Table 6.76). A Fisher's exact test showed no significant differences in the frequency of LEH of the mandibular lateral incisor between males and females in Phase I (p=0.3333).

Table 6.76. Counts for mandibular lateral incisor LEH by sex in Phase I

	Present	Absent	Total
Phase I Male	0	6	6
Phase I Female	1	2	3
Total	1	8	9

For the sex-specific analysis, individuals of indeterminate sex are left out. In Phase II, linear enamel hypoplasia of the mandibular lateral incisor is present in 12 out of 27 males (44.4%) and ten out of 25 females (40%) (Table 6.77). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular lateral incisor between males and females in Phase II (p=0.7852). Consequently, males, females, and individuals of indeterminate sex were pooled for the overall analysis comparing frequency of LEH of the mandibular lateral incisor between Phases I and II.

Table 6.77. Counts for mandibular lateral incisor LEH by sex in Phase II

	Present	Absent	Total
Phase II Male	12	15	27
Phase II Female	10	15	25
Total	22	30	52

In the Phase I pooled sexes sample, LEH of the mandibular lateral incisor is present in one out of nine individuals (11.1%). In the Phase II pooled sexes sample, LEH of the mandibular lateral incisor is present in 23 of 53 individuals (43.4%) (Table 6.78). A Fisher's exact test showed no significant differences in the frequency of mandibular lateral incisor LEH between Phase I and Phase II (p=0.1353).

Table 6.78. Counts for mandibular lateral incisor LEH by Phase, pooled sexes

	Present	Absent	Total
Phase I	1	8	9
Phase II	23	30	53

## Linear Enamel Hypoplasia: Mandibular Central Incisor

Of the total sample of 160 adults, 53 (33.1%) had data for Phase and at least one scorable mandibular central incisor in order to evaluate presence or absence of linear enamel hypoplasia. Of these, seven (13.2%) date to Phase I and 46 (86.8%) date to Phase II. Of the individuals from Phase I, four are male (57.1%) and three are female (42.9%). Twenty-four of the 46 Phase II individuals are male (52.2%), while 21 (45.7%) are female and one is of indeterminate sex (2.2%) (Table 6.79).

Table 6.79. Individuals with data for Phase and mandibular central incisor LEH

	Male	Female	Indeterminate	Total
Phase I	4	3	0	7
Phase II	24	21	1	46
Total	28	24	1	53

In Phase I, linear enamel hypoplasia of the mandibular central incisor is present in zero out of four males and zero out of four females (Table 6.80). A Fisher's exact test showed no significant differences in the frequency of LEH of the mandibular central incisor between males and females in Phase I (p=1.0).

Table 6.80. Counts for mandibular central incisor LEH by sex in Phase I

	Present	Absent	Total
Phase I Male	0	4	4
Phase I Female	0	3	3
Total	0	7	7

For the sex-specific analysis, individuals of indeterminate sex are left out. In Phase II, linear enamel hypoplasia of the mandibular central incisor is present in five out of 24 males (20.8%) and six out of 21 females (28.6%) (Table 6.81). A Fisher's exact test showed no significant difference in the frequency of LEH of the mandibular central incisor between males and females in Phase II (p=0.7302). Consequently, males, females, and individuals of

indeterminate sex were pooled for the overall analysis comparing frequency of LEH of the mandibular central incisor between Phases I and II.

Table 6.81. Counts for mandibular central incisor LEH by sex in Phase II

	Present	Absent	Total
Phase II Male	5	19	24
Phase II Female	6	15	21
Total	11	34	45

In the Phase I pooled sexes sample, LEH of the mandibular central incisor is present in zero out of seven individuals (0%). In the Phase II pooled sexes sample, LEH of the mandibular central incisor is present in 12 of 46 individuals (26.1%) (Table 6.82). A Fisher's exact test showed no significant differences in the frequency of mandibular central incisor LEH between Phase I and Phase II (p=0.3291).

Table 6.82. Counts for mandibular central incisor LEH by Phase, pooled sexes

	Present	Absent	Total
Phase I	0	7	7
Phase II	12	34	46

#### CHAPTER SEVEN: DISCUSSION AND CONCLUSION

This study had two primary goals: 1) to explore biodistance within *Rusellae* and between *Rusellae* and contemporary sites using cranial measurements; and 2) to examine variation in skeletal stress indicators over time and between affinities. As compared with archaeological and historical studies, osteological research from this region and period are relatively sparse.

Therefore, these research avenues contribute to a better understanding of the demography and environment of a Late Antique and Early Medieval Italian city.

Four research questions were developed to address these goals. Overall, the results showed that, despite exhibiting an overall greater affinity with contemporary European samples than African references, *Rusellae* was a heterogeneous site comprising several craniometrically distinct groups. Further, the presence of individuals of African affinity was preliminarily attested at the site. Notably, the individuals with a greater affinity to African reference samples showed no significant difference in frequencies of the skeletal stress markers analyzed, with the exception of the maxillary central incisor. In addition, there were no statistically significant differences in either skeletal stress indicators or cranial measurements between the earlier and later phases of the cemetery. This chapter will address each research question in turn, including the key findings, their interpretations, implications, and potential alternate explanations. Then, I will discuss the broader impact of this research, acknowledge its limitations, and recommend future research directions. Finally, I will briefly conclude by describing the key takeaways from this project.

#### **Research Question 1**

The first research question asked: how does craniofacial variation at *Rusellae* resemble or differ from that of geographically and temporally proximate reference samples, and what might

these relationships suggest about population continuity, migration, or gene flow? Further, what variables drive craniometric variation within *Rusellae*, and does there appear to be heterogeneity or homogeneity in overall craniometric variation at the site?

This research question has three key findings. First, based on the Mahalanobis distance analysis, *Rusellae* is statistically distinct from the Berg, Zalavar, Norse, Mis Island 3-J-10, Mis Island 3-J-11, and Dogon samples. It is closest to the other European references (Zalavar, Norse, and Berg), and more distant from the African reference samples (the two Mis Island cemeteries and the Dogon sample). Second, in the seven-group analysis, the first two principal components separate the African from European samples, indicating that craniometric variation across the groups is patterned geographically, with more geographically proximate groups clustering together. Third, in the intra-cemetery principal components analyses at *Rusellae*, seven PCs were identified as contributing most to the craniometric variation within the site. Hierarchical cluster analysis on these seven PCs identified three phenotypically distinct subgroups, indicating craniometric heterogeneity in the study sample.

I argue that the totality of these findings suggests *Rusellae* experienced overall population continuity with central, northern, and eastern Europe during Late Antiquity and the Early Middle Ages. This means that most gene flow was occurring between *Rusellae* and other geographically proximate individuals on the European continent. The results also indicate *Rusellae* interacted with diverse groups, including some from Africa, through its position in the Tuscan landscape and its connection with the broader Mediterranean region. Using a biocultural approach, this argument situates biological variation within the archaeological and historical context through an exploration of how migration and invasion, trade and economic networks, urbanization, and sociocultural mating practices may have influenced the observed craniometric heterogeneity.

From a population genetic perspective, these results are generally consistent with the expectations of an isolation-by-distance model with consistent gene flow. Gene flow refers to the transfer of genetic material between populations. In humans, gene flow is typically caused by migration and the resulting exchange of genes, which increases genetic variation in the receiving population. In this study, cranial measurements are used as a phenotypic proxy for the underlying genetic data. One explanation for the craniofacial variation observed at Rusellae is the migration of non-local groups to the site, including through invasion or conquest. Notably, the first phase of the cemetery overlaps with the "Migration Period," also known as the "Barbarian Invasions," which occurred between roughly AD 300 - AD 700 (Rubini, Libianchi, Gozzi, & Zaio, 2022). The Migration Period refers generally to the movements of Germanic groups from central and Eastern Europe into the Italian peninsula during and after the fall of the Roman Empire in the West (Rubini et al., 2022). The Lombards moved into Rusellae and overtook the city in approximately AD 593 (Sebastiani et al., 2015), roughly coinciding with the earliest dates of the cemetery. The presence of Lombard-style funerary objects such as a sax and a belt dating to the 7th century AD at *Rusellae* suggest the presence—or at least the imitation—of Lombard culture at Rusellae (Celuzza et al., 2002). Therefore, the archaeological and historical evidence indicates the presence of Germanic migrants at Rusellae; this non-local genetic contribution to Rusellae would have increased the genetic-and thereby craniometric-variation within the site, potentially contributing to the observed craniofacial variation.

The results are also congruent with the participation of *Rusellae* in trade and economic networks connecting far-flung regions throughout the Mediterranean and facilitating movement of people and goods. In addition to the archaeological indication of *Rusellae* being a multicultural site, it is also unsurprising that a site with proximity to a system of Roman roads,

particularly the via Aurelia, which connected Rome to Pisa (Sebastiani, 2017), would be composed of several cultural groups, as opposed to being isolated and homogenous. Previous biodistance studies have shown the construction of Roman roads reduced geographic barriers to migration (such as the Apennine mountains), thereby increasing gene flow within the Italian peninsula, as well as southern and central Europe (Rubini et al., 2022). Similarly, a biodistance analysis of nonmetric traits in the Abruzzo region of Italy also showed greater trait variation in sites along the Roman road system, in contrast to more biological isolation in sites distant from the roads (Cilli, 2021). Rusellae's connection—by the via Aurelia—to the famously cosmopolitan city of Rome was, of course, a direct route for migrants from across the Mediterranean to travel to Rusellae, but Rusellae's connection to Pisa is also noteworthy. During the Late Antique and Early Medieval periods, Pisa was an active port city, which, like Rusellae, was also under control of the Lombards during the 7th century AD. During the 9th century AD, Pisa also became a major defense outpost against Muslim raids from North Africa, with fleets navigating between Pisa and Tunisia (Alberti, 2023), thereby facilitating gene flow and leading to greater genetic diversity. The phenomenon of coastal cities in Italy exhibiting greater diversity than inland cities, has been demonstrated in the Roman era (Emery, Stark, et al., 2018) and appears to continue throughout the occupation of Rusellae. Additionally, recent aerial and magnetometer surveys have revealed the remains of a road roughly 200 meters from the city walls, connecting Rusellae to the surrounding countryside (Campana, 2021). Therefore, Rusellae would inevitably have been visited by merchants and migrants using the ports and roads that connected Rusellae to the rest of the Mediterranean, further increasing the potential for gene flow and genetic variation at the site.

Additionally, the transition between the Roman to Medieval settlement structure of Tuscany would have influenced the genetic variation at the site. Between the early 4th and mid-5th centuries, settlement density in the region markedly declined, including a substantial decrease in the number of rural sites (Vaccaro, 2008). By the mid-6th century AD, sites near Rusellae, including Scoglietto, Spolverino, and Umbro Flumen, in Alberese, Italy, had experienced demographic decline and were ultimately abandoned (Sebastiani, 2016, 2017; Sebastiani et al., 2015). A declining Mediterranean economy, Germanic invasions, the Gothic Wars, and the plague of Justinian caused further population decline and abandonment of rural sites between the 5<sup>th</sup> and 7<sup>th</sup> centuries AD (Brogiolo et al., 2017; Francovich, 2007). By the 7th-8th centuries AD, lowland settlements in Tuscany were infrequent and fortified hilltop settlements such as Rusellae were favored (Francovich, 2008). Rusellae maintained its occupation despite the decline of the surrounding Tuscan countryside, which may have instigated a convergence of diverse peoples in the urban center. This constriction of the population would have thereby increased the genetic effect of any migration on the overall variation at the site. In sum, the concentration of people from the surrounding region at Rusellae may be responsible for introducing genetic variation into the sample, producing the observed craniometric heterogeneity.

Sociocultural factors such as kinship, status, and ethnicity may help explain the persistence of diverse subpopulations within the sample. Assortative mating based on status, ethnicity, or religion is a common human phenomenon (Diggelmann, 2016; Sebro, Peloso, Dupuis, & Risch, 2017). Genealogies within Medieval Europe were important for support networks, models of inheritance, establishing alliances, legitimizing power and status (Pohl, 2016). At *Rusellae*, the importance of recognizing kinship and maintaining and consolidating

status could have led to endogamous subpopulations of individuals who primarily interbred within their group. Kinship was particularly important for Lombards. Studies have shown that Lombards in Hungary and northern Italy structured their cemeteries around pedigrees and that biological relatedness was an important component of their society (Amorim et al., 2018). For example, marriage between elite individuals is attested in Lombard society (Amorim et al., 2018). It is hypothesized that the Phase I burials at *Rusellae* may comprise elite individuals buried closer to the church due to their higher social status. These burials are also the ones that include Lombard-style grave goods (Celuzza et al., 2002). Genetic isolation of this elite cultural group within the site may have contributed to the variation seen within the sample. Therefore, if different ethnic or social groups were identified by occupants of *Rusellae* based on different geographic origins, cultural practices, status, language, or religion, assortative mating may have occurred along these lines. If assortative mating occurred, this could maintain genetic diversity, in contrast to a system of random mating which would drive genetic homogeneity at the site over time.

In general, the results of this research question are consistent with the findings of previous distance and mobility studies in the Late Antique and Early Medieval Mediterranean. As a reminder, the key finding for this question is that the cemetery generally comprised individuals most similar to other European groups, but maintained heterogeneity within the site that could be attributed to migration, invasion, trade, shifts in regional settlement structure, or sociocultural barriers to mating. Distance studies have identified Lombard cemeteries in Hungary and Italy wherein people with central and eastern European ancestry coexisted with those of southern European ancestry (Amorim et al., 2018). Further isotopic analysis of the Hungarian cemetery at Szólád indicates a wide heterogeneity in the geographic origin of individuals interred

there; approximately 31% of the sample was non-local according to strontium isotope ratios (Alt et al., 2014).

The results of the present study are also consistent with recent genetic findings. In a large-scale analysis of genetic ancestry for individuals from Rome with known historical and archaeological contexts, researchers found that Late Antique individuals have an average ancestry most similar to modern central European populations. This was probably facilitated by a reduction in Roman population combined with moderate amounts of immigration, primarily from Germanic tribes from central and Eastern Europe, that would have influenced the average genetic ancestry (Antonio et al., 2019). However, Antonio and colleagues also found high interindividual heterogeneity in Late Antique Roman individuals with respect to their geographic ancestries. They attributed this "persistent genetic diversity" to the remnants of trade, migration, slavery, and conquest in the Imperial era, along with the maintenance of trade networks within the western Mediterranean and the movement of Germanic tribes into the Italian peninsula (Antonio et al., 2019).

Researchers also found that Medieval individuals can be modeled as a two-way combination of Late Antique Romans and a central and northern European component, including Lombards from Hungary, Saxons from England, and Vikings from Sweden (2019). They hypothesize that this result is likely due to the connection between Medieval Rome and mainland Europe through the Holy Roman Empire, which connected a loose confederation of states under a Christian emperor, beginning with the coronation of Charlemagne in AD 800 (Antonio et al., 2019). Their summary suggests that the overall ancestry of Roman individuals generally coincided with the changes in the political affiliations of Rome; however, individuals maintained highly diverse genetic ancestries, including contributions from the Near East, Europe, and North

Africa through the medieval period, making Rome a "genetic crossroads" of peoples from Europe and the greater Mediterranean (Antonio et al., 2019).

I argue that the results from *Rusellae* show that it, too, represented a "genetic crossroads." The overall biodistance analysis showed the greatest affinity between *Rusellae* and other medieval European references, as expected within an isolation-by-distance model. However, the interconnectedness of *Rusellae* with its hinterlands and large port cities, its conquest by the Lombards in the late 6th century AD, and its use as an urban center facilitated a degree of genetic diversity that is identifiable from the crania in its cemetery.

#### **Research Question 2**

The second research question asked: do any individuals at *Rusellae* show a closer affinity to African reference samples than European reference samples (Phases I and II)?

The key finding from this research question is that, based on the LDFA and the reference samples used in this analysis, 11 individuals (42.3%) in the *Rusellae* sample showed greater affinity with African craniometric references, while 15 individuals (57.7%) showed greater affinity with European craniometric references. When the *Rusellae* individuals are plotted against the reference group centroids for the first two linear discriminant functions, it is clear that the crania exhibit overall heterogeneity. However, the model appears to be separating the African from the European reference samples well on the first linear discriminant axis, while the second linear discriminant axis appears to be separating the group based on temporal variation, with the earlier reference samples (Norse, Zalavar, and Mis Island) having positive values and the later reference samples (Berg and Dogon) having negative values.

I argue that these findings suggest the presence of individuals with African affinity at *Rusellae*, supporting the existing historical, archaeological, genetic, and anthropological

evidence of African migration to Italy during the Roman Imperial era, Late Antiquity, and the Early Middle Ages. These results likely reflect a much more complex biological and cultural reality than the binary affinity classification can illuminate. Therefore, I posit that the greater affinity of some individuals from *Rusellae* to African references is likely either a product of genuine gene flow caused by migration and admixture through historical population interactions, methodological limitations of craniometric classification statistics, or both.

The first possible explanation for several individuals from *Rusellae* showing a greater affinity with African references that individuals either of African origin or African descent lived at *Rusellae*. Given Italy's geographic and cultural position, it is expected that the results would primarily indicate affinity with European samples. However, there is still some degree of African influence within *Rusellae*. The results indicate at least a small degree of gene flow from Africa into the site, which may be influencing the observed patterns of population affinity in the cemetery. A biocultural approach can be used to account for the relationship between human behavior and mobility and the observed biological variation in the sample.

This gene flow is expected based on extensive historical and archaeological documentation. Italy was undeniably a crossroads of trade, migration, and invasion for the entire Mediterranean, including North Africa, before, during, and after the use of the cemetery at *Rusellae* (Holmes, 1988; Wickham, 2006).

Overseas trade was particularly important during the Imperial era, and there is clear evidence of trade relationships between Italy and North Africa during this period as well. One of the wrecks at the island of Giglio on the Tuscan coast carried North African amphorae, indicating trade between North Africa and the North Mediterranean (Bound, 1987). Small merchant ships and large vessels traversed the open seas between Carthage and Rome, and

Roman wrecks at the Skerki Bank between Tunisia and Sicily reveal varied cargo, including goods from throughout the Mediterranean. Shipwreck archaeology provides clear evidence of trade relationships between North Africa, particularly Carthage, and the western coast of Italy during the Roman era (McCann, 2008).

African Red Slip, or ARS, wares played a crucial role in trade between Egypt, North Africa, and Italy, so much so that the density, location, and dating of these wares is a useful proxy for trade due to their widespread distribution and almost exclusive use in some parts of the empire (S. Mitchell, 2007; Wickham, 2023). Importantly, ARS wares have been identified in Tuscany during the 4th-5th centuries AD, suggesting an ongoing trade relationship between Tuscany and North Africa during this time (Vaccaro, 2008). Despite a decline in trade with North Africa during the mid-fifth century AD, due in part to the Vandal conquest of Carthage in AD 439, by the 9th and 10th centuries AD, coastal cities in southwest Italy show clear evidence of resumed trade with North Africa (Horden & Purcell, 2000; Wickham, 1994, 2006). These examples provide evidence for a circum-Mediterranean economy involving Italy and North Africa, which would have naturally required human agents to move the goods, thereby providing a vector for gene flow between the continents. Additionally, other cultural variables such as occupation, military membership, or enslavement may have contributed to migration patterns (Redfern et al., 2016).

The results of this research question also align well with previous skeletal studies, which have consistently identified the presence of individuals with African affinity in Imperial, Late Antique, and medieval European cemeteries using several distance measures and biogeochemical methods (Emery, 2018; Emery, Duggan, et al., 2018; Emery, Stark, et al., 2018; Fentress, 2003; Leach et al., 2010; Leach et al., 2009; Redfern et al., 2023; Redfern et al., 2016; Redfern &

Hefner, 2019; Salesse et al., 2021). Individuals hypothesized to have African affinity were identified in Roman York through craniometric discriminant function and stable isotope analysis, suggesting African origins for roughly 10% of their study sample (Leach et al., 2010; Leach et al., 2009). Crania with macromorphoscopic traits consistent with African affinity were identified in Roman and Medieval London; non-White European ancestry was hypothesized in as much as 29% of the 41-person sample (Redfern et al., 2016; Redfern & Hefner, 2019).

In Italy, several studies have identified the possible presence of African individuals in cemeteries contemporary with *Rusellae*. In the rural southern Italian necropolis at Vagnari (1st-4th centuries AD), oxygen and strontium isotope analysis revealed that 7% of the individuals at the site were non-local; further analyses of the non-local individuals revealed isotopic and mtDNA evidence of North African origin (Emery, Duggan, et al., 2018; Emery, Stark, et al., 2018). At the Imperial sites of Velia and Isola Sacra, isotope analysis also suggests possible North African origin for several individuals (Prowse et al., 2007; Stark et al., 2020). Moreover, Salesse and colleagues use multiple isotopes, dental morphology, and ancient DNA to identify an individual in the Roman catacombs of Saints Peter and Marcellinus who they suggest was likely born in the Nile Valley or the central Sahara Desert. The authors hypothesize this individual may have endured forced migration as a slave Salesse et al. (2021). Finally, the tentative presence of individuals with African affinity was also reported at the Early Medieval (11th century AD) cemetery at Cosa, near *Rusellae*, based on craniofacial morphology (Fentress, 2003).

Genetic studies have also highlighted the complexity of the modern and archaeological Italian genome, which appears to be a product of regular multidirectional gene flow (Arauna, Hellenthal, & Comas, 2019). Evidence of North African influence has been detected in the Italian genome and hypothesized to be a result of Arab conquests, including migration to Italy

from Tunisia (Arauna et al., 2019; Busby et al., 2015; Fiorito et al., 2016). North African male DNA has also been identified in Iberia, Sicily, and the Italian peninsula as a result of Arab and North African incursions in these regions during the medieval period (Capelli et al., 2009). Additional genetic investigation of haplotype data found that gene flow from North Africa into Tuscany primarily came from the North African coast of the Mediterranean Sea as opposed to the Atlantic coast; the authors reported that the estimated date of admixture for the Tuscan and North African samples suggests movement of North Africans into Tuscany around the fall of Rome, in approximately the 4th century AD (Arauna et al., 2019). Botigue and colleagues further note that the genetic contributions to the Italian genome from North Africa are more substantial than those from sub-Saharan Africa (2013).

The other possible explanation for the observed presence of individuals with African affinity at *Rusellae* is that the results are, in part, a product of the methodological limitations of classification statistics and issues with extrapolating forensic methods to a bioarchaeological context. Although the results obtained in the present study are consistent with historical, archaeological, anthropological, and genetic studies that show the presence of African affinity individuals throughout Imperial and post-Roman Europe, this dissertation shows a much higher proportion of African affinity in the sample than any other comparable study. Therefore, it is likely that at least some of the individuals with African affinity in the *Rusellae* sample are misclassified.

This misclassification, if present, is probably due to the following causes: the clinal nature of human variation, missing data, complex genetic history, applying forensic methods to bioarchaeological samples, and poor reference samples. Human populations do not fall into discrete categories or types with respect to any genetic or phenotypic variable. Therefore,

overlapping craniometric variation of the reference samples is expected regardless of which groups are included in the analysis. This means that even if an individual shows the greatest affinity with one reference sample, they may still fall within the expected variation of a different reference group and may simply represent an atypical member of that group. The overall correct classification rate of the model used in this study is 77.2%, meaning that approximately 23% of individuals will be misclassified when the function is applied. Importantly, the concepts of "correct classification" and "misclassification" are relative to the reference samples used. Linear discriminant function analysis, by its nature, will classify every unknown individual into one of the reference samples used to build the model, regardless of whether or not the unknown individual actually belongs to one of the reference groups. This "forced classification," so-tospeak, will inevitably lead to misclassifications, especially if the true affinity group for an unknown individual is not included in the model. That is why the term "affinity" is so useful in this context; an unknown individual may accurately be said to show a greater affinity to a particular reference sample, without the assumption that the individual actually belongs to that population.

Another factor that may contribute to misclassification is missing data. Using data imputation to complete a dataset, while useful for improving sample size, lowers classification accuracy because it minimizes variation within the sample (Kenyhercz & Passalacqua, 2016).

The complexity of the Italian genome suggests regular gene flow, and it is almost certain that misclassification occurs as a result of this complexity, with individuals classifying as African when, in reality, they belong to a diverse European group. Misclassification is not surprising in *Rusellae* specifically, as several genetic studies have identified a complex and admixed Italian genome with contributions from throughout the Mediterranean (Arauna et al.,

2019; Busby et al., 2015; Fiorito et al., 2016). The phenotypic proxy of this complex genetic data is therefore also expected to exhibit a heterogeneity that may be challenging to classify.

Moreover, studies have shown issues with misclassification when applying discriminant function analysis to bioarchaeological samples. Using the forensic anthropology classification software, FORDISC 2.0, Williams and colleagues found that the program misclassified Meroitic Nubian individuals into a variety of the Howells' reference samples included in the software; they suggest, and I agree, that this misclassification stems from the inability for a statistically-defined sample to capture the extent of variability in a given human population. The clinal nature of human variation, as well as cultural and environmental factors that contribute to deviation from the expectations of an isolation-by-distance model, will always pose challenges to classification (Williams, Belcher, & Armelagos, 2005). Previous studies have also shown misclassification of affinity when using macromorphoscopic traits as a genetic proxy (Redfern & Hefner, 2019). The authors also argue that the lack of temporally and geographically representative reference data leads to misclassification of affinity and "false positives" of individuals with non-local origin or affinity (Redfern & Hefner, 2019).

One way to improve classification accuracy, and thereby increase confidence in the results, is to refine reference datasets. The more representative a reference sample is of the potential affinity of any given unknown individual, the more likely the model will be able to correctly classify that unknown. This can be achieved by collecting or collating additional data from contemporary populations with a wider geographic range and including more samples that are potentially related to the unknown sample or better correspond with the "sending" populations when attempting to "identify" a migrant. Including more geographically or

temporally consistent samples may alter classifications, but will not affect the underlying group relationships and will also improve confidence in results.

### **Research Question 3**

Research Question 3 asked whether there is evidence of differential life experience between individuals with African affinity, as compared with individuals with European affinity with respect to skeletal stress indicators (Phase II).

The key finding of this research question is that no significant differences were found in any of the skeletal stress indicators analyzed by sex within affinity or overall affinity, except for linear enamel hypoplasia of the maxillary central incisor. This tooth had a higher frequency of LEH in the African affinity sample compared with the European affinity sample. Generally, *cribra orbitalia* and porotic hyperostosis exhibited relatively low frequencies, while periosteal reaction was present in all individuals, and linear enamel hypoplasias of the anterior maxillary and mandibular dentition varied in frequency by tooth, ranging from 25% to 88.9%.

Before delving into this discussion, it is important to acknowledge the limitations of this research question up front. The dataset for this analysis is exceptionally small, severely limiting the power of the statistical analyses and potentially introducing sampling bias, making any conclusions drawn from the results tentative at best. Therefore, this discussion serves as a preliminary exploration of the data with the caveat that further data will be necessary to confirm the findings. With these limitations in mind, I argue that the observed patterns of physiological stress in the cemetery provisionally suggest broadly similar exposure to stressors across affinity groups, potentially resulting from shared sociocultural practices and environmental conditions among individuals with varying affinities during the later phase of the cemetery. However, there is a weak indication that early life developmental stressors were more frequent in the African

affinity group, reflecting the potential for a bioculturally complex life experience in Late Antique and Early Medieval Italy.

The combined results for *cribra orbitalia* and porotic hyperostosis suggest a relatively low prevalence of both porosities across the sample. Caution should be taken in interpreting these lesions due to their complex etiologies. A constellation of variables has been proposed that can lead to porotic hyperostosis and *cribra orbitalia*, including inadequate nutrition, vitamin deficiencies, inflammation, poor sanitation, and infectious disease (Larsen, 2015; Walker et al., 2009). Since it is impossible to determine the cause of these lesions based on the data collected here, it can simply be said that the absence of affinity-related differences suggests a relatively similar nutritional status, hygienic environment, and disease load across the samples.

Similarly, lack of significant differences in frequency of periosteal reaction also suggests similar exposure to infection, trauma, or inflammatory conditions between affinity groups. This result may be indicative of similar labor conditions or a similar propensity to injury among all adults in the later phase. Periosteal reactions are not limited to the period of growth and development, and can therefore provide insight into later-in-life insults (Weston, 2011).

With respect to linear enamel hypoplasia, results indicate a similar degree of developmental stress between affinity groups for most teeth, especially during the period between one year and six years old, during which time enamel forms and records developmental insults (Reid & Dean, 2006). The maxillary central incisor shows a significantly higher frequency of LEH in the African affinity sample. This tooth tends to begin forming slightly earlier than the others, at around six months old (Liversidge, 2000), tentatively suggesting a higher proportion of very early childhood stress in the African affinity sample.

A variety of factors could result in a higher frequency of LEH in the maxillary central incisor for the African affinity group. For example, weaning practices in non-local individuals in a Late Roman/Early Medieval Roman frontier province in Southern Bavaria differed from those of local individuals, as identified by isotopes. LEHs were most frequent in the post-weaning interval, suggesting that children were at increased risk of developing LEH after breastfeeding had ceased (Velte et al., 2023). It is possible that earlier weaning, mediated by variable social norms and diverse weaning practices among differing affinity groups, may have contributed to a higher frequency of LEHs in the maxillary central incisor for the African affinity sample. A higher proportion of childhood illness, poor diet and nutrition, or psychosocial stressors related to migration or integration with the local population may all lead to the observed pattern.

Overall, these results may suggest at least some degree of inequity in life experience within the sample. A more likely explanation, however, is that the results are a product of sampling issues.

As previously noted, the findings for this question should be interpreted cautiously, as the results may be an artifact of several underlying methodological limitations. Therefore, I will now discuss several potential alternate explanations for the observed results: sample size and bias, misclassification of affinity, variation within an affinity group, and the osteological paradox.

First, the lack of significant differences among most of the indicators may be a product of small sample size or random sample bias rather than a true absence of differences. This has been suggested as a cause for similar results in Redfern and Hefner's analysis of the same stress markers between different affinity groups in Medieval London (2019). Further, age-associated differences in stress indicators and age-at-death distributions of the groups may compound to create a false depiction of differential stress exposure (Redfern et al., 2023). For example, periosteal new bone formation has been shown to increase with age (DeWitte, 2014), so the high

frequency of periosteal reaction could indicate a greater proportion of older adults in the sample (Redfern et al., 2023).

A second consideration stems from compounding issues with the results of the previous research question. It is almost certain that some of these individuals are misclassified with respect to affinity, making the affinity groupings themselves tenuous. Therefore, it is completely possible that the observed results are a product of misclassifications and consequent faulty data partitioning as opposed to being reflective of the biocultural reality of life at *Rusellae*.

Third, lumping together individuals into continent-wide affinity groups flattens the variation within each group; importantly, there are known cultural and ancestral distinctions within the "European" affinity group (i.e. Germanic central European groups vs. autochthonous Italians) present at *Rusellae*. These individuals may have had heterogeneous life experiences, resulting in further hidden heterogeneity within each broadly-assigned affinity group that is masked by the present research design.

Finally, it is important to consider alternative interpretations of the data within the framework of the osteological paradox. The cemetery sample represents the individuals who survived any insults long enough for them to leave markers in the bones; since there is some indication of high infectious load (high frequency of periosteal reaction) and high developmental metabolic stress (high frequency of LEH), I argue for at least some degree of resilience in the face of chronic illness, infection, and childhood developmental stress in the individuals studied. Similarly, the higher frequency of LEH observed in the African affinity sample may indicate decreased frailty and increased resilience in this group, especially since the adults included in this study would have lived for many years beyond whatever insult caused the LEH during their

early life. All of these options—or a combination thereof—should be considered as potentially contributing to the observed pattern of physiological stress.

Despite these legitimate concerns, it is notable that the results obtained herein are consistent with the limited comparable studies comparing affinity to skeletal stress in Late Antique and Early Medieval contexts. Redfern and colleagues examined the relationship between the Black Plague, physiological stress, and population affinity (estimated using macromorphoscopic traits) in a 14th-century plague cemetery in London (2023). Although the plague cemetery contained a higher proportion of individuals with estimated African affinity as compared with non-plague cemetery, no statistically significant differences were found between African affinity and European affinity in the frequency of linear enamel hypoplasia, cribra orbitalia, or periosteal reaction (Redfern et al., 2023). In a separate study, Redfern and Hefner found a higher frequency of LEH, periosteal reaction, and *cribra orbitalia* in individuals with White European ancestry (2019). They argue that the complete absence of some stress indicators, particularly in Black males, may suggest an increased mortality risk in those individuals within the context of a racialized society. Although these results are not directly comparable to the present findings due to the lack of statistical testing, it is notable that the authors propose the influence of a racialized society as underpinning their results, supporting a worse health outcome in individuals with estimated African affinity. As their study site post-dates the Rusellae cemetery by several centuries and is located in England instead of Italy, the sociocultural context of their conclusions differs from the expectations of Rusellae.

I argue that in the context of *Rusellae*, the lack of distinction in frequency between affinity groups for nearly all the stress indicators under study might be the product of a more expansive view of ethnicity that incorporates religious affiliation into the concepts of self and

otherness in Late Antique and Early Medieval Tuscany. Therefore, it is important to consider how residents of *Rusellae* might have constructed a concept of ethnicity that would have produced an environment wherein different affinity groups appear to have been exposed to similar degrees of infection, comparable diets, and similar sanitary conditions. Heng (2011) and Lopez-Jantzen (2019) argue that Late Antique and Early Medieval "race" was not necessarily based on skin color or origin, but that religious affiliation served as a sociocultural and biopolitical marker of medieval racialization. Importantly, the incorporation of Christianity into Roman identity inextricably linked religion with "Romanness." In the 6th century AD, Justinian limited full membership in the Roman community to those identifying as Christians (Lopez-Jantzen, 2019). In the 7th century AD, the Byzantine Emperor Heraclius ordered all Jews to convert to Christianity to attain religious conformity; this decree was also enforced in North Africa (Bat-Sheva, 2010). Therefore, as Christianity proliferated, so did marginalization along religious lines, with Jews and pagans viewed as "other" (Lopez-Jantzen, 2019).

Beginning in the 8th century AD, the church represented not only a sacred building, but also the node of a district, having baptismal and burial rights as well as responsibility for collecting tithes. In Tuscany, churches were historically located in hilly areas and distributed in strategic locations near rivers or roads, representing a pole of attraction for diverse occupants of the region (Riccomi, 2021). Given the nature of churches as religious and civic centers in rural Tuscany, it is also possible the church not only attracted diverse people from throughout the region, but that the shared sense of Christianity, and thereby an adoption or reiteration of Roman identity, may be a greater unifying force than any affinity-based social division. In support of this assertion, Ristuccia posits that baptism and burial in the same place produced a social and ritual community (2018). Therefore, it is possible that individuals buried within the same

constructing a social environment with similar stressors. The burial of individuals with differing affinities in the same cemetery is especially surprising since North Africa was predominantly Muslim during this period (Fenwick, 2013), but the individuals interred at *Rusellae* were given Christian burials. Given the trend at *Rusellae* towards Christian burial tradition in Phase II (Celuzza et al., 2002) it appears that individuals with differing affinities may have been subsumed under the "Christian Roman" umbrella, thereby forming a sense of "self" and "other" that was less reliant upon concepts of affinity or origin and more in line with religious affiliation.

This argument does not, of course, exclude the possibility that marginalization occurred on the lines of skin color or geographic origin during this time period or in this part of the world; the Early Medieval era was by no means "pre-racial," but the concepts of race, self, and otherness were not necessarily drawn along modern Western lines. In fact, there is a general consensus that socially-constructed race was an important factor in Late Antiquity through the Middle Ages and that the production and reproduction of race and racism from classical antiquity through the central Middle Ages contributes to modern concepts of racism (Lopez-Jantzen, 2019). However, colonization in the Mediterranean was thought to not have had as harmful a biocultural effect as that observed in other contexts because racism in the Mediterranean was considered to be less severe due to long histories of trade and interaction (Kyle, Shehi, Koçi, & Reitsema, 2020). Rusellae was the seat of a diocese and a fortified hilltop settlement that would have attracted a diverse population in the wake of the collapsed rural Roman settlement system in Tuscany. Given the results of this research question, it seems that the Christian church was a unifying sociopolitical force, potentially outweighing distinctions based on sex or affinity.

# **Research Question 4**

Research Question 4 asked whether detectable craniofacial differences exist between Phase I (6th-7th centuries AD) and Phase II (8th-12th centuries AD) burials at *Rusellae*, suggesting either shifting population demographics or relative population continuity. Further, it sought to understand whether differences exist in the frequency of *cribra orbitalia*, porotic hyperostosis, periosteal reaction, and linear enamel hypoplasia between phases, suggesting changing environmental stressors, diet, disease, or other cultural factors over time.

The key findings of this question are twofold. First, no significant differences were found between Phase I and Phase II individuals with respect to any of the 12 craniometric variables included in the study. Second, no significant differences were found in any of the skeletal stress indicators analyzed by sex within Phase or overall Phase: *cribra orbitalia*, porotic hyperostosis, periosteal reaction, and linear enamel hypoplasia of the maxillary and mandibular canines and incisors. Generally, *cribra orbitalia* and porotic hyperostosis exhibited relatively low frequencies, while periosteal reaction was present in a high proportion, and linear enamel hypoplasias of the anterior maxillary and mandibular dentition varied in frequency by tooth, ranging from entirely absent in the mandibular central incisor to 80% in the mandibular canine.

The caveats from Research Question 3 also apply here with respect to small sample size, limited statistical power, and consequent interpretive limitations. Thus, these findings should be considered tentative and as forming the basis for future studies. With these constraints in mind, I argue that the observed diachronic craniometric variation may preliminarily indicate an overall population continuity at the site between the earlier and later phases of the cemetery; there is not strong evidence for secular change or a sudden influx of new phenotypes that would alter the overall craniofacial complex at *Rusellae*. Further, the combined results of physiological stress

indicators support the presence of a generally consistent diet, nutrition, and exposure to environmental, developmental, and infectious disease stressors over time.

Although it is nearly impossible to establish a specific cause-and-effect relationship for the studied lesions due to their nature as primarily non-specific stress indicators, the unique historical, cultural, and environmental circumstances of *Rusellae* may help us understand the observed pattern of cranial variation and physiological stressors, which are characterized by a relative degree of continuity in the midst of an otherwise transitional period. Three primary drivers are likely contributing, with various degrees of importance, to the observed outcome: demographic and epidemiological stressors, environmental and ecological factors, and diet and weaning practices. Overall, these sociocultural and environmental variables can help explain the consistency and frequency of stress indicators found in this study.

The context of *Rusellae* as a continuously occupied site within a declining Roman settlement system is crucial. Historical evidence suggests that disease and stress were widespread during the sixth century AD, as a result of the Gothic Wars, the plague of Justinian, leprosy, famine, malaria, and economic decline during this time (Belcastro et al., 2007; McCormick, 2001). Additionally, the population of Italy declined by half between the 3rd and 6th centuries AD (Belcastro et al., 2007). This reduction in population coincided with the 6th-century transition from traditional Roman settlement patterns, such as the villa system, to fortified hilltop sites that were military and civil centers of a territory (Chirico & Sebastiani, 2023). As one of these sites during Late Antiquity, *Rusellae* was surrounded by cities either abandoned or in crisis, including nearby Cosa, Heba, Vetulonia, and Saturnia. In contrast, *Rusellae* became a bishopric and constructed a church from the ruins of a Hadrianic bath. Despite the abandonment of parts of the site, a theme of its occupation was reuse, transformation, and rebuilding. Similarly, *Rusellae* 

was valued for its interconnectedness, including accessibility to Roman roads, control of the coast of the Tyrrhenian Sea, and proximity to the northern frontier of the territory of Rome (Celuzza et al., 2023).

In combination, these factors would have led to a coalescence of people within Rusellae, which may have resulted in the urban penalty phenomenon, or an increased pathogen or disease load for individuals in urban vs. rural settings. This "urban penalty" has been identified in Late Antiquity and the Early Middle Ages throughout Europe as a failure to adapt to diseases common in urban contexts (Meinzer, Steckel, & Baten, 2019) and has been linked to proliferative bone reactions, such as periosteal new bone formation (Riccomi, 2021). Zoonotic diseases, such as those transmitted from cattle to humans, and parasites may have been common, despite efforts made to improve sanitation (P. D. Mitchell, 2017); parasites including whipworm and roundworm, fleas, lice, and bed bugs are all vectors of infection, possibly from contamination of food resulting from poor hand hygiene and fecal contamination during this period (P. D. Mitchell, 2017). Therefore, it is possible that the process of urbanization led to higher population density within the city walls, resulting in issues with sanitation and waste management, creating the conditions for outbreaks of infectious disease (Riccomi, 2021). An increased pathogen load may contribute to the high frequency of linear enamel hypoplasia and periosteal reaction observed in the sample (Larsen, 2015). Since the site is held constant in this study, it is unsurprising that the stressors potentially linked to urbanization would also remain consistent in frequency, even over time.

In addition to shifting settlement structure and the impact of urbanization, environmental and ecological factors could also have contributed to the skeletal stress markers at this site. Most importantly, environmental conditions favorable to the spread of parasitic infections, such as

malaria, likely influenced the sample. As the Roman Empire declined, marshes and coastal swamps likely expanded due to an inability to maintain waterway infrastructure, in concert with rising sea levels, facilitating mosquito breeding (Celuzza et al., 2023; Fornaciari, Giuffra, Ferroglio, Gino, & Bianucci, 2010; Fornaciari & Mallegni, 1985). During the Early Middle Ages, a brackish lagoon extended northwest of *Rusellae*, providing ideal breeding conditions for the *Anopheles* mosquito, which spread the malaria-causing *Plasmodium falciparum*, *P. vivax*, and *P. malariae* parasites across the landscape (Bellotti et al., 2004; Celuzza et al., 2023; Fornaciari et al., 2010; Sallares, Bouwman, & Anderung, 2004). In fact, malaria remained hyperendemic in the region of *Rusellae* until the mid-20th century (Boccolini et al., 2012; Celuzza et al., 2023).

Cribra orbitalia has been linked to malaria-induced anemia (Gowland & Western, 2012; Schats, 2023); although these lesions are by no means pathognomonic for malaria, their presence in a region of known malarial infection should be considered, as malaria cannot be ruled out as causing them based on historical data. Additionally, differing ecological conditions (and the resulting variation in parasitic infections) have been suggested as a cause for different frequencies of cribra orbitalia in different regions of Italy (Belcastro et al., 2007). Further, recent application of Smith-Guzman's (2015) paleopathological approach to identifying malaria using cribra orbitalia, femoral cribra, humeral cribra, spinal porosity, and periosteal reaction was applied to the Rusellae sample; results indicated that malarial infection was present at Rusellae based on Smith-Guzman's criteria (2015), potentially in up to 66% of the individuals with similar frequencies across phases, sexes, and age cohorts (Celuzza et al., 2023). Notably, many of the lesions showed signs of healing, and their presence in older individuals indicates a degree of endemicity in the sample, wherein malarial infection was not acutely fatal. Therefore, the low

frequency of *cribra orbitalia* lesions at *Rusellae* may be linked to the environment, as a result of exposure to parasites causing endemic, but not acutely fatal, malarial infection. The hyperendemicity of malaria in the region of *Rusellae* until relatively recently also supports the consistency in lesions over time.

Another component of life at *Rusellae* that may provide context for the lesion frequencies is diet, nutrition, and weaning practices. After the fall of the western Roman Empire, diet in the Roman world was characterized by bread, oil, and wine, while hunting, cattle breeding, and fishing were common of Germanic groups (Belcastro et al., 2007). In the 5th and 6th centuries, there was an integration of the two dietary cultures, as a result of migration, that persisted for the following centuries (Belcastro et al., 2007). Early Middle Ages historical sources suggest the importance of cereals along with uncultivated vegetable and animal products. A typical meal for most people of the time, including peasants, consisted of a meat stew with beans and vegetables, along with bread and wine. Animal products appear to have contributed to the diet even of the poor class during this period, which was somewhat unusual in the context of human dietary history (Belcastro et al., 2007). The role of meat consumption in the Late Antique and Early Medieval Italian diet, largely as a result of German influence, might have increased iron reserves, thereby contributing to the relatively low frequency of iron-related porosities such as *cribra orbitalia* and porotic hyperostosis.

Additionally, weaning practices and the post-weaning diet would likely have influenced early-life stress, thereby affecting the frequency of linear enamel hypoplasias in the sample.

LEHs occur after the first year of life on the anterior teeth; insults prior to this are unlikely to be recorded in the enamel (Reid & Dean, 2000; Reid & Dean, 2006). This period corresponds to the weaning period for Roman and Early Middle Age groups in Italy, based on historical documents.

Early Middle Ages medical literature on infant feeding suggested that a diet of exclusively breast milk was fed until approximately 6 months, or when the infant cuts their first tooth. This phase was to be followed by gradually introducing solid foods such as bread and milk, cereal, and porridge. Weaning was complete by approximately 3 years of age; historical documentation suggests this general trajectory did not change between Roman and Early Middle Ages periods (Belcastro et al., 2007). Therefore, a possible explanation for the lack of significant difference in LEH is that weaning practices did not change significantly over time, with a protein-deficient and possibly unsanitary post-weaning solid diet leading to disturbances in enamel formation across much of the population (Belcastro et al., 2007). However, increased protein consumption at a later age may have contributed to greater resilience and a lower overall frequency of porotic hyperostosis and *cribra orbitalia*.

Given these examples, it is possible that the aforementioned elements would have contributed to a relatively stable living environment over time—imperfect, but consistent. Therefore, the lack of significant diachronic metric change in the cranium is relatively unsurprising. Secular change in skeletal measurements is influenced by environmental factors and changes in living conditions, as well as variables such as social class, income, family size, urban vs. rural location, region, health and hygiene, and overcrowding (Cole, 2000). The consistency in environment and living conditions that appears to be influencing the stasis in stress markers is also likely exerting similar selection pressures across time, minimizing the influence of cranial secular change in the sample. Another possible explanation is that the high degree of craniometric heterogeneity observed in the sample is maintained over time, thereby masking small temporal differences.

These data are consistent with previous findings that have suggested a regional variation within Italy in life experience for the transition between Late Antiquity and the Middle Ages. A study of the frequency of LEH, cribra orbitalia, and periosteal reaction in Molise, Italy show similar frequencies from the Roman to the Early Medieval period (Belcastro et al., 2007). Further, isotopic analysis revealed no substantial shift in diet between Early Medieval and Medieval occupations at Cosa, a geographically and temporally contemporary site to Rusellae; this dietary stability may help explain the lack of difference between nutrition-related stressors, such as porotic hyperostosis, cribra orbitalia, and LEH (Scorrano et al., 2014). In addition, no difference in frequency was found for *cribra orbitalia* or porotic hyperostosis among one Late Antique and two Early Medieval sites in Tuscany (Riccomi, 2021). Notably, although their study was conducted in Late Antique and Early Medieval Albania, Kyle and colleagues found that stress indicators did not increase in frequency through time, despite historically attested waves of colonization and social turmoil (2020). However, there is some evidence for worsening conditions (using stress indicators as a proxy) from the Roman to Medieval period in Latium, near Rome (Belcastro et al., 2007); this finding suggests that the periphery of Rome may have experienced greater devastation, but the impact of the Classical to post-Classical transformation may have been felt less strongly with increasing distance from Rome proper, such as the Tuscan countryside.

The diachronic analysis shares many of the limitations as Research Question 3 with respect to sample size and statistical power, so I will not go into too much detail here.

Importantly, the sample sizes are very disparate between Phase I and Phase II, with much smaller samples in Phase I. Not only is the extremely small Phase I sample in the craniometric analysis (n=4) unrepresentative of the overall variation in the cemetery during that period, there are also

statistical concerns associated with comparing a small sample to a substantially larger one using MANOVA. Therefore, it is possible that the observed lack of difference is a byproduct of the sample and statistical limitations. Further, porotic hyperostosis and linear enamel hypoplasia of the mandibular central incisor are entirely absent in Phase I, making those analyses especially tenuous. Finally, the Phase categorization may be a semi-arbitrary delineation that does not adequately align with the biocultural shifts occurring at the site, consequently limiting the capacity for a fine-grained diachronic analysis.

# **Broader Implications**

This research has a number of methodological lessons and contributions that can be applied to future studies. First, the results highlight the value in partitioning datasets to uncover hidden heterogeneity. The significant difference found in linear enamel hypoplasia between African and European affinity groups for the maxillary central incisor may indicate some degree of different life experience between affinity groups. Although methods for estimating affinity undoubtedly need improvement, this study provides preliminary evidence for the value of considering the influence of affinity on susceptibility to skeletal stressors.

Second, this study stresses the importance of taking a regional perspective, specifically with regard to Late Antiquity and the Early Middle Ages. Riccomi notes the value in a regional approach, as it is understood that the impact of the end of the Western Roman Empire differs, in part, based on proximity to the core of the empire versus the periphery (2021). The present research, in conjunction with several similar studies, contributes unique, regional, osteological evidence for varied living conditions throughout the Late Antique and Early Medieval eras, rather than supporting a universal collapse or stagnation, which has been hypothesized by Ward-

Perkins (2005). Thus, this dissertation shows that there is value in employing a regional level of analysis when attempting to understand the "big picture."

Additionally, the findings related to estimating affinity within the cemetery present a "cautionary tale" for the utility of classification methods; as described previously, these methods will always produce a classification, even if the unknown does not belong to one of the reference samples in the model. Consequently, it is crucial to use caution and avoid overinterpreting the results of classification statistics, especially when the reference samples used are unlikely to represent the true affinity of the unknown. In the present study, classification methods should be viewed as a heuristic device that provides a framework for interpreting sample variation as opposed to a binary classification of African or European ancestry.

This study also has modern relevance, despite its focus on the past. In an analysis of race and religion in Medieval England, Lewis introduced the idea of Africans as "officially absent although actually present" (2016). This concept was acknowledged by Redfern and Hefner as guiding their study of ancestry in Medieval London, noting that anecdotal evidence of African presence in Medieval cemeteries was not supported by published evidence (2019). The broad failure of bioarchaeologists to identify and record the presence of Africans in Europe during the Classical and Post-Classical periods perpetuates the invisibility and erasure of these migrants and their descendants, contributing to further marginalization, exclusion from academic discourse, and ignorance of their lived experiences. Perhaps even more nefariously, a whitewashing of the Middle Ages has been used by the Far Right to support White supremacist ideas of national identity and cultural heritage; excluding the non-White "other" from history thus legitimizes their modern racism and xenophobia from their perspective (Elliott, 2018). For Italy–a country where Mussolini took great pains to instill a national identity and a connection to the Romans–

populism, xenophobia, and far-right thinking are again on the rise (Dotti, 2020; Favero, 2010; Fazzi, 2015). Despite the modern Italian genome showing contributions from the broader Mediterranean, including North Africa (Antonio et al., 2019), there are still factions who legitimize their racism and xenophobia with concepts of a White Rome and a White Middle Ages. Although the present study is relatively small in scope, it is my hope that it provides an opportunity to record the "official presence" of Africans in Late Antique and Early Medieval Italy.

#### **Future Directions**

The limitations of the samples and methods used in this dissertation have been detailed in previous chapters (Chapter Two: Bioarchaeological Approaches to Biological Distance and Skeletal Stress and Chapter Five: Materials and Methods), and this discussion has included an acknowledgement of the methodological and sample-related issues specific to each research question and dataset that may lead to alternate interpretations. In light of these constraints, I recommend several avenues of future research that may help overcome these limiting factors. These including improving reference samples and comparative data, expanding analytical approaches, integrating additional lines of evidence, and providing a more in-depth analysis of stress indicators.

In order to expand reference samples and comparative data, future research should include additional skeletal material. Excavations are currently underway in and near *Rusellae*, which have uncovered additional burials in both the urban and suburban contexts (Campana, 2022). Incorporating this material into future biodistance and stress studies will not only improve sample size, potentially allowing for more rigorous statistical analysis, but also may allow for a comparison of urban, suburban, and rural contexts of *Rusellae*. Additionally, adding reference

samples from more contemporary datasets near *Rusellae* will allow for a more fine-grained biological distance analysis. Nearby sites, including Cosa, have shown preliminary evidence for individuals with African affinity (Fentress, 2003), which would provide a useful craniometric comparison with *Rusellae*. Finally, should Late Antique or Early Medieval North African craniometric reference samples become available, particularly from Roman Tunisia, these would be invaluable comparative material, providing a better approximation of the possible range of homelands for migrants in Tuscany.

Additionally, future research could expand analytical approaches. For example, geometric morphometric methods have been utilized to study craniofacial variation in Roman Italy (Hens & Ross, 2017). Three-dimensional cranial shape data are available for Rusellae, as the craniometric variables in this study were collected using a 3-D digitizer. This avenue of research would allow for a more nuanced shape analysis of the crania, as opposed to the size data used here. This may allow for an evaluation of more subtle morphological differences between individuals and between phases, and the wireframe models produced through geometric morphometric analyses provide a good visual representation of any variation. A spatial analysis of the cemetery would also be interesting, as it could contribute additional data towards an understanding of how age, sex, affinity, stress, and phase relate to cemetery orientation. Finally, incorporating a paleodemography approach and including subadults in the analysis would allow for a finer analytical resolution regarding age-at-death distributions, sex ratios, and mortality curves. Some skeletal stress indicators have shown a relationship to age (Redfern et al., 2023), so evaluating the age distribution of the site will provide important context. Further, examining mortality in addition to skeletal stress indicators could illuminate hidden shifts in health patterns

and may also reveal sex- and age-specific differences that are invisible in the paleopathology data.

Another way to overcome some of this study's limitations would be to integrate additional lines of evidence. Adding an ancient DNA analysis, particularly for those individuals with estimated African affinity, could help illuminate whether the genetic data are concordant with craniometric information and provide further evidence for long-distance migration in Late Antiquity and the Early Middle Ages. Analyzing pathogen DNA could also provide insight into the disease environment in which these individuals lived; malarial parasite DNA has successfully been identified in archaeological populations (Celuzza et al., 2023). Similarly, stable isotope analyses for understanding both migration and diet would provide critical information to this study. Not only can stable isotopes help identify instances of ancient mobility, they can also help understand whether individuals are first-generation migrants or, perhaps, are the descendants of migrants. Stable isotope analyses of dietary behavior would also provide important context for understanding stress indicators in relation to nutrition as well as sociocultural differentiation based on dietary patterns. Finally, it would be useful to incorporate nonmetric data, including macromorphoscopic traits, to add to an analysis of population affinity; ascertaining whether the metric and morphological traits are concordant in this sample is broadly relevant to biodistance and forensic research programs.

A further avenue of research involves a more in-depth avenue of stress indicators. First, additional indicators of stress and disease can be included, such as caries and antemortem tooth loss, which might provide more information regarding dietary behaviors and the degree of carbohydrates in the diet. Additionally, acknowledging and analyzing the differences between active, healing, and healed lesions would help understand what phase of life the stress event

occurred in, which would help narrow down whether most stressors are occurring during development or as a result of disease processes later in life. The sample size for the present study was too small to partition the data this way, but should more skeletal material become available, it will be important to analyze the pathology data using this approach.

#### Conclusion

The present dissertation contributes preliminary data towards a better understanding of the nature of the transition between Classical and Post-Classical Rusellae through an analysis of biological distance and skeletal stress indicators. The key findings ultimately paint a picture of a diverse and resilient town that was influenced by, but did not succumb to, conquests and political upheaval, migration, changing settlement structure, and environmental and ecological stressors. The lack of diachronic change in skeletal stress indicators and craniometric variation suggests a degree of biological, ecological, environmental, and psychosocial stability throughout Late Antiquity and the Early Middle Ages. Further, it seems that migration, both before and during the cemetery's use, introduced biological diversity into the population, while a general continuity with Roman ideals and the growing importance of Christianity seem to have encouraged assimilation instead of social stratification. These outcomes are consistent with Brown's conception of a "long Late Antiquity" that emphasizes cultural continuity with Roman times through the Middle Ages (1971), and Horden and Purcell's notion of the Mediterranean region as comprising ecologically diverse microregions between which exchange and connectivity ebbed and flowed, but never entirely ceased (2000). Thus, the totality of the evidence supports a view of the transitional period between the 5th and 12th centuries, not as a "Dark Age" characterized by collapse, but as an era of continuity and resilience for Rusellae.

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