AN ANALYSIS OF MORPHOLOGICAL DIFFERENCES IN THE FEMORAL DIAPHYSEAL MIDSHAFT BETWEEN FOSSIL AND MODERN HUMANS

A Thesis

by

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ABSTRACT

The understanding of morphological variation between fossil and modern humans is critical to the discussion of evolutionary processes within the *Homo* lineage. Neanderthals are recognizably distinct in their morphology from Late Pleistocene *Homo sapiens* and recent modern humans, especially in the femoral midshaft cross-section. These lineages are often assumed to be independent when applying statistical methods to account for these morphological differences. The shared evolutionary history of fossil and modern humans, however, increases the likelihood that the distinction in observable traits cannot entirely be attributed to divergent selective pressures; morphological variation is obscured by phylogenetic signal and therefore violates assumptions of statistical independence. In order to understand the observed variation between these groups, phylogenetic signal must be taken into account.

To test for phylogenetic signal in the femoral cross-section in recent human evolution, geometric morphometric shape data was taken from fossil groups (Neanderthals and Late Pleistocene *Homo sapiens*) and compared to a global recent modern human sample. This shape data was isolated from other morphological constraints through Procrustes superimposition and mapped onto a phylogeny created from mitochondrial genomes from geographic and temporally comparable populations. The trends in femoral midshaft cross-sectional shape variation were examined through Principal Component Analyses and Canonical Variate Analyses and showed concentrated shape change in the region of the pilaster. A permutation test indicated that phylogenetic signal is present in the femoral midshaft shape. The presence of this signal between fossil and modern human groups stresses the importance of taking into account, and controlling for, shared evolutionary history in comparative analyses. In order to understand the nature of the phylogenetic signal present in the femoral midshaft, independent contrasts were calculated and a multivariate regression was performed to test for the impact of evolutionary allometry.

The results showed that allometric changes throughout modern and fossil human evolutionary history had an insignificant impact on changes in shape, explaining only 20% of shape variation. This, therefore, suggests that the observed shape changes at the femoral midshaft between these groups are not due to evolutionary allometry but can be attributed to other factors, such as behavioral, genetic, or environmental pressures.

For Goldia.

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CHAPTER I

INTRODUCTION

Since their first recognized discovery in the Neander Valley, Germany in 1856, Neanderthals have been the center of controversy in both academic and social circles (Trinkaus and Shipman, 1992). While the question of whether or not Neanderthals are members of our own species is often argued on morphological, genetic, and behavioral grounds, what cannot be argued are the marked distinctions in morphology that indicate unique evolutionary histories. Given the problematic application of species concepts on fossil groups, Trinkaus et al. (2002) have argued that the question of speciation is no longer important as compared to what can be understood about evolutionary processes through studying the morphological variation of fossil and modern humans.

One critical way in which Neanderthals and modern humans are distinct is in their postcranial morphology. Understanding the evolutionary processes that impact postcranial changes in a population can shed light on behavior, cultural practices, life history traits, overall health, and environmental adaptations within that population or between that population and another. Studies into the biomechanics of Neanderthals have also shown that their range of movement, long bone strength, and dexterity is within the range of human variation (Trinkaus, 1983; Trinkaus and Rhoads, 1999; Niewochner et al., 2003; Cowgill, 2010). Postcranial studies taking into account environmental adaptation have suggested variation among Neanderthals, especially in regards to body proportions and robusticity, is primarily due to their interaction with a cold environment (Churchill, 1998; Tilkens et al., 2007; Pearson, 2000; Weaver, 2009; Churchill, 2008). This hypothesis has been called into question, however, suggesting that Neanderthal body proportions may also be due to a decrease in their mobility when compared to other modern humans and possible changes in the endocrine system, though these latter hypotheses are arguably less supported (Holliday and Falsetti, 1995; Churchill, 1998; Weaver and Steudel-Numbers, 2005). Through postcranial studies of

Neanderthals and modern humans, much has been discerned about both groups' evolutionary histories and adaptations to their environments, including possible interactions between them.

While both Neanderthal and modern human femoral midshaft shapes have been the topic of comparative analyses before (Trinkaus, 1975; Trinkaus and Ruff, 1999: Holt, 2003), the underlying sources of the observed variation have been difficult to discern and have been attributed to behavior (Holt, 2003; Ruff et al., 2006), climate (Weaver, 2003), and developmental processes (Cowgill and Hager, 2007). Notably, the role that shared evolutionary history plays between these groups in their observed morphological similarities and differences has never been tested.

The majority of statistical methods used in comparative analyses often assume independence between the traits that are in question. This becomes especially problematic when individuals, populations, or species are closely related. Due to the shared evolutionary history, these groups display traits that have been shaped by the same processes, violating this assumption of independence (Felsenstein, 1985; Cheverud et al., 1985; Gittleman and Kot, 1990; Harvey and Purvis, 1991). Felsenstein (1985) suggests that prior to comparing traits between or within groups (or between a group as compared to its environment), this shared evolutionary history, or phylogenetic signal, must be accounted for. The effect of shared evolutionary history can be observed by comparing the differences in these traits to a phylogenetic tree, which provides the evolutionary context to test for signal (Purvis et al, 2004; Cheverud et al., 1985; Felsenstein, 1985).

Phylogenetic signal has been part of the scientific discourse in the biological sciences since Cheverud et al. (1985) and Felsenstein (1985) brought the problem of assumed independence forward and suggested methods to resolve them. Since the 1980's these methods have been refined and adapted to be applied to different types of traits (Smith and Cheverud, 2002; Cardini and Elton, 2008; O'Neill and Dobson, 2008; Young, 2008). Tests for phylogenetic signal, however, are rarely applied in Paleoanthropology, usually due to a dearth of ancient genetic information on which to create a reliable phylogeny. However, given that the strength of phylogenetic signal is significantly increased between closely related groups, it becomes critical to understand its impact in such closely related groups as Neanderthals and modern humans (Harvey and Purvis, 1991). Once this phylogenetic signal is accounted for, true morphological differences, such as femoral midshaft shape, can be examined in respect to evolutionary processes and meaningful applications of the comparative method.

The comparative method uses statistical analyses to establish similarities and differences between groups of populations and species in order to understand their relationship to one another, their environment, and possible adaptive processes acting on the observed variation. Studying this variation, how it changes, and what impacts it, can also aid in the understanding of fossil groups where this data may not be readily available (Sanford et al., 2002). This is arguably the most important method in Paleoanthropology, as fossil groups are typically compared to recent human groups in order to make inferences about evolutionary change, selective processes, and adaptive strategies in human evolutionary history.

Phylogenetic signal in the context of the comparative method is particularly critical in shaping our understanding of femoral midshaft morphology. Previous studies have shown that fossil humans, specifically Neanderthals, exhibit a rounder midshaft shape with an increased thickening of the cortical bone at the cross-section (Ruff et al., 1993; Trinkaus and Ruff, 1999; Trinkaus et al., 2002). The morphology of modern humans as observed today is somewhat different, with the most significant difference showing the presence of a pilaster, a bony posteriorly oriented protrusion for muscle attachment (Pauwels, 1980; Trinkaus, 1976) and a reduction in the thickness of the cortical bone (Ruff et al., 1993). The change in shape of the femoral midshaft has important implications for the femur due to the biomechanical stresses placed on the lower limb during locomotion. The changes in the pilaster, namely its appearance in modern humans, suggest that the pressures placed on the femur from the associated musculature have changed, accentuating the importance of resisting anterioposterior stress (Pauwels, 1980; Amtmann, 1971; Ruff et al., 1993). Variation in the shape of the femur, especially in diaphyseal thickness, has also been associated with possible changes in activity levels as well as in structure in overall Neanderthal postcrania (Trinkaus 1976; Trinkaus and Ruff, 1999; Stock and Pfeiffer, 2001; Ruff et al., 2006). Therefore, it is important to understand the extent to which the observed shape of fossil and modern humans truly reflects the evolutionary processes acting on the lower limb.

The purpose of this study is to examine the variation found in femoral midshaft shape between fossil and modern humans and to examine these data for phylogenetic signal present between these groups. The understanding of the effect of phylogenetic signal on femoral midshaft shape is critical to the interpretation of the observed variation between these groups and can shed light on the impact of the evolutionary processes shaping this variation.

In order to account for phylogenetic signal in the femoral shape of fossil and modern humans, I use midshaft shape data from European and West Asian Neanderthals, Late Pleistocene *Homo sapiens* (LP*Hs*), and a Neolithic archaeological sample as well as corresponding mitochondrial DNA sequences to account for evolutionary history in a phylogeny. This research will also address the phylogenetic relationship between evolutionary allometry and the observed femoral midshaft shape; in other words, the impact that changes in size have had on the observed femoral shape once their shared history has been accounted for. The results will clarify the presence of phylogenetic signal among these closely related groups and whether or not their morphology at the midshaft is independent of one another and from changes in body size. Overall shape comparisons will allow for an understanding of shape change between fossil and modern humans that can aid in the understanding of what processes have shaped the variation in morphology observed between and within these groups.

CHAPTER II

POPULATION DIFFERENCES BETWEEN NEANDERTHALS AND LATE PLEISTOCENE *HOMO SAPIENS* WITH EMPHASIS ON THE POSTCRANIA

Prior to the examination of the variation within and between fossil and modern human populations, it is important to explain the classifications used here. In this study, Late Pleistocene *Homo sapiens* will be used to refer to the fossil specimens that share morphological characteristics with recent modern humans and have been classified as *Homo sapiens* taxonomically. This terminology includes individuals from Middle and Early Upper Paleolithic populations, both of which are included here. Additionally, the term "fossil human(s)" refers to all fossil specimens outlined in this study and includes Neanderthals and LP*Hs*. Specimens taken from Neolithic or recent archaeological sites who fall within the same range of variation as is shared by recent populations of humans are referred to as modern humans or recent modern humans.

Although the controversy surrounding the species designation of Neanderthals is far from resolved, the distinctiveness in both their genetic and morphological aspect is undeniable (Schwartz and Tattersalll, 1996; Harvati et al., 2003; Serre et al., 2004; Smith et al., 2005; Briggs et al., 2007; Fu et al., 2014; Fu et al., 2015). With the addition of ancient nuclear DNA and the discovery of the presence of Neanderthal variation in modern human non-African populations, it can be argued that questions of admixture related to species designations have changed to focus on the importance of the contribution of Neanderthals to modern human evolutionary history (Green et al., 2010; Prüfer et al., 2014; Fu et al., 2015). This paper takes the suggestion of Trinkaus et al. (2002) that one should focus on the evolutionary histories and morphology of Neanderthals and LP*Hs,* rather than on the significance of a species designation.

This study will focus on postcranial morphology, specifically on aspects of the cross-section of the midshaft of the femur. It is important to address the evolutionary history of each group outlined above to better understand the relative importance of measures of similarity and difference in all aspects of their morphology. I will emphasize those postcranial characteristics which relate to theoretical perspectives associated with the unique shape of the Neanderthal femur as compared to modern humans, specifically its circular cross-sectional shape at the midshaft. These perspectives include cold-adaptation, behavioral implications, resource exploitation, and several biomechanical implications of postcranial differences between Neanderthals and modern humans. Models and hypotheses such as these are critical to the understanding of midshaft variation once shared evolutionary history has been accounted for. Taking into account evolutionary histories as well as theoretical models and hypotheses to understand femoral midshaft shape can aid in uncovering the unique evolutionary processes and selective pressures acting on these populations, such as why the suite of characters that make up the Neanderthal morphological pattern disappeared nearly 30,000 years BP.

Morphological Trajectories in Fossil Human Evolutionary History

Neanderthal morphology is distinct from modern humans in both the crania and the postcrania. Cranial distinctions include a large brain case with a sloping forehead with pronounced and divided supra-orbital tori, mid-facial prognathism, a large nasal aperture, the lack of a mental eminence, and a retromolar gap. In terms of the postcrania, Neanderthal morphology includes a marked increase in overall robusticity, enlarged joint surfaces, barrel-shaped chests, curved femora, and a broad pelvis (Smith, 2013). The earliest evidence of the emergence of Neanderthal morphology has been proposed in Europe as early as 600 kya BP in a cranium found in Reilingen, Germany. Dean et al. (1998) suggest that the specimen shares morphological affinities with both Neanderthals and fossil forms of the genus *Homo*. The suite of morphological characters found in this specimen does not indicate that it is entirely a Neanderthal, but it does exhibit a high frequency of characteristics similar to what is found in Neanderthal populations (divided supraorbital tori, pronounced suprainiac fossa, and an occipital torus) (Dean et al., 1998).

Though these arguments have been made for Neanderthal features appearing in Europe as early as the late Pleistocene, the place of this and similar specimens in

Neanderthal emergence and human evolution is still somewhat unclear (Stringer, 1994; Smith, 2013). Specimens from Gran Dolina in Sierra de Atapuerca in Spain, have been cited as presenting with cranial features similar to modern humans and Neanderthals, but with unique autapomorphies (Bermúdez de Castro, 1997; Arsuaga et al., 1997; Arsuaga et al., 2014). This led to the introduction of a new species, *Homo antecessor,* which has been suggested to be the common ancestor of Neanderthals and modern humans (Bermúdez de Castro, 1997). While not widely accepted as a new species or a common ancestor, the features found in *H. antecessor* lend credence to an accretion model of Neanderthal emergence, trending towards the development and eventual appearance of Neanderthals on the landscape (Smith, 2013). This is countered by another model suggesting that Middle Pleistocene *Homo* is the last viable common ancestor between the two groups (Stringer, 1994; Rosas et al., 2006; Hublin, 2009; Smith, 2013). Though it is difficult to definitively say which model is correct, especially given what and when one defines the emergence of a new bauplan on the landscape, it is clear that the evolutionary history between these groups is both recent and closely intertwined.

Evidence for the first Neanderthals, referred to as the “Early Neanderthals,” that begin to show a suite of distinct characteristics, dates to MIS 7-6, or approximately 230 kya BP. By MIS 5, the full suite of the Neanderthal traits (including changes to the cranial vault) can be seen in the fossil record (Hublin, 2002; Hublin, 2009; Smith, 2013). This is the development of the “Classic” Neanderthal and is the group that comprises the morphological sample in this study. The earliest Neanderthals first occupied Europe and moved into West Asia approximately 170 kya BP. This migration has often been cited as the reason for cold adaptation in Western Asian Neanderthals (Smith, 2013). Modern humans moved into Europe far later than in Asia (approximately 40 kya BP) and as Neanderthals’ last footholds were in Europe until approximately 30 kya BP, sufficient temporal overlap in this region increases the likelihood of interaction and interbreeding between them (see below) (Autunes and Cunha, 1992; Trinkaus et al., 2003; Higham et al., 2011; Smith, 2013); the ability for considerable Neanderthal contribution still holds with estimates of earlier disappearance dates (around 40kya) of their populations from some parts of Europe (Higham et al., 2014).

The geographic range of the Neanderthals has been suggested to be restricted to European and Western Asia, specifically, below 55° N latitude and north of Africa, with an Eastern border marked by Teshik Tash in Uzbekistan, which, if accurate, greatly limits their ability for interaction and contribution to the evolutionary lineage of modern humans (Cartmill and Smith, 2009; Smith, 2013). This range has been challenged by recent discoveries, not only through the reassessing of the taxonomic classification of Teshik Tash as a Neanderthal (Glantz et al, 2009), but through the presence of a genome showing admixture with Neanderthals in the Denisovan cave in Siberia (Reich et al, 2010). The eastern range of Neanderthals has also been recently called into question by Li et al. (2017), who have described two crania that share Neanderthal characteristics discovered in Xuchang, China. These crania from western China have not been classified as Neanderthals, but have been reported to share some of the suite of traits that identify Neanderthal morphology, specifically those found in the occipital and temporal regions of the cranium. The expansion of the Neanderthal range can have significant implications for the adaptive strategies and techniques associated with Neanderthals. Given the estimated age for the Xuchang 1 and 2 crania (125 to 105 kya BP per Li et al. (2017)), it is possible that Neanderthals were capable of moving and interacting across all of Europe prior to the arrival of modern humans. This could also call into question the role that cold adaptation plays on Neanderthal evolutionary history and survival, as well as the interaction and impact of Neanderthals on modern humans and vice versa.

Contributions of Neanderthals to modern human evolutionary history are especially important in terms of analyzing phylogenetic signal, and this has been demonstrated in both morphological and genetic contexts. These contributions can be best represented by fossil remains that shows evidence of hybridization between these two groups, even before genetic data could weigh in on the question. The first of these individuals, Lagar Velho I, was categorized as a hybrid between LP*Hs* and Neanderthals through the assessment of the morphology alone. An approximately 4-year-old child was uncovered in the Lapedo Valley in the Abrigo do Lagar Velho in Portugal in 1998 (Zilhão and Trinkaus, 2002). The child has been dated to approximately 24,500 years BP and is buried intentionally with ochre and shells pointing to the Gravettian (Duarte et al., 1999). The skeleton shows a remarkable association with LP*Hs* as well as Neanderthals. Duarte at al. (1999) associate it with LP*Hs* through the features of the dentition (especially the proportions), the mandibular ramus, specific features of the radius (the curvatures and radial tuberosity), as well as the breath of the pubic bone. The authors also align Lagar Velho 1 with the Neanderthals primarily through its hyperpolar body proportions and the relative lengths of the femur to the tibia (Duarte et al., 1999). This association is further supported by the lack of hyperpolar body proportions in other LP*Hs* at the time, especially given the amount of time modern humans had occupied the region and the lack of plasticity in this trait. With the young age of the burial relative to the last Neanderthals in the region (approximately 30 kya BP), this child is likely the result of many generations of hybridization between these groups (Duarte et al., 1999; Zilhão and Trinkaus, 2002). The child of Lapedo Valley demonstrates that the shared evolutionary history of Neanderthals and LP*Hs* is even more complex than previously thought, and along with a common evolutionary lineage, there is more recent admixture that may inflate phylogenetic signal between them.

Another individual that demonstrates Neanderthal contribution to modern human evolution initially was argued as a hybrid between the two groups on the basis of morphological grounds. Trinkaus et al., (2003) analyzed the morphology of one of the oldest specimens representing modern human movement into Europe, Oase 1 from Romania, directly dated to around 35 kya cal BP. The authors show that the morphology of this individual is varied and contains features similar to modern humans and Neanderthals, as well as intermediate structures, perhaps indicative of its hybrid ancestry. Several features of the mandible associate the individual with modern humans (the narrow corpus, a medially located condyle, and no retromolar gap), while analyses of the proportions related to the size and shape of the mandible associate it with Neanderthals (Trinkaus et al, 2003). The mandibular symphyseal cross-sectional measurements are not similar to either group but fall in-between them (Trinkaus et al., 2003). The mixed morphology continues onto the cranium and is demonstrated by Rougier et al. (2007). Unlike the mandible, the proportions of the cranium are more similar to that of modern humans rather than Neanderthals (the lack of a supraorbital torus and the clear presence of canine fossae), but there are traits associated with Neanderthals as well (the juxtamastoid eminence and a more Neanderthal-like sagittal frontal arc). The presence of both Neanderthal and modern human morphologies led to hypotheses that this was a hybrid between the two groups and is evidence of Neanderthal impact on modern human evolution (Trinkaus, 2005). This hypothesis was tested genetically and Fu et al. (2015) found a significant amount of Neanderthal DNA in the Oase 1 nuclear genome. The amount of Neanderthal ancestry was great enough to indicate this individual was a 4th-6th generation hybrid. Though the genetic variants found in Oase 1 have not been found in modern humans, this shows that Neanderthals likely did contribute in some way to modern human history and that these offspring were viable.

Genetic Trajectories in Fossil Human Evolutionary History

Ancient DNA has, in the last 20 years, become critical in the examination of shared history between modern and fossil human groups, both in their recent contact and deeper in evolutionary time. Initial studies of ancient Neanderthal DNA centered around the mitochondria due to its higher rate of recovery in the cell (Krings et al., 1997). Comparisons of Neanderthal mtDNA to that of modern humans showed a lack of similarities between the genomes, which led to conclusions that Neanderthals did not contribute meaningfully to modern human evolution (Krings et al., 1997; Serre, 2004). Mitochondrial studies have also estimated Neanderthal and modern human divergence dates, some of which suggest Neanderthals and modern humans diverged before admixture could take place, as far back as 500,000 years, though the dates from genetic data vary greatly (Krings et al., 1997; Ho et al., 2005). Mitochondrial studies are supported by Y-chromosomal DNA studies, which also show a lack of Neanderthal contribution to modern human Y-chromosomes (Mendez et al., 2016). As Mendez et al. (2016) point out in their analysis, the lack of Neanderthal Y-chromosomal DNA in modern humans does not necessarily indicate a lack of admixture or contribution to modern human evolutionary history, but could be due to a lineage that has died out, thus the haplotypes are no longer visible in current populations (though, the authors also show that this is complicated by the discovery of specific mutations in the Neanderthal Y-chromosome that may indicate infertile male offspring, therefore inhibiting Neanderthal contribution to the modern human genome, even within extinct lineages). This can be applied to mtDNA observations as well, and highlights a limitation of analyzing haplotype sequences transmitted through one parent; both mitochondrial and Y-chromosomal DNA Neanderthal contributions could be eliminated as early as the F2 generation in a single lineage and nuclear DNA information would still be observed.

Nuclear DNA of Neanderthals was sequenced after the initial studies of mtDNA and yielded surprising results given the consistent absence of Neanderthal mtDNA in modern human sequences. Green et al. (2010) showed that non-African populations of recent modern humans shared approximately 1-4% of their nuclear DNA with Neanderthals, suggesting that their genetic influence is still present in modern human genomes. Though this discovery has changed the conversation in the field of Paleoanthropology from whether or not Neanderthals and LP*Hs* admixed to how much contribution could be attributed to the former, the Neanderthal individuals analyzed are still more closely related to each other than they are to modern humans, demonstrating a unique evolutionary lineage; however, they are more distinct from African modern humans than non-African individuals (Green et al., 2010). Studies like this reinforce the need for understanding phylogenetic signal in groups with recent evolutionary divergence and even more recent admixture. It is important to mention that Neanderthals show genetic variation across their range, indicating reduced gene flow between Eastern, Southeastern, and Western Asian Neanderthals (Fabre et al., 2009). This coincides with morphological variation between these groups, with Western Asian Neanderthals exhibiting variation in the expression of traits, such as cold adapted body proportions

(Holliday and Trinkaus, 1991). Recent studies have examined genetic similarities between fossil and modern humans in greater depth and have even begun to analyze the impact of specific gene variants on modern human adaptation, including genes that control the formation of keratin (Sankararaman et al., 2014) and genomic patterns associated with health risks like depression (Simonti et al., 2016). As Neanderthals' contribution to modern and fossil humans becomes more recognized, assuming independence in comparative studies between these groups becomes increasingly difficult.

Neanderthals and modern humans have a complex history involving genetic and morphological distinctions as well as a significant, and even surprising, number of similarities. Smith (2013) has even suggested that the perseverance of Neanderthal genes in modern human populations not only impacts the interpretation of the evolutionary history of both groups, but also changes the narrative of the extinction of Neanderthals, suggesting that they are not, in fact, extinct. The persistence of Neanderthal elements in modern genomes reinforces the intersection of these evolutionary lineages, making the impact of their evolutionary history on fossil and modern human morphology more likely.

Postcranial Models on Femoral Morphology

The understanding of evolutionary factors impacting the morphology of the femur is critical when assessing midshaft cross-sectional shape once the shared history described above has been accounted for. Studies of postcranial variation throughout human evolutionary history have often been complicated by the lack of preservation of postcranial elements, especially given variable preservation environments and the deep evolutionary time involved with the genus *Homo.* While these limitations apply to the study of Neanderthal postcrania as well, the relative recent history of this group of humans allows for a more detailed examination of their postcrania. In conjunction with cranial analyses, paleoanthropologists can better understand the evolutionary pressures acting on the lineages of modern and fossil humans that not only led to their differential survival, but also made modern human morphology uniquely derived from patterns of earlier members of the genus *Homo* (Trinkaus, 2006).

Regardless of whether or not Neanderthals and LP*Hs* are considered members of the same species, the morphological distinctions between the postcrania of these two groups has been well established (**Figure 2-1**). Though initially described as pathological (see Trinkaus and Shipman (1992) for a detailed description of the history of the Neanderthals' interpretation and acceptance), it is now understood that Neanderthal postcranial morphology is similar to modern humans in regards to their biological capabilities and ranges of motion, with minor distinctions due to recent changes in the evolutionary histories between this group and modern humans (Trinkaus, 1983). Although Neanderthals have been characterized as sharing traits with fossil members of *Homo,* emphasizing the derived traits in modern humans, the Neanderthal suite of postcranial traits is distinct, and is often associated with a "hyper-robusticity," large muscle attachments, low crural and brachial indices associated with cold-climate adaptation, barrel-shaped torsos, broad pelves, curved and round femoral diaphyses with a low neck-shaft angle (Trinkaus, 1976; Trinkaus, 1983; Trinkaus, 1993; Holliday, 1997; Trinkaus, 2006; Churchill, 2008).

Cold Adaptation

Evidence for cold adaptation in Neandertals is a long-standing hypothesis that began with the examination of their cranial morphology (Coon, 1962). Postcranial examinations of cold adaptation in Neanderthals, especially those from the western end of their range in Europe, has often been based on Allen (1877) and Bergmann's (1847) rules regarding climatic adaptation as related to body proportions. The former states that warmer adapted populations will have shorter limbs, specifically in their distal limb segments; while the latter states that the torso, or trunk, of colder adapted populations will be larger than those in warmer climates. These adaptive principles serve to reduce, in cold-adapted populations, the surface area to volume ratio in order to preserve heat. These principles have been used in the study of climatic variables on modern humans and their ancestors in conjunction with brachial and crural indices (Trinkaus, 1981). Low brachial (radius relative to humerus) and crural (tibia to femur) indices are associated with colder adapted populations (indicating longer proximal to distal limb segments) and have long been observed in Neanderthals (Fraipont and Lohest, 1887; Trinkaus, 1981). Ruff (1994) has also shown that the most correlated relationship between trunk size and cold-climate is that of the bi-iliac breadth: the broader the measure, the more positively correlated a group is with colder environments. He has used this to measure the relationship between known colder climates and the measures of bi-iliac breadths in fossil populations to determine human evolutionary trends (see below).

Trinkaus (1981) has regressed crural and brachial indices with climate to show that they are, in fact, positively associated. He has shown that Neanderthals have low crural and brachial indices indicative of a cold-adapted population and that Neanderthal indices are consistently below those of cold-adapted modern humans. When the proximal and distal limbs are compared to trunk height in Neanderthals, the trend of cold adaptation continues, though it is most significant in European Neanderthals (Holliday and Trinkaus, 1991). Western Asian Neanderthals do show cold-adapted relationships between trunk height and limb segment length, but it is not as pronounced as in European Neanderthals, further emphasizing the cold adaptation inherent in these traits. Holliday (1997) was able to further support the findings from Holliday and Trinkaus (1991) with a larger comparative modern human sample, though it did not include separate analyses for European and Western Asian Neanderthal groups.

Additionally, it is important to mention that Neanderthal body proportions have been explained through hypotheses emphasizing behavioral changes and activity in conjunction with cold adaptation (Weaver and Steudel-Numbers, 2005). This association is currently difficult to support due to a lack of correlation between body proportions and activity levels in modern humans and if it plays a role in selective pressures acting on Neanderthals, it is likely a much less significant one than cold climate.

Figure 2-1. A composite Neanderthal skeletal reconstruction shows the overall morphological differences between Neanderthals (left) and modern humans (right). (Reprinted from Sawyer and Maley, 2005; Figure 4).

Ruff (1994), as mention above, has specifically studied the bi-iliac breadth of Neanderthals and other fossil and modern humans in relationship to climate and has shown that this is a highly-correlated measurement, indicating that populations and individuals with cold-adapted bauplans have wider breadths than those who are warm-adapted. Though the sample size of Neanderthal pelves are small and sex-biased (La Chapelle-aux-Saints 1 and Kebara 2 are both male individuals), both indicated a wide bi-iliac breadth that clustered with the highest cold-adapted ranges in the modern human sample, in this case Alaskan Inuit individuals. The measures of bi-iliac breadth strongly support the cold adaptive hypotheses for the bauplans of Neanderthals, but this is difficult to extend to the rest of the Neanderthal trunk.

While estimations of the large, barrel-shaped Neanderthal chests have been associated with thermoregulatory processes, they have also been associated with high levels of activity requiring larger lung capacities (Churchill, 2008), or larger internal organs due to possible higher protein intake in their diet (Ben-Dor et al., 2016). This reinforces the importance of using a bi-iliac measure in relationship to cold-adaptive hypotheses as well as the difficulty in isolating biomechanical pressures in the hip and femur from the heavy weight of a large Neanderthal trunk.

Postcranial Robusticity

Studies of Neanderthal postcranial robusticity are frequent and well-established in the literature and have often centered around this distinction in the femur (Trinkaus, 1976; Trinkaus, 1983; Ruff et al. 1993; Pearson, 2000). Robusticity, as defined by Ruff et al. (1993), refers to the biomechanical reinforcement or additional strength given to a portion of the skeleton when bone tissue is added to it. Measures of long bone robusticity have often included comparisons of diaphyseal size to overall bone size, and more recently, have included cross-sectional properties in order to test bone resistance to biomechanical loading (Stock and Shaw, 2007). The increased robusticity, or hypertrophy, of the Neanderthals can be seen throughout their skeleton, and particularly in their large muscle attachments (suggesting a heavily muscled form supported by the robust bones) (Trinkaus, 1983). Not only does this indicate an increase in overall muscle mass in a Neanderthal frame, but this robusticity could aid in reinforcing and strengthening the lower limb. The impact of overall robusticity is important to the morphology and understanding of Neanderthal and modern human evolution, but for the purposes of this study and the discernment of the changes in shape in the femoral midshaft, the focus has been placed on robusticity in the lower limb.

Trinkaus (1983) emphasized the increased robusticity throughout the Neanderthal lower limb, stressing the importance of the femoral head in strengthening the hip joint, the robusticity of the knees in aiding in increased power relative to modern humans, strengthened tibiae to resist torsional stress, and even increased robusticity in the foot. The significance of this robusticity, however, has been called into question in more recent studies. While overall robusticity has been declining throughout the history of the genus *Homo* (Ruff, 2008), comparisons of robusticity and measures of strength between Neanderthals and modern humans have shown comparatively little difference between them once body size has been controlled for. Trinkaus and Ruff (1999) found that lower limb robusticity, especially in the tibia, can be greatly impacted by body proportions. When body mass was taken into account, differences in femoral and tibial shaft strength were no longer significant. Subsequent studies of the femora and tibia between Neanderthals and modern humans continue to support a lack of increased strength and robusticity using this method (Trinkaus et al., 2002).

Trinkaus and Rhodes (1999) used biomechanics to test the strength of the knee and previous assumptions of increased Neanderthal strength in that joint relative to modern humans. As with previous studies of the femur and tibia, when body mass is controlled for, the hypothesized increase in strength of the joint (as observed through patellar thickness and measurements of the tibial condyles) is no longer supported. It is important to note that fossil humans (both LP*Hs* and Neanderthals) appear to have stronger knee joints than recent modern humans, suggesting a cause for these postcranial changes unrelated to the differences between them.

The observed robusticity in the Neanderthal foot, however, has been supported in comparative analyses. Trinkaus and Hilton (1996) examined the biomechanical properties of the proximal phalanges of the Neanderthal foot and found that the shape of the phalanges indicated a broader medio-lateral reinforcement with shorter shaft lengths. This robusticity was significantly larger than modern humans, though LP*Hs* comparisons where not nearly as divergent. Trinkaus and Hilton (1996) suggest that these changes in phalangeal morphology indicate a greater strength in reinforcement against biomechanical pressures created by the tendons; the more medio-laterally expanded cross-section would also resist both medio-lateral and tensional forces acting on the foot during habitual behavior, such as locomotion. Contrary to comparisons on the knee, however, this distinction suggests changes in the patterns of biomechanical forces between Neanderthals and modern humans rather than possible differences (such as behavior or activity level) between LP*Hs* and recent modern humans.

While studies of robusticity are dependent upon multiple factors, such as body size and mass, a general trend of decreasing robusticity throughout the history of the genus *Homo* can be seen, especially in recent populations (Ruff, 2008). Recent studies, such as Shackelford (2007), however, have supported analyses mentioned above in the importance of recent modern human changes in robusticity as opposed to LP*Hs* and Neanderthals. In understanding the importance of robusticity in human evolution, it may be more important to look at global events, such as the Last Glacial Maximum, where changes in lower limb robusticity and strength may be more significant in human evolutionary history (Shackelford and Trinkaus, 2002; Shackelford, 2007).

Bio-behavioral Factors

Certain bio-behavioral factors can indirectly impact femoral midshaft morphology, specifically the interaction between Neanderthals and their environment. Long-standing views of Neanderthal subsistence strategies identified them as carnivores at the top of the food chain with a high protein diet, and it has even been suggested that a high intake of protein required a larger liver, resulting in a larger chest cavity (Ben-Dor et al., 2016). Recent studies have shown a wide range of Neanderthal resources exploited across their geographic distribution. In Gibraltar, Neanderthals have been shown to exploit marine resources (Stringer et al., 2008) and Neanderthals in Western Asia ate a variety of plant and processed foods (Henry et al., 2011), calling into question their reliance on primarily animal protein and a narrow diet. Exploiting a wider variety of resources would require variations in their subsistence strategies, and their overall larger body size could place additional strain on those strategies, impacting Neanderthal biology (See Chapter III).

Churchill (2008) was able to estimate, using bioenergetics, that the overall BMR for a Neanderthal was at higher levels than modern human Inuit populations (approximately 3500-5000 kcal/day). It is difficult to estimate whether or not this increased caloric intake can be attributed to heat regulation or requirements from internal organs and muscle output (such as a larger lung capacity or liver), but it does suggest a large requirement from the environment in terms of carrying capacity for Neanderthal groups. This is compounded by the lack of an increase in significant thermoregulatory advantages in the bauplan of Neanderthals (Aiello and Wheeler, 2002), estimated at less than 1ºC, which may have required more of a cultural buffer (such as clothing) than is typically attributed to them. This would have required more environmental resources, especially in colder climates. When combined with estimates of low population densities for Neandertals (Briggs et al., 2009; Fabre et a., 2009), it is likely that Neanderthals had to increase their range in order to find enough calories to maintain their large form. The distance and type of terrain covered could greatly impact the bone and soft-tissue structures frequently used in locomotive activities, even in populations with similar biomechanical capabilities (Stock and Pfeiffer, 2001).

Summary and Application

Because Neanderthals and modern humans have separate evolutionary lineages at least as far back as 230 kya BP, and in view of more recent evidence of admixture in the Late Pleistocene, the importance of examining shared evolutionary history when making morphological comparisons between these groups is clear. As their shared history is varied through time and is geologically recent, it is likely there is high prevalence of phylogenetic signal throughout many aspects of their morphology. However, it is important to recall that Neanderthals and LP*Hs* have statistically distinct evolutionary histories that, when signal is accounted for, can shed light on the selective pressures shaping each population. The postcranial differences between them and their evolutionary implications for affecting femoral midshaft cross-sectional shape are critical in the evaluation of the processes forming these differences once shared evolutionary history is accounted for. Femoral midshaft shape is impacted by a suite of biomechanical forces throughout the body which have various selective pressures acting on them. It is important to take into account these possible secondary impacts when considering the shape changes associated with the femoral midshaft. The hypotheses regarding the direct selective pressures on the femoral midshaft shape will be discussed below, and many of them are based in the over-arching adaptive strategies observed throughout fossil and modern human postcrania.

CHAPTER III

CROSS-SECTIONAL FEMORAL MORPHOLOGY

Among the differences in postcranial morphology between fossil and modern humans, the distinctions in the femoral midshaft cross-section have important evolutionary, biomechanical, and behavioral implications. The most noticeable, and arguably most meaningful, difference in the variation on femoral morphology at the midshaft is in the properties of the cross-sectional shape (Trinkaus and Ruff, 1999). Neanderthals are characterized by a round femoral midshaft shape that noticeably lacks the pilaster, a trait well-defined in modern humans (Hrdliča, 1934; Trinkaus, 1976; Trinkaus et al., 1991; Trinkaus et al., 2002). The pilaster is a bony protrusion in the posterior portion of the femur that is most pronounced at the midshaft (**Figure 3-1**). The presence of a pilaster has been attributed to the muscle attachments along the linea aspera in conjunction with biomechanical forces acting on the bone, and runs the majority of the length of the diaphysis creating a tear-drop shape in cross-section (Trinkaus, 1976; Ruff et al., 1993; Trinkaus and Ruff, 1999). Many studies have addressed these differences in femoral midshaft cross-sectional shape morphology, both in Neanderthal and modern human ranges of variation, attributing them to biomechanical loading patterns and muscle structure (Trinkaus, 1976; Churchill et al., 2000; Trinkaus et al., 2002), activity levels (Holt, 2003), terrain (Bridges, 1989; Ruff, 1999; Stock and Pfeiffer, 2001), climatic variation (Weaver, 2003), and even some ontogenetic factors (Cowgill and Hager, 2007; Cowgill, 2010; Michopoulou et al., 2015).

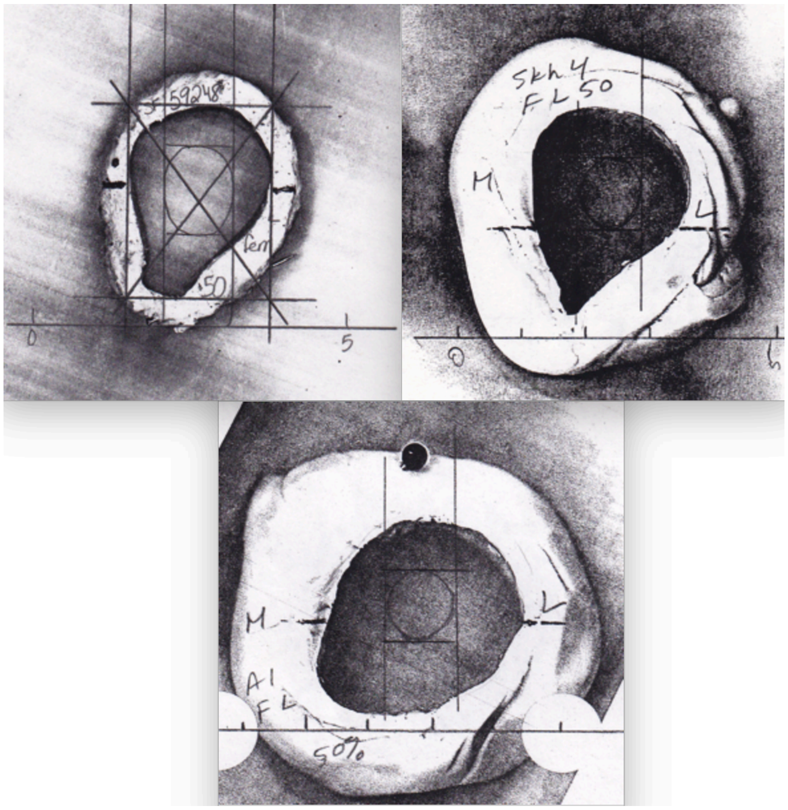

Figure 3-1. Left femoral diaphyseal midshaft cross-sections exhibiting common diaphyseal shapes between fossil and modern humans. Top left: Pre-Dynastic Egyptian, top right: Skhūl 4, Bottom: Amud 1.

Biomechanical Loading Patterns

The importance of different biomechanical loading patterns, and consequently variation in muscle and soft-tissue attachments, on cross-sectional shape, is stressed in studies of both Neanderthal and modern human morphology, spanning more than 100 years in the literature (Wolff, 1892; Amtmann, 1971; Trinkaus, 1976; Wolff, 1986; Ruff and Hayes, 1983; Churchill et al., 2000; Trinkaus et al., 2002). The biomechanical stressors along the femoral shaft, however, are not consistent. For example, a greater impact of body breadth and the type and frequency of certain activities (such as intense, infrequent behaviors like extreme jumping) have been found to alter proximal femoral shaft shape (Niinimäki et al, 2016), whereas the distal femur appears to be correlated with bipedal movement (Tardieu and Trinkaus, 1994). This reinforces the importance of isolating each area of the femur individually to understand the factors acting on it. As previously stated, the pilaster, of significant importance to the morphology of the portion of the femur addressed in this study, is most pronounced at the midshaft, defined as approximately 50% of the diaphyseal, or biomechanical, length (see Chapter V (Methods)). It is maintained due to the anterio-posterior bending stresses placed on the femur during everyday locomotion (e.g., walking and running) as well as the stresses placed on it by the anterior musculature of the thigh, for which the linea aspera serves as a point of attachment. Studies of the presence and importance of the pilaster have shown a consensus regarding femoral variation, often trending toward a less-round diaphysis throughout human history, associated with a decrease in anterio-posterior bending stress on the bone (Trinkaus, 1976; Churchill et al., 2000; Trinkaus et al., 2002; Ruff et al., 2006).

The femoral midshaft morphology in Neanderthals reflects characteristics found in earlier hominin groups and when compared to modern humans follows a similar pattern of decreasing absolute robusticity throughout the genera (Ruff, 2008). The pilaster, specifically, is a trait unique to modern humans and is absent, not only from Neanderthals, but from earlier members of the genus *Homo* (Trinkaus, 1976; Trinkaus, 2006). Trinkaus (1976) also notes a thickening of the bone on the medial aspect of the femoral midshaft in Neanderthals. He attributes this to increased compressive stress during habitual movement (such as locomotion) and a greater medio-lateral expansion in the cross-section, which may result in a lack of pilaster due to a greater surface area for muscle attachments. Additionally, Ruff and Hayes (1983) show that a round femoral cross-section could be biomechanically resistant to two types of stress acting on the bone: torsional stress and perpendicular bending stresses (in both an anterio-posterior and a medio-lateral direction). The authors suggest that the latter is the most likely as torsional stresses in the lower limb are more pronounced in the tibia and both directions of bending stress have been observed in bipedal locomotion (Eberhart, 1954; Paul, 1971; Ruff and Hayes, 1983). If this is applied to the Neanderthal and modern human comparative femoral diaphyses, it can be extrapolated that a medio-lateral stress experienced by Neanderthals was not as critical (or as frequently experienced) in shaping modern human femora.

As mentioned above, Trinkaus and Ruff (1999) reinforced this distinction by emphasizing that most markers of robusticity become insignificant once body mass is controlled for, leaving the significant difference between Neanderthals and modern humans as the absolute shape of the femoral diaphyseal cross-sectional, a finding supported by Trinkaus et al. (1998) and Shaw and Stock (2011). The increase in medio-lateral stress has also been suggested to be, in part, due to the broad trunk of the Neanderthals, specifically the bi-iliac breadth (Shaw and Stock, 2011; see below). As this stress is distributed throughout the femur, and even reflected farther down the lower limb, it can change biomechanical forces acting along the bone even as far as the midshaft (Trinkaus and Ruff, 1989). It becomes clear that biomechanical pressures acting on the femoral midshaft are critical in determining what factors may impact the remodeling of the bone and thus variation on bone structures, but it can be difficult to distinguish between them (Trinkaus, 1976; Trinkaus and Ruff, 1999; Churchill et al., 2000).

Mobility Patterns and Activity Levels

Given the importance of biomechanical changes on the remodeling of weight-bearing bones, such as the femur, it follows that associations of activity levels would be examined for correlations between femoral cross-sectional properties, such as shape, and behavior related to movement, such as subsistence. While predicting behavior from cross-sectional shape can be difficult, it is more reliable when comparing closely-related groups and/or populations, such as fossil and modern humans (Ruff et al., 2006). Due to an increase in data on recent modern human activity patterns, these groups are frequently used as a proxy for fossil human behavior and while this comes with inherent assumptions of similarity of movement, it is the best estimation that can be given to understand possible behavior patterns of fossil hominins, such as Neanderthals.

Changes in behavior and mobility patterns have been observed in femoral midshaft morphology and compared to groups of known subsistence in order to better understand the impact on femoral shape. Bridges (1989), for example, measured cross-sectional properties (cortical area and minimum/maximum moments of inertia) to determine relationships in the observed differences in the lower limb between agriculture and hunter-gatherer Mississippian populations. She found an increase in both sexes in the lower limb for the agricultural society with a change to processing maize. Femoral midshaft changes related to even the smallest differences in subsistence can be seen in a study by Stock and Pfeiffer (2001) who showed that changes in patterns of loading between hunter-gatherer groups can alter lower limb morphology, specifically the proximal limb, through comparing two groups: the Andaman Islanders who exploited marine resources and the terrestrial-only resource exploitation of Late Stone Age modern humans. The more intense mobility and lower population density of the Late Stone Age humans resulted in a higher dependence on mobility for subsistence and in a sharper pilaster (Stock and Pfeiffer, 2001). This demonstrates, not only the importance of the pilaster in modern human movement, but how small changes in behavior (including those behaviors that can change population density) among similar subsistence strategies can impact cross-sectional shape. In more recent modern human populations, especially in the Holocene, a lack of robusticity in the femur, with a less-pronounced pilaster, has been associated with less mobile societies and increased sedentism, and can even been seen reflected in sexual division of labor within subsistence strategies (Ruff, 2008; Shackelford, 2007; Macintosh et al., 2014; Ruff et al., 2015).

Studies supporting a difference in activity levels based on cross-sectional shape have shown an increase in size of muscle attachments for the Neanderthal gluteal muscles (Trinkaus, 1976) as well as an increase in the *gluteus maximus* muscle correlating with high-impact, heavy lifting, and irregular loading patterns, in addition to increased muscle size and body mass (Niinimäki et al., 2016) (which has been associated with Neanderthals (Churchill, 2008)). Chapman et al. (2010) support variation in activity between modern humans and Neanderthals as well a 3D simulation showing comparable joint movement and a possible muscular advantage in the strength of the hamstring muscles for Neanderthals.

Ruff et al. (2006) caution, however, that moving from cross-section morphology to inferring a type of biomechanical pressure, referred to as a strain, or a level of activity can be problematic. Habitual activities, for instance, can be less stressful on the bone and show smaller modifications than an activity done with less regularity but that causes more intense bone remodeling, especially if a movement is not consistent in the type of loading it causes (such as torsional versus bending stress). Ruff et al. (2006) also caution that the variation of loading stresses, or strains, within the same movement, but in different individuals can differ; what causes a specific osteological reaction in one person may be different in another. Aging can also play a part in the amount of reloading seen with behavior, in addition to the age children assume adult behavior roles within a society (Kannus et al., 1995; Pearson and Lieberman, 2004). While this casts significant concerns on the extrapolation of behavior from (or to) changes in cross-sectional shape, increases in the understanding of both how activity can impact bone and the limitations that must be accounted for can lead to more accurate analyses. Additionally, the use of technological advances, such as computer the computer simulations outlined in Chapman et al., (2010), may aid in the understanding of the nuances of behavioral pressures on bone.

Ontogenetic Factors

An increasing number of studies have been done addressing the impact that age has on cross-sectional morphology and its relationship to overall robusticity within a society. Pearson and Lieberman (2004) address many factors that can impact variation seen within cortical bone, including the idea that behavior impacts cross-sectional shape. Specifically, they note that the highest impact that activity has on bone deposition is in childhood and during adolescence with increased skeletal growth. It is important to note that while the degree of modification is greater in adolescents, behavior can change bone shape in adults, though less drastically (Ruff et al., 2006). The impact of ontogenetic factors may also play a role in femoral midshaft shape, complicating the information that can be extrapolated from biomechanical and behavioral models. Cowgill (2010) addresses femoral robusticity using cross-sectional properties and shows that adult ranges of robusticity are present in many societies early in development, some even before the onset of ambulatory behavior. This is further supported in Cowgill and Hager (2007) in which levels of robusticity based on cross-sectional data seen in an urban versus agricultural societies develop early and at varying rates between groups. While the trend in robusticity supports patterns of decreased strain with less daily mobility as outlined above, the high variability of onset and young ages of the individuals (six years of age in this study), suggest a larger component than activity patterns to cross-sectional morphology. This supports the hypothesis that aspects of cross-sectional morphology may be attributed to factors impacting bone growth other than subsistence activity, such as genetics and/or nutrition (Cowgill and Hager, 2007). Therefore, the estimation of behavior from cross-sectional data must be done with caution, understanding the limitations and complexities affecting varying morphological types.

Terrain

The effects of subsistence strategies to estimate loading patterns on femoral midshaft shape have also been called into question due to the impact of varying terrains on different locomotion requirements. Ruff (1999) compared femoral midshaft morphology of Prehistoric Native American populations from the Great Basin region of North America, selecting hunter-gatherer populations with similar subsistence strategies but occupying different terrains. He found that the populations from a mountainous region, as opposed to the coast or the plains, had greater robusticity in their femora based on their cross-sections. As Ruff (1999) was able to control for sex and subsistence and still see a significant difference between populations, including the foraging range and type of ground covered within a biped’s daily behavior, he showed that the impact of terrain is a critical consideration in femoral midshaft shape, especially given biomechanical principles of bone remodeling. This further supports the importance of careful interpretation of biomechanical patterns of bone distribution, as seen above, as small differences in resources exploited within a similar subsistence “type” can alter what is reflected on the bone.

Climatic Variation

It is important to mention another hypothesis that has been presented to account for Neanderthal cross-sectional shape, specifically the impact that climate and cold-adapted lower limbs may have had on determining this morphology. Weaver (2003) addressed this question using geometric morphometrics and measurements of the os coxa, femoral head, and femoral midshaft of fossil and modern humans. He found that a rounder femoral shape (and a less pronounced pilaster) appeared to be associated with a wider bi-iliac breadth, associated with more cold-adapted populations (Ruff, 1994). Though he uses the femoral midshaft in comparison with other biological climatic variables, subsequent studies have shown that when factors of climate and mobility are compared to femoral shaft shape, at the midshaft specifically, the morphology is more directly correlated with mobility patterns rather than climate (Stock, 2006). Stock (2006) also showed that the lower limb does not correlate equally to climate or mobility patterns (having a stronger correlation to climate in the proximal end), which again stresses the caution that needs to be made when discussing which portion of the femur is being evaluated in conjunction with evolutionary processes.

Summary and Application

Given the myriad of factors that can affect the morphology of the femoral diaphysis, it is important to understand how each section of the shaft interacts with biological, environmental, and ontogenetic pressures. The morphology of the femoral midshaft is of critical importance to the evolution of the lower limb and several explanations for its morphology have been given here. As shown above, it can be difficult to discern between different selective pressures acting on the midshaft, emphasizing the need for cautious interpretation as well as a better understanding of how selection impacts the shape, especially in comparative studies between fossil and modern humans. It is critical to be able to test for the impact that shared evolutionary history has on the shape of the femoral midshaft in order to account for this in comparative studies. With a greater understanding of how phylogeny acts on femoral midshaft cross-sectional shape similarities, the more meaningful comparative studies attempting to understand the nature of this shape become.

CHAPTER IV

MATERIALS

In order to test for the presence of phylogenetic signal between fossil and modern humans as well as the effect it has on femoral morphology, both morphological and genetic data were collected from a sample of Late Pleistocene *Homo sapiens*, a sample of Neanderthals, and a sample of global recent modern humans. Though it is unrealistic to expect that human populations have ever been independent of the impacts of gene flow due to long-distance migration and admixture, populations were selected for this study with long-standing occupants of the region in which they reside in order to minimize these effects as much as possible.

Morphological Sample

Late Pleistocene Homo sapiens

The LP*Hs* in the morphological sample consist of 25 individuals whose femoral midshaft cross-sections were provided by Dr. Erik Trinkaus. The sample includes individuals from varied geographic regions, from Western Europe to Western Asia. The femoral specimens used in this study are listed in **Table 4-1** with their reported ages outlined below.

European Late Pleistocene *Homo sapiens*

Paviland 1, the Red Lady (though actually a male), may represent one of the oldest early modern human remains from the United Kingdom and his femur is included in this study (Trinkaus and Holliday, 2000). This specimen was initially discovered in 1823 in Goat's Hole Cave (Paviland, South Wales) and has a long history of being dated, using both direct and indirect dating methods (Oakley, 1971; Jacobi and Higham, 2008). Direct AMS radiocarbon dates from Paviland 1 initially yielded the dates 29.4-27.4 kya cal BP[1] (Hedges et al., 1989; Aldhouse-Green and Pettitt, 1998). With more recent ultrafiltration methods, Jacobi and Higham (2008) reported a slightly older AMS date, on both a rib and scapula fragment of 29.5-32.1 kya cal BP[1]. Given the careful resampling and attention to contamination, the dates given by Jacobi and Higham (2008) are likely the most accurate radiocarbon dates for this specimen and, given the articulated provenience of the rest of the partial skeleton, are accepted here as the dates for the Paviland 1 femur.

Six of the femora in the LP*Hs* sample come from the site of Dolní Věstonice in the Czech Republic (Dolní Věstonice (DV) 3, 13, 14, 15, 16, and 35). Found in the Pavlov hills, Dolní Věstonice is broken into two sites (Dolní Věstonice I and II), both of which are represented here. The first of the six femora comes from the specimen DV 3, which was found at Dolní Věstonice I and was excavated with the postcrania in articulation, making it ideal for sampling femoral cross-section. Dated indirectly through the radiocarbon dating of archaeological layers associated with the burial, DV 3 is estimated to be approximately 29.2-26.9 kya cal BP[1] (Trinkaus and Jelínek, 1997). Also recovered from Dolní Věstonice I is the partial femoral diaphysis DV 35, which was directly radiocarbon dated by Trinkaus et al. (1999) to 25.5-24.6 kya cal BP[1] (although the authors considered this an underestimation of the actual age of the specimen and suggested the youngest age possible of 25 kya cal BP[1] due to contamination from marine resources).

Perhaps the best-known remains from Dolní Věstonice are the individuals from the triple burial at Dolní Věstonice II (DV 13, 14, 15). Well-preserved, these three individuals, 2 males and a female, each retained both femora, the left of which is sampled here. The estimated geologic ages of these individuals based on radiocarbon dates from charcoal near the burial are between 29.1-24.6 kya cal BP[1] (Svoboda et al., 2002). The last femoral sample taken from Dolní Věstonice, DV 16, was the single

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[1] Calibrated by the author using OxCal 4.2, IntCal13 calibration curve (Reimer et al., 2013, Bronk Ramsey, 2009).

burial of an adult male from Dolní Věstonice II. The femora of DV16 were more fragmentary than those of DV13, DV14, and DV15, but both were present and the left cross-section was sampled (Svoboda, 1987; Trinkaus and Svoboda, 2006). The dates for DV 16 were reported using associated charcoal, similar to the other specimens at Dolní Věstonice. DV 16, however, was also buried in close proximity to a hearth, which yielded radiocarbon dates as well. The approximate date for DV 16 is around 26 kya BP[1] (charcoal: 29-27 kya cal BP[1]; hearth: 28.6-27.4 kya cal BP[1]) (Svoboda et al., 2002; Trinkaus et al., 1999). Though the dates at Dolní Věstonice II would be much more secure if taken from the bone directly, the charcoal associated with the remains appears reliable given the preservation of the remains and the stratigraphy associated with them.

Pavlov 1 is another specimen from the southern Czech Republic, in the same region as Dolní Věstonice (and these sites are often referred to together). Pavlov 1 was found in a depositional context that had evidence of erosion; however, part of the individual had been trapped under a mammoth bone that likely protected it from some of these environmental processes. Though it is highly unlikely that this individual remained in the same location as its original burial, a radiocarbon date of 28.8-28.3 kya cal BP[1] was reported by Trinkaus and Svoboda (2006). The date was taken from a portion of the site not associated with Pavlov 1, and as it is likely the remains have shifted since deposition, the date should be taken with some caution; it is, however, the only estimated date for this individual and is taken here as a best estimate and approximation of the Pavlov 1 remains.

This study also includes three femora from the Upper Paleolithic site of Předmostí in Moravia, Czech Republic, Předmostí 3, 4, and 14. Due to the skeletal remains' destruction in a fire in 1945, the femoral cross-sections were collected from casts recreated from photographs (Svoboda, 2008). Předmostí 3 and 4 were originally recorded as nearly complete skeletons, while Předmostí 14 was fragmentary. The original archaeological context and provenience of these specimens, however, was lost due to insufficient description of their location at the site, and had to be reconstructed through notes and subsequence excavations (Svoboda, 2008). The burials, associated with the layer Předmostí Ib were most recently dated via radiocarbon from excavated bone at the same layer as the original burials and yielded dates of 29.2-28.7 kya cal BP[1] for the lower layer and 26.7-26.1 kya cal BP[1] for the upper layer (Svoboda, 2008). Given the agreement of these dates with previous radiocarbon dates at the site (Svoboda, 2003; Jöris and Weninger, 2004), this range is accepted as the best estimate for these skeletal remains as they cannot be directly tested.

Mladeč is another cave site from Moravia in the Czech Republic with early modern human femoral remains; the individual used in this study is Mladeč 27. Wild et al. (2005) analyzed the age of the human remains through direct C14 radiocarbon dating of four teeth and an ulnar fragment (specimens Mladeč 1, 2, 8, 9a, 25c, respectively). All but two of the samples gave approximate dates of 33 kya cal BP[1] with the exception of the ulnar sample, Mladeč 25c (29-28.2 kya cal BP[1]) and one of the samples taken from the canine, Mladeč 9a (29.6-29 kya cal BP[1]), though the authors present possible contamination of these last two specimens (Wild et al., 2005). These direct dates do not come from the femoral remains used in this analysis, but they are the most current and available direct dates for the Mladeč site and are used as the estimates for the other modern human remains at Mladeč here, specifically Mladeč 27.

Three femoral specimens in this analysis are from the skeletal remains found at the rock-shelter of Cro-Magnon at Les Eyzies-de-Tayac, Dordogne in southwestern France and include Cro-Magnon 4322, 4323, 4325. The archaeology of the site, in conjunction with the skeletal remains and the radiocarbon dates from the comparable Aurignacian levels at nearby rock shelter of Pataud (300 m away), initially resulted in an estimated date of approximately 32 kya cal BP[1] for these individuals (Movius, 1969). In 2002, Henry-Gambier reported a 30.3-29.1 kya cal BP[1] radiocarbon date from a shell described as ornamentation in association with these individuals, arguing that the initial Aurignacian association was incorrect (the authors report the date of 31,324-32,666 cal BP using the IntCal09 calibration curve; see Reimer et al. (2013)). Mellars (2004), however, argues that the levels of C14 in the atmosphere were not constant around 40-30 kya BP[1] and that dates given around this time-frame are likely underestimated, leaving the Cro-Mangon individuals likely closer to their original estimate of 32 kya cal BP[1].

An extremely young date of 690±39 cal BP was reported by Fu et al. (2013) for the date of Cro-Magnon 1, but was argued by Henry-Gambier et al. (2013) to have been obtained from a sample with no contextual data. The authors further argued that no collagen has been recoverable from the original fossils and that the change in patina on the sampled specimens used by Fu et al. (2013) further support a different depositional context than the other human remains (the tested individuals were quoted as CM 4252-4345) (Henry-Gambier et al., 2013). Henry-Gambier et al. (2013) have also compared ivory pendants and shell species from Cro-Magnon to those of Level 5 at the Pataud rock shelter, which may indicate and support their previous claim of an Early Gravettian association with these individuals. If correct, resampled and calibrated dates at level 5 from Pataud have yielded dates of approximately 30.3-29.1 kya cal BP[1] (Higham et al., 2011). While there are only two ivory pendants recovered from the skeletal layer at Cro-Magnon, they do bear some resemblance to those found at Pataud, Level 5; one of the pendants is arguably made in the same fashion (Henry-Gambier et al., 2013). Additionally, *Littorina littorea* has been found at both Pataud, Level 5 and Cro-Magnon, though the latter has a higher frequency as well as higher biodiversity. Though it is difficult to find an exact date at Cro-Magnon given the lack of direct dating from the human specimens and the complex history of excavation at the site, Henry-Gambier et al. (2013) make a compelling argument for the earlier date. Due to these recent studies on the associations and dating of Cro-Magnon and Pataud, a conservative date of 30 kya BP is being used in this analysis.

Willendorf I is a site that makes up a portion of a larger group of sites attributed to Willendorf, located in Austria, on the west bank of the Danube River. The modern human remains found at Willendorf were few (a femoral and mandibular fragment) and the femur, of importance here, was designated as Willendorf I by Teschler-Nicola and Trinkaus (2001). Though enough of the femoral shaft was preserved to estimate the midshaft for cross-sectional characteristics to be recorded for this study, the femur has limited reliable provenience. In order to place the femur in a stratigraphic context, Teschler-Nicola and Trinkaus (2001) performed AMS radiocarbon dating and directly dated the bone. The age 26.7-25.9 kya cal BP[1] was obtained from the specimen, suggesting the femur belongs to a similar age as the Gravettian Layer 9 in Willendorf II (and thus providing context for Willendorf I) (Teschler-Nicola and Trinkaus, 2001).

West Asian Late Pleistocene *Homo sapiens*

Ohalo II is a once-underwater site that is located near Tiberias (9km to the south) in Israel. It was discovered during a drought when the Sea of Galilee water levels dropped enough to expose the site (Nadel and Hershkovitz, 1991). The femur used in this study is from the skeletal remains of the same name, Ohalo II. This was an adult male who was found lying on his back with bent knees with well-preserved femora (Nadel and Hershkovitz, 1991). The dating for this site has been done via radiocarbon using charcoal, barley, and various other flora (including grains and fruit) and has yielded 26 different dates ranging from 23.6-19 kya cal BP[1], with an approximate mean of 19,400 years BP (21 kya cal BP[1]) (Nadel et al., 1995). Given the lack of indirect dating of Ohalo II, the currently accepted associated average of 21 kya cal BP is used in this study as the best date of the site currently available.

Qafzeh is a cave site that is also located in Israel, east of Nazareth. The specimen used here is Qafzeh 8, one of the 27 Late Pleistocene *Homo sapiens* remains recovered from the Middle Paleolithic levels found at the site (Shea, 2003; Shea and Bar-Yosef, 2005). The MP layers at the site where Qafzeh 8 was found have been dated by flint associated with the human remains to a thermoluminescence (TL) date of approximately 92,000 Kya BP (Valladas et al., 1988). The 20 flint samples were taken from 6 layers and had a date range of 109,900±9,900 – 82,400±7,700 years BP. This surprisingly early date was later supported through Electron Spin Resonance (ESR) dates, performed on macrofauna teeth from the same layer as the skeletons, of 115,000±15,000 – 96,000±13,000 years BP, with an emphasis on the older date; this is likely less accurate than TL due to the unknown rate at which the calcium in the teeth is replaced by uranium (Schwarcz et al., 1988). As the error of margin is large (especially with the ESR dates), the conservative average reported by Valladas et al. (1988) with TL, 92,000±5,000 years BP, appears to be the best estimated date of the specimen.

The Middle Paleolithic site of Skhūl, located in the Nahal Me'arot Park in northern Israel, is a cleared, collapsed rock shelter with 10 human fossils recovered from the excavation Level B (Shea, 2003; Shea and Bar-Yosef, 2005). These specimens were first described by McCown and Keith (1939) and four femora are used in this study (Skhūl 3, 4, 5, 7). The site of Skhūl was first dated by ESR on bovid teeth on the same level as, but not associated directly with, the human skeletons, yielding estimated dates of 101,000±12,000 – 81,000±15,000 years BP (Stringer et al., 1989). The site was redated by TL through associated flint (6 burned specimens) with an average date of 119,000±18,000 years BP. Given the limitations regarding ESR uranium uptake and the more stable decay rates in flint of uranium, potassium 40, and thorium, the age of 119 kya BP is likely a better estimate of the age of these specimens and is used here (Mercier et al., 1993). This date is further supported by recent U-series and ESR dating of associated fauna remains and direct samples of Skhūl II by Grün et al. (2005), resulting in an estimated age range for the site of 120 – 95 kya BP, and a reported mean of 102,000±26,000 years BP.

Nahal Ein Gev 1 is another site in Israel with well-preserved femoral remains included in this study (specifically, the complete left femur). Nahal Ein Gev 1 is located to the east of the Sea of Galilee and is known for Levantine Aurignacian lithic material as well as the relatively complete skeletal remains of an adult female (Nadel and Hershkovitz, 1991; Belfer-Cohen et al., 2004). The radiocarbon date of 18-16.1 kya cal BP[1] was calculated using burned bone in associated with, but not directly from, these remains (Vogel and Waterbolk, 1972; Arensburg and Bar-Yosef, 1973).

The femora from Sunghir used in this study are from two Upper Paleolithic graves found at the site, located northeast of Moscow, in Vladimir. Sunghir 1 is a nearly complete skeleton with both femora preserved (as previously mentioned, only the left will be used here) and was found in an individual burial. Sunghir 4 was found in a second grave with three individuals and is represented by a single, adult left femur; both graves contained grave goods and ochre (Dobrovolskaya et al., 2012). Dobrovolskaya et al., (2012) reanalyzed the previously reported dates from these burials using a femur from Sunghir 1 and the humerus of Sunghir 3 for radiocarbon AMS dating. Sunghir 1 yielded a date of 29.3-28.8 kya cal BP[1] and Sunghir 3 a date of 29-27.3 kya cal BP[1]. Due to the close proximity and shared grave of Sunghir 3 and Sunghir 4, the date of the former is an acceptable estimate for the femur used in this analysis. Given the carbon/nitrogen levels reported for the tested samples and the care taken to reduce contamination, the collagen from these samples is preserved enough to give reliable dates and, therefore, are the dates reported here (Dobrovolskaya et al., 2012; Trinkaus et al., 2014).

Neanderthals

Due to the morphological differences between European and Asian Neanderthals, the femoral samples included in this study are divided into two respective geographical populations. Of the 12 Neanderthal femora cross-sections analyzed here, eight of them belong to the subgroup of fossil humans, European Neanderthals. As with the LP*Hs*, these specimens were provided by Dr. Erik Trinkaus. As above, the European Neanderthal femoral specimens used in this study are listed in **Table 4-1** with their reported ages outlined below.

European Neanderthals

La Chapelle-aux-Saints 1 is a partial adult male skeleton recovered from a cave site at La Chapelle-aux-Saints in the department of Corrèze, southwest France (Rendu et al., 2014). Both femora are partially preserved and the left is used here due to the retention of the midshaft (Trinkaus, 2011). The skeletal remains have not been directly dated; however, ESR has been used to indirectly date the level with the remains to 56,000±4,000 years BP and 47,000±3,000 years BP (Raynal, 1990; Gómez-Olivencia, 2013). Due to the wide range and limited dating for this specimen, an approximate median of 50 kya BP will be used in this analysis.

The second Neanderthal femur sampled for this study is a partial left femur, Fond-de-Forêt 1, from the cave site of the same name close to Liège, Belgium in the Vesdre Vally. The specimen was initially discovered by J. Tihon in 1895 and is likely a male based on previous studies of sexual dimorphism conducted using Neanderthal long bones (Trinkaus and Ruff, 1989; Trinkaus, 1980). The specimen, complete but missing its proximal diaphysis, has not been dated but is associated with Mousterian lithic technology at the site which suggests that it is Middle Paleolithic in age (Stringer and Hublin, 1984; Trinkaus and Ruff, 1989).

La Ferrassie 1 and 2 were also excavated in the department of Dordogne in France, specifically in Savignac-de-Miremont. La Ferrassie 1 was excavated from the collapsed rock shelter and is relatively complete adult Neanderthal male with both femoral diaphyses intact (only the left is used in this analysis) (Fennell, 1997). La Ferrassie 2 is an adult female Neanderthal partial skeleton that is not as complete as La Ferrassie 1, but does retain both femora (the left of which is used here) (Trinkaus, 1980). The skeletons were found in a level with Mousterian artifacts (Level C) and Fennell and Trinkaus (1997) suggested that the associated macrofauna can be attributed to a cold climate, which indicates an age of these specimens on the border of OIS 3 and 4. Recent AMS radiocarbon dates have put the lower Aurignacian levels at La Ferrassie at 36.8-34.9 kya cal BP[1], establishing, at the minimum, that the Neanderthal specimens are older than 34.9 kya cal BP[1] (Higham et al., 2006). Given the evidence presented by Fennell and Trinkaus (1997) and that the specimens are older than the Aurignacian levels, it is estimated that the specimens at La Ferrassie are approximately 60 kya, near the transition between OIS 4 and OIS 3.

Saint Césaire 1 is another partial adult male Neanderthal skeleton from the southwest coastal region of France, specifically the Charente-Maritime department. The site, La Roche à Pierrot, is a collapsed rock shelter found near Saint Césaire (Zollikofer et al., 2002). Though the femora were preserved, both are fragmentary. The right femur is preserved enough in the mid- and proximal diaphysis that a reliable 50% cross-sectional mold could be taken and is included here (Trinkaus et al., 1998). Saint Césaire 1 has been indirectly dated through the TL dating of six burned flints found in the same archaeological layer (defined as Châtelperronian). The dates range from 37,400±5,200 - 33,700±5,400 years BP, with a mean of 36,300±2,700 (Mercier et al., 1991). These are currently the best dates estimated for the St. Césaire Neanderthal as it has been suggested that there is not enough preserved collagen to justify radiocarbon dating (reported by Mercier et al. (1991) as a personal communication).

From the Belgium Middle Paleolithic site of Grotte de Betche-aux-Rotches (Grotte de Spy), the right, nearly complete, femoral remains of the Spy 2 Neanderthal were recovered, and given the complete diaphysis and nearly complete epiphyses (with the exception of a portion of the greater trochanter), it is an ideal candidate for a midshaft measurement (Trinkaus, 1978; Trinkaus and Ruff, 1989). Semal et al. (2009) reported direct radiocarbon dates of the Spy 2 Neanderthal through the examination of the dentition and a phalanx. The dates of the dentition centered around 39-38.3 kya cal BP[1], with the phalanx yielding a somewhat younger age (approximately 35.5-34.6 kya cal BP[1]) that was called suspect by the authors due to problematic ratios of nitrogen to carbon (Semal et al, 2009). It is entirely possible that the cranium of Spy 2 and the femur do not belong to the same individual. Trinkaus and Ruff (1989) show that, based on the morphology of the femur, it could have belonged to a male, which differs from the cranial morphology suggesting the individual was a female. Given their association and their classification as Neanderthal, the dates reported by Semal et al. (2009) likely represent an estimated date for the femoral cross-section and are reported as such below.

Another French Neanderthal femoral specimen used in this study was found in the cave site of Les Rochers-de-Villeneuve in the western department of Vienne near Lussac-les-Chāteaux. The femur was found with a small amount of lithic material (primarily scrapers) as well as burned faunal remains related to human activity. The femur itself is a partial shaft fragment (missing both diaphyses and the distal end), characterized by carnivore gnaw marks (Beauval et al., 2005). The femur is sufficiently preserved near the midshaft that a 50% diaphyseal mold can be reliably made. Additionally, Beauval et al. (2005) estimate that the impact of cryoturbation on the site was limited to within each stratigraphic level. In order to approximate the age of the femur, AMS C14 radiocarbon dates were taking form a similarly sized, similarly deposited hyena radius in relatively close proximity (143cm). The resulting age of the radius, and thus the age attributed to the Neanderthal femur, was 40.9-44 kya cal BP[1] (Beauval et al., 2005).

One of the few Neanderthal specimens that has yielded both a femoral cross-section as well as a viable mtDNA sequences (both of which are used in this study) is the individual Feldhofer 1 from the Kleine Feldhofer Grotte in the Neander Valley close to Düsseldorf, discovered in 1856 (Schmitz et al., 2002; Sawyer and Maley, 2005). The partial skeleton has both femora well preserved and the right midshaft cross-section was provided for the analysis performed here. Schmitz et al. (2002) performed AMS C14 dating directly to the remains of Feldhofer 1 (right humerus) yielding 42.7-40.7 kya cal BP[1]. As Feldhofer 1's original depositional context may never be known (due to its discovery during blasting for limestone quarrying), a direct date of a portion of the fossil and other remains (found at an approximate location of the Grotte), represent the best possible date for this specimen (Schmitz et al., 2002).

Western Asian Neanderthals

The second group of Neandertals includes those from western Asia and is represented by four femoral specimens. As outlined above, these cross-sections were provided by Dr. Erik Trinkaus and their location, side, and date are listed in **Table 4-1**. The justifications for the dates in the table are outlined below.

The first of the specimens is from the partial skeleton of Amud 1 and is represented here by the left femur. Amud 1 was located in a cave site in the Nahal Amud Valley near the Sea of Galilee in Israel. The site is characterized by three archaeological horizons, the lowest of which (B_4) is separated from the other two by a layer that has not yielded human remains or lithic artifacts. Though three Neanderthal individuals were recovered from the Amud Cave, they were not found in the same depositional levels: Amud 1 was recovered from the upper-most layer of the cave (B_1) with Amud 7 and 9 recovered from B_2, below it (Suzuki and Takai, 1970; Rink et al., 2001). For the femoral specimen used here, only the dates of layer B_1 in relationship to Amud 1 will be considered. This layer has been dated indirectly using ESR, TIMS (Thermal Ionization Mass Spectrometry) $^{230\text{Th}}/^{234}$U, and TL methods which have yielded similar results (Valladas et al., 1999; Rink et al., 2001). The TL dates, provided by Valladas et al. (1999) were taken from burned flints in the upper levels (from B_1/B_2) and provided a wide range of dates from 70,600±6,900 – 44,100±3,100 years BP. This wide range of variation was cited as possibly a reflection of the mixed sediments at the site affecting the absorbed radiation. The levels closest to Amud 1 (level K, squares 3a/b) gave date ranges of 70,600±6,900 – 49,000±4,600 years BP (Valladas et al., 1999). The reported TIMS/ESR dates of were taken from faunal remains (the tooth of a gazelle from layer B_1, Level L, square B) and reported as a combined date of 53,000±7,000 years BP (Rink et al., 2001). Unfortunately, none of the reported dates are directly associated with Amud 1, which was found in levels G and H, and therefore can only give the youngest possible date for the individual (Rink et al., 2001). Due to the possible problems with the radiation levels on the TL dating mentioned above, as well as the stratigraphic discrepancies between the fossil and the dated remains, it is prudent here to take the most conservative estimate of 53,000±7,000 years BP for the oldest possible age of Amud 1.

Two femoral specimens are included from the Shanidar sample from the Shanidar Cave found in the Zagros Mountains in Kurdistan, Iraq. The Neanderthal remains at Shanidar consist of several adults and infants found in the Middle Paleolithic layer, D. The femora represented here are from the individuals Shanidar 5 and 6, found at different levels of this layer (Trinkaus, 1983; Reynolds et al., 2015). Shanidar 5 is an adult male, partially preserved (including his femoral diaphyses); the right femoral midshaft diaphysis is included here. Shanidar 6, an adult female, is also partially preserved with enough of her right femoral diaphysis for inclusion in this study

(Trinkaus and Zimmerman, 1982). Shanidar 5 was found in the upper layers of level D (in a layer of increased archaeological material), whereas Shanidar 6 was found near the middle of layer D (above another layer of increased archaeological activity) (Trinkaus, 2014). The upper level of layer D was estimated through radiocarbon dating charcoal to 48.8 – 42.3 kya cal BP, with an estimate of 61.8 – 43.8 kya cal BP[1] from charcoal to date Shanidar I (and, therefore, the best approximate date for Shanidar 5, found at the same level) (Solecki, 1963; Vogel and Waterbolk, 1963; Trinkaus, 2014). Solecki (1963) used comparative site data, climate estimates, and palynology to estimate the time frame for older ages at Shanidar Cave; giving the estimate of 60,000 kya BP for Shanidar 6. It is reasonable to give the date of 61.8-43.8 kya cal BP[1] for Shanidar 5 given the radiocarbon dates; however, though Shanidar 6 is older than Shanidar 5 based on the stratigraphy at the site, a conservative date of 61.8-43.8 kya cal BP[1] is reported for this individual with the understanding that it is the youngest age limit.

The final specimen included in the femoral sample is Tabun C1, a partial Neanderthal skeleton from Mount Carmel, Israel (near the Skhūl site). There is some debate as to the provenience of the individual concerning which layer (Tabun B, C, or D) it truly belongs to (Garrod and Bate, 1937; Grün and Stringer, 2000). The right femur is relatively intact with some distal deformation (Garrod and Bate, 1937). Due to the fragmentary preservation of the left femur, the mid-section was taken on the right femur and is included here. Schwarcz et al. (1998) used U-series dating to directly date both the right femur and the mandible from Tabun C1. Given the different assumptions the authors make on the rate of uranium uptake, the estimated ages change drastically. For the mandible, an assumption of constant uptake versus absorption shortly after burial changes the date from 70,000 ± 25,000 – 34,000±5,000 years BP; for the femur this date changes from 33,000±4,000 – 19,000±2,000 years BP (Grün et al., 2000). As Millard and Pike (1999) point out, U-series dating can be extremely problematic given the highly variable and difficult to predict rates of uranium uptake (even between bones of the same individual), casting doubt on the young age of Tabun C1.

Mercier and Valladas (2003) give TL dates on burnt flint from Layer C yielding 165,000±16,000 years BP, while Grün and Stringer's (2000) combined U-series/ESR dates on dentine from faunal remains gave dates from Layer B (104,000 +33,000/-18,000 years BP) and C (135,000 +60,000/-30,000 years BP). Given the limitations and the disparities between ages from the associated flint and fauna and the direct dating for Tabun C1, it is important to mention that the archaeological context of the layer C has been compared to the lithic technologies at Qafzeh, while layer B is similar to that of Amud. It is impossible to associate skeletal remains with technocomplexes with complete certainty, however with the similarities between Tabun and other sites in the region as well as the updated methods used by Grün and Stringer (2000), it seems more likely that the dates for Tabun C1 are between 135-104 kya BP.

Recent Modern Humans

The recent modern human morphological comparative sample consists of 146 individuals from a global sample of limited gene flow populations. These populations include samples from Alaskan Inuit, Pre-Dynastic Egyptian, Catalhöyük, and Libyan Saharan populations. Details of each of these samples are outlined in the sections below and in **Table 4-2**.

Alaskan Inuit

The Alaskan Inuit population included in this study consists of 53 adults of mixed sexes from the Point Hope peninsula in Alaska, USA. The cross-sectional data used here was originally collected by Dr. Laura Shackelford (Shackelford, 1995). The Point Hope excavations revealed several different occupational levels, two of which make up the sample used in this analysis: the Ipiutak (870-56 years cal BP[1]) and the Tigara (300-400 years BP[1]) (Shackelford, 1995). Though these populations differ in their exploitation of resources (caribou vs. whales, respectively) they are included in this study as one population; it has been suggested that this site may represent cultural change of one group over time (Larsen and Rainey, 1948; Costa, 1982; Shackelford, 1995).

Table 4-1. Fossil Morphological Specimens. All fossil specimen midshaft measurements were collected by, and analyzed with, permission from Dr. Erik Trinkaus. Where a range of dates is given for calibrated radiocarbon dates, the approximate center of the range is reported here (see text for further details). LP*Hs*: Late Pleistocene *Homo sapiens.*

Population	**Geographic Location**	**Specimen**	**Side**	**Estimated Age**
LP*Hs*	Wales, UK	Paviland 1	Left	31 kya cal BP
LP*Hs*	Czech Republic	Dolní Věstonice 3, 13*, 14*, 15, 16*, Pavlov 1*	Left	27-28.5 kya cal BP
LP*Hs*	Czech Republic	Dolní Věstonice 35	Right	25 kya cal BP
LP*Hs*	France	Cro-Magnon 4322, 4325	Left	30 kya cal BP
LP*Hs*	France	Cro-Magnon 4323	Right	30 kya cal BP
LP*Hs*	Israel	Ohalo 2	Left	21 kya cal BP
LP*Hs*	Israel	Qafzeh 8	Right	92 kya BP
LP*Hs*	Czech Republic	Předmostí 3, 4, 14	Left	29.2-26.1 kya cal BP
LP*Hs*	Israel	Skhūl 3, 4, 5	Left	119 kya BP
LP*Hs*	Israel	Skhūl 7	Right	119 kya BP
LP*Hs*	Israel	Nahal Ein Gev 1	Left	17 kya cal BP
LP*Hs*	Austria	Willendorf 1	Right	26 kya cal BP
LP*Hs*	Russia	Sunghir 1, 4	Left	28-29 kya cal BP
LP*Hs*	Czech Republic	Mladeč 27	Right	33 kya cal BP
European Neanderthals	France	La Chapelle-aux-Saints 1	Left	50 kya BP
European Neanderthals	Belgium	Fond-de-Forêt 1	Left	undated
European Neanderthals	France	La Ferrassie 1, 2	Left	60 kya BP
European Neanderthals	France	St. Césaire 1	Right	36 kya BP
European Neanderthals	Belgium	Spy 2	Right	38 kya cal BP
European Neanderthals	France	Villeneuve	Left	42 kya cal BP
European Neanderthals	Germany	Feldhofer 1*	Right	41.7 kya cal BP
Asian Neanderthals	Israel	Amud 1	Left	53 kya BP
Asian Neanderthals	Iraq	Shanidar 5, 6	Right	52.8 kya cal BP
Asian Neanderthals	Israel	Tabun C1	Right	135-104 kya BP

*These individuals are represented in the morphological and genetic data, with both the genome sequence and femoral measurements from the same individual.

Pre-Dynastic Egyptian

The Pre-Dynastic Egyptian population included 39 adult individuals that were collected by and assessed for age by Dr. Laura Shackelford (Shackelford, 1995). These specimens are of mixed sexes, but given the lack of evidence for the impact of sexual dimorphism on the asymmetry of the lower limb, sexual determinations are not included here (Genovés, 1967; Auerbach and Ruff, 2006). These individuals were excavated from the Keneh and Mesaeed Middle Egyptian archaeological sites, near the Great Bend of the Nile, and dated to before 5050 BP (Bard, 1994; Shackelford, 1995).

Libyan

The modern human sample also includes 31 adult individuals of mixed sex determination from the archaeological sites of Wadi el Agail and the Tanezzuft Valley in the Fezzan region of Libya. These sites were dated to 500BC-500AD and 5,000-6,000 years BP, respectively (Shackelford, 1995; reported personal communication in Shackelford (1995) for the Wadi el Agail dates). These populations have been estimated from stress markers on bone to be semi-nomadic in nature, with evidence for a sexual division of labor (Arrighetti et al., 2002). Shackelford (1995) showed there was no significant difference in the lower limb morphology between these two populations and included them in one group in her analysis of postcranial robusticity; these populations were combined into one sample in this analysis as well.

Çatalhöyük

The modern human population includes a sample of 22 adults from the Çatalhöyük site in the Konya Plains, Anatolia, Turkey (Mellaart, 1962; Cowgill and Hager, 2007). This site has been dated to approximately 8.2-7.4 kya cal BP (via AMS C14) and included a long-term occupation of a population exploiting both domesticated plans and animals (Cowgill and Hager, 2007; Cressford, 2001; Atalay and Hastorf, 2006). The original sample was recorded by Cowgill and Hager (2007) and included children and adults of mixed sexes; only the adults were used in this analysis.

Table 4-2. Recent Modern Human Morphological Sample**.** All modern human midshaft measurements were collected by and reproduced with permission of Dr. Laura Shackelford and Dr. Libby Cowgill. As before, when a range of dates can be given for calibrated radiocarbon dates, the approximate center of the range is reported here (see text for further details).

Population	Geographic Location	Number of Individuals	No. Left	No. Right	Estimated Age
Alaskan Inuit	Point Hope, Alaska, USA	53	27	26	460 cal BP 300-400 BP
Pre-Dynastic Egyptian	Nile Valley, Upper Egypt	39	20	19	>5050 BP
Libyan	Southwestern, Libya	31	18	13	5-6 Kya BP, 500 BC-500 AD
Çatalhöyük	Anatolia, Turkey	22	13	9	8.2-7.4 kya cal BP

Genetic Sample

The genetic sample used in this analysis consists of mitochondrial DNA sequences representative of each group of modern and fossil humans in order to create a phylogenetic tree on which to base inferences of shared evolutionary history (as outlined below in Chapter V (Methods)). In order to capture sufficient variation to create a well-supported phylogenetic tree, the mtDNA sequences given the highest preference were those with full mitochondrial genomes. Additionally, the sequences selected were chosen due to their collection from geographical and temporal populations congruent to those recorded in the morphological data. Where full mitochondrial genomes were not available, those sequences with optimal geographic and temporal placement with partial mtDNA sequences were selected and taken from the hypervariable D-loop of the control region. All samples, both partial and complete, were obtained from the open-access database of submitted and published sequences, GenBank (Benson et al., 2005, 2013).

In order to test for phylogenetic signal between fossil and modern humans, one must examine these groups as populations rather than as separate species. This is important in when studying late *Homo* population dynamics as the species delineation can either difficult to assess (such as with Neanderthals and modern humans) or the number of species is statistically too small to use in many analyses (especially in the Late Pleistocene). Given the observable variation between modern and fossil human groups in the femoral midshaft, it is also valuable to understand this inter-group variation, which could not be done if this study were conducted at a solely species level distinction. Using populations instead of species, as is done in this study, can be essential in understanding phylogenetic relationships between these closely related groups of humans, as long as gene flow can be minimized (Garland et al, 1992).

In order to preserve distinctions between populations and to minimize the effects of gene flow on geographic and temporal variation, the selection of mtDNA sequences in this study were chosen by the same criteria as the morphological sample and were limited to populations with long-term occupation of a region and, when possible, those that occupy similar geographic and temporal regions as the femoral sample. If an individual included in the femoral sample also had a complete mtDNA sequence available, it was included in the genetic sample in order to yield a 1:1 mapping of their morphology to their genome in the phylogenetic tree. Complete genomes were used when possible to prevent including identical partial sequences from some of the fossil humans with femoral samples (Beauval et al., 2005). The genetic sample used to create a comparative phylogenetic tree and model evolutionary relationships between these populations is outlined in **Table 4-3**, along with the GenBank accession numbers associated with each sequence. The justification for the use of each sequence is detailed below.

Late Pleistocene Homo sapiens

Several specimens from the site of Les Eyzies-de-Tayac (Southwestern, France) were sampled in the femoral data (Cro-Magnon 4322, 4323, and 4325). Cro-Magnon 1 from the same site has a completely sequenced mtDNA genome reported by Fu et al. (2013). Given that Cro-Magnon 1 (reported above as 30 kya cal BP) is from a similar time frame as several of the femoral specimens (several of which are near or just under 30 kya cal BP), is an early modern human from a similar location to many of the femoral specimens (specifically those in France), and has a complete mitochondrial genome, it was chosen as an ideal candidate for phylogenetic representation of the LP*Hs*.

Dolní Věstonice 13, 15, and 16, as well as Pavlov 1 reported above, are individuals with a femoral and a mitochondrial DNA sample. Due to this 1:1 correspondence between these data, the three complete Dolní Věstonice mtDNA sequences as reported in Fu et al. (2013) and in a submitted publication by Fu et al. (2016) are also represented in the phylogenetic tree as terminal taxa for LP*Hs*. Dolní Věstonice 15 was also sequenced and reported by Fu et al. (2013), but was identical to Dolní Věstonice 14, and therefore was not included in the phylogenetic tree. Pavlov 1 is a nearly complete mtDNA genomic sequence reported by Fu et al. (2016) and reproduced here as a terminal taxon to correspond to the femoral outline reported above. Dr. Qiaomei Fu provided the sequence in a personal communication.

Mitochondrial DNA sequences have also been recovered from individuals at Sunghir and from Paviland 1 (Alexeeva, 2000; Sykes, 2000); however, these sequences have a high likelihood of contamination. The Paviland 1 aDNA extraction did not use cloning which aids in the control for contaminated ancient sequences, nor did they test their sample to see if cloning made a substantial difference in accounting for problems with the aDNA sequence (Sykes, 2000; Winters et al., 2011). The authors of the Sunghir sequences have been highly criticized for not following proper aDNA precautions, such as a lack of cloning, repeated sampling, or accounting for appropriate preservation (Alexeeva, 2000; Ovchinnikov and Goodwin, 2003). Due to the suspect nature of these sequences, they have not been included here.

Neanderthals

European Neanderthals

The European Neanderthal sequence used to map the femoral data on the phylogenetic tree is El Sidrón 1253. Briggs et al. (2009) reported the full mitochondrial genome for this individual. El Sidrón is a cave site located in the northwest coastal principality of Asturias, Spain. In the most recent study to date the site, Wood et al. (2013) used careful tests for suitable collagen and contamination in order to directly radiocarbon date a Neanderthal specimen (00/46) and yielded a date range of 61.3-41.5 kya cal BP[1]. This result coincides with the upper end of estimates from de Torres et al. (2010) where a date range of 28,000±2,500 to 46,900±5,200 was obtained through direct and indirect dating of the fossil specimens, faunal remains, and sediments using the methods of ESR, OSL (Optically-Stimulated Luminescence), and AAR (Amino Acid Racemization). De Torres et al. (2010) also reported radiocarbon dates on charcoal, but due to a high amount of variation (ranging from approximately 4.9-56.4 kya cal BP[1]) likely caused by contamination and insufficient preservation, these are highly suspect and not reported here (Wood et al., 2013). Given the wide range of dates estimated for the site, (centering around 37,000±3,000 (ESR) and 61.3-41.5 cal BP (radiocarbon) for direct Neanderthal remains), the youngest date for the site is approximately 41.5 kya cal BP. Given the earlier reported dates for the European Neanderthals, the El Sidrón 1253 specimen can be used as a representation for that sample because it is in the center of the temporal range with a fully sequenced mitochondrial genome. With the 1:1 representation of Feldhofer 1 (see below), the geographic distribution of the femoral samples can roughly be estimated to fall within the range of these two terminal taxa.

Feldhofer 1, the Neanderthal type specimen, has also had a complete mtDNA genome reported for this individual and is therefore used as a terminal taxon in the phylogenetic tree per the criteria outlined above (Briggs et al., 2009). It is important to note that two other specimens used in the femoral sample have published mitochondrial DNA sequences. Specifically, Fond de Forêt 1 and Villeneuve have yielded small, partial sequences (less than 300 base pairs) that are identical to the Mezmaiskaya 1 complete sequences used as a reference sequence for the Western Asian Neanderthals (as outlined below) (Beauval et al., 2005; Lalueza-fox et al, 2005). Due to the redundancy in these sequences, they are not included in this study.

Western Asian Neanderthals

Unlike the previous groups of fossils outlined above, the Western Asian Neanderthal genetic sample does not have an individual with a 1:1 representation in both genetic and femoral samples. With that said, the representative chosen as the best available proxy for the Asian Neanderthals is Mezmaiskaya 1. Mezmaiskaya 1 is a Neanderthal from the northwest Caucasus region of Russia and has a complete mtDNA sequence as reported in Briggs et al. (2009). The excavations at the site led to the discovery of two Neanderthal infants, in separate stratigraphic layers. Mezmaiskaya 2 is the younger of the two from Layer 2 and Mezmaiskaya 1, the individual from whom the sequence used here was taken, is from Layer 3. Mezmaiskaya 1, in a layer with Mousterian artifacts, has been directly dated through radiocarbon dating to 33.2-29.3 kya cal BP[1], which is considerably younger than indirect ESR dates for associated teeth at these levels (approximately 40 kya BP for Layer 2 and 70-60 kya for Layer 3) (Ovchinnikov et al., 2000; Skinner et al., 2005). The rib from which the original radiocarbon date was derived showed significant collagen preservation and the authors explain the discrepancy between the bone and other dates of Layer 3 through problematic stratigraphic identification at the site (Ovchinnikov et al., 2000).

However, a second radiocarbon date extracted from Mezmaiskaya 2 of 43.4-40.2kya cal BP[1] using newer methods (Pinhasi et al., 2011), suggests that the initial direct date of Mezmaiskaya 1 is suspect and may be due to recent contamination, as is suggested by Skinner et al. (2005). Skinner et al. (2005) estimated through ESR that Layer 3 ranged between 67,600±5,4000 – 64,500±5,200 years BP, linear and early uptake models, respectively. These dates are consistent with the estimated radiocarbon date from Pinhasi et al. (2011) for Layer 3 (>47 and >46 kya cal BP). Give that the age estimate for Mezmaiskaya 1 is significantly younger than Mezmaiskaya 2 and the later was calculated with improved radiocarbon methods, it can be concluded that the younger age estimate is due to contamination as it is in a lower stratigraphic position than Mezmaiskaya 1. Therefore, the older age of 70-60 kya BP for Mezmaiskya 1, based on indirect ESR dating of associated faunal remains, is used here.

As Mezmaiskaya 1 is of a comparable age to the Asian Neanderthals in the femoral sample and is the closest geographic specimen with a full mitochondrial DNA sequence (north and northeast of the femoral specimens). It is used here as the individual to represent the terminal taxon for this group.

Recent Modern Humans

Four different mitochondrial sequences, two complete and two partial, represent the recent modern human sample used to create the phylogenetic tree (see the complete list of sequences used in in **Table 4-3**). Though not sampled directly from the same populations as the femoral data, the individuals represented here are taken from geographical and/or temporal regions similar to them. Where a comparable archaeological sample was unavailable, a recent geographically comparable population was used. Partial sequences were collected instead of complete mtDNA sequences when the individuals closely met both criteria for data collection.

The first sequence is from On Your Knees Cave (OYKC) collected from the Prince of Wales Island in Southern Alaska, USA. This individual's sequence includes the tRNA-Pro and D-loop sequences and was recognized as that of an Alaskan Inuit (unpublished sequence, Genbank accession number EU719667.1). While the skeletal remains recovered from OYKC were initially radiocarbon dated to 9.3-8.8 kya cal BP[1] (taken from a mandible) and 9.6-9.2 cal BP[1] (from a pelvis) (Dixon et al., 1997), it has been suggested that the depletion of radiocarbon in marine resources exploited at OYKC artificially inflates the dates from the skeletal remains recovered there (Southon and Fedje, 2003; Kemp et al., 2007). Kemp et al. (2007) suggest that the corrected date for the adult remains found in Alaska should be approximately 9.2 kya BP, which coincides with the dates from the archaeology of the site (Dixon et al., 1997). Given the time frame of this individual’s occupation at OYKC, this represents an acceptable proximity to the femoral data from Point Hope. Though Point Hope is a considerable geographic distance from Prince of Wales Island (near the northwestern and southeastern coastal boarders of the state, respectively), it represents a coastal occupation from the same temporal range (OYKC at 9.2 kya BP falls within the range given for the femora: 870-56 years cal BP and 300-400 years BP).

In order to best match the Pre-Dynastic Egyptian femoral data temporally, a partial sequence from the control region (D-loop) of the mitochondria was used from the individual Nekht-ankh (Pääbo, 1989). Nekht-ankh was a Pre-Dynastic Egyptian buried in a tomb with another individual (called The Tomb of the Two Brothers) at the site of Dier Rifeh and recovered archaeologically in 1906 (Petrie, 1907; David, 1979; Petersen et al., 2003). His remains, found in a marked coffin, were archaeologically attributed to the Twelfth Dynasty, approximately 4 kya BP (Petrie, 1907; David, 1979). The mtDNA sequence was taken from the preserved soft tissue, specifically the liver (David, 1979; Pääbo, 1989). Dier Rifeh, like Mesaeed and Keneh, is located in Middle Egypt, farther north along the Nile near Asyut (see Figure “Map of District and Plan of Cemeteries,” in Petrie (1907) for the estimate of the site location) (Petersen et al., 2003). Given the location of all three of these sites in Middle Egypt and their Pre-Dynastic ages (though the femoral sample has an earliest date of approximately a thousand years before the genetic sample), Nekht-ankh’s mitochondrial partial sequence is chosen as a reasonable estimation of the temporal and geologic ages represented by the femora above.

The final two sequences are complete mitochondrial genomes from recent populations, previously published and submitted to GenBank. The sequence used to represent the Çatalhöyük femoral sample is a modern, recent Turkish Kurd individual (Kur84) from the eastern part of Turkey reported and collected by Fernandes et al. (2012). The temporal and geographic distance is greater in this population than in previous populations in this study; however, it is the best distinct mitochondrial sequence representative currently available with the closest geographic proximity to the femoral sample.

The last genetic sequence used here to complete the phylogenetic tree has been paired with the Libyan Saharan population from the Wadi el Agail and the Tanezzuft Valley archaeological sites. The individual sequence, Tah 13, is from a recent modern population found in southwestern Libya from the Tahala village in the southwest Fezzan region, as reported by Ottoni et al. (2010). Although the most recent specimen from the archaeological femoral sample is approximately 1,500 years BP, the Tanezzuft Valley region is less than 100km from Tahala Village (see Fig1 in Tafuri et al. (2006) for the distance estimation) and Tahala is near the Alwanat/Al Awaynat village (Ottoni et al., 2009), making it geographically representative of the sample. While Wadi el Agail is substantially farther away in the Fezzan region (near the Ubari Lakes (Belmonte et al, 2002)), the two populations are morphologically similar and are therefore referenced by the same phylogenetic taxon.

Table 4-3. Fossil and Modern Human Mitochondrial DNA Genetic Sample. The majority of the sequences were retrieved from the online database GenBank. Sequences are submitted to this database by original authors of papers publishing the sequences or by those who originally analyzed the data. The exception is that of Pavlov 1 which was received directly from the first author of the associated publication (see text). No sequences were collected directly from specimens for this study. LP*Hs:* Late Pleistocene *Homo sapiens.*

Population	**Geographic Location**	**Specimen**	**mtDNA Region**	**GenBank Accession No.**	**Estimated Age**
Asian Neanderthals	Northwest Caucasus, Russia	Mezmaiskaya 1	complete genome	FM865411.1	70-60 kya BP
European Neanderthals	Northern Spain	El Sidrón 1253	complete genome	FM865409.1	> 41.5 kya cal BP
European Neanderthals	Germany	Feldhofer 1*	complete genome	FM865407.1	41.7 kya cal BP
LP*Hs*	France	Cro-Magnon 1	complete genome	KC521456.1	30 kya cal BP
LP*Hs*	Czech Republic	Dolní Věstonice 13*	complete genome	KC521459.1	27 kya cal BP
LP*Hs*	Czech Republic	Dolní Věstonice 14*	complete genome	KC521458.1	27 kya cal BP
LP*Hs*	Czech Republic	Dolní Věstonice 16*	complete genome	KU534949.1	28 kya cal BP
LP*Hs*	Czech Republic	Pavlov 1*	partial sequence	Personal Communication	28.5 kya cal BP
Alaskan Inuit	Alaska, USA	On Your Knees Cave	tRNA-Pro and D-loop	EU719667.1	9.2 kya BP
Pre-Dynastic Egyptian	Egypt	Nekht-ankh	control region, D-Loop	L36834.1	4 kya BP
Kurdish	Turkey	Kur 84	complete genome	JQ245757.1	recent population
Tahala (Taureg)	Libya	Tah 13	complete genome	HM171280.1	recent population

*These individuals are represented in the morphological and genetic data, with both the genome sequence and femoral measurements from the same individual.

CHAPTER V

METHODS

In order to test for phylogenetic signal in the femoral midshaft, morphological data must be first be collected from the specimens outlined above using silicon molds that are then digitally processed and digitized into landmark coordinate shape data. In this way, the morphological variation captured in the femoral molds can be compared to the mitochondrial sequences in the context of inferred evolutionary relationships (Garland et al., 1992). In order for the femoral data to map appropriately onto the evolutionary history of fossil and modern humans represented by a phylogenetic tree, and to accurately compare and understand observed variation between and among populations, the femoral cross-sections must be analyzed using principal component analyses of Procrustes superimposed shape differences. The phylogenetic signal can then be examined for correlation with allometric changes though independent contrasts and multivariate regression to further describe the factors attributing to shape change.

Morphological Data Collection

The cross-sections of the femoral diaphyses of the morphological sample were collected using the methods outlined by Trinkaus and Ruff (1989). The fossil femoral samples were collected by, and used with, permission from Dr. Erik Trinkaus; the recent human samples were collected by Dr. Laura Shackelford and Dr. Libby Cowgill and used here for analysis with their permission (Trinkaus and Ruff, 1989; Trinkaus and Ruff, 1999; Shackelford, 1995; Mednikova and Trinkaus, 2001; Teschler-Nicola and Trinkaus, 2001; Beauval et al., 2005; Cowgill and Hager, 2007; Trinkaus and Ruff, 2012).

The cross-sectional measurements of the femoral diaphyses were taken at the midshaft. The femoral midshaft is defined as the halfway point of the diaphyseal, or biomechanical, length (the total length of the femur, without the neck and head, divided in half). The biomechanical length is measured from the average of the distal-most point of each condyle to the end of the shaft, medial to the greater trochanter (Ruff and Hayes, 1983). This has also been defined by Trinkaus and Ruff (1989) as the intersection of the sagittal and coronal planes running though the center of the diaphysis of the femur at the proximal end of the bone. In order to obtain a reliable, noninvasive, and comparable estimate of the femoral midshaft morphology, a method using silicone putty (e.g., CutterSil Putty Plus Silicone Impression Material) was used. This method, as outlined in Trinkaus and Ruff (1989) and referred to as a Latex Cast Method (LCM) by Stock (2002), is performed by aligning the femoral specimen on x- and y-axes drawn on 1 mm graph paper. The femur is placed posterior-surface down on the paper, with the distal end facing the origin (0,0). Foam props are used to keep this orientation given the specimen's specific morphology and state of preservation. The average bicondylar measurement, defined as the distal end of the biomechanical length above, is calculated using architectural triangles, and is subsequently placed at the origin of the graph on the 1 mm paper. When the femur is lined up along the positive x-axis with the point medial to the greater trochanter along the axis (as the proximal point of the length), the biomechanical length is determined as the distance between these two points (as measured in mm along the graph paper). This distance is then divided in half to calculate the 50% diaphyseal cross-section.

Once the 50% cross-section is calculated, a piece of tape (a non-invasive tape with limited adhesive properties on bone, such as Scotch® Magic™ Tape) is wrapped around the diaphysis and the midshaft point is marked without writing on the bone directly. At this marked point, silicon putty is placed on the tape and is flattened on the distal end with a wooden tongue depressor (to prevent damage to the bone), until the flat surface of the putty is aligned with the marked midshaft point. The medio-lateral orientation is then indicated for future orientation once off of the bone. These cross-sectional molds, once set, are removed from the bone with a sharp blade and later are photocopied, using the flattened surface, to capture the morphology of the midshaft from the molds. **Figure 5-1** shows a representation of the midshaft measurement as well as the biomechanical length as measured on a modern human femur.

These photocopies can then be scanned into a computer to be oriented, scaled, and digitized using the appropriate software (as outlined below); this was the state at which the current samples were received. An example of the photocopied femoral midshaft scans is given in **Figure 5-2.** The LCM used to collect them has been compared to direct sectioning of the diaphysis and was found to have comparable accuracy in estimating the properties of long bone morphology in cross-section (Stock, 2002). Stock (2002) estimated that the LCM had an error rate of approximately 5% from a direct, invasive, sectioning method (called DSM) and performed better overall than other methods (such as eccentric elliptical methods).

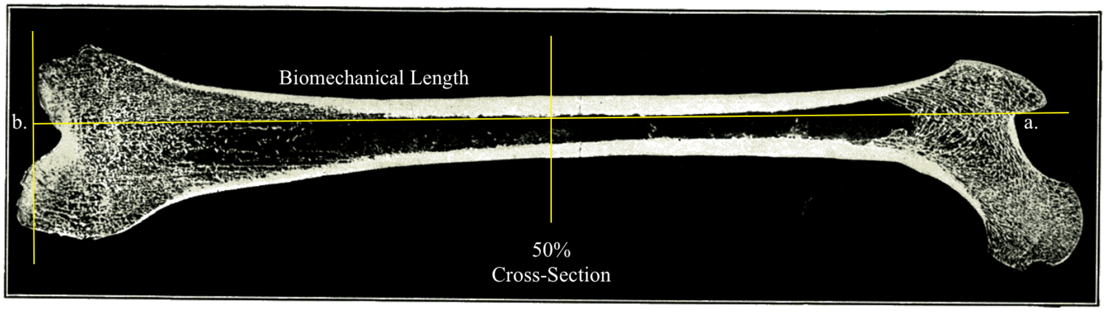

Figure 5-1. Graphical Representation of the calculation of 50% Femoral Diaphyseal Length**.** The figure above shows a representation of the femoral biomechanical length and location of the 50% cross-section on a sagittal cross-section of an adult femur. The distal and proximal borders of the biomechanical lengths are denoted by (a.) at the medial point of the greater trochanter and (b.) at the mean distal bicondylar distance. Reprinted and adapted from Gray (1973).

In all populations used in this study, several individuals had bilateral representation of their femora. Though Genovés (1967) showed that differences between both sides and sexes are statistically insignificant in the lower limb, Auerbach and Ruff (2006) demonstrated, using a more diverse sample than Genovés (1967), that femoral diaphyseal breadth tends to be larger in the left side of the lower limb, though sexually dimorphic differences are relatively small. Therefore, sexual determinations of the samples with represented femora are not taken into consideration here (Trinkaus, 1976). However, in order to minimize any effects of asymmetry when examining femoral cross-sections, only one femur from each individual was used in the analysis of phylogenetic signal. The left femur was chosen if available due to a higher number of left femora in individuals with only one side represented. Those individuals that were represented by only the right femur were not excluded from the sample in order to preserve sample size, which is especially critical in the fossil sample due to a lack of preservation and access to the lower limb bones.

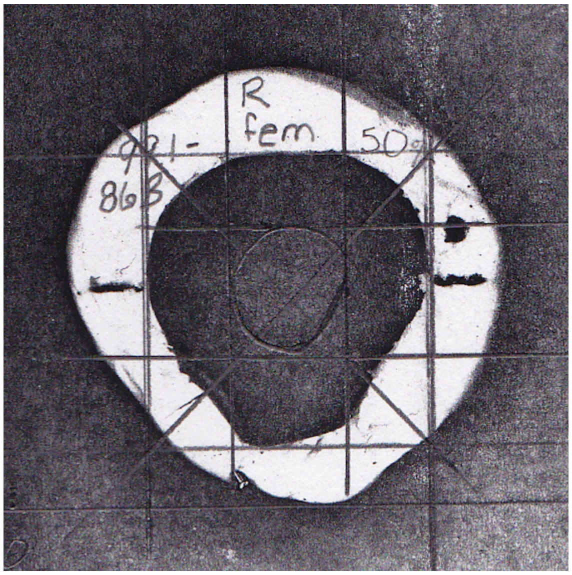

Figure 5-2. Example of 50% Diaphyseal Femoral Cross-Section**.** Above is an example of the scanned midshaft femoral cross-sections taken from the silicone putty molds of the right femur of an adult Alaskan Inuit individual from Point Hope, Alaska.

Phylogenetic Tree Model based on Mitochondrial DNA

In order to create a phylogenetic tree on which to base the estimation of shared evolutionary history between fossil and modern humans in the femoral midshaft, populations are used in lieu of species. When using populations instead of species in phylogenetic analyses, it is critical that gene flow be controlled for as much as possible. If it is not, or if it is poorly understood between the groups, a relationship present in the phylogeny may appear to represent differentiation by natural selection when it is truly reflecting migration and gene flow (Felsenstein, 2002). This may show an artificially high association of the trait with phylogenetic signal. Aside from the problematic interference from gene flow, phylogenetic comparative methods have been shown to reveal important evolutionary relationships which may otherwise be obscured when assuming independence between groups in statistical analyses, especially where populations are genetically distinct but evolutionarily closely related (Garland et al., 1992; Edwards and Kot, 1995; Avise, 2000; Ashton, 2004). In order to control for migration as much as possible in this analysis, groups were selected that were long-term occupants of a region, often isolated by geographic barriers (such as mountain ranges, seas, and oceans) or temporal barriers (such as the 4,000-year-old separation between the modern human individual from Libya and the recent individual from Libya).

As outlined above, mitochondrial DNA sequences were collected for the creation of the phylogeny (**Table 4-3, Figure 6-20**). While mtDNA has limitations in its expression of lineages, such as only giving information on surviving maternal lines, it has several strengths that make it idea for the comparison of population genomes. Principle among these is the lack of evidence of sequences from Neanderthal mtDNA in early and recent modern human mitochondrial DNA (Serre et al., 2004; Ghirotto et al., 2011). This does not mean that these two groups did not admix at some point, but it does indicate that the mtDNA lineages did not survive in the early and recent modern human lineages presented here; there is no *mitochondrial* gene flow evident between these individuals.

Early ancient DNA studies of modern humans and Neanderthals concentrated on the extraction of mitochondrial DNA (e.g., Krings et al., 1997). Mitochondrial DNA (mtDNA) was ideal for these early studies given the higher frequency of mitochondria in the cell (from 100 -1000 per cell) when compared to the nucleus, allowing for a better recovery rate of reliable sequences (Witas and Zawicki, 2004). Additionally, the mitochondrial genome of recent and fossil humans contains just over 16,500 base pairs as compared to the nuclear genome, which contains around 3 billion. Mitochondrial DNA mutates faster than nuclear DNA (and does so at an even higher rate in the D-loop region) (Brown et al., 1979, 1982; Howell et al., 1996), allowing for the observation of change over a shorter amount of evolutionary time, and, given the lack of recombination, the changes observed in these sequences can be attributed to mutation; this can reveal a high level of variation between populations (Merriwether et al., 1991; Witas and Zawicki, 2004). As mitochondrial DNA is inherited through the mother rather than through both genetic parents, there are fewer problems in analysis than with diploid data (Giles et al., 1980).

Mitochondrial DNA does have limitations in its use in phylogenetic studies. Due to its materially inherited haploid nature, there is no paternal information accounted for in studies using mtDNA. This can greatly obscure population dynamics by only informing on the survival of maternal lineages. Mitochondrial DNA has also been known to show the presence of phantom mutations, impacted by the sequencing equipment, which appear to create sequence distinction when there may not be any, leading to an inflated interpretation of variation within the sequences (Pusch and Bachmann, 2004; Brandstätter et al., 2005). Despite these limitations, the availability of full mitochondrial genome sequences for appropriate temporal and geographic samples as well as the aforementioned positive attributes of ancient mtDNA, make it ideal for use in this study.

Preliminary explorations into the phylogeny of fossil and recent modern human mitochondrial genomes performed by this author revealed limited differentiation between populations of fossil and recent modern humans with only partial sequences of mtDNA. In order to limit the noise and create greater resolution in the tree, the entire mtDNA sequences were included where possible. Additionally, these early phylogenies consistently showed Neanderthals as an outgroup, which was then used to root the tree. The monophyly of Neanderthals has also been supported in other published phylogenies, including those using nuclear DNA (Ovchinnikov et al., 2000; Green et al., 2008; Krause et al., 2010).

Following precedent from previously published recent modern human phylogenetic studies, representative sequences for each population (as outlined in **Table 4-3**) were selected that would allow for mapping of average Procrustes femoral shapes of each specific group onto the corresponding terminal taxon (Green et al., 2008; Krause et al., 2010). One genetic sequence is unlikely to represent the full range of genetic variation within each population; however, a representative sequence is the best way to summarize a phylogeny to map femoral shape to genetic data for each specific population. The exception concerns the individuals for whom both a femoral cross-section and a genetic sequence were available. These individuals were included as terminal taxon even if they could be included in another population. The 1:1 mapping of these individuals allow for a more direct relationship between the genetic and morphological components of the test for phylogenetic signal in this portion of the tree.

The sequences described in **Table 4-3** were aligned in MAFFT (Multiple Alignment using Fast Fourier Transform), a program for the alignment of amino acid and nucleotide sequences (Katoh, 2013), using the online server and the L-INS-i iterative refinement method. The L-INS-i method was ideal for these sequences due to its ability to deal with flanking sequences (as is expected in the Pre-Dynastic Egyptian sequence that contains part of a tRNA-Pro sequences) and small numbers of sequences accurately. The alignment was visually inspected in Mesquite (Maddison and Maddison, 2015) to verify that the alignment completed using the entire genome contained enough variation to create a reliable phylogenetic tree. Once this variation was verified, the distance matrix of the alignment was examined in PAUP* (Swofford, 2003) to ensure that there were no identical sequences present, which is especially important when using partial mitochondrial DNA sequences. Only one sequence was included when several sequences appeared to be identical. Where possible, especially in the Neanderthal population where this was especially prevalent, partial sequences that were identical were replaced with a full mitochondrial DNA sequence for that individual.

Initial analyses of the alignment to create a phylogenetic tree were run using Bayesian, Parsimony, and Maximum Likelihood methods. Maximum Likelihood methods create trees with the highest likelihood of estimating the observed data (here, an alignment of mtDNA sequences) and use parameters describing patterns of molecular evolution. Bayesian analyses use *a priori* information about the observed data in order to create a distribution of possible trees, or a posterior probability distribution, using an algorithm, such as the Markov Chain Monte Carlo algorithm, from which a best estimate phylogeny can be chosen. Parsimony methods infer the smallest number of evolutionary changes along branches to create a phylogenetic tree (Lemey et al., 2009). As the Bayesian and Parsimony analyses did not distinguish between modern human populations (most nodes were collapsed together), the Maximum Likelihood analysis was used in order to create the most informative tree for phylogenetic analysis (**Figure 6-10**). Maximum Likelihood methods use parameters within specified models of evolution (parameters such as branch lengths, tree topology, the ratio of transversions/transitions, etc.) in order to yield the tree with the highest probability of resulting in the sequences provided from the alignment (Lemey et al., 2009).

In order to determine the best-fit Maximum Likelihood evolutionary model to use to create the phylogenetic tree, JModelTest (Darriba et al., 2012; Guindon and Gascuel, 2003) was used to on the aligned sequences to infer an optimal model using a likelihood ratio test. The evolutionary model with the highest overall weighted score was the TrN+I model. The Maximum Likelihood phylogenetic tree analysis was run on the CIPRES Science Gateway using RAxML-HPC2 (Miller et al., 2010; Stamatakis, 2014). As RAxML does not use the TrN+I model, the next most complex model was chosen, GTR. The GTR, or general time reversible, model of evolution is the most complex model commonly used in Maximum Likelihood methods and allows for the highest number of parameters, representing all possible types of nucleotide substitutes individually. This is acceptable as the TrN+I model is nested within the GTR model of evolution, a requirement for comparing Maximum Likelihood methods (Lemey et al., 2009). As the consensus tree from the CIPRES output had a number of collapsed nodes (and therefore did not have enough distinction between groups), the tree with the highest likelihood, the best tree, was used. As previously stated, Neanderthals were consistently monophyletic and were used as an outgroup to root the tree. The Maximum Likelihood best tree is reported here and is the basis for the evolutionary relationships to test phylogenetic signal **(Figure 6-20)**.

Digitizing Femoral Cross-Sections and Applying Landmarks

In order to test phylogenetic signal against femoral shape, the femoral midshaft cross-sections detailed in Chapter IV (Materials) need to be transformed into morphometric data (in this case, x and y axis coordinates for 2-dimensional cross-section shape data). In this way, geometric morphometrics (the study of shape and shape changes in relationship to other evolutionary pressures) can be used to map evolutionary relationships onto the shape space of morphological data (Klingenberg and Gidaszewski, 2010; Adams et al., 2013). In order to compare and understand the processes behind shape change, morphology must be described in concrete terms, specifically landmarks. Landmarks can be defined as congruent, or homologous, anatomical structures shared between specimens in 2- or 3-D shape space that can give information on the evolutionary question that is being asked. Defining morphology in the context of landmarks is critical as it allows for a better understanding of covarying variables and their impact on shape change without requiring *a priori* assumptions about those variables. Instead, the analysis itself will yield this critical information as long as the landmarks chosen are relevant to the hypothesis being tested (Zelditch et al., 2004).

Traditional landmark analysis cannot be used, however, in the determination of homologous points on femoral outlines. Zelditch et al. (2004) outlined four qualifications of landmark analysis on a 2-dimensional specimen (such as femoral cross-sections): homology, consistent order of points on each specimen, repeatability, and that all important aspects of the shape in question are visible ("comprehensive coverage" per Roth (1993)). While the femoral pilaster is easily the most identifiable feature on a cross-section of a femur, it can be difficult and variable to determine its defining landmarks. Additionally, outlining the beginning or end (and even most posterior projection) of the pilaster alone does not encompass the complete shape of the cross-section. Therefore, the requirements of homology and "comprehensive coverage" are not met.

In order to capture the shape variation of a cross-section under these conditions, semilandmarks can be used. Semilandmarks can be defined as points along a curve, such as a femoral outline, that can be correlated to one another to create the illusion of homologous landmarks. Once semilandmarks are established, they must be correlated in the same way for each specimen; they can then be used to replace, or in conjunction with, landmark data (Klingenberg, 2008). Similar to the principals used in eigenshape analysis (Lohmann and Schweitzer, 1990; MacLeod, 1999), the cross-sectional femoral data are described wherein a consistent number of semilandmarks are identified that share an initial starting point (see the description below) (Klingenberg, 2008). In this way, homology is inferred, and sufficient data are recorded to summarize the outline of the cross-section for meaningful comparison between specimens/populations.

The femoral data for this study were received as photocopied cross-sections made from silicone molds as outlined above (also see **Figure 5-3**). The molds were marked with information denoting their side, specimen, and orientation. Thick black lines on either side of the cross-section indicate the medio-lateral orientation of the femur at the time it was sampled, with an indicator of the medial or lateral side (sometimes denoted by a black dot or by the letters "M" or "L"). Prior to digitizing the femoral data, each specimen first had to be oriented and scaled to one another. This process was accomplished using the freeware program, GIMP (Kimball and Mattis 1995-2014). The cross-sections were scanned and cropped to a 2-inch by 2-inch section and oriented so that the medio-lateral plane was on the horizontal axis and the medial side was on the left. In order to ensure that the digitized points were comparable across all samples, it was critical to make sure that the specimens taken from right femora were processed as mirror images. In this way, all medial and lateral aspects were directly comparable to each other and conclusions drawn from the data analyses would reflect true trends in variation and not the side from which the femur was sampled.

In order to locate the centroid of the 2-D representation of the femoral cross-section, tangential planes at the medial, lateral, anterior, and posterior points were identified. Where these planes intersected, straight lines were drawn across the femur (the anterio-medial intersection was connected to the posterio-lateral intersection, and the medio-posterior with the anterio-lateral). Where these diagonal lines met represented the center of the cross-section, or the centroid (see **Figure 5-3**).

In order to digitize the outline of the femoral cross-section and establish semi-landmarks for Procrustes transformations to test for phylogenetic signal, the centroid-marked cross-sections were input into the digitizing program MLMetrics (Lestrel and Wolfe, 2003-2014). To create homologous semilandmark data, the femoral cross-sections (roughly circular in size with the pilaster projection near the posterior portion) were divided into 36 different points in order to get "complete coverage" of the femoral shape, with the first point established at the anterior-most point of the femur along the axis perpendicular to the medio-lateral axis used for the original orientation of the femur. Each semilandmark was marked in ascending numerical order along the external-most edge of the cross-section, in a clockwise orientation, every 10 degrees radiating from the centroid (**Figure 5-4**). The x and y coordinates of each semilandmark gave a digital representation of each homologous point and were exported as the character traits to be used in the analysis of phylogenetic signal. The digitized femoral data was imported into the Geometric Morphometrics program MorphoJ and PAST3 so the femoral shape data could be analyzed for Procrustes superimposition, Principal Component Analysis (PCA), Canonical Variate Analyses (CVA) and a permutation test for phylogenetic signal (Hammer et al., 2001; Klingenberg, 2011).

Figure 5-3. Example of a cropped and oriented femoral cross-section with the centroid identified by the intersection of the red lines. The specimen below is Dolní Věstonice 16.

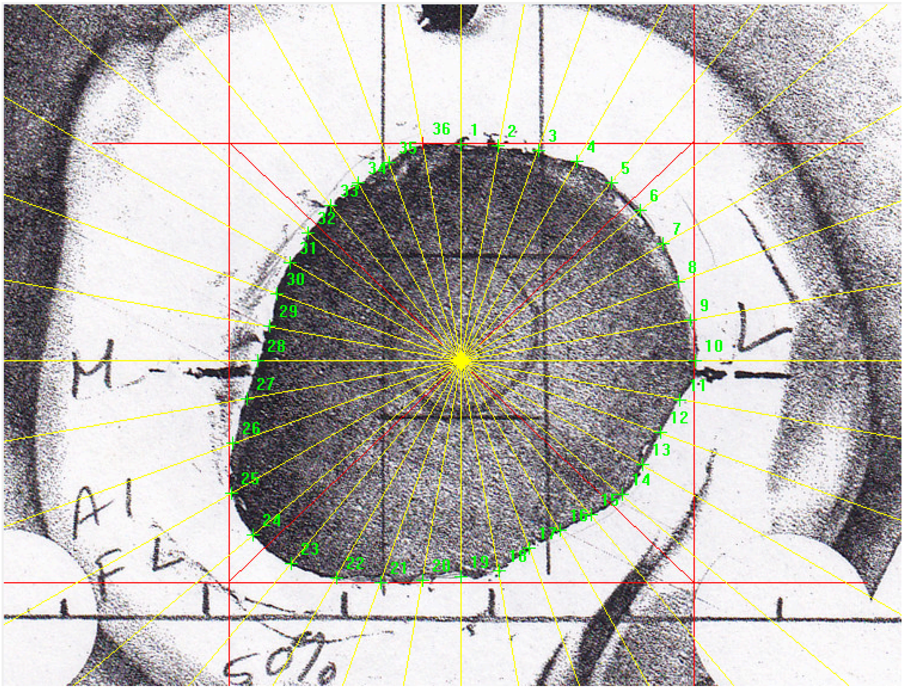

Figure 5-4. Example of a femur with the outline digitized into 36 points. Each point highlighted in green will yield (x,y) coordinates. The centroid and 10 degree increments are indicated in yellow. The specimen below is Amud 1

Procrustes Transformation and Principal Component Analysis

Generalized Procrustes Analysis (GPA) is used in geometric morphometrics to aid in the comparison of shape data and other selective factors by allowing for the isolation and examination of shape data from other traits influencing apparent morphological variation (such as size and orientation) (Lohmann and Schweitzer, 1990; Adams et al., 2013). GPA uses landmark (and semilandmark) coordinate data to adjust, or superimpose, each specimen onto the same shape space. This includes moving all shapes to the same set of coordinates (the centroid is placed at the origin, either (0,0) or (0,0,0) depending upon if the coordinate data supplied are from a 2- or 3-D digitized specimen). The specimens are then scaled so that they have the same relative size (or "unit centroid size") and rotated (Adams et al., 2013). The rotation of the specimens is performed by minimizing the sum of squares distances of each specimen's landmarks from the mean. Once this is performed, the resulting shape data (Procrustes shape coordinates) can then be used in statistical analyses to understand the relationship between the isolated shape of the specimens and other factors, specifically in this study, the understanding of the evolutionary relationships between fossil and recent modern humans and the impact of shape when compared to size, or allometric, changes between specimens (Adams et al., 2013; Mitteroecker et al., 2013).

After Procrustes superimposition, a principal component analysis was conducted using the Procrustes shape coordinates to better understand the variation found in the shape data. PCA can be used to show the relationships of size and shape, also called form, of each specimen. This is measured using the Procrustes shape coordinates from the aforementioned analysis. Each point in the PCA represents the form of a specimen and can reveal information on the relationship between shape and size on femoral midshaft variation (Mitteroecker et al., 2013). The variation in shape explained by the PCA is measured in the eigenvalues and the eigenvectors, the first of which explains the variance found in the different principal components, with PC1 usually accounting for the largest amount of variance and attributed to allometric changes in shape. The latter components, the eigenvectors, explain the direction of shape change as outlined by the eigenvalues (Klingenberg, 2013a). In the case of femoral outlines, for example, eigenvectors can indicate if shape change in a population is centered around the pilaster or anterior medio-lateral extensions.

Examining femoral shape change and the differences between the populations prior to correcting for signal was done through a canonical variate analysis. Using the populations outlined below as the *a priori* CVA group classifications, the femoral shape variables can be analyzed to best visualize the between-group differences, and, as expressed in a permutation test for the Mahalanobis p-value, can evaluate if the differences found are significant (Klingenberg, 2011).

In order to best visualize the morphological changes of the femoral midshaft between fossil and modern humans, the analyses were assigned to the following populations: LP*Hs*, Asian Neanderthals, European Neanderthals, Alaskan Inuit, Pre-Dynastic Egyptian, Libyan, and Turkish. Those individuals with a 1:1 correspondence of morphological and genetic data were treated initially as a unique group, but were included with the overall population they could be associated with in combined analyses (e.g., Feldhofer 1 in the European Neanderthals), such as in Procrustes superimposition, PCA, and CVA analyses. For further comparison, these groups have been combined into larger populations: LP*Hs*, Neanderthals, and Recent Modern Humans. Finally, the large groups of All Fossil specimens and a group of All Modern Humans (recent and fossil) were combined for shape comparison. For final analysis of phylogenetic signal and overall shape and size comparison, all specimens were analyzed together.

Phylogenetic Signal for Shared Evolutionary History

The test for phylogenetic signal between fossil and modern humans in the size and shape of their femoral cross-sections was performed in MorphoJ (Klingenberg, 2011). Phylogenetic signal in femoral size was measured by a permutation test after projecting the morphological data onto the aforementioned phylogeny (though squared-change parsimony). The relationship of this signal was then tested against size using independent contrasts to see if evolutionary allometric changes have impacted the variation observed in femoral midshaft shape (Felsenstein, 1985).

It is important to mention the difficulty in assessing multivariate traits in traditional phylogenetic comparative methods. Due to the high number of variables, recorded as the coordinates to retain the characteristics of the femoral cross-sectional shape, as compared to the number of individuals in the morphological sample size, traditional multivariate methods cannot be used for assessing shape data. For example, femoral outlines have 36 semilandmarks with two trait dimensions each (the x and y coordinates), resulting in 72 variables to represent a femoral cross-sectional shape. In this phylogeny, there are 9 terminal taxa which function as the sample size for phylogenetic comparison. As the number of terminal taxa is significantly smaller than the number of variables in the coordinate data, the basic assumptions of multivariate methods, which require the opposite scenario, are violated (Gunz and Mitteroecker, 2013: Adams, 2014).

In order to address this and other problems in traditional phylogenetic comparative analyses, such as assumptions of trait independence or influencing results through the selection of how shape characters relate to one another, Klingenberg and Gidaszewski (2010) view shape as a single character with multidimensionality. They argue that shape is complex in its changes and moves with both the magnitude of a scalar trait and direction. When viewing shape in this way, an entire shape can be treated as the state of a single character, reducing, drastically, the number of variables in the analysis (Klingenberg and Gidaszewski, 2010).

In order to view the changes in shape in relationship to the shared evolutionary history between fossil and modern human groups, the evolutionary history, as represented by a phylogeny, must be projected into the same shape space as the morphometric data. In MorphoJ, this is done by a squared-change parsimony method adopted from Maddison (1991) (Klingenberg, 2011). The permutation test for phylogenetic signal using squared-change parsimony takes the multidimensional shape characters and assigns, or maps, them to specific taxa on the phylogenetic tree in order to recalculate tree length. This is done in such a way to minimize the number of changes along the branch of the tree and within the coordinate data (measured in squared-change parsimony as the squared Procrustes distances). Species that have shared evolutionary history (and thus exhibit signal) will have fewer changes along their branch lengths. The squared-change parsimony method follows a Brownian motion model of evolution and is robust to rotations within the shape space (Klingenberg and Gidaszewski, 2010).

Phylogenetic signal is then evaluated through a permutation test run on the phylogeny as it is projected into shape space (Klingenberg and Gidaszewski, 2010). As mentioned above, phylogenetic signal is present if species that have recently diverged (and therefore have a long shared evolutionary history) are more closely related and resemble each other than those who do not have a shared history. In morphometric data, this is translated to more closely related species occupying the same region (or more similar regions) of the shape space than those species who do not share evolutionary history (Klingenberg and Gidaszewski, 2010). The permutation test used in MorphoJ estimates phylogenetic signal in the data by comparing the shape data to the terminal taxa in the phylogenetic tree to see if any specific iteration will impact the estimated shape changes along the branch lengths (Klingenberg, 2011). Due to the number of terminal taxa in this study (12), all permutations possible (12! or 479,001,600) were not run on the tree due their high number; instead 100,000 permutations were chosen as Klingenberg and Gidaszewski (2010) recommended more than 10,000 for adequate resolution. If phylogenetic signal is present, the estimated branch lengths from a tested permutation will be longer than in the tree calculated from mtDNA data, indicating an influence of shared history that is greater than expected (Klingenberg and Gidaszewski, 2010); in other words, the observed phylogeny has shorter branch lengths than the morphometric data alone would estimate. In this way, the null hypothesis of independence between morphometric traits is tested: signal is represented by a low number of character state changes and shorter branch lengths (Klingenberg and Gidaszewski, 2010).

If the presence of phylogenetic signal is detected, evolutionary allometric changes can also be measured through a multivariate regression and independent contrasts. If signal is present, it is important to understand whether or not the observed variation in shape is due to a shared evolutionary history of allometric changes. A multivariate regression can demonstrate the changes in shape based on the measurement changes in size. The regression was run here on the Procrustes shape coordinates against centroid size in the context of the phylogeny. The resulting vector of regression coefficients and centroid size indicates how much a change in allometry impacts changes in the observed morphometric shape (Klingenberg, 2013a; Mitteroecker et al., 2013). The amount of shape change based on allometry can also be measured by the total sums of squares which indicates approximately how much of the observed variation can be attributed to changes in size.

Finally, in order to understand the impact of shared history between size and shape, a comparative method, independent contrasts, was run using the provided phylogeny and Procrustes superimposed data (Felsenstein, 1985). The null hypothesis in independent contrasts tests the independence of two evolutionary traits and relies on known branch lengths and assumes a correct phylogeny. This method tests the evolutionary relationship of two traits by comparing adjacent terminal taxa with an immediate shared common ancestor. This is repeated along the tree, removing each of the compared nodes and exposing the ancestor as a new terminal taxon, until the comparisons reach the final two nodes near the root of the tree (Felsenstein, 1985). If the resulting p-value is significant, the two compared traits show phylogenetic signal and are not evolutionarily independent of one another. In this case, changes observed in shape could not be observed or compared directly without compensating for evolutionary allometric changes.

CHAPTER VI

RESULTS

Procrustes Superimposition

Though Procrustes superimpositional data can show the changes between an individual specimen and Procrustes shape coordinate data, the transformation of landmarks yields limited information on the overall analysis of shape. It is possible, however, to get a basic understanding of the data through the examination of initially superimposed landmarks (Klingenberg, 2013b). These data are included here to give a general assessment of the shape of femoral diaphyseal midshafts in each population.

For the recent modern human populations, as shown in **Figure 6-1**, the overall shape of each population is similar: a pronounced pilaster can be seen with gently sloping medio-lateral borders. As expected, this trend is continued when these populations are combined (see **Figure 6-2a**). LP*Hs* show a similar trend to that observed in the cross-section of recent modern humans, a clear pilaster, though possibly with a sharper slope and point. All groups of Neanderthals show a distinctly round femoral shape, though there may be evidence of a pinching among the more gracile Asian group at the posterior end; however, it is so slight it cannot be attributed to the presence of a pilaster or even the beginning of one (see **Figure 6-3** and **Figure 6-2c**). The aforementioned trends in shape can be observed though the examination of each group prior to superimposition.

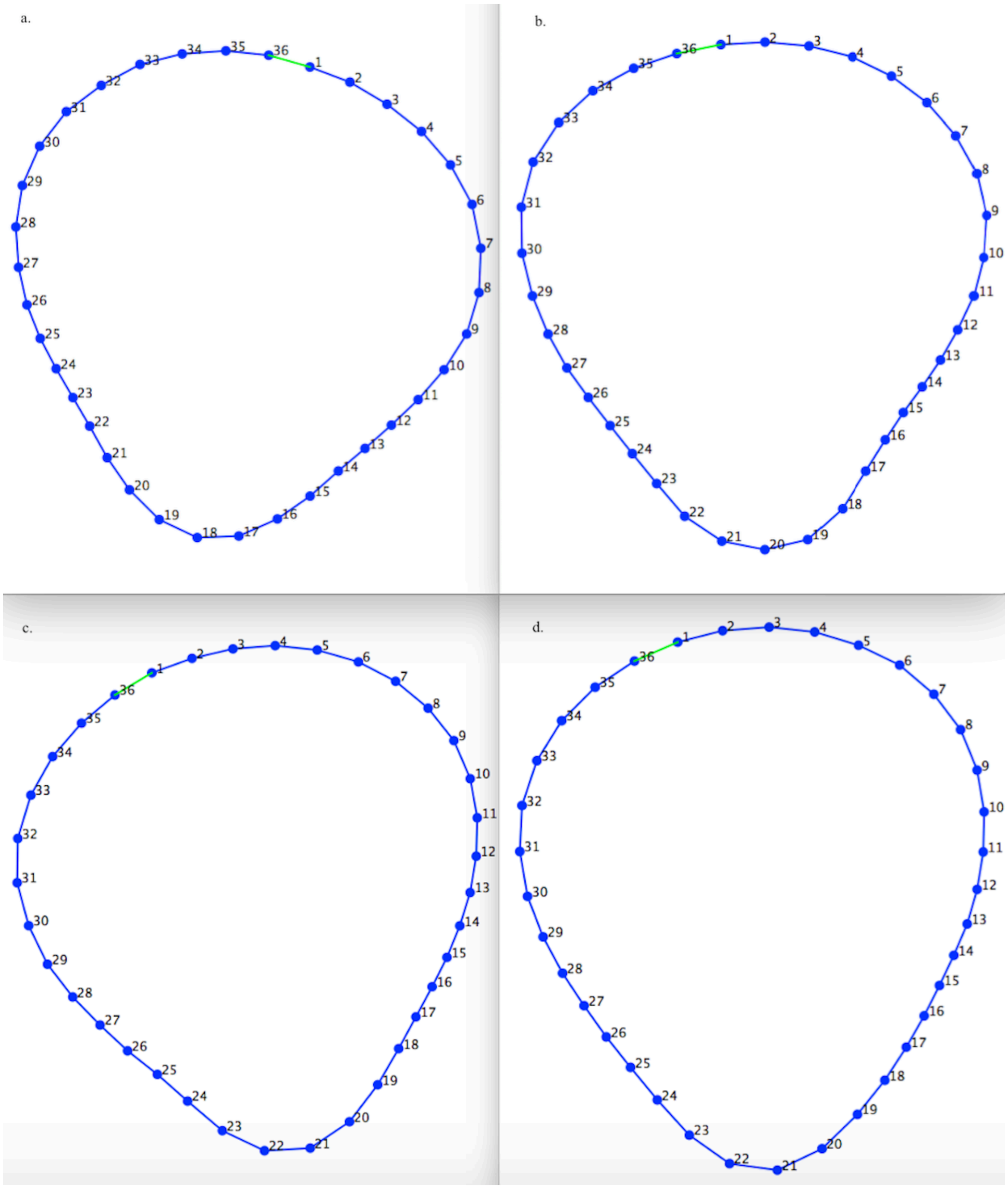

Figure 6-1. Procrustes shape coordinate data for each recent modern human population (a. Turkish, b. Alaskan Inuit, c. Pre-Dyanstic Egyptian, d. Libyan).

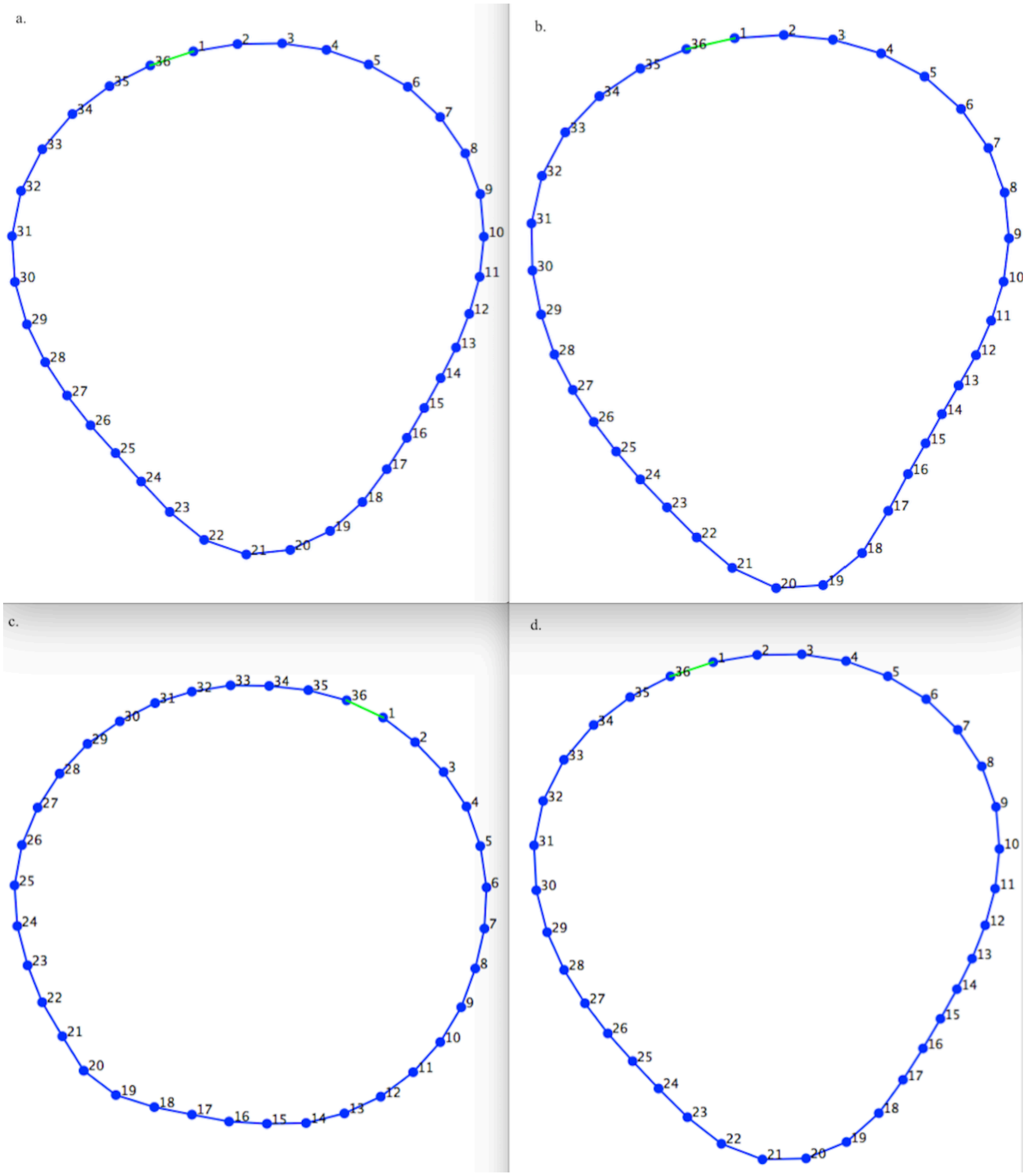

Figure 6-2. Procrustes shape coordination data for combined populations (a. all Recent Modern Humans, b. all LP*Hs*, c. All Neanderthals, d. Total Specimens Combined)

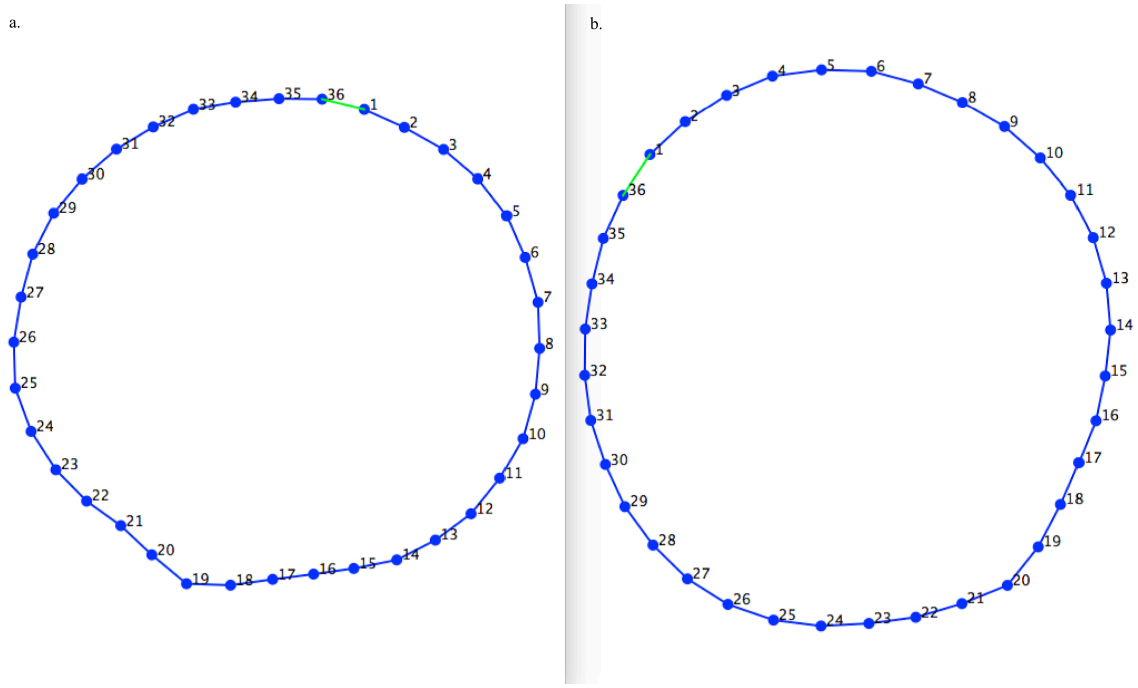

Figure 6-3. Procrustes shape coordination data for Neanderthal populations (a. Asian Neanderthals, b. European Neanderthals)

Principal Components Analyses

Recent Modern Humans

A principal component analysis on the combined Recent Modern Human group showed 91.21% of the variation was explained in the first five principal components (PCs), with 45.58% on PIC1 and 23.97% on PC2 (**Table 6-1**). The majority of the variation in shape on PC1 is centered around the pilaster with medio-anterio movement on the lateral portion of the pilaster and posterio-medial movement on the medial portion of the pilaster (**Figure 6-4a**). PC2 (**Figure 6-4b**) indicates variation associated with the pilaster as well as the anterior medio-lateral femoral breadth. Though similar to PC1, it is less pronounced and the shape variation on the anterior slope of the pilaster is in an outwardly direction, suggesting less emphasis on the pilaster. In **Figure 6-5**, the scatterplot of the first two PC's with recent modern human femoral cross-section forms is shown. There is one significant outlier, the bottom-most specimen, which represents an individual from the Libyan population with a shape that is the most divergent from the mean.

Table 6-1. The first five Principal Components for the combined Recent Modern Human populations. Only Principal Components that contribute to more than 5% of the variance are shown.

Principal Components	Eigenvalues	Variance (%)
1	0.00306095	45.58
2	0.00160967	23.97
3	0.00058517	8.71
4	0.00052486	7.82
5	0.00034494	5.14

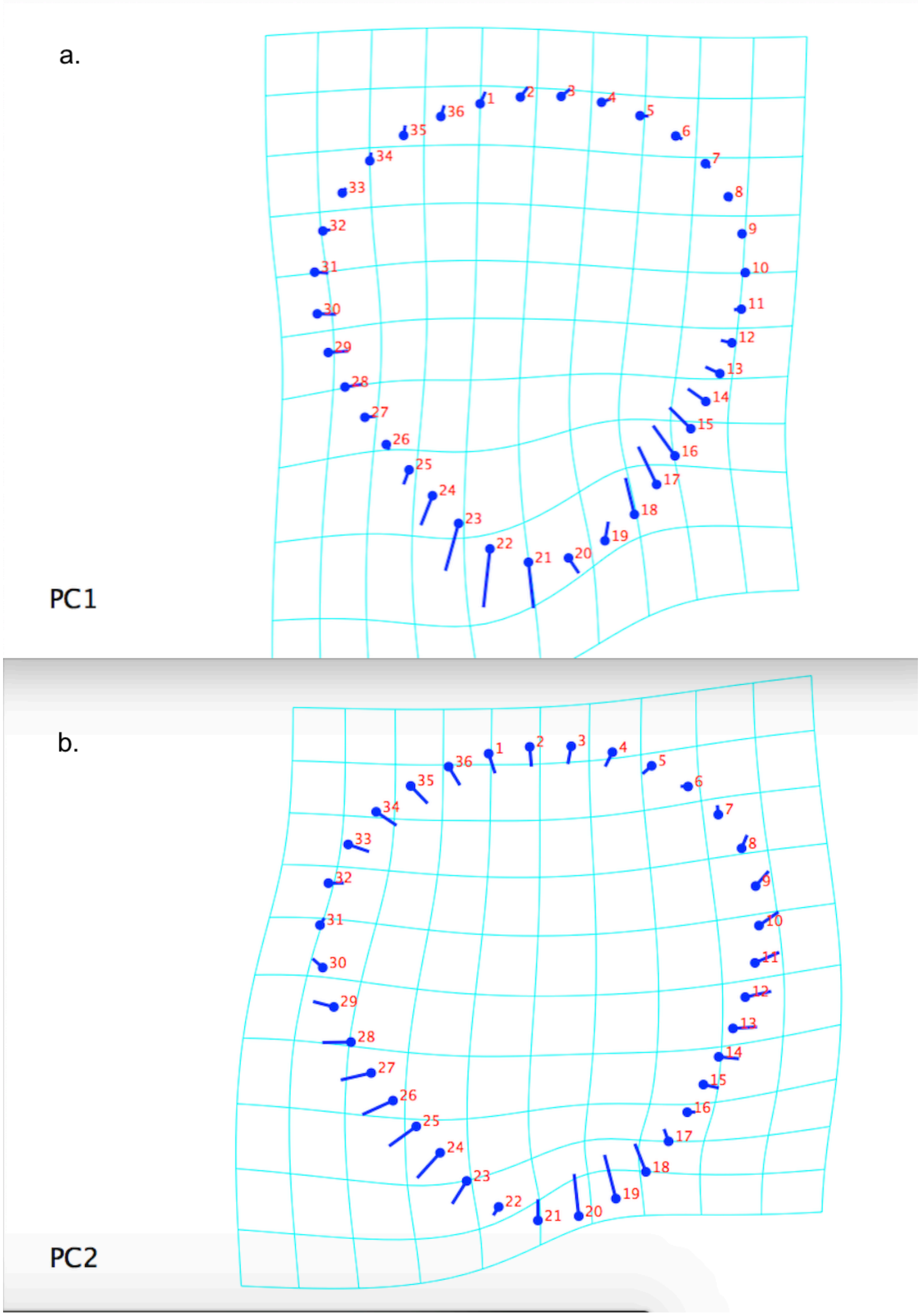

Figure 6-4. PC1 and PC2 shown in Transformation Grids for the combined Recent Modern Human Population.

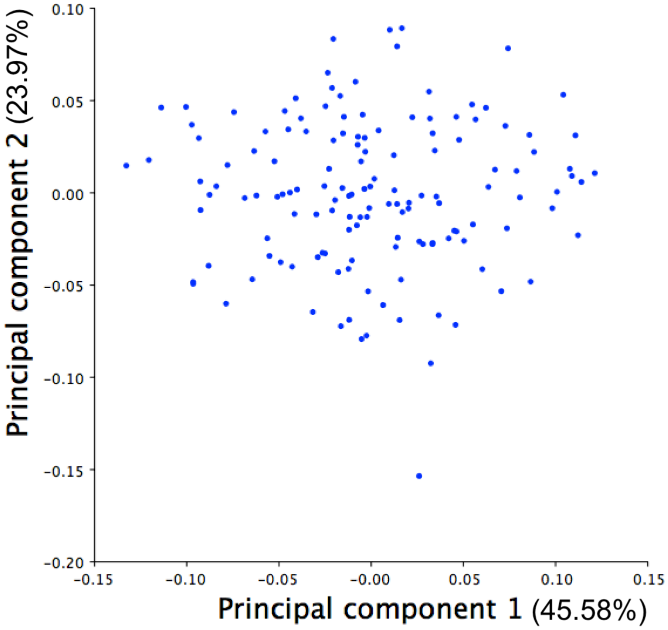

Figure 6-5. Scatterplot of the first two PCs showing the shape changes of the Recent Modern Human population. The bottom-most outlier is an individual from the Libyan population.

Late Pleistocene Homo sapiens

The next PCA was performed on the Late Pleistocene *Homo sapiens* population. Most of the variation in shape in this population was centered in the first three PCs, which accounted for 85.25% (see **Table 6-2**). PC1 accounted for 45.56% of the variation and PC2 accounted for 32.2% of the variation. The shape ranges in PC1 are centered around the pilaster, although with this population the variation indicates an inward movement of the pilaster on both the medial and lateral sides with a posterior projection at the point. There is a small amount of variation in the shape ranging toward the anterior portion of the femur indicating an anterior movement suggestive of an elongating shape anterio-posteriorly throughout this group (**Figure 6-6a**). PC2 is also associated with variation in the pilaster, specifically with a posterio-medial movement on the medial side and an anterio-medial movement on the lateral side (**Figure 6-6b)**. The scatterplot of the Late Pleistocene *Homo sapiens* specimens is a loose cluster with one significant outlier, Sunghir 1, which shows a deviation from the mean for PC2 (**Figure 6-7)**.

Table 6-2. The first three Principal Components for the combined Late Pleistocene *Homo sapiens* populations. Only Principal Components that contribute to more than 5% of the variance are shown.

Principal Components	Eigenvalues	Variance (%)
1	0.00379667	45.56
2	0.00268571	32.23
3	0.00063315	7.47

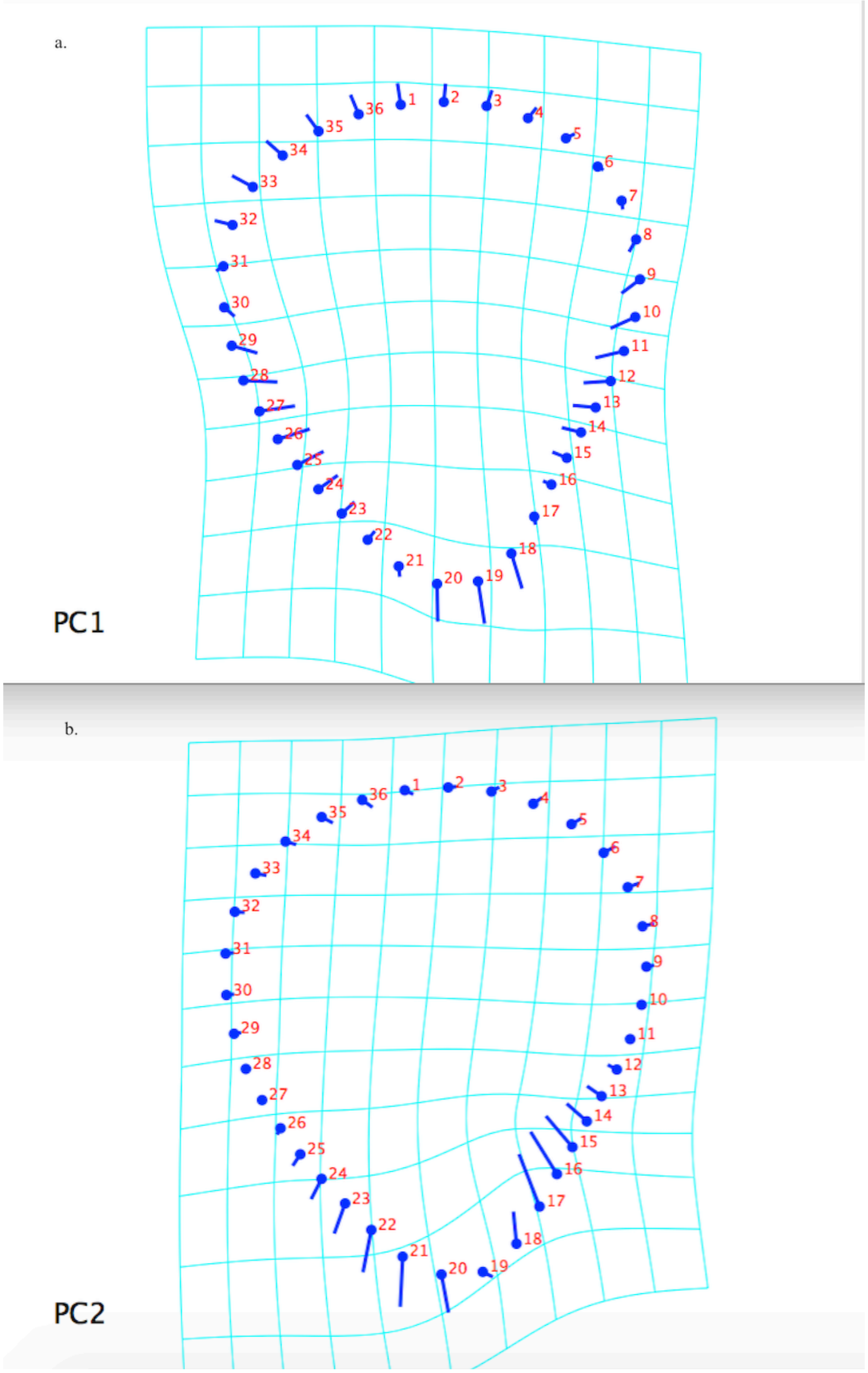

Figure 6-6. PC1 and PC2 shown in Transformation Grids for the Late Pleistocene *Homo sapiens* Population.

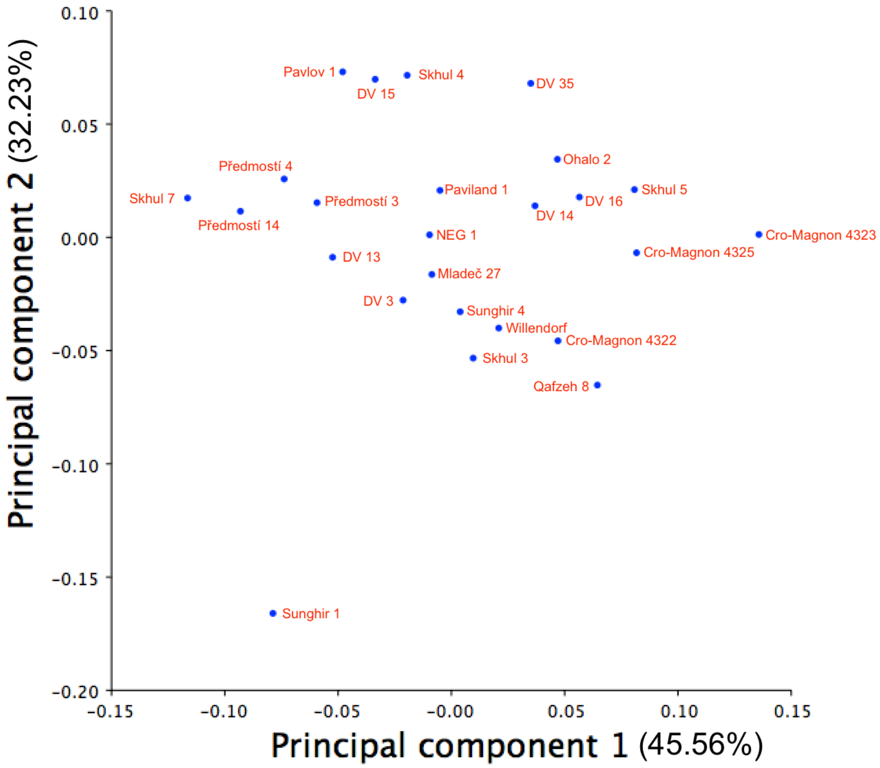

Figure 6-7. Scatterplot of the first two PCs showing the forms of the Late Pleistocene *Homo sapiens*. DV: Dolní Věstonice, NEG: Nahal Ein Gev.

Neanderthals

Given the genetic and morphological differences between Western Asian and European Neanderthals, two separate PCAs were run on these two groups prior to running the analysis on Neanderthals as a whole. The PCA for the Western Asian Neanderthal group shows the majority of shape variation within the first 2 PCs (**Table 6-3**). PC1 accounted for 69.06% of the variance and PC2 accounted for 26.6%. The shape variation for PC1 is associated with a posterio-medial elongation and an anterio-medial constriction (**Figure 6-8a**). PC2 shows more general shape variation with a medial and posterio-lateral constriction toward the centroid and an anterio-lateral expansion away from the centroid. There does appear to be an unusual range in the shapes moving towards a sharp pilaster-like projection posteriorly (**Figure 6-8b**). The scatterplot of PC1 and PC2 shows no cluster around a mean shape reflecting the small sample size and large amount of variance that the specimens account for (**Figure 6-9**).

Table 6-3. The first two Principal Components for the Western Asian Neanderthal population. Only Principal Components that contribute to more than 5% of the variance are shown.

Principal Components	**Eigenvalues**	**Variance (%)**
1	0.00289631	69.06
2	0.00222564	26.60

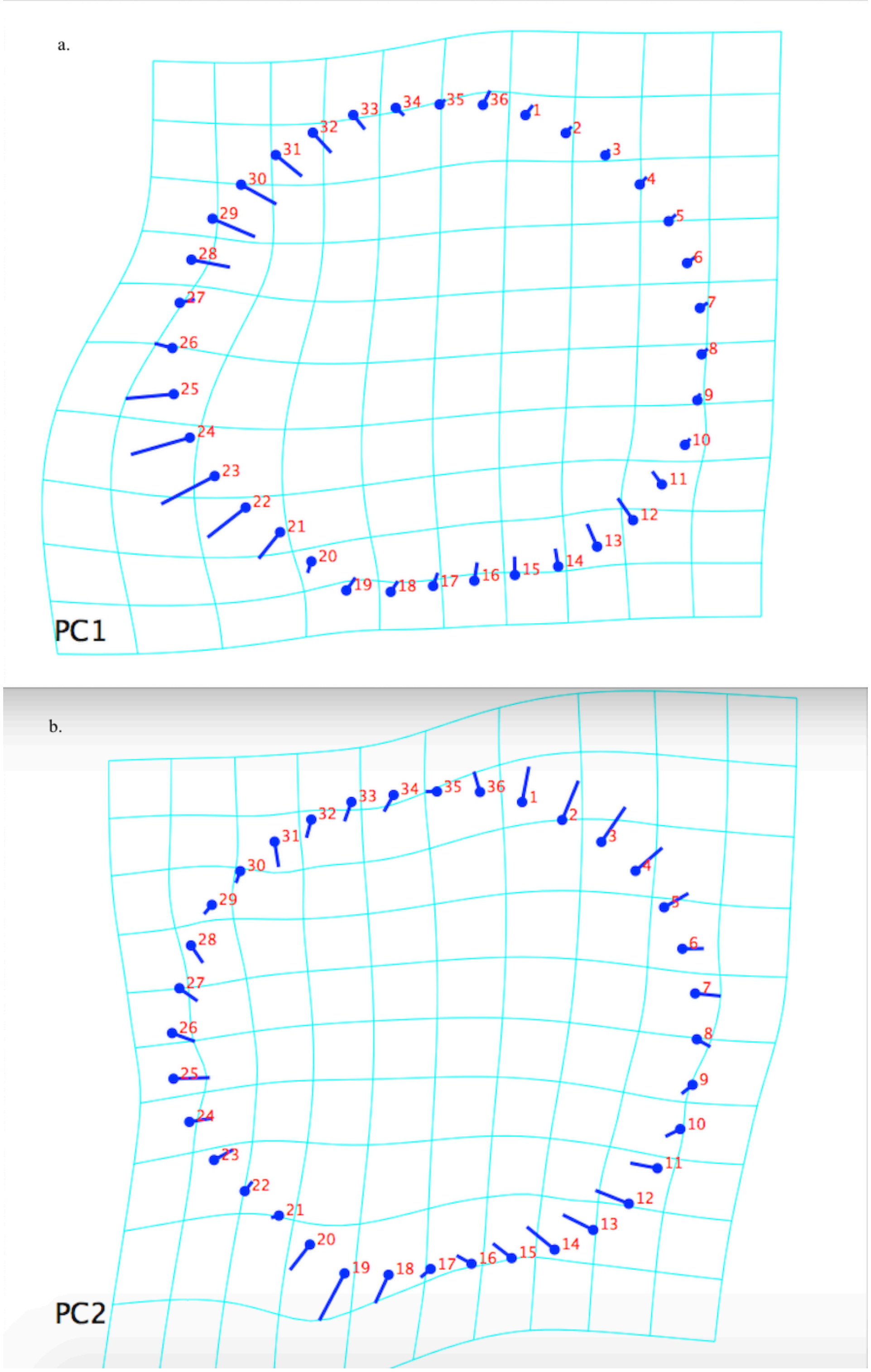

Figure 6-8. PC1 and PC2 shown in Transformation Grids for the Western Asian Neanderthal Population.

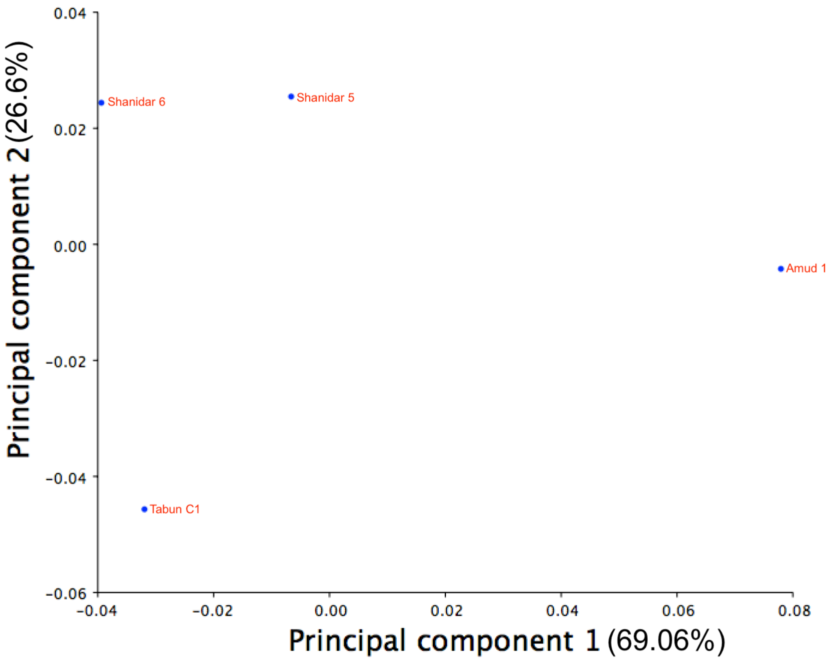

Figure 6-9. Scatterplot of the first two PCs showing the forms of the Western Asian Neanderthal population.

The PCA for the European Neanderthals contained 96.25% of the variation in the first four PCs. PC1 accounted for 56.41% of the variation and PC2 and PC3 accounted for 21.2% and 13.61%, respectively (**Table 6-4).** The shape variation related to PC1 surrounds the pilaster and shows a medial constriction toward the centroid and a posterior expansion away from the centroid. PC1 also shows an anterio-lateral constriction with a medio-anterior expansion on the anterior aspect of the femur (**Figure 6-10a**). PC2 shows a posterior constriction as well as a medio-lateral expansion, reinforcing the movement away from a pilaster and to a rounded shape characteristic of Neanderthal cross-sections (**Figure 6-10b**). The scatterplot of the European Neanderthal PC1 and PC2 scores is show in **Figure 6-11.** As with the Asian Neanderthals, the European Neandertals show a wide dispersal and a small sample size with one possible outlier, the right-most specimen, St. Césaire 1.

Table 6-4. The first four Principal Components for the European Neanderthal population. Only Principal Components that contribute to more than 5% of the variance are shown.

Principal Components	**Eigenvalues**	**Variance (%)**
1	0.00241956	56.41
2	0.00090915	21.20
3	0.00058365	13.61
4	0.00021607	5.04

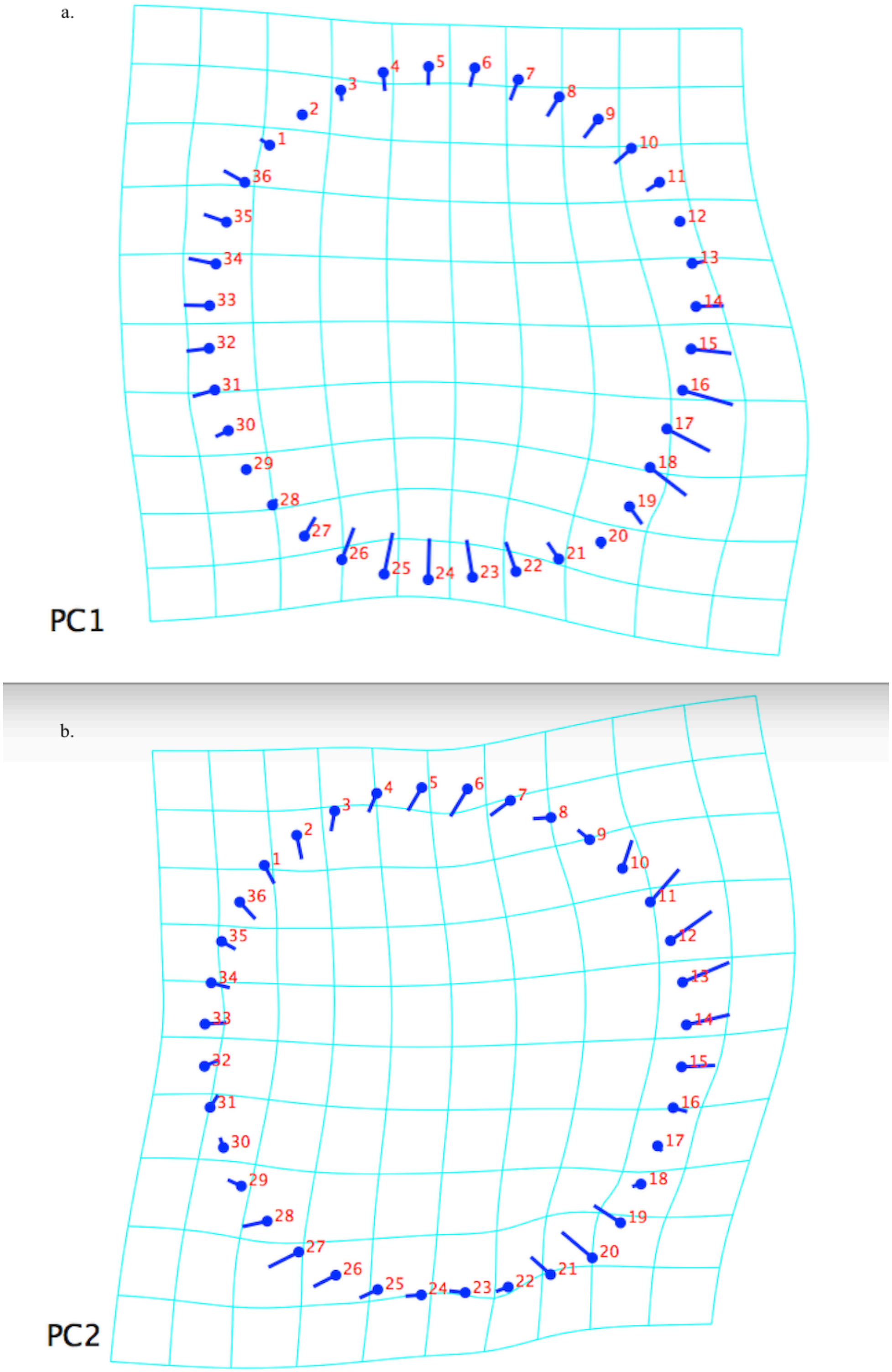

Figure 6-10. PC1 and PC2 shown in Transformation Grids for the European Neanderthal Population.

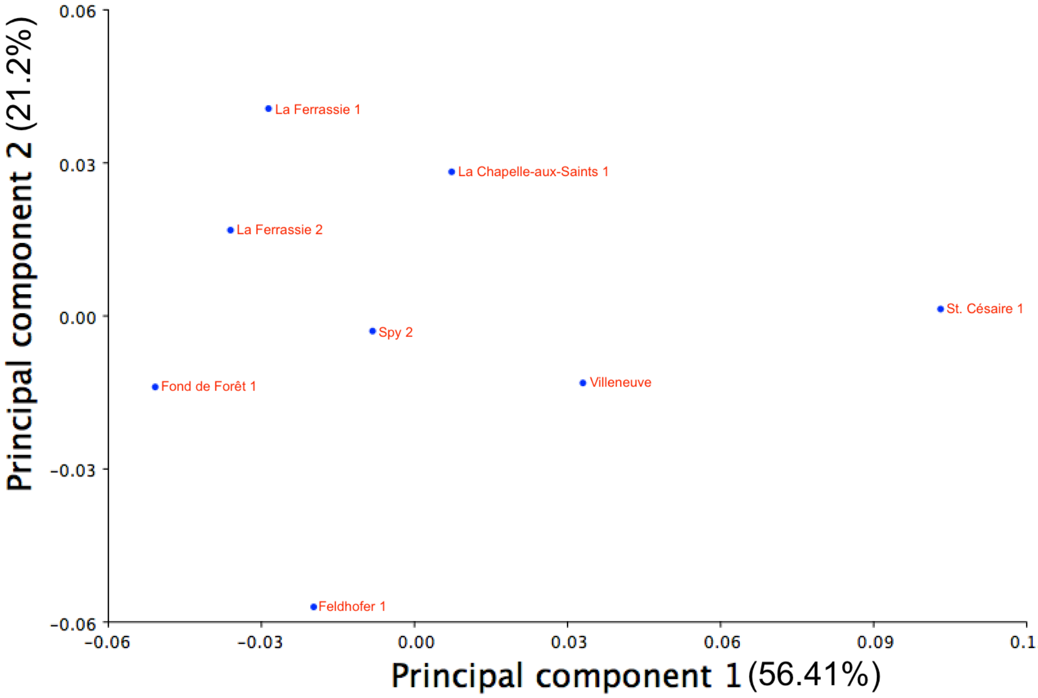

Figure 6-11. Scatterplot of the first two PCs showing the forms of the European Neanderthal population.

For the combined Neanderthal group, the PCA showed 92.56% of the overall variation within the first four PCs. PC1 accounted for the most variation (48.25%), which PC2 and PC3 accounted for 22.9% and 15.89%, respectively (**Table 6-5**). PC1 shows changes in shape medially that are associated with a medial expansion at the anterior aspect with a constriction at the posterior aspect. The lateral side of PC1 shows the opposite trend of a posterior expansion and an anterior constriction (**Figure 6-12a**). PC2 shows a trend toward expansion in the posterior and anterior aspects and constriction medio-laterally (**Figure 6-12b**). The scatterplot of PC1 and PC2 shows a wide range of variation with the majority of shapes clustering around the mean for both PCs. The most divergent individuals are La Ferrassie 1 and Amud 1 on opposite ends of the shape changes demonstrated in PC1, though arguably La Ferrassie 1 appears to be the farther from the mean of any individual (**Figure 6-13**).

Table 6-5. The first four Principal Components for the combined Neanderthal population. Only Principal Components that contribute to more than 5% of the variance are shown.

Principal Components	Eigenvalues	Variance (%)
1	0.00196095	48.25
2	0.00093067	22.90
3	0.00064573	15.89
4	0.00021193	5.22

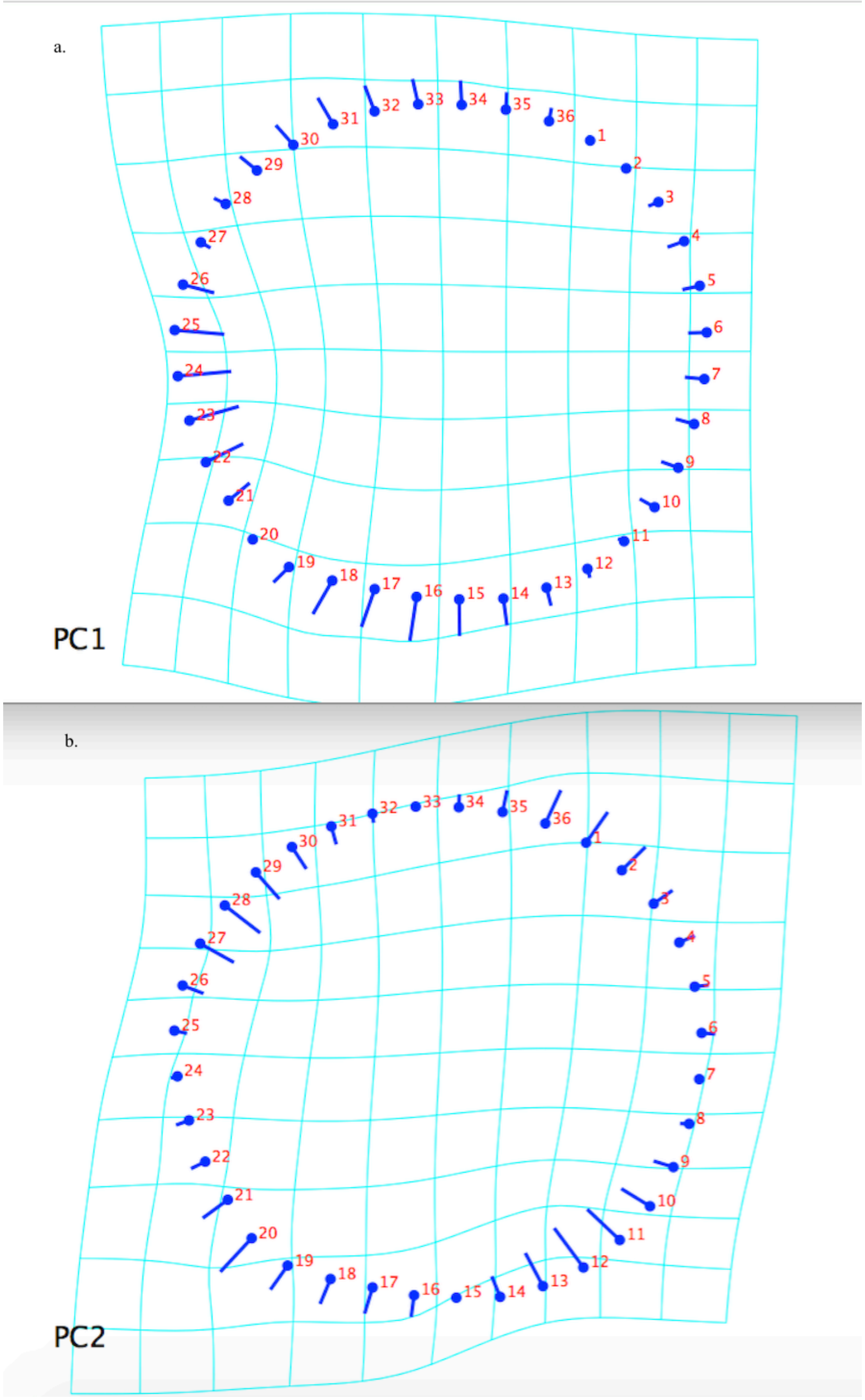

Figure 6-12. PC1 and PC2 shown in Transformation Grids for the combined Neanderthal population.

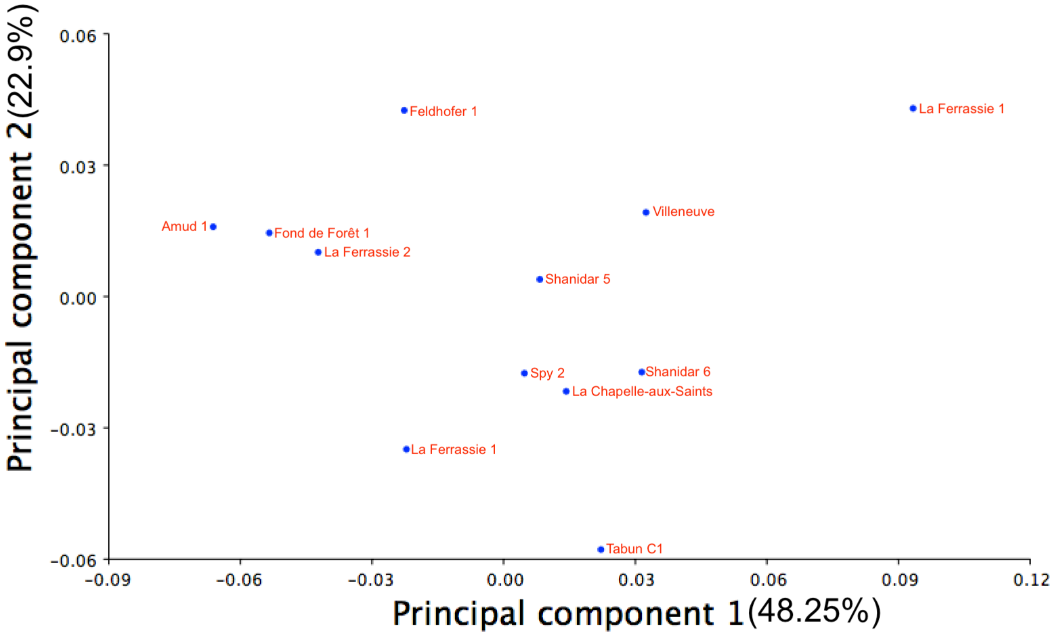

Figure 6-13. Scatterplot of the first two PCs showing the forms of the combined Neanderthal population.

Combined Fossil Data

To examine the shape changes in the combined fossil data, a PCA was run (using LP*Hs* and both groups of Neanderthals). The PCA showed 85.64% of the variation within the first three PCs (**Table 6-6**). PC1 accounts for 55.96% of the variation and PC2 accounts for 23.72%. The change in shape between the groups in the first PC can be described as a posterior constriction along the both sides and an expansion at the apex towards the shape of a pilaster. Additionally, the anterior portion of the medial side shows changes moving away from the centroid (**Figure 6-14a**). PC2 also shows shape changes associated with the pilaster, however the constriction is only on the lateral side while the medial side of the pilaster is expanding. The anterio-medial side of PC2 has a slight constriction as well and the anterio-lateral side shows an expansion (**Figure 6-14b**). **Figure 6-15** is the scatterplot data for the first two PCs for the between-group fossil data. The Late Pleistocene *Homo sapiens* show clear distance from both Neanderthal groups with little overlap. The Neanderthals, while clustering closer together across PC1, show marked separation from each other across PC2, indicating that the shape change occurring there may differentiate between them.

Table 6-6. The first three Principal Components for the combined fossil data.

Principal Components	Eigenvalues	Variance (%)
1	0.00535280	55.96
2	0.00226901	23.72
3	0.00056917	5.95

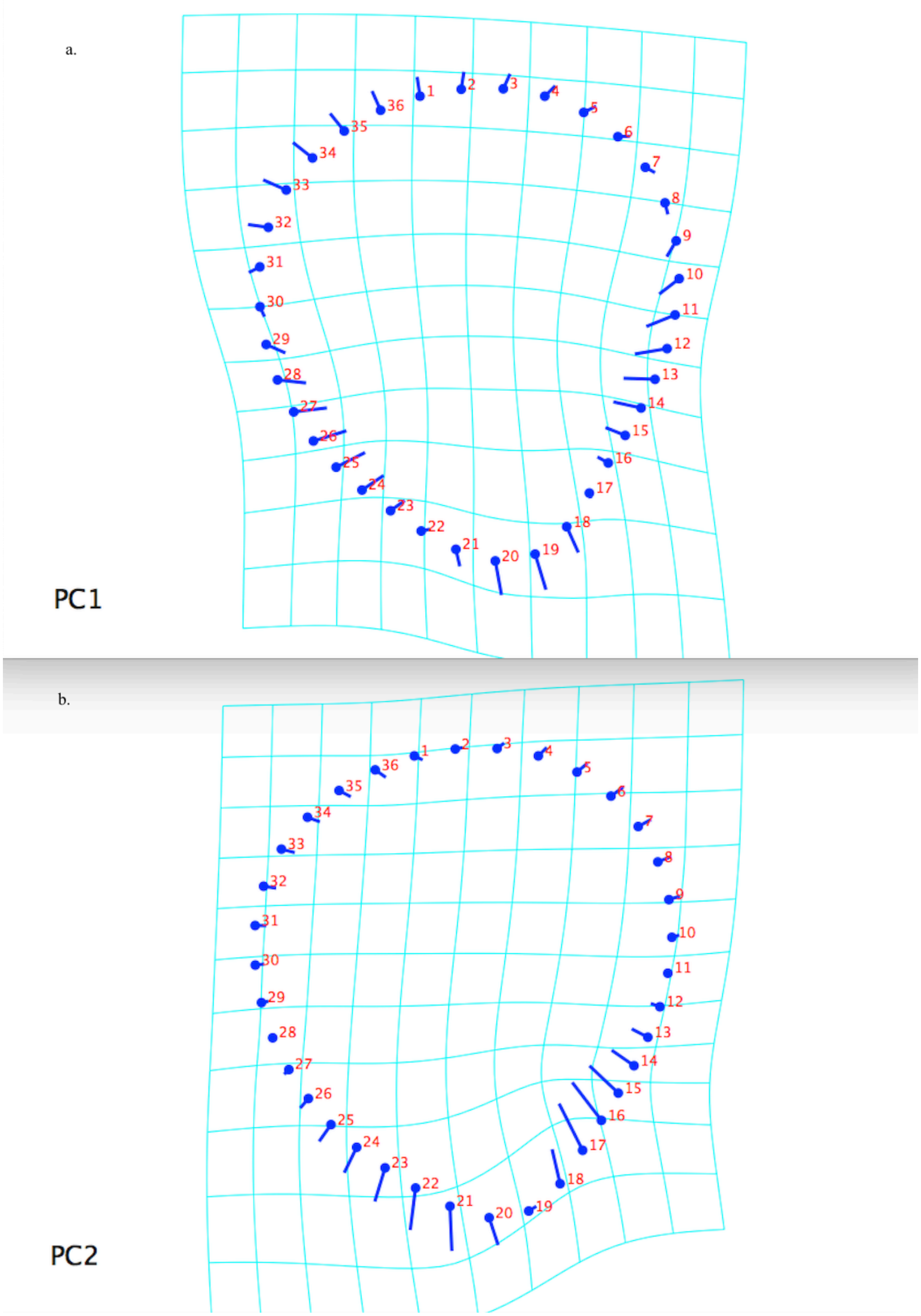

Figure 6-14. PC1 and PC2 shown in Transformation Grids for the combined fossil data.

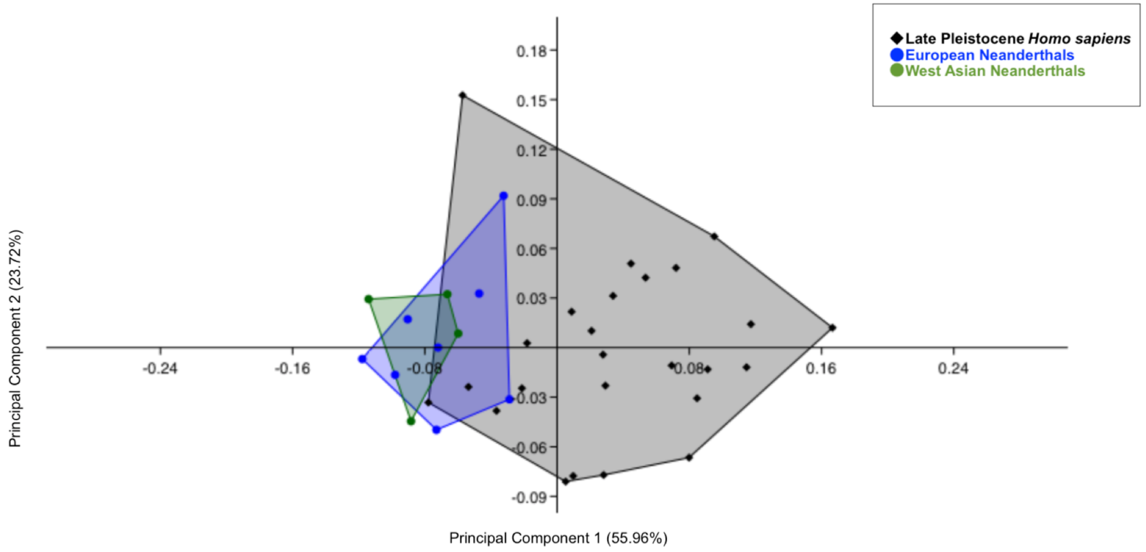

Figure 6-15. Scatterplot of the first two PCs showing the forms of the combined fossil data.

Combined Modern and Fossil Human Data

The final PCA analysis was conducted on the combined fossil and modern human specimens. In this analysis, 91.15 % of the variation in shape was accounted for in the first five PC's, with 38.91% attributed to the first PC and 31.31 % attributed to the second (**Table 6-7**). The changes accounted for by PC1 are, as expected, centered on the pilaster. There is a posterior projection on the medial side of the pilaster and an anterio-medial constriction on the lateral side. In addition, there is a small constriction just above the pilaster medially (**Figure 6-16a**). PC2 also shows shape change at the pilaster with a posterio-medial expansion all along the medial side of the cross-section and a posterio-lateral expansion located near the lateral side of the end of the pilaster. Additionally, the change in shape indicates a slight anterior constriction with an expansion on the center of the lateral side (**Figure 6-16b**). The scatterplot of PC1 and PC2 shows considerable overlap between the Late Pleistocene *Homo sapiens* and the Recent Modern Human populations, especially along PC1. The Neanderthals show more differences from the modern human populations along this axis, but have little distinction between their own European and Asian groups. PC2 follows a similar pattern for the Neanderthals, but shows less overlap than PC1, suggesting the shape changes here may be important in differentiating between them (**Figure 6-17**).

Table 6-7. The first five Principal Components for the combined Fossil and Modern Human populations.

Principal Components	Eigenvalues	Variance (%)
1	0.00287276	38.91
2	0.00231165	31.31
3	0.00057975	7.85
4	0.00057071	7.73
5	0.00039721	5.38

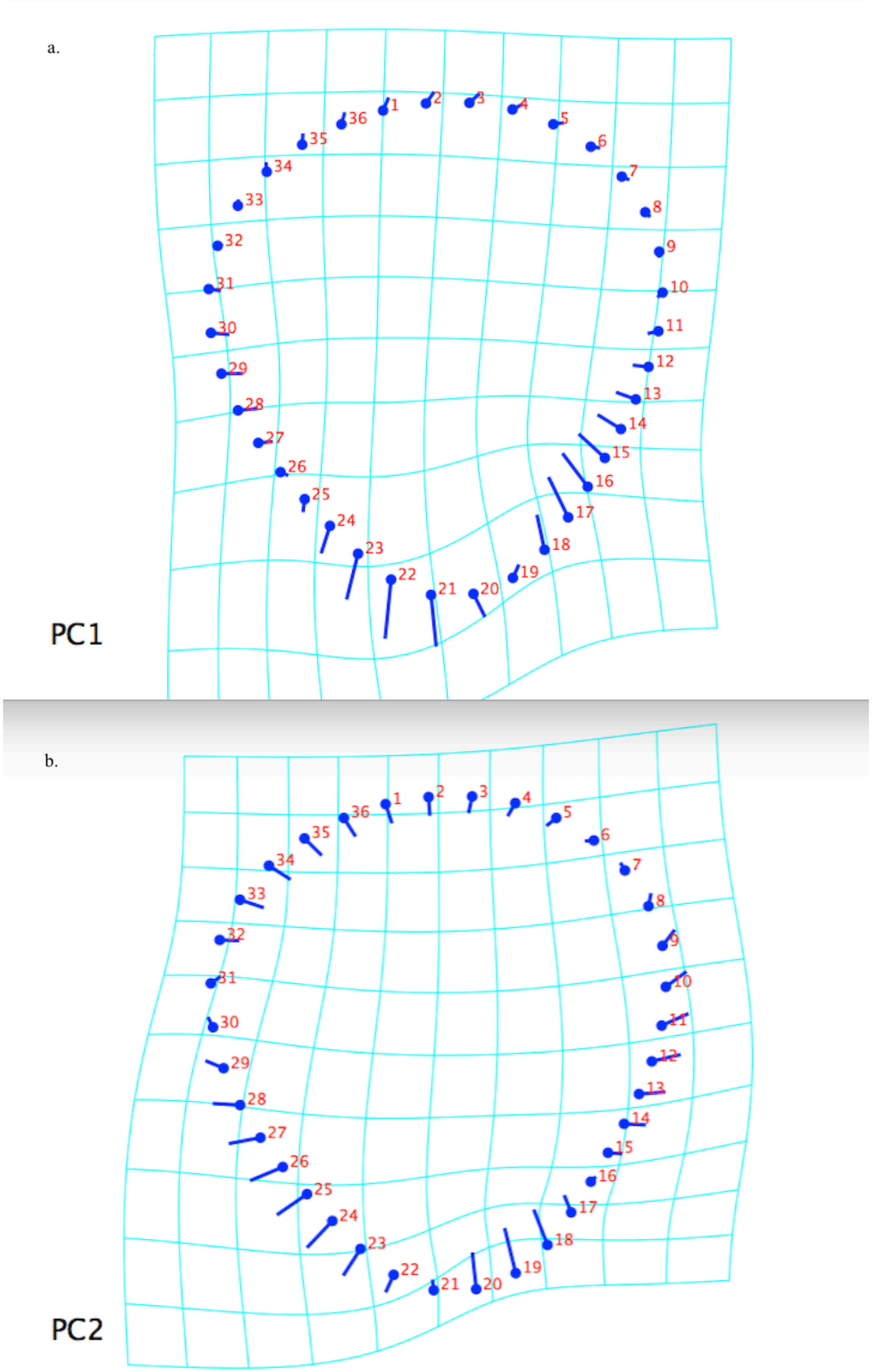

Figure 6-16. PC1 and PC2 shown in transformation grids for the combined populations.

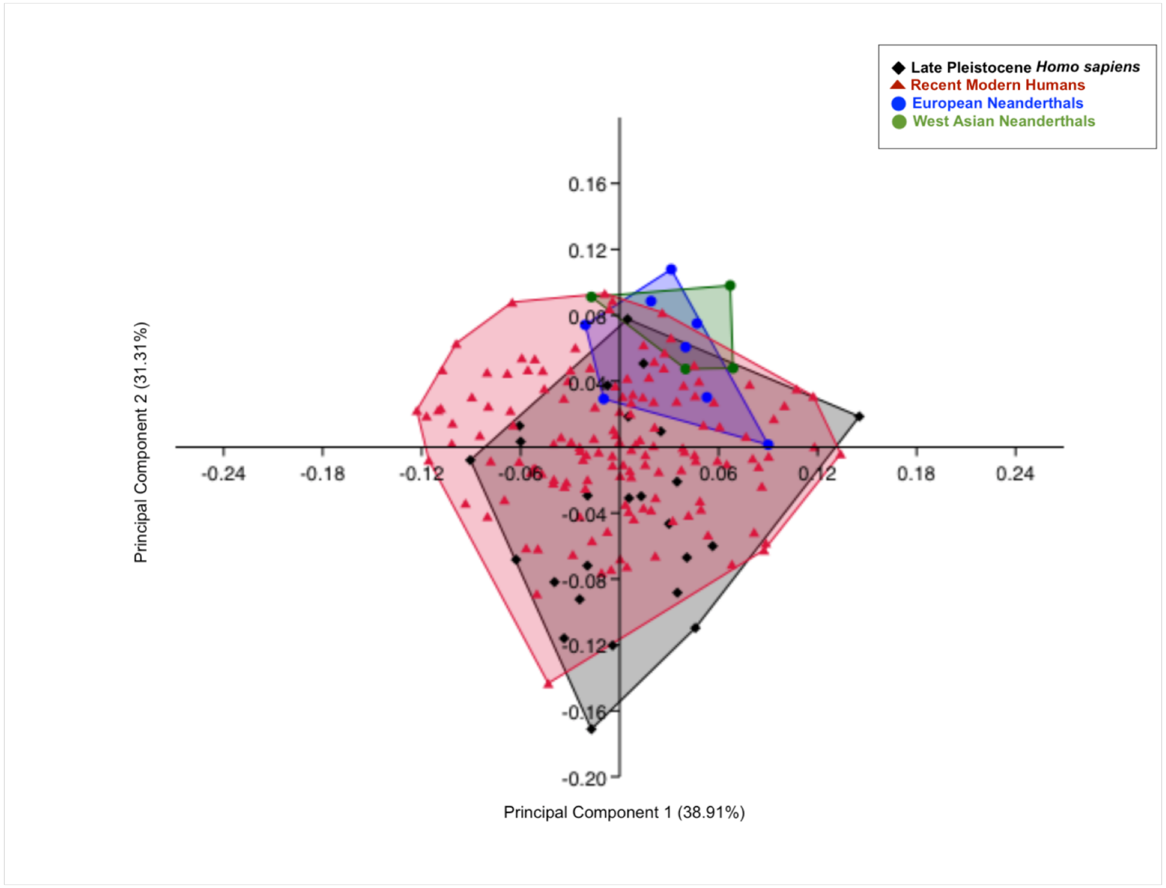

Figure 6-17. Scatterplot of the first two PCs showing the forms of the combined populations in this study separated by color.

The PCA analysis from the total, combined sample of all data, averaged by populations denoted as terminal taxa was also conducted as this analysis is critical for the phylogenetic signal permutation test. This PCA used the coordinate data averaged into one set of coordinates for each population represented as a terminal taxon on the tree. This analysis shows femoral midshaft variation found in the most recent members of the genus *Homo* and is briefly summarized below. In this combined analysis, the first 3 PCs accounted for 92.36% of the variation in the sample: PC1 accounted for 58.45%, PC2 accounted for 25.12%, and PC3 accounted for 9.78% (**Table 6-8)**. As expected, the first PC accounts for shape change in the pilaster, with a medio-lateral constriction at the posterior moving to the development of a pilaster shape. There is an anterio-medial expansion outward as well as a slight anterio-lateral projection and clockwise rotation. The second PC accounts for shape change primarily located in the pilaster as well. The medio-posterior portion of the pilaster is moving away from the centroid and the posterio-lateral portion of the pilaster is toward the centroid (**Figure 6-17)**. The distribution of these averaged populations is widely dispersed across both the first and second PC, though the sample size is significantly reduced due to the averaging of the populations (**Figure 6-18)**.

Table 6-8. The first three Principal Components for the combined, averaged Fossil and Recent Modern Human populations. Only Principal Components that contribute to more than 5% of the variance are shown.

Principal Components	**Eigenvalues**	**Variance (%)**
1	0.00269936	58.45
2	0.00116019	25.12
3	0.00040562	8.78

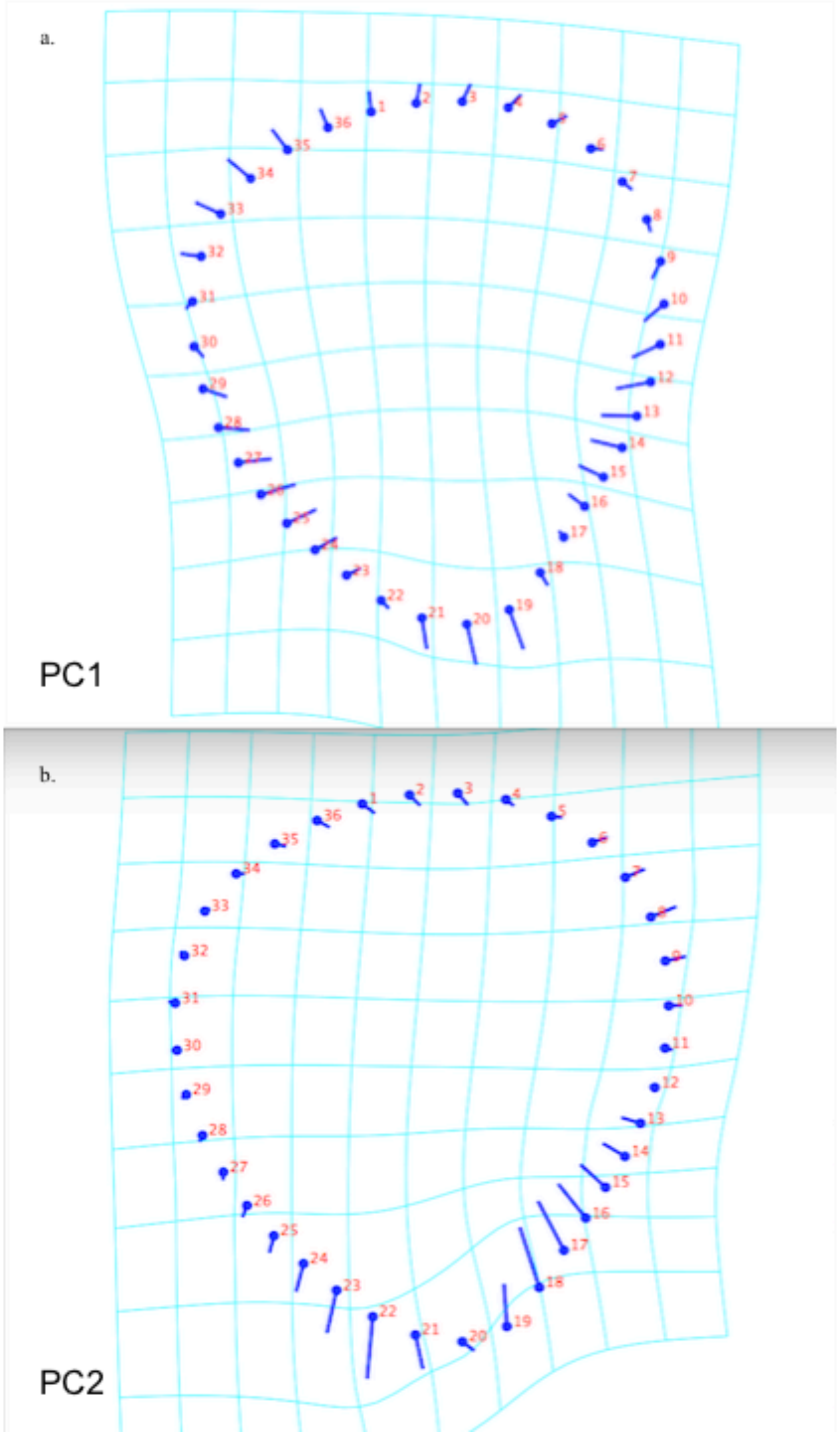

Figure 6-18. PC1 and PC2 shown in Transformation Grids for the Combined, Averaged Populations.

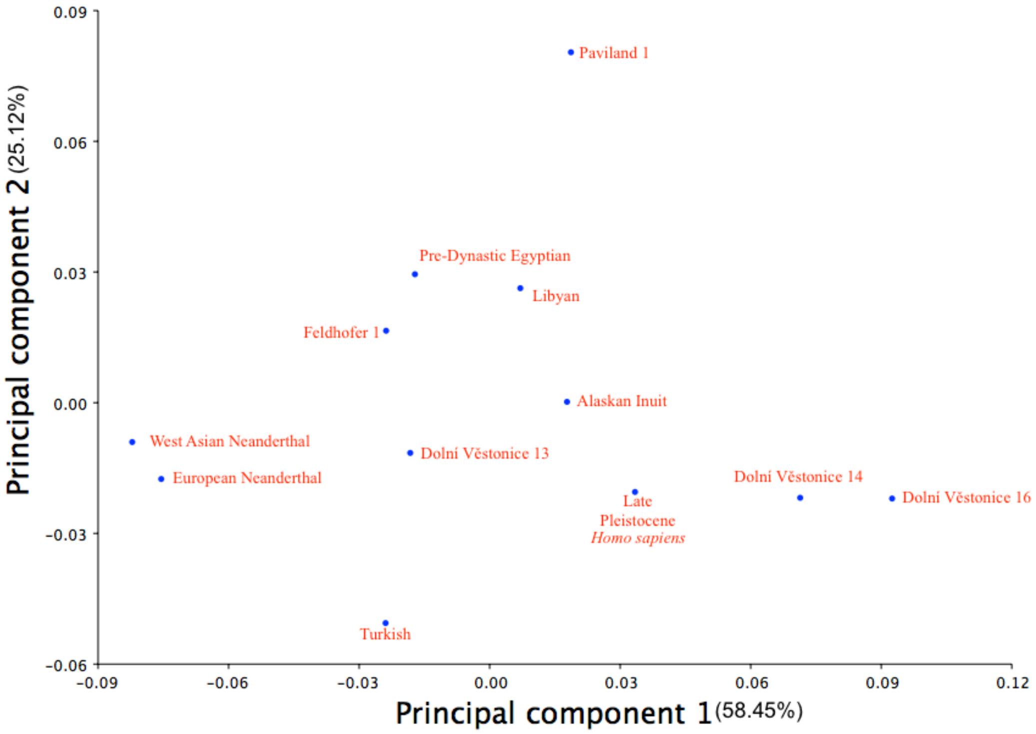

Figure 6-19. Scatterplot of the first two PCs showing the combined, averaged fossil and modern human populations.

Phylogenetic Signal Permutation Test

In order to test for phylogenetic signal, the femoral data first was mapped onto a phylogenetic tree. The Maximum Likelihood phylogenetic tree used for this analysis is outlined in **Figure 6-20**. As previously discussed, the Neanderthal populations form a monophyletic mitochondrial lineage, distinct from the Recent Modern Human and LP*Hs* groups. This was expected as phylogenetic trees comparing Neanderthals with other human groups consistently result in a distinction between them (Ovchinnikoc et al., 2000; Green et al, 2008; Krause et al, 2010). Due to the monophyletic nature of the Neanderthal lineage, it was used to root the tree. The LP*Hs* populations clustered together creating a monophyletic group that included the Libyan population. The Alaskan Inuit population was basal to both the Recent Modern Human and LP*Hs* populations. Despite the limited genetic samples, this phylogeny best represents the femoral populations chosen to have as little admixture as possible between them, and it shows a strong distinction between modern human and Neanderthal groups.

This phylogeny (**Figure 6-20**) was used to test for phylogenetic signal in the femoral cross-sectional data between these populations (Klingenberg, 2011). In order to map the specimens onto the phylogeny, the average Procrustes shape coordinates for each group were used as a representation of the femoral shape for that population (see previous chapter). The groups that were used were the same as those outlined as the terminal taxa in the phylogeny: Asian Neanderthals, Feldhofer 1, European Neanderthals, LP*Hs*, Dolní Věstonice 13, Dolní Věstonice 14, Dolní Věstonice 16, Pavlov 1, Pre-Dynastic Egyptian, Turkish, Libyan, and Alaskan Inuit. Using the squared-change parsimony method, the phylogeny can be projected into the shape space of the first two principal components of the averaged total population data (**Figure 6-21)**. Along the first PC, there is a marked separation between two groups: Neanderthals and Fossil/Recent Modern Humans. Distinction within these groups becomes more apparent along the axis of the second PC, which further separates modern human groups. The permutation test for phylogenetic signal resulted in a p-value of 0.0549 against the null (that the data do not show phylogenetic signal). Using this p-value, the hypothesis that closely related groups such as fossil and modern human populations (and species) are independent of the effects of their shared evolutionary history in their femoral midshaft shape can be rejected.

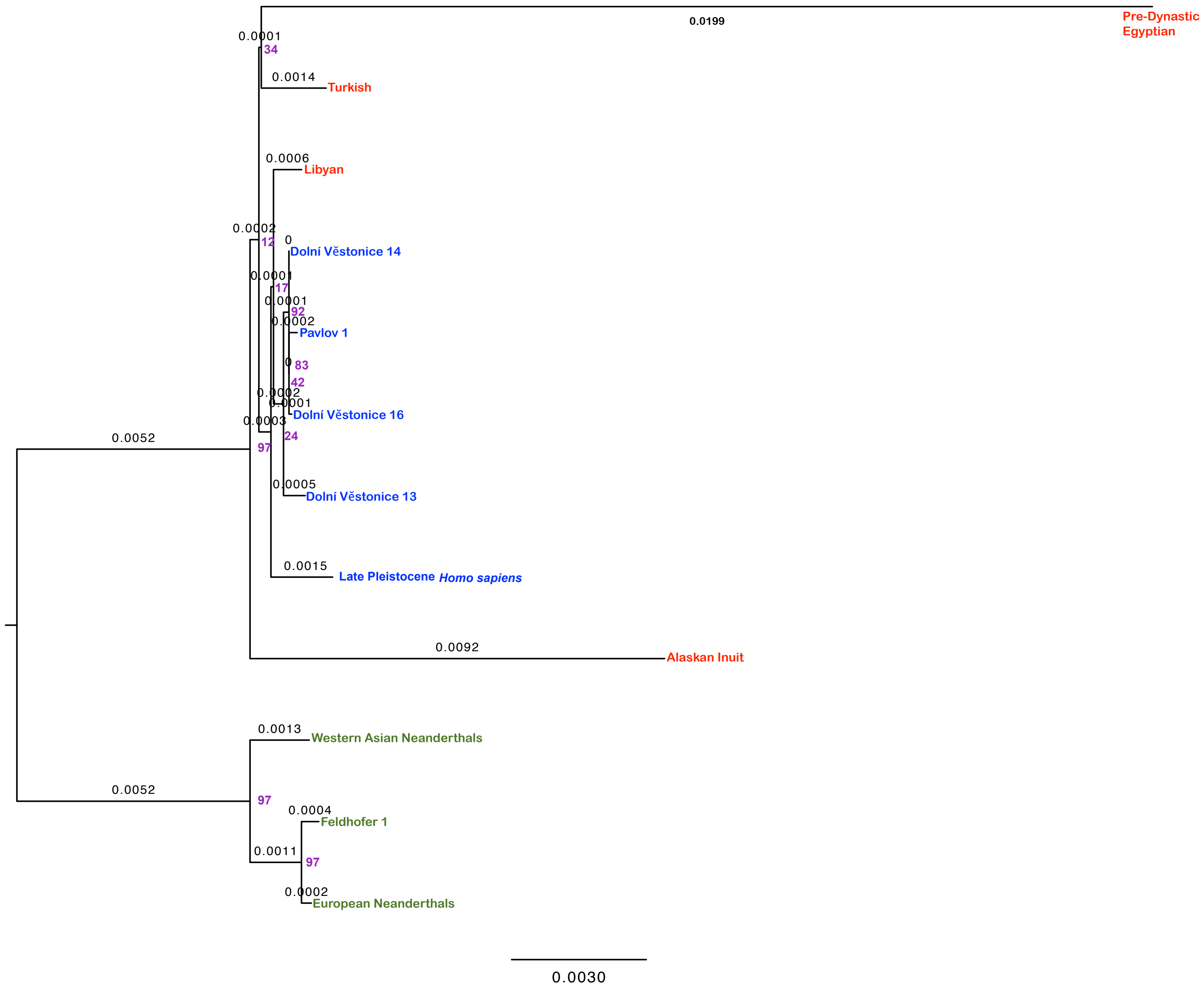

Figure 6-20. Phylogenetic tree created by maximum likelihood analysis of mitochondrial sequences outlined in **Table 4-3.** Branch lengths are show in black with clade support boot strap values in purple. Neanderthals are shown in Green. LP*Hs* are shown in Blue. Recent Modern Human groups are show in Maroon.

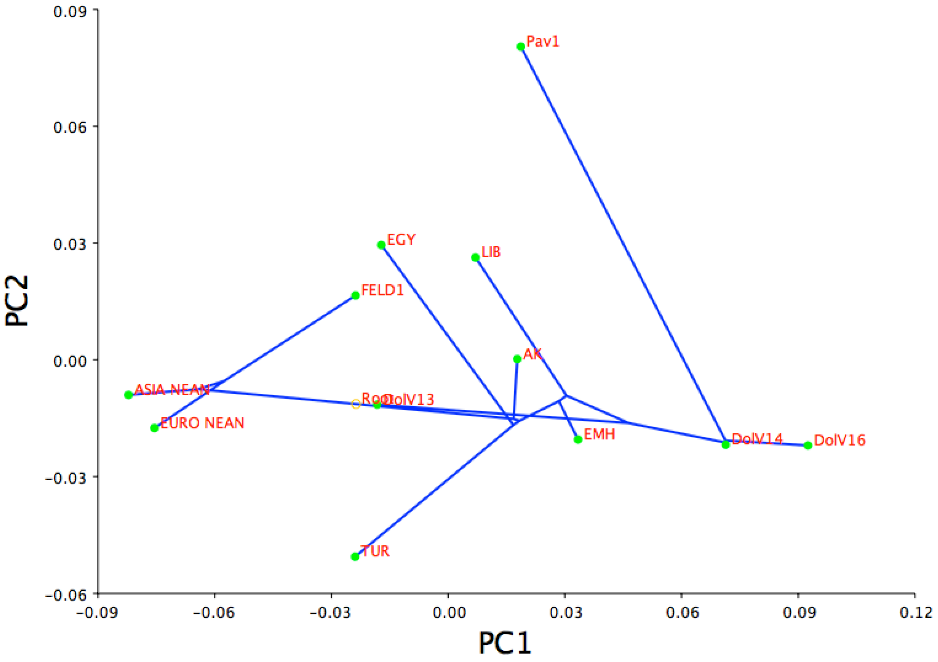

Figure 6-21. Phylogeny from Figure 6-20 projected onto the femoral shape space of the first two PCs using the averaged Procrustes shape coordinates. (ASIA NEAN: Western Asian Neanderthals, EURO NEAN: European Neanderthals, FELD1: Feldhofer 1, EMH: LP*Hs*, DolV13: Dolní Věstonice 13, DolV14: Dolní Věstonice 14, DolV14: Dolní Věstonice 16, Pav1: Pavlov 1, AK: Alaskan Inuit, EGY: Pre-Dynastic Egyptian, LIB: Libyan, TUR: Turkey).

The phylogeny was also mapped onto the centroid size of each group in order to inspect changes between terminal nodes and the root of the tree (**Figure 6-22**). In this analysis, the greatest displacement from the root of the tree was the LP*Hs* population. Given the divergence of this group, it can be suggested that there is a large amount of evolutionary distinction between Neanderthals and LP*Hs* given their relative distances from the root of the tree. Additionally, the terminal taxa can be roughly divided into three groups of similar distances from the root: Recent Modern Humans, LP*Hs*, and Neanderthals.

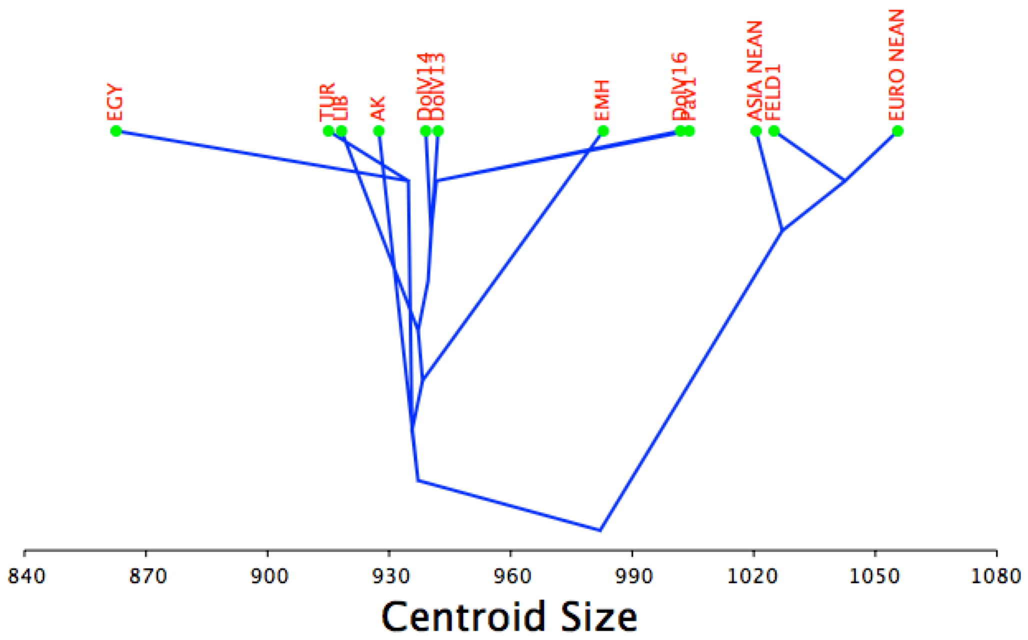

Figure 6-22. Phylogeny from Figure 6-20 mapped onto the centroid size of the averaged total populations.

Evolutionary Allometry

In order to understand the differences in femoral shaft shape, especially given the presence of phylogenetic signal, a test for evolutionary allometry, independent contrasts, was run to determine if changes in size over evolutionary time have had bearing on the observed variation in shape at the midshaft cross-section. To calculate the impact of evolutionary allometry, a multivariate regression was performed using the Procrustes shape coordinates from the averaged total population data calculated in the independent contrasts, against the centroid size. The resulting vector showed no correlation between femoral midshaft shape and allometric size changes. A permutation test was then performed to test the null hypothesis that allometric and shape changes are independent. The percent of predicted shape changes based on allometric changes is approximately 20%. The p-value for the permutation test is p=0.1518 resulting in a failure to reject the null that size and shape are independent of one another at the femoral midshaft. This suggests that size changes in the femoral midshaft, as represented by evolutionary allometry, do not have a significant impact on the observed shape difference between these groups.

Trends in Femoral Midshaft Shape Variation

Principal Component Analysis

Due to the presence of phylogenetic signal at the femoral midshaft, but the lack of significant impact of evolutionary allometry on cross-sectional shape, the trends in shape variation observed in the PCA can be assumed to accurately represent shape changes across and within fossil and modern human groups. The results from the PCA, estimated as the true observed variation at the femoral midshaft, are summarized in **Figure 6-23**. In order to view the location of the primary variation for PC1, the extreme values were mapped onto a wireframe graph of the average shape data (from -0.08 to 0.09), as represented by the x-axis femoral outlines in **Figure 6-23.** The variation observed in PC1 shows the primary difference between the shapes of the groups measured in this study: the Neanderthals presenting with a lack of a pilaster, and the *LPHs* and Modern Humans showing a pronounced pilaster. For the second principal component, the variation is expressed along the y-axis of **Figure 6-23.** The extreme outlier values for the y-axis ranged from 0.08 with Pavlov 1 (0.03 without) to -0.05**.** The variation expressed in PC2 includes the range of the recent modern human groups and is related to the orientation of the pilaster.

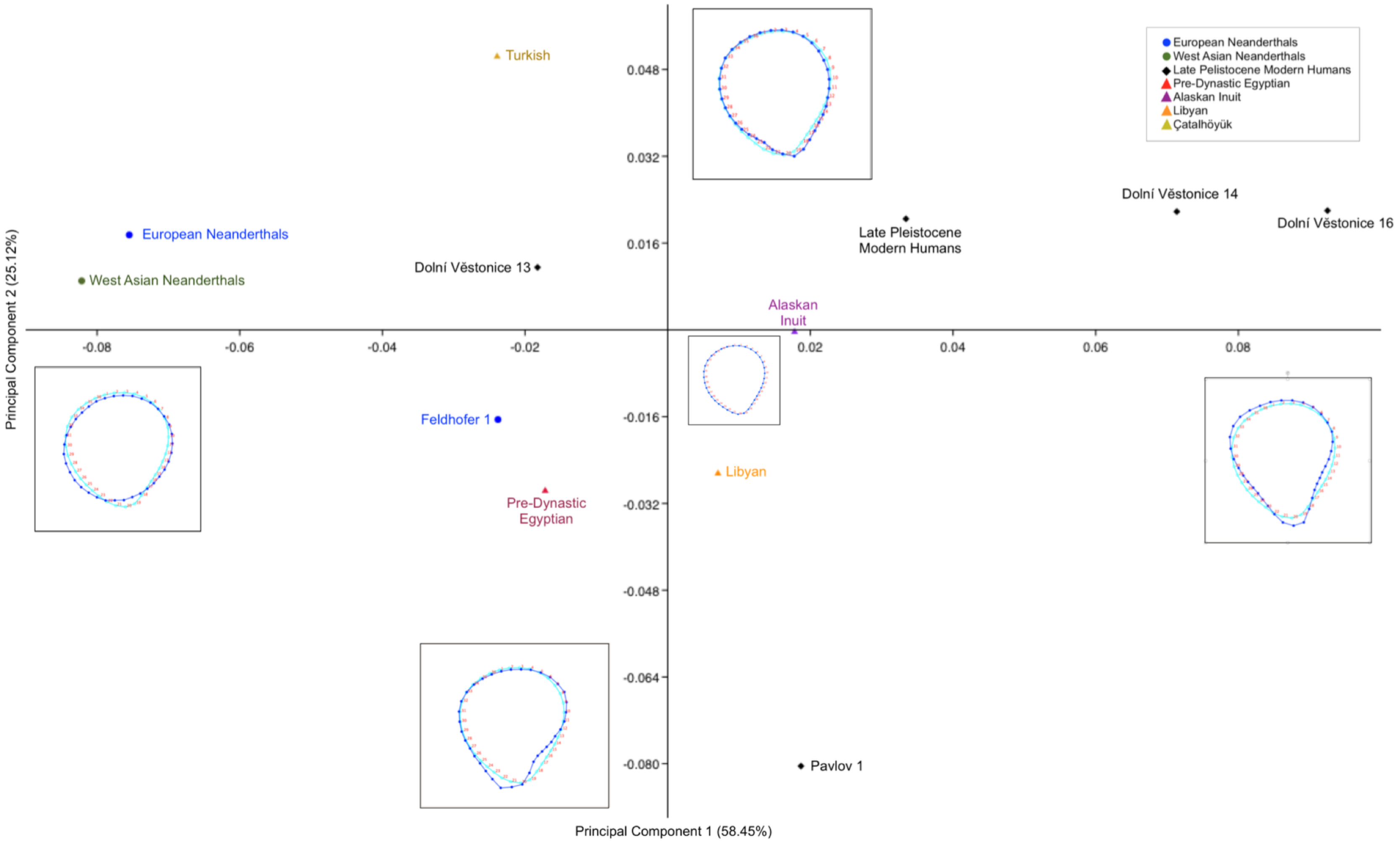

Figure 6-23. Principal component shape variation for all groups. The extreme ends of shape the variation can be seen at the end of each x- and y- axis in a wireframe graph. The light blue represents the farthest point along the axis while the dark blue represents the average overall shape at the femoral midshaft.

Canonical Variate Analysis

In order to better understand the variation present in the femoral midshaft shape between these groups, a canonical variate analysis was conducted using the results from the PCA. Given the presence of signal in the data but the lack of allometric impact, the results from the Mahalanobis distance p-values are assumed to reflect a close approximation to the true between-group differences in the shape variables. The p-values from the Mahalanobis permutation tests (using 100,000 permutations) showed a significant difference between all groups with values less than 0.0001. Due to the small sample size of the individual Western Asian and European Neanderthal groups (four and eight, respectively), the CVA was run with these groups combined. When the groups were separated, however, the distances remained significant, though the Manalanobis p-value changed from <0.001 to 0.0279. The results for the first and second canonical variates (CV) can be seen in **Figure 6-24.**

The primary differences captured in CV1 are demonstrated in **Figure 6-25** and show the range along the x-axis, differentiating between modern human populations from values -4 to 5. The primary differentiation between these groups, as reflected in the PCA results for shape variation, is in the orientation of the pilaster. The difference captured in CV2 are shown in **Figure 6-26** and show the range along the y-axis from 3 to -7 with the upper values parsing out modern humans and the lower values, Neanderthals. The primary differences in these extremes, as with the captured variation in PC1, is in the presence of the pilaster.

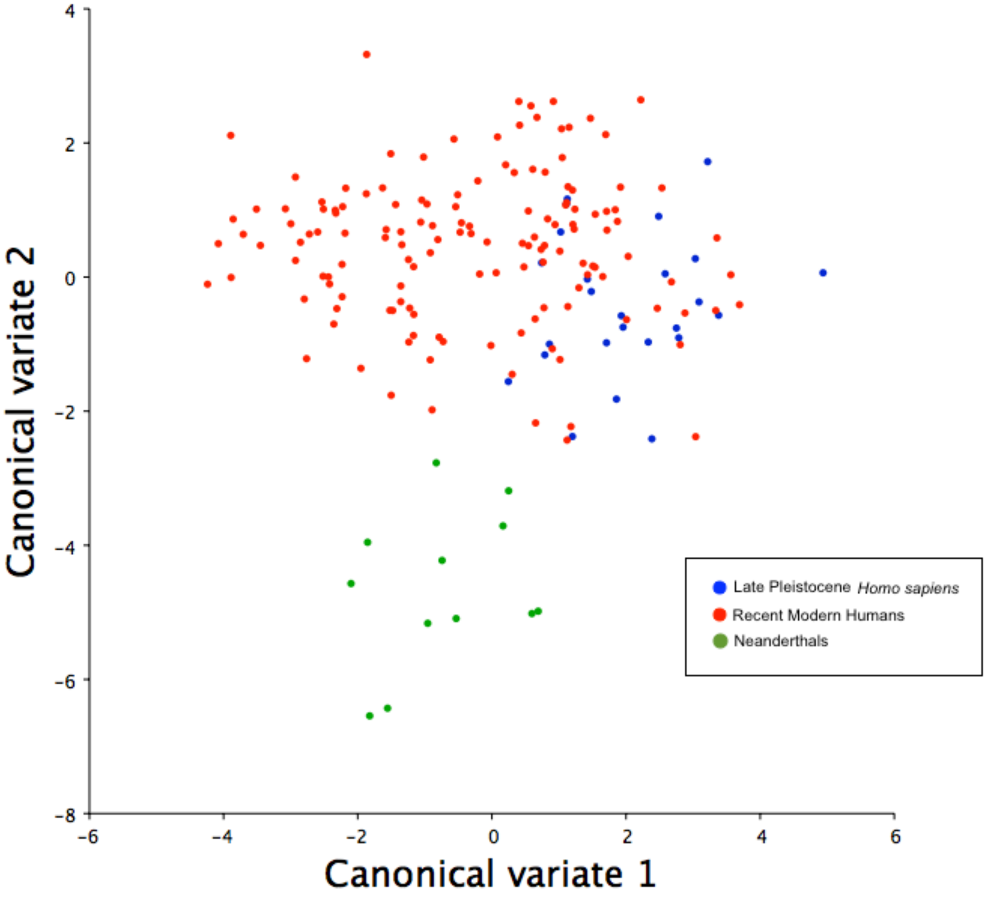

Figure 6-24. CVA results for the first two canonical variates for each specimen, colored by group.

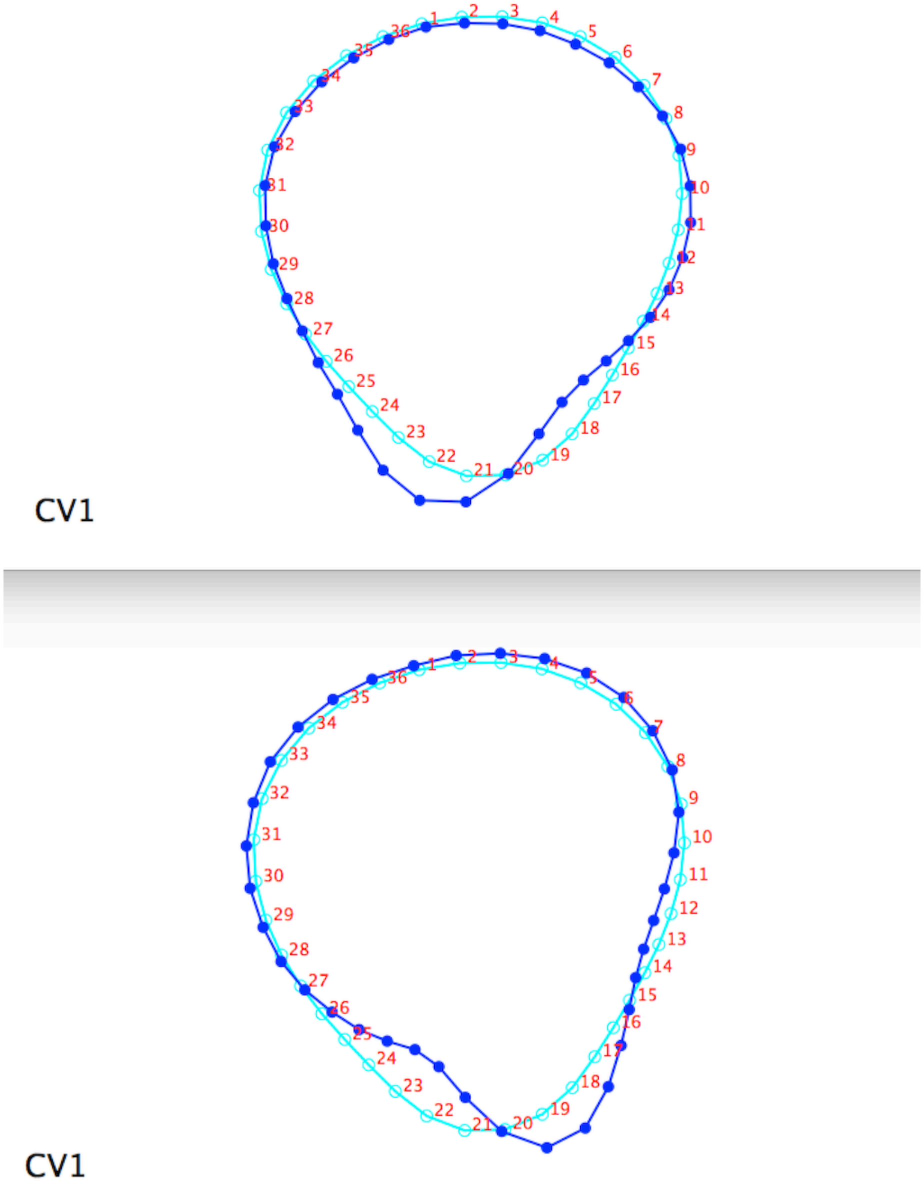

Figure 6-25. CV1 population differences. The top graph shows the extreme left end of the x-axis the bottom graph shows the extreme right end of the x-axis. This axis shows difference between modern human groups centering on the orientation of the pilaster.

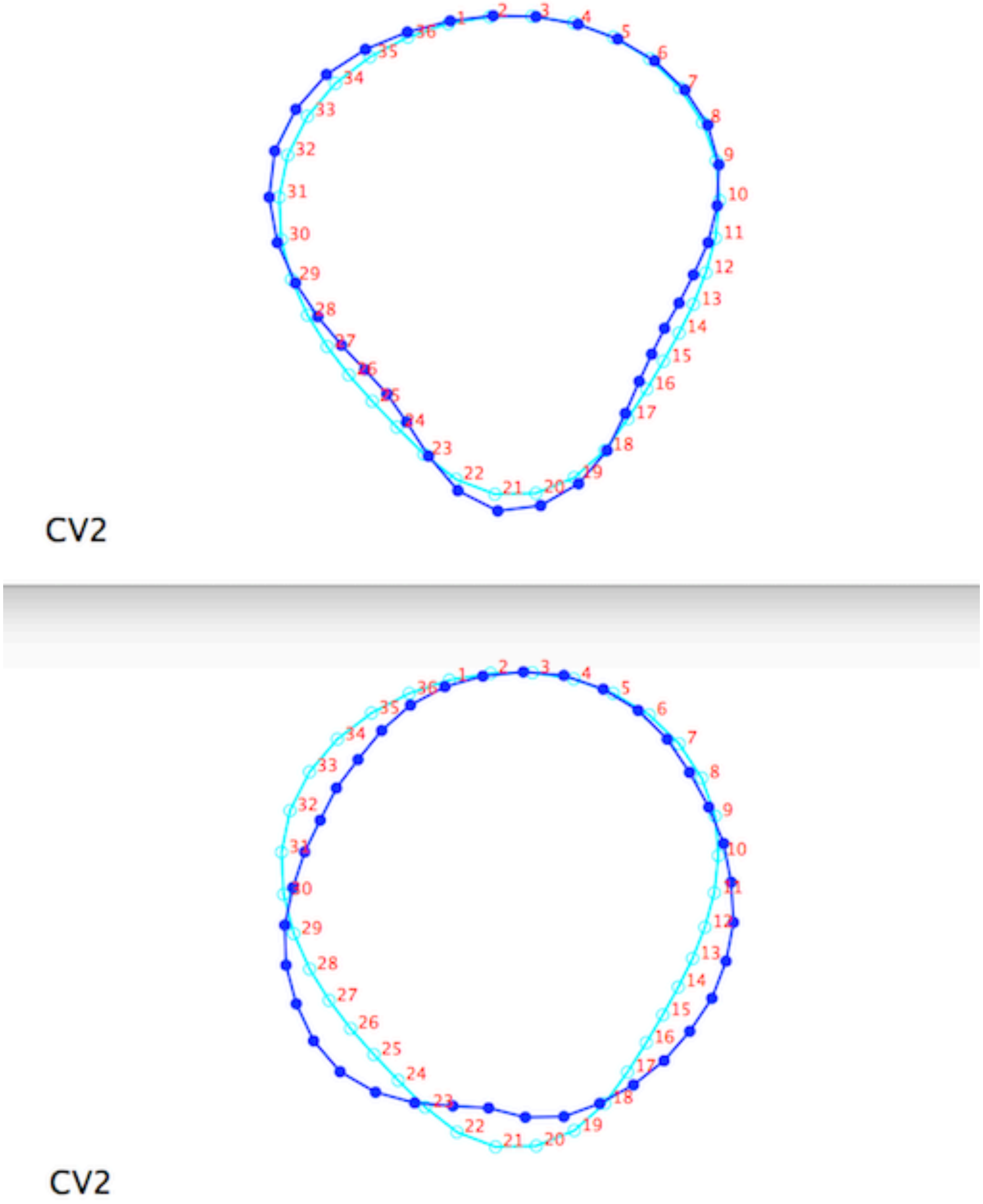

Figure 6-26. CV2 population differences. The top graph shows the extreme upper end of the y-axis the bottom graph shows the extreme lower end of the y-axis. This axis shows difference between modern human groups and Neanderthals centering on the presence of the pilaster.

CHAPTER VII

DISCUSSION

Femoral midshaft cross-sectional morphology has been used to estimate shifts in adaptive strategies in the human lineage. It has been suggested that the loading patterns exhibiting changes on the femoral shaft between Neanderthals and modern humans may be due to biomechanical responses to behavioral differences (Holt 2003), terrain variation (Ruff, 1999), ontogenetic factors (Cowgill, 2010), climatic adaptation (Weaver, 2003), or a combination of several of these pressures (Ruff et al., 2006). In order to understand these morphological differences and their evolutionary implications, the comparative method was used as a cornerstone of these analyses. This study has addressed the long-standing assumption that shape differences at the femoral midshaft are independent and instead has shown that shared evolutionary history may play some role in this shape variation. However, when femoral midshaft shape data are tested against evolutionary allometry, there is no significant correlation between changes in size and changes in shape though time. This indicates that the observed shape differences between these groups are likely due to other factors that are evolutionarily significant and that the observed variation reflects true changes in morphological shape due to selective processes.

Observed Femoral Cross-Sectional Midshaft Morphology

In the PCA and CVA of this study, and as expected given the biomechanical analyses and previous studies of femoral shaft shape, the majority of the shape changes observed among fossil and modern human groups involves mediolateral expansions or constrictions and changes to the shape and presence of the bony protrusion for muscle attachments, the pilaster (Trinkaus, 1976; Ruff and Hayes, 1983; Churchill et al., 2000; Stock and Pfeiffer, 2001). The majority of shape change observed among the recent modern human populations represented here (Alaskan Inuit, Turkish, Pre-Dynastic

Egyptian, and Fezzan Libyan) is focused in the region of the pilaster, with variation moving inward and outward on the medial and lateral aspects. When each group is examined in isolation after Procrustes transformation, it can be seen that the most distal projection of the pilaster is variable, with a presentation of medial, lateral, and posterior orientations for the most extreme anterio-posterior distance.

This range in variation of the pilaster between these populations is most likely caused by multiple factors. Given the breadth of variation in location, subsistence, sexual division of labor, terrain, and temporal occupation of these populations, it is difficult to state definitively which evolutionary pressures have affected the greatest amount of cross-sectional change between them. Ruff et al., (2006) have stressed the importance of caution in extrapolating behavior from biomechanical pressures acting on the femoral cross-section; the impact of small changes in terrain (Ruff, 1999), subsistence exploitation (Stock and Pfeifffer, 2001), and ontogenetic onset of adult patterns of morphology (Cowgill, 2010) make it responsible to take a conservative estimate of shape change observed here. It is highly likely that many of these factors act on femoral shape within and between these recent modern human populations. The pilaster does account for most of the shape change among these groups; however, these changes do not indicate that the pilaster is being selectively removed from any of these populations and instead suggests that the orientation is the important factor. While the differences in femoral morphology between these recent modern human populations do appear to be subtle when viewed initially (**Figure 6-2**), the CVA analysis run on all populations indicate that these differences are statistically significant and reliably differentiate between modern human populations as categorized in this study. Even more importantly, the recent modern human groups are significantly different from all other groups, including LP*Hs*.

Variation in the LP*Hs* population, unsurprisingly, is similar to the recent modern human population as the range of variation within this group is centered around the pilaster with consistent presence within the population. The variation is different, however, as the primary direction of change is in how pronounced the pilaster is, rather than in its orientation. This can be seen in the range of shapes in the population, centered around the posterior movement of the most distal portion of the pilaster and a central movement of both the posterio- medial and lateral aspects. In LP*Hs* populations, a greater presence of the pilaster (versus a rounded cross-section) has been associated with an increase in biomechanical pressures due to higher activity levels (Shackelford, 2007; Holt, 2003), but as with the recent modern human populations, this explanation is difficult to support based on the wide range of temporal and geographic associations within the group. As stated above, terrain (Ruff, 1999) and population density, as well as increased travel distances related to subsistence strategies (Stock and Pfeiffer, 2001), can greatly impact variation in modern humans. Evolutionary trends in human femoral shape can also compound the interpretations for the LP*Hs* group. It has been suggested that changes in the femur, specifically in the robusticity and cortical area, could support a decrease in activity correlating with the emergence of modern humans (Holt, 2003). In terms of robusticity determined from cortical area, LP*Hs* specimens have actually been more closely related to fossil, rather than modern, groups (Ruff et al., 1993). While this has been proposed to be correlated with activity (Stock and Pfeiffer, 2001; Holt, 2003), studies accounting for body size have also shown no change in robusticity, and therefore no change in behavior significant to activity level decrease, until 30 kya (Trinkaus, 1997). This compounds interpretations of activity as it relates to shape, and reinforces the need for caution, especially as the archaeological evidence from the record does not always support these correlations, as is the case with the hypothesis of increasingly sedentary behavior when LP*Hs* appear on the landscape (Trinkaus, 1997). Therefore, while we have seen activity change femoral shape in modern humans' proximal shaft, this cannot necessarily be extrapolated to the midshaft shape (Niinimäki et al., 2016).

What can be definitively supported from this study is the clear difference in femoral shaft shape in LP*Hs* from the other groups outlined here. The CVA run on the groups outlined in this study show statistically significance p-values against the null of similarity in shape between all human groups, reliably parsing LP*Hs* from both Neanderthals and recent modern human populations. Clearly, this movement of LP*Hs* femora away from the centroid in the posterior aspect is evolutionarily significant, and is likely related to changes in habitual or heavily loaded activities, variation in the environment and/or exploitation of it, ontogenetic activity, or even a combination of several of these factors. Additionally, these factors have changed significantly from those impacting recent modern humans, which center on the orientation, rather than definition of the pilaster.

Distinctions between European Neanderthals and Western Asian Neanderthals have been observed through both morphological and genetic analyses (Trinkaus 1981; Green et al., 2010). While European Neanderthals exhibit the characteristic “classic” Neanderthal bauplan, such as hypertrophy and cold-adaptation in their post-crania, Western Asian Neanderthals exhibit a more gracile morphology (Trinkaus, 1981; Trinkaus and Ruff, 1999; Hublin, 2002). It is important to note, that while Western Asian Neanderthals exhibit a form that appears less-constrained by cold-adaptation (also called a “hyper polar” form), their morphology is distinctly Neanderthal (Franciscus and Churchill, 2002). Although the variation observed in the Western Asian Neanderthal sample has been argued to have increased with features more like LP*Hs* (Arensburg and Belfer-Cohen, 2002), genetic studies have shown that both European and West Asian Neanderthals are more closely related to one another than they are to modern humans, and this is supported in the phylogenetic analyses conducted in this study (Green et al., 2010). Due to this variation in morphology and genetics, the Neanderthals were broken up into two groups for analysis: Western Asian and European.

The European Neanderthals’ overall shape variation, as observed in the PCA, demonstrates a marked lack of a pilaster. The circular femoral midshaft shows no evidence of a pilaster what-so-ever and the region of shape variation shows a restriction toward the centroid at the posterio-medial and anterio-lateral aspect of the cross-section, with a anterio-medial and posterio-lateral expansion away from the centroid on the horizontal plane. Despite this range in shape differences within the group, the average shape is still characteristically rounded. This coincides with observed and hypothesized biomechanical interpretations of the Neanderthal femur, stressing the importance of a medio-lateral distribution of cortical bone and lack of requirement of a pilaster to support the skeleton or muscles in their movement.

The Western Asian Neanderthals' primary direction of shape variation is also in the region of the pilaster and the in the medio-lateral plane, similar to the European Neanderthals, but different in direction. The most extreme range of shapes is to the medial side of the pilaster which shows a somewhat mini-pilaster-like expansion away from the centroid; a small pinching of the bone can be seen in the average Procrustes transformed outline as well. This is evident in the first principal component. Shape variation in this group includes a slightly counterclockwise-rotated mediolateral expansion with a slight anterioposterior projection. While this cannot be called a true pilaster, there is a clear distinction that can be observed in the overall circular shapes of the Neanderthals: European Neanderthals appear to have an even more pronounced circular cross-section.

These differences become even more clear when a CVA is run on both the combined and separated Neanderthal populations, as well as the LP*Hs* and recent modern humans. As expected, the combined Neanderthal morphology is significantly different from either modern human groups and can be reliably parsed out together. However, when European and Western Asian Neanderthals are separated, the p-values of the CVA between Neanderthal groups is larger (0.0379 versus <0.001), but still significant. As with the modern human populations, the reason for the slight increase in the cortical bone on the posterior aspect of the Western Asian Neanderthal individuals compared to that of the European Neanderthals is not clear. While differences in terrain and climate across the Neanderthal range have been observed between these two groups for quite some time (Hublin, 2002; Smith, 2013), the extent of their subsistence strategies and, therefore, the interaction they had with their environment is still being uncovered (such as an exploitation of marine (Stringer et al., 2008) or plant resources (Henry et al., 2014)).

When combining all populations and examining shape changes between fossil and modern humans, the primary direction of shape change is in the pilaster. This is expected given the discussion of the importance of the pilaster in distinguishing between these groups, as well as the PCA analyses in the individual populations. The overall primary direction of the shape change is away from the centroid, directly medially to the posterior-most aspect, and toward the centroid on the posterio-lateral aspect of the cross-section. From the previous analyses, it would follow that the posterior aspect would be the most significant change, but the orientation in the direction of the pilaster is important to mention, though it does not account for as much variation.

When the extreme values of the PCA analyses are taken into account, one of the most significant shape changes is found in the Western Asian Neanderthal group which represents a round cross-section with medio-lateral reinforcement (while this seems contrary to the description of the changes in shape above, the European average shape may have been impacted by Fond-de-Forêt 1, an undated Neanderthal from Belgium with a surprisingly pronounced pilaster-like posterior protrusion). The other end of the extreme variation in shape change is represented by the Dolní Věstonice specimens and exhibits a significant pilaster. Secondary to the presence of a pilaster (as represented by PC2), the shape change is centered around the orientation of the pilaster, from a medial to lateral position of the posterior-most aspect of the cross-section. Pavlov 1 represents an outlier for the orientation of the pilaster in the medial direction; however, when excluded, a medial orientation (though less severe) can still be found driving the secondary component of shape change.

The importance of these shape changes can be observed in the extreme values of the CVA as well. The greatest difference between groups in the CVA, however, is the orientation of the pilaster, rather than its presence. The extreme of the medially oriented group is represented by the Catalhöyük population with its counterpart (a lateral location of the pilaster from the posterior-most aspect) in the LP*Hs* population. These are the same groups at the extremes of the PC2 in the PCA analysis. The second discerning characteristics of shape between populations is the presence of the pilaster, again ranging from extremely round (Western Asian Neanderthals) to pronounced (this time identified as the Alaskan Inuit population). Through these analyses, it is clear that not only the presence of the pilaster is significant, but the orientation plays an important role, especially among modern human groups. Variation in the pilaster represents a change in the femoral anatomy concerning muscle attachments as well as skeletal and behavioral loads on the femur. These shape data are consistent with previously established patterns in Neanderthal and modern human morphology, indicating varying adaptive strategies in the use and loading patterns of the lower limb; however, it is critical to understand what true shapes are reflected through phylogenetic analyses prior to attempting to understand the processes acting on these sources of variation.

Phylogenetic Signal and Evolutionary Allometry

The permutation test for phylogenetic signal showed that signal is present at the femoral midshaft between recent modern human groups (Alaskan Inuit, Catalhöyük, Libyan, and Pre-Dynastic Egyptian populations), LP*Hs*, and European and Western Asian Neanderthals. The p-value of 0.0549 allows for the rejection of the null hypothesis that shape changes at the femoral midshaft are independent of shared evolutionary history. This is further supported by the close evolutionary relationship and short evolutionary time to divergence between fossil and modern human groups (Felsenstein, 1985; Cheverud et al., 1985). It is important to note, however, that the observation of the presence of phylogenetic signal between these populations only applies to the midshaft of the femur and could vary in other morphological aspects of both the crania and the postcrania.

In order to understand the nature of this signal, multivariate regression and phylogenetic independent contrasts were performed to examine how size changes throughout evolutionary history may have impacted changes in shape; in other words, to examine the correlation of the shape data with evolutionary allometry. These results showed that size and shape were not significantly correlated and only 20% of shape variation could be attributed to changes in size. This is significant in the understanding of past and current models of evolutionary history based on femoral midshaft morphology. When comparing morphology between fossil and modern humans, the shape variation reflects the evolutionary processes acting on this aspect of femoral morphology and not apparent changes in shape through changes in allometry.

It is important to address that although the sequences used to represent the femoral populations in the morphometric data were able to clearly distinguish between Neanderthal and modern human populations, the distinction between LP*Hs* and recent modern humans was less clear. This may be due to a use of partial sequences for some archaeological populations (specifically the Pre-Dynastic and Alaskan Inuit individuals), a lack of sufficient separation between lineages (unaccounted for admixture), or even modern contamination into ancient DNA strands (though recent methods have worked to reduce this problem). If the populations used here were not genetically distinct enough to create high resolution on the modern human portion of the tree, one would expect an inflated amount of phylogenetic signal. However, there was clear delineation between the LP*Hs* and modern human groups with the Neanderthal populations in the phylogenetic tree, further supporting the presence of signal between these branches.

The lack of the presence of a correlation between allometry and shape changes indicates that the previously described shape changes from the PCA and CVA results above likely reflect true changes between fossil and modern human femoral midshaft shape, and that evolutionary hypotheses for these differences are not weakened by the presence of phylogenetic signal. As with distinctions between recent modern human and LP*Hs* populations, the exact causes for differences between fossil and modern human femoral midshaft shape can be difficult to discern. Though climatic variables have been shown to have a reduced, if any at all, impact on the midshaft shape in modern human groups (Stock, 2006), they have impacted other aspects of the Neanderthal postcrania and their overall morphology. From a biomechanics perspective, the reinforcement of the medio-lateral breadth in Neanderthals certainly indicates a necessary and sustained skeletal support against pressures in that direction, with a circular shaft likely indicating that both planes (medio-lateral and anterio-posterior) are under biomechanical remodeling, especially during growth (Ruff and Hayes, 1983; Ruff et al, 2006; Cowgill, 2010).

Given that the resources exploited by both groups are varied (Stringer et al., 2008; Henry et al., 2014) and that small changes in subsistence can make variable changes in femoral cross-sections (Bridges, 1989; Stock and Pfieffer, 2001; Shackelford, 2007), it is difficult to attribute fossil and modern human differences to an increase or decrease in activity. This is compounded by a lack of archaeological evidence for substantial behavioral differences between earlier members of both fossil groups, especially in the Levant (Churchill et al., 2000; Trinkaus et al., 2002). It is possible, however, that greater energy requirements due to a larger absolute bauplan may have caused Neanderthals to require higher rates of return in their subsistence, making their interaction similar to the one found in by Stock and Pfieffer (2001) in modern humans, where an increase in requirements for foraging range may have put more strain on the femoral shaft shape (Sorensen and Leonard, 2001; Churchill, 2008). Developmental patterns, especially in relationship to adult levels of robusticity and strength vary between modern human populations; however, Cowgill (2010) shows that Neanderthals are not outside the range of modern human variation for lower limb strength. This study, in conjunction with studies of femoral robusticity, show that once size is accounted for, modern human and Neanderthal hypertrophy and strength are not statistically different (Trinkaus and Ruff, 1999; Churchill et al., 2000; Shaw and Stock, 2011). Therefore, it is unlikely that a drastic change in strength or activity levels differentiate Neanderthals from LP*Hs* or modern human groups, especially since increases in activity in humans tend to increase the presence of the pilaster, rather than medio-lateral breadth (Shackelford, 2007; Stock and Pfeiffer, 2001).

While there are undoubtedly differences in modern human and Neanderthal lower limb function, such as a slight hamstring advantage in Neanderthals (Chapman et al., 2010), or a calcaneus more adapted for running in modern humans (Raichlen et al., 2011), it is difficult to discern whether adaptive pressures lead to these advantages, or if they are not adaptive, but rather reactive to another change in the bauplan of one of these groups. It is important to note that the Neanderthal femoral shape is similar to that of earlier members of the genus *Homo* (Ruff, 2008). The trend to the development of the pilaster in modern humans appears to be a derived trait, and places emphasis on the need to understand what processes changed modern human biomechanics from an already established shape and why it continued to develop over time (Trinkaus, 2006). It is also likely that the differences observed between the femoral midshaft shapes are due to equifinality and represent two distinct morphologies in the human lineage toward the same end, but due to half a million years of evolutionary isolation, resulted in the variation we observe today.

The understanding of the role of phylogenetic signal between modern and fossil groups aids in the ability to test the observed shape variation at the femoral midshaft and the hypotheses attempting to explain this variation. By establishing that the observed differences are affected by phylogenetic signal but not by evolutionary allometry, current studies and observations in the variation between modern humans and Neanderthals in this aspect of their morphology are supported. In the case of modern and fossil humans, the shape variation at the midshaft is likely due to selective pressures as opposed to their recent divergent date.

CHAPTER VIII

CONCLUSION

The research presented in this study has examined the evolutionary relationships between fossil and modern humans, specifically the presence of their shared evolutionary history, or phylogenetic signal, and its impact on the observed variation found in femoral midshaft shape. Using geometric morphometrics, a permutation test for phylogenetic signal, and phylogenetic independent contrasts, it is demonstrated that, while phylogenetic signal is present in the femoral midshaft diaphyseal shape, allometric changes do not significantly impact the observed shape changes. The analyses performed here have also tested the assumption of independence for the characteristics at the femoral midshaft shape that is often critical in comparative studies. The lack of correlation between allometric changes and observed shape changes in these groups, despite the presence of signal, suggests that the causes of femoral variation at the midshaft is due to other evolutionary factors rather than shared history.

Additionally, the study has shown through principal components and canonical variate analyses that the variation in femoral shaft shape between Neanderthals, LP*Hs*, and recent modern humans follows previously established patterns of variation. This variation demonstrates that Neanderthals exhibit the fossil morphology of a circular femoral midshaft shape, with the lack of the bony projection, or pilaster, found in modern humans. Neanderthals also show a distinct broadening of the medio-lateral diameter of the shaft, which in some cases can mark the broadest portion of the midshaft. A sample of Late Pleistocene *Homo sapiens* individuals and a global sample of recent modern humans present a distinct presence of the pilaster and a narrowing of the medio-lateral aspect of the shaft, though they are still statistically different from one another. Recent modern human groups are additionally significantly differentiated by the orientation of the pilaster, whether in a medial or lateral direction.

Although it is difficult to directly associate the causes of the variation in the midshaft femoral cross-section to a single specific evolutionary cause, the distinctions are clear between the populations tested here. While the selective pressures of the femoral midshaft shape are likely multifactorial, the observed differences are likely due to achieving the same general type of locomotion with slightly different bauplans and evolutionary histories. This emphasizes the need for examining modern human derived traits to understand their morphology and to view Neanderthals in the context of their own evolutionary history, regardless of species or population associations. Being able to test for phylogenetic signal to isolate these evolutionary changes that led to modern human distinctiveness, including the femoral midshaft shape, may shed light on adaptive strategies that lead to the elimination of the Neanderthal phenotype approximately 30,000 years BP.

The implementation of phylogenetic comparative methods to test for phylogenetic signal in Paleoanthropology is rare due to methodological constraints caused by the fossil record. However, with the increase of access to ancient DNA and with the improvement of extraction methods, studies such as this can be used to test statistical independence of other changes in morphology between these groups. Understanding the levels of phylogenetic signal among fossil and modern humans will allow for a better view of the human evolutionary lineage as well as strengthen the hypotheses and models used to test autapomorphies by parsing apart the impact shared history has on the traits in question.

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