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**SKELETAL ASYMMETRY WITHIN THE NEW LISBON COLLECTION:
IMPLICATIONS FOR ENVIRONMENTAL AND MECHANICAL STRESS**

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BRIEF ABSTRACT

The purpose of this research is to investigate bilateral asymmetry within the New Lisbon Skeletal Collection, and to correlate observed levels with demographic and socioeconomic variables. The New Lisbon Collection is a historic skeletal assemblage of birthdates spanning 1805 – 1975, sourced primarily from Lisbon, Portugal. Metric long bone measurements of length and dimension were taken on a juvenile subsample ($n = 82$) and an adult subsample ($n=248$), and asymmetry indices were calculated incorporating both right and left and scaling for size.

For the juvenile samples the objective was to investigate lateralization during growth and development. No significant difference could be found between the sexes, suggesting that males and females were responding similarly to external stressors. Close correlation was found between age and clavicle and humerus asymmetry, which may be related to increasing participation in unimanual activities. Moderately differences in asymmetry between cause-of-death categories, coupled with the presence of both directional and fluctuating asymmetry suggests a population experiencing environmental instability and/or scarcity.

In the adult subsample, the main objectives were to examine difference in asymmetry between groups, and track temporal change in asymmetry. Mechanical loading stress appears to be the source of adult asymmetry. This is supported by higher asymmetry observed in manual laborers, and in comparison against modern skeletal material. No difference could be determined between the sexes, which may be attributed to high physical workloads recorded for all historic Portuguese. In temporal analysis, asymmetry significantly increases during years 1890 – 1919, coinciding with an era of significant socioeconomic and political transition in Portugal. Ultimately, bilateral asymmetry in the New Lisbon Collection reflects a population experiencing moderate to high levels of environmental and mechanical stress.

FULL ABSTRACT

Bilateral asymmetry, defined as metric and morphological differences in paired biological structures, may be used to infer various forms of environmental and mechanical stress imposed upon the skeletal system. The New Lisbon Collection is a historic skeletal assemblage of birthdates spanning 1805 – 1975, sourced primarily from Lisbon, Portugal. Highly conscribed cemetery records provide extensive demographic data and allow direct examination of environmental, social, and occupational variables affecting this population, all factors that potentially influence levels of bilateral asymmetry. Two patterns are typically observed in human skeletal material: fluctuating asymmetry (FA) is irrespective of side and is closely associated with environmental instability affecting development, while directional asymmetry (DA) occurs when heavy mechanical loading induces bone modification on a particular side experiencing the greatest stress. Adult and juvenile skeletal material was analyzed separately owing to hypothesized differential environmental stressors experienced by the groups and dissimilar stages of asymmetrical development represented in the skeletons.

For the juvenile samples (defined as individuals 20 years or younger) the primary objective was to investigate lateralization during growth and development. Variable levels of directional asymmetry were expected within this subset, with the potential for observations of fluctuating asymmetry. Metric measurements of length and dimension were taken on all major long bones, and a single asymmetry index was calculated to represent the difference between the right and left elements in a single value. Data were subjected to multivariate analysis (with permutation randomization tests) to evaluate differences between right and left elements, and between the sexes. Furthermore, levels of asymmetry were analyzed for correlation with age and known causes of death, as well as overall pattern of asymmetry.

Individuals in the juvenile subset demonstrated significant bilateral asymmetry in the majority of investigated skeletal areas. No significant difference could be found between the sexes, suggesting that males and females were responding similarly to external stressors. Close correlation was found between age and asymmetry of the clavicle and humerus length. This may be related to the onset of more unimanual mature activity patterns, which probably commenced at a relatively early age in this population. Furthermore, significant difference was determined between infectious and sudden causes of death in terms of clavicle asymmetry. This may partially result from a low number of samples in the latter category, but may potentially be related to the detrimental effects of tuberculosis (the most frequently noted infectious pathogen) on clavicle morphology.

Finally, examples of both fluctuating and directional asymmetry were observed in the juvenile subset. Humerus length asymmetry demonstrated a directional pattern, with a significant and consistent right side bias observed in most samples. The frequency curve assumes a very narrow distribution, with little variation away from a mean positioned significantly above the zero value. Alternatively, radius diameter asymmetry most closely represents of a fluctuating pattern. The frequency histogram reveals a very wide distribution with inherently large amounts of variation, although the peak of the mean is clearly right-biased. However, the leptokurtic value demonstrated by this variable suggests the presence of selective forces and fluctuating asymmetry. Clavicle length asymmetry produced a complex pattern of both fluctuating and directional asymmetry. This variable appears to assume a fluctuating pattern earlier in life, when developmental stability is the primary determinative force, and becomes increasingly directional later in life as the clavicle encounters greater mechanical loads. The presence of fluctuating asymmetry in immature skeletal samples suggests a population experiencing environmental instability and/or scarcity, preventing the normal trajectory of growth and development. This supposition is confirmed

by historical documents and ethnographic data describing a period of socioeconomic transition in Portuguese history.

The primary objective in the adult sub-sample is to examine significant differences between groups (sexes, cause of death categories, and occupational types), to track temporal change in directional asymmetry, and compare levels of asymmetry in the New Lisbon Collection with levels observed in modern Americans skeletal samples. Adult human populations generally follow a directional model of lateralization. Similar to the juvenile subsample, metric measurements of length and dimension were taken from long bones and a single asymmetry index was generated for each variable.

Significant difference was found between right and left elements of the clavicle and upper limb, but little discrepancy was found in the lower limb. Presumably, the arms perform more unimanual operations, while the legs primarily function in more equilateral bipedal locomotion. No statistically significant difference could be determined between the sexes, which may be attributed to the high physical workload documented for historic Portuguese women. Although insignificant, males had slightly higher asymmetry in variables of the upper limb (clavicle and humerus measurements), while females were more asymmetrical in variables of the forelimbs. This suggests that males may have been using the shoulder girdle more heavily in actions involving lifting, pulling, and carrying weight; while females may have performed more duties requiring dexterity of the forearms and wrists, applying pressure to the elbow joint. Furthermore, individuals employed in manual occupations had notably higher levels of asymmetry than non-manual workers.

In temporal analysis, levels of long bone length asymmetry demonstrated a significant increase during years 1890 – 1919. Because linear growth terminates in early adulthood, this finding suggests that high mechanical loads were encountered in adolescence and early adulthood for this group. The most conspicuous increase was found in humerus

and clavicle length. Males possessed higher values in these variables, implying that over time males experienced greater change in their activity and asymmetry patterns than females. This period coincides with an era of significant socioeconomic and political transition in Portugal. Additional skeletal evidence of high mechanical stress in the New Lisbon Collection includes osteoarthritis and healed fractures. Finally, Lisbon samples demonstrated a significantly higher degree of asymmetry than modern samples at all indices of the upper limb, a result ascribed to greater unimanual activities performed in historic Lisbon.

Bilateral asymmetry in the New Lisbon Collection generally reflects a population experiencing moderate to high levels of environmental and mechanical stress. Historical documentation associated with the skeletal material further suggests a period of significant social and political instability within the Lisbon environment. Greater nutritional and mechanical loading stress was placed upon individuals during crucial life stages of growth and development and adult livelihood, consequently creating higher lateralization in the long bone morphology.

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INTRODUCTION

Physical asymmetry refers to morphological and metric differences in paired biological structures (Dangerfield 1994). Differential growth of paired body tissues occurs in response to significant environmental perturbations (Van Valen 1962). Thus, individuals experiencing a stress-related insult may fail to develop both right and left sides equally. Although several types of asymmetry have been defined and identified in various organisms, fluctuating asymmetry (FA) and directional asymmetry (DA) are most frequently observed in human skeletal material. These forms of asymmetry may be determined through individual examination and population-level comparisons (Van Valen 1962). Fluctuating asymmetry is random with respect to sides: either the right or left member may experience greater development, sometimes oscillating or fluctuating between the two over the course of an organism's development. The magnitude of lateralization is significant, rather than side of dominance. Fluctuating asymmetry (FA) is closely correlated with environmental stability during crucial stages of growth, and implies a barrier preventing equal development of paired structures. To diagnose FA, researchers typically investigate tissues which create a permanent record of the developmental environment. In human populations, such tissues include the teeth and dermatoglyphic patterns of the hands and feet. In addition, skeletal fluctuating asymmetry may be examined utilizing radiographic data, or direct analysis of metric measurements taken from the bones. An adequate sample size of remains which have not, or have recently achieved skeletal maturity is necessary when investigating potential environmental stressors,

Directional asymmetry (DA) is manifested by the greater development of one particular side in a pair. Greater lateral development occurs in a consistent and predictable direction. DA is closely related to mechanical loading stress, inducing bone response and

modification on the side receiving the heaviest burden. Mechanical loads capable of inducing significant changes in bone maintenance are not typically encountered until adolescence or early adulthood, although commencement of loading may be population-specific. The adult metabolic patterns of bone repair and maintenance lack canalization processes, making them more susceptible loading stress and resulting directional asymmetry. DA investigations primarily focus upon changes in long bone measurements and their dimensional areas, although weight-bearing joints have also been studied. DA has a venerable history in anthropology, and has been applied in biomechanic, anthropometric, bioarcheological, and modern forensic contexts.

The New Lisbon Collection, housed at the Bocage Museum in Lisbon, Portugal, provides a unique opportunity to investigate both fluctuating and directional asymmetry. The entire collection currently consists of over 1700 identified individuals recovered from Lisbon cemeteries, with birthdates ranging from 1805 – 1975 (Cardoso 2006). This summation includes one of the largest historic samples of subadult skeletons, totaling 92 individuals of both sexes (Cardoso 2005). In addition to exceptional sample size, the collection boasts extensive documentation provided by mortuary records. Biographic information collected at the time of death has been maintained throughout the interment process and for each set of remains. Demographic data include: name, sex, date of birth and death, nationality, place of residence at birth and death, occupation, marital status, and cause of death. The highly conscribed nature of the skeletons in the New Lisbon Collection allows direct examination of environmental, social, and occupational variables affecting this group, all factors that potentially influence levels of bilateral asymmetry. Furthermore, this collection is extraordinary in the long time depth represented in a small, rather geographically isolated European population. While morphological and craniometric secular change has been

previously documented in modern American populations (Jantz 2001; Jantz and Meadows Jantz 2000), less research has been conducted on temporal change prior to the twentieth century. Even fewer studies have specifically focused on change in levels of asymmetry through historic periods.

The present research will evaluate the prevalence of skeletal asymmetry in the New Lisbon Collection, and correlate this pattern with environmental variables and activity levels as inferred via cemetery records. This study utilizes both juvenile and adult skeletal material. The subsets were separated for analysis, owing to differential stages of development, hypothesized environmental stressors, and diverse levels of mechanical loading stress experienced between the groups. Fluctuating asymmetry may be observed in (incompletely developed) juvenile subjects, because it is easily masked by the presence of strong lateralization typically encountered later in life. Directional asymmetry is ideally analyzed in adult samples that have engaged in mature activity patterns for a significant amount of time. Skeletal remains in the New Lisbon Collection exemplify the conditions necessary to investigate both forms of bilateral asymmetry.

Juvenile Subsample

For juvenile material, the primary objective is to investigate lateralization and the onset of DA during growth and development. Although dental odentometrics are often utilized in studies of juvenile asymmetry, lack of preservational quality and availability in the New Lisbon Collection prevented this type of study. Therefore, skeletal asymmetry was exclusively investigated using long bone metric data.

The “juvenile” sample was defined as individuals 20 years at-death or younger, to ensure skeletal immaturity (terminal epiphyseal closure incomplete). In any mortuary

context, juveniles represent arrested development and a compromised growth pattern. If fluctuating asymmetry is detectable in the New Lisbon Collection, it would be observed in the juvenile subset. However, this pattern may change with increasing age. Commencement of puberty and the adoption of adult occupations and gendered-roles initiate a new set of asymmetric stressors on the skeletal system. Older individuals may demonstrate a transition to directional asymmetry. For this reason, the juvenile subset is analyzed with respect to sex, age, and environmental indicators, such as cause of death. Specific questions to be addressed within the juvenile sub- sample include:

1. Is skeletal asymmetry observable in the long bones of the juvenile subset?
2. Which long bones contribute the greatest asymmetry and are the departures for equality significant?
3. Is there a significant sexual dimorphism in juvenile asymmetry?
4. Does bilateral asymmetry change with increasing age? For example, do younger remains represent lower developmental stability, resulting in higher asymmetry?
5. Does asymmetry demonstrate a correlation with certain causes of death?
6. Is asymmetry correlated with other indicators of development stress, such as irregular/bilateral epiphyseal closure?
7. What asymmetry pattern or combination of patterns is observed in the juvenile subset, and is this pattern associated with a particular variable or concentrated within a specific skeletal area?

Given the potential for biological mortality bias, it is hypothesized that the subadults in the New Lisbon Collection represent a stressed population. Higher (than average) levels

of skeletal asymmetry are assumed relative to the living population. High asymmetry is anticipated especially among very young individuals whose development was arrested very early in life. The majority of juveniles in the sample population have not entered puberty, the life stage at which populations begin to exhibit sexual dimorphism. Therefore, significant difference between the sexes is not anticipated among the juvenile subset. Finally, high correlation is hypothesized with chronic morbidity (indicated by mortuary records), and irregular maturation (as inferred via epiphyseal fusion).

Adult Subsample

The primary objective in the adult sub-sample is to examine and to track temporal change in directional asymmetry (DA). Adult human populations generally follow a directional model; lateralization is related to activity and habitual use of the dominant side (Dangerfield 1994; Steele 2000). Samples in the adult subset may retain some degree of fluctuating asymmetry, reflective of their developmental environment. However, documented high levels of unimanual activity suggest DA as the primary form of asymmetry expected within this group.

Directional asymmetry has previously been used in anthropological studies to establish changes in the activity patterns and socioeconomic status of various groups. For example, in Native American skeletons, DA has been used to explain the change in activity patterns during the shift from hunting-and-gathering subsistence to maize agriculture. Similarly, class-related differences in asymmetry may be examined in stratified societies. Lower socioeconomic groups tend to perform more manual labor, presumably producing more modification and asymmetry (Larsen 1997). The present work intends to follow these bioarcheological models, correlating skeletal asymmetry observed in the long bones with

historical events and environmental conditions. Specific questions investigated in the adult subpopulation include:

1. Is skeletal asymmetry observable in the bones of the adult subset?
2. Which long bones contribute the greatest asymmetry and are the departures for equality significant?
3. Is there a significant sexual dimorphism in adult asymmetry?
4. Is there a significant difference in asymmetry between types of occupation?
5. Does skeletal asymmetry demonstrate a correlation with certain causes of death, particularly chronic or infectious conditions?
6. Do levels of asymmetry change through time? Are they associated with the significant socioeconomic transition experienced by Portugal during the last century?
7. How do levels of asymmetry in the New Lisbon Collection compare to that found in a modern skeletal population of twentieth century Americans?

The adult skeletal material is hypothesized to exhibit a high degree of directional asymmetry, consistent with high levels of mechanical occupational loading. Occupational stress in this group is considered high, similar to pre-modern agricultural groups, as is sex-specific stress. Disparate occupations and activities produce differential loading stress, and therefore, significant sexual dimorphism in asymmetry is expected. This may be especially true for bones of the upper extremities, which support the majority of mechanical stress. In addition, it is hypothesized that occupations which require extensive manual labor and chronic morbid conditions will elevate skeletal asymmetry. Finally, the New Lisbon Collection is expected

to demonstrate higher levels of asymmetry than a modern comparative population with less stressful occupational patterns.

Individuals in the New Lisbon Collection represent a significant period of economic and social transition in Portugal. Political instability, resource scarcity, and a declining economy contributed to a marginalized environment. Portugal endured this oppressed condition for several decades, roughly spanning the turn of the century until the Second World War. While these factors have been historically documented through public records, mortality levels, literacy rates, and economics, there have been few attempts to investigate the biological consequences in human skeletal remains. This thesis will demonstrate the relationship between bilateral asymmetry and environmental, socioeconomic, and occupational stressors in the context of historic groups.

CHAPTER 1: ASYMMETRY DEFINED

Morphological symmetry requires nearly identical development of both right and left sides, and may be regarded as the standard for most organisms in a natural environment (Dangerfield 1994). This is true of humans, who generally achieve morphological symmetry to within 1 part in 100 parts (dependant upon scale of measurement) for paired elements such as ear size, knee width, and maximum foot length (Trivers et al. 1999). Asymmetry represents a departure from equilaterality, resulting in quantitative and qualitative differences in paired biological structures. Asymmetrical phenomena are the topic of multi-disciplinary interest, studied in various structures among a wide variety of organisms. Diverse investigations have documented the location, degree, and patterns of asymmetry, linking it to environmental, functional, pathological, and genetic factors (Dangerfield 1994; Hallgrímsson 1998; Møller 1997; Parsons 1990; Van Valen 1962).

Van Valen's seminal 1962 work identified three distinct types of asymmetry: (1) Directional asymmetry (DA), which occurs when a character becomes augmented on a single side. The difference between sides (right – left) deviates significantly from a mean of zero (in a sample) consistently favoring one direction. (2) Antisymmetry (AS) exists infrequently in nature, but may be detected where asymmetry is the natural state, but variable as to which side (right or left) demonstrates greater development. Antisymmetry often shows a tendency for bimodality in a large population, favoring both extremes around a mean zero. (3) Fluctuating asymmetry (FA) is ubiquitous among organisms and sensitive to a number of causative environmental agents. Fluctuating asymmetry is random in direction and normally distributed about a mean zero. Side bias is irrelevant; rather, the magnitude of divergence from equality is significant (Hallgrímsson et al. 2002; Van Valen 1962).

1.1 Asymmetry and Developmental Stability

Asymmetry reflects a high degree of variability in measurable, quantifiable traits. While a range of variation is inherent for any character, developmental homeostasis typically favors an average phenotypic value (Livshits and Kobylansky 1991). The effect of developmental stability is the tendency for a genotype to follow a given trajectory under constant conditions, and produce a standard phenotype (Hallgrímsson et al. 2002). The process of canalization refers to the buffering of developmental systems against internal and external influences, such as environmental perturbations and mutations. Canalization allows the production of similar and viable organisms, even in the presence of instability (Hallgrímsson et al. 2002). When stressors exceed the buffering capacity of canalization, various forms of asymmetry (chiefly fluctuating asymmetry), pathology, and morbidity may arise. In an exhaustive literature review, Møller (1997) found a significant relationship between developmental stability and the fitness of an organism. Measured in terms of fluctuating asymmetry (also termed “phenodeviance”), Møller found the relatively more symmetrical individuals demonstrated generally faster growth, higher fecundity, and longer survival than their asymmetrical counterparts. Comparatively asymmetrical individuals were also less competitive in mate acquisition, susceptibility to parasitism, and predation (Møller 1997).

The relationship between asymmetry and fitness may be directly or indirectly inferred, essentially involving energetic transfer and metabolic pathways (Møller and Swaddle 1997). The basic tenet is as follows: energy and other vital nutrients – such as amino acids, trace elements, and regulatory factors – are limiting resources. During stressful periods, materials commandeered for repair or maintenance cannot be allocated towards growth, reproduction, or survival (Hallgrímsson 1998). Lab experimentation has revealed

several mechanisms, including energy (ATP) generation, collagen synthesis, and control of growth factor concentrations, which are extremely sensitive to deteriorated external conditions (Hallgrímsson 1998; Møller 1997). This theory is consistent with the observation by Siegel and Doyle (1975) of a strong association between stress-induced fluctuating asymmetry, and an overall decrease in skeletal size. They proposed that compromised calcium transport reduced the total mass and homeostasis of osteological structures.

In contrast, the cause and consequence of directional asymmetry is often more readily identifiable than that of fluctuating asymmetry. Directional asymmetry is the adaptive response of the skeleton to repeated mechanical loading stress on a particular side (Dangerfield 1994). Responses may include increased bone strength due to augmented bone density and cross-sectional area, increased mechanical efficiency through altered shape, and resistance to avulsion by increasing the surface areas of muscle-attachments (Ruff 2000; Steele 2000). The effective bone mineral formation is typically localized to the site of muscle-bone interaction, or concentrated endosteal development of cortical layers (Steele 2000; Steele and Mays 1995). In each case, mechanical response facilitates improved function.

1.2 Environmental and Genetic Factors

Biological, physical, and genetic environments all effectively produce stress, and influence asymmetry (Hallgrímsson 1998). Stressors encountered in the external environment are regularly studied with non-mammalian organisms, where populations are sufficiently large, reproductive generations are rapid, and extreme conditions may be simulated in the laboratory. These conditions include (but are not limited to) exposure to parasites, pollutants, and toxins like mercury, DDT, acidified water, and salinity, each having significant impact on asymmetry levels and fitness (Clarke 1993; Hallgrímsson et al. 2002;

Parsons 1990; Valentine and Soulé 1973). Similarly, natural populations existing at marginal positions of temperature, altitude, and aridity for example, show much higher levels of metric and morphological asymmetry (Leary et al. 1992; Parsons 1990; Zakharov 1992).

Environmental marginalization and scarcity among human groups produces comparable results. Bailit et al. (1970) studied geographic distribution of dental FA, finding the greatest FA values among the isolated islanders of Tristan da Cunha (Bailit et al. 1970). Perzigian (1997) compared levels of FA among several prehistoric Native American skeletal populations. He determined that the earliest group of hunter-gatherers, who seemingly experienced the greatest resource scarcity and physical loading stress, had the highest levels of asymmetry (Perzigian 1977). Also, the prenatal developmental environment plays an equally significant role. Mothers in higher “risk” categories (older age, smokers, no prenatal care, etc.) have a higher percentage of premature and asymmetrical infants (Livshits et al. 1988).

An interesting yet unresolved debate is the role of heterozygosity in determining asymmetric levels. Heterozygosity, possessing different alleles in a given pair at one or more positions, is generally accepted as a fitness advantage in natural populations. Proposed mechanisms for hybrid vigor include: providing a greater number of adaptive responses (through protein synthesis), co-dominance of alleles, or by preventing the expression of deleterious homozygous recessive traits (Livshits and Kobylansky 1984). Individuals with multiple homozygous loci are typically characterized as phenotypic extremes, and are normally eliminated by stabilizing selection, one of the most powerful evolutionary mechanisms (Hallgrímsson et al. 2002; Waddington 1957). Developmental instability produces greater variation, and asymmetrical individuals usually fall near the susceptible ends of the continuum (Hallgrímsson et al. 2002)

Several studies support an inverse relationship between heterozygosity and asymmetry, including a variety of vertebrates and invertebrates (Livshits and Kobylansky 1989; Livshits and Kobylansky 1991; Parsons 1990). Homozygosity is associated with various detrimental traits, presumably due to the dual expression of recessive alleles. Individuals who are homozygotes at multiple alleles also tend to exhibit higher levels of bilateral asymmetry, although reasons for this relationship are not fully understood (Livshits and Kobylansky 1984).

Another study utilizing human subjects compared morphological traits between individuals heterozygous and homozygous for five biochemical (blood-group) loci. The majority of diheterozygotes demonstrated extreme morphological phenotypes, coupled with the highest FA. Conversely, diheterozygotes had the lowest coefficient of variation values, and the lowest FA (Livshits and Kobylansky 1984). Additionally, distinct heterozygote and homozygote groups could be classified through discriminant and principle components analyses (Livshits and Kobylansky 1991). The influence heterozygosity has over asymmetry remains inconclusive, however, as a few studies have produced contradictory results. Graham and Felley (1985) cite several examples where developmental homeostasis and fitness was considerably lower in hybrid populations (Graham and Felley 1985).

Some genetic factors, like levels of heterozygosity, have complicated effects upon asymmetry. However, chromosomal aneuploidy is a genetic environmental factor with clear and predictable consequence. Aneuploidy is defined as an abnormal number of chromosomes in a given chromosomal pair. The consequences of which is “a generalized disruption of the evolved genetic balance in cells of affected individuals, leading to decreased developmental and physiological buffering against genetic and environmental forces” (Shapiro 1983). Therefore, sex chromosome aneuploidies, such as Klinefelter (XXY)

syndrome, XXY males, and Turner (XO) syndrome are relatively rare in the greater population. Autosomal aneuploidies are extremely rare, with the exception of Down syndrome (trisomy 21) (Shapiro 1983). Fluctuating dental asymmetry increases significantly in individuals with chromosomal abnormalities, reflecting the disruption in their developmental homeostasis (Parsons 1990; Shapiro 1983; Townsend et al. 1986). Interestingly, Turner (XO) individuals typically do not demonstrate elevated laterality, although there is a slight reduction in overall permanent tooth size (Townsend et al. 1986).

1.3 Historical Investigations of Asymmetry

Lateralization in the human body and skeleton has been noted throughout history. Some of the earliest translated writings on asymmetry predate the Aristotelian period (Kieser 1990). In the 19th century, Charles Darwin published several primary observations on asymmetry among various faunal species. The first documented work on human skeletal asymmetry was undertaken by anatomist Arnold in 1844 (surname only listed in publication), creating the first account of humeral right-dominance (Stirland 1993). These early works followed the naturalist tradition, creating detailed descriptions and recording variations in natural species for the purposes of classification rather than comparative analysis.

Organized study of anthropoid asymmetry was initiated in France and Germany during the early 20th century, and later the United States. Medical professionals performed the first research in the field of asymmetry. Working with disarticulated limbs, doctors analyzed skeletal structures and nerve endings to perform more efficient amputations on the battlefield. They noted consistent right – left disparity in length, dimension, and weight of limbs. Interest in this type of work later expanded, including skeletons from museum collections and medical cadavers donated for the purpose of science (Stirland 1993). One of

the first studies by Gustav Guldberg on several anthropoid species (including orangutans, gibbons, chimpanzees, and humans) found asymmetry favoring the right extremities (Guldberg 1897). Employing human skeletal material from France, Rollet (1902) demonstrated inequality in upper limb length, with the right limb dominating in an unprecedented 99 out of 100 samples (Rollet 1902). Hasse and Dehner (1893) found a similar result in their study of 5141 German soldiers (Hasse and Dehner 1893). In America, Hrdlička (1932) examined humeral length and midshaft dimensions, as well as humeral-femoral index in a sample 4432 American white, American blacks, and American Indians of both sexes. He determined humeral length and humeral-femoral index to be slightly greater in the right side for all ancestral groups and sexes, although variable in magnitude among different groups (Hrdlička 1932).

Looking at lower limbs, Garson (1879) found a reverse pattern of dominance, with left limbs longer in 54.5% of all cases (Garson 1879). This model was replicated in the works of Hasse and Dehner (1893), Schwertz (1912), and Schultz (1937). Asymmetries of the clavicle are functionally related to that of the upper limbs, and were historically documented in the early works of Martin (1928), and Terry (1932). Both authors determined the left clavicle characteristically dominates in terms of length.

These initial studies established many of the basic principles in human asymmetric research, including Schultz (1937):

- (1) Humans possess a larger degree of asymmetry, and range of asymmetric variation than any other anthropoid
- (2) The upper limb (humerus, radius, and ulna, measured individually and in conjunction) tend to dominate on the right side in length and weight.
- (3) Lower limbs (femur and tibia specifically) favor the left side in length and weight.

- (4) Asymmetries of the clavicles typically favor the left side in length
- (5) On average, asymmetric values of the upper extremities are twice that of the lower extremities

In the centuries following these initial studies, bilateral asymmetry has been used to infer environmental stability, biomechanical stress, stabilizing selection intensity, heterozygosity, patterns of sexual selection, ontogenic constraints, and the process/limits of canalization and homeostasis (Hallgrímsson et al. 2002). The present study focuses on long bone asymmetries of the arms and legs. Therefore, studies reviewed in the following section are examples which draw a relationship between limb asymmetry and environmental instability and/or mechanical stress.

CHAPTER TWO: REVIEW OF PREVIOUS RESEARCH

2.1 Fluctuating Asymmetry

The presence of fluctuating asymmetry is best studied in skeletal structures which may be influenced during development, but lack any inherent side bias. Work with laboratory subjects (typically rodents) reveals that increased stress during the growth period induces fluctuating asymmetry in long bone length (Siegel and Doyle 1975). Fluctuating asymmetry (FA) is more difficult to assess in human skeletal structures, particularly in archeological contexts. Directional asymmetry is more readily discernable in adult remains and often masks the presence of fluctuating asymmetry. Subadult skeletal material generally lacks the confounding effects of strong lateralization, but remains are scarce in prehistoric and historic skeletal collections due to preservational and cultural factors. Consequently, fluctuating asymmetry is most often studied in soft tissue structures like dermatoglyphics (the characteristics number and pattern of ridges on the dermal surface of hands and feet) of living groups, or well-preserved dental metric data (odontometrics). These fields began as a way to characterize groups and make population-level comparisons; however, each has progressed as a means to understand internal variation and the determinative relationship with environmental conditions.

Anthropometrics

A relatively small number of anthropometric studies have been performed investigating fluctuating asymmetry among living populations. Livshits and Kobylansky (1989) assessed fluctuating in a set of 12 selectively neutral metric measurements in a large sample of Israeli individuals. They found an inverse relationship between age and FA, with the youngest sample, perinatal infants (<73 hours) had the highest observed asymmetry,

followed by the subadult groups (ages 5 – 18 years), and adults having the lowest degree (Livshits and Kobylansky 1989). Given the close relationship between FA and environmental stability, adults represent the most stable and complete developmental pathway.

Trivers et al. (1999) executed a preliminary study of fluctuating asymmetry using longitudinal data from a cohort of Jamaican public school children. They similarly found a negative correlation between age and asymmetry, but a positive association between asymmetry, height, and weight (for age). The interaction between these variables and FA may be directly related to metabolism. Wilson and Manning (1996) also noted that resting metabolic rate has a pattern similar to FA in terms of height and weight. At early ages, the fastest growing children (and therefore the largest) have high metabolic rates (Wilson and Manning 1996). The youngest cohorts in the Jamaican study similarly had the highest FA. As development concludes and metabolic rate declines as does levels of fluctuating asymmetry (Trivers et al. 1999). Children recorded to be shorter and smaller than average reach this state at an earlier age. Further research is needed on the effects of metabolism, development, and resulting anthropometric asymmetry.

Odontometrics

Early odontometrics work was inspired by the desire to demonstrate noninheritence of FA among dental structures. Garn and colleagues was one of the earliest research teams in this field, working with the Fels Institute Longitudinal Growth Study. Data was derived from dental casts of 239 native Ohio adolescents, primarily of middle-socioeconomic status and northwestern European ancestry (Garn et al. 1965; Garn et al. 1966; Garn et al. 1968). Researchers measured buccolingual and mesiodistal metric diameters of the second molar.

They determined that variation was randomly distributed between right and left sides, consistent with a fluctuating pattern (Garn et al. 1968). Additional conclusions reached: (1) larger teeth produce correspondingly larger asymmetric values; (2) FA increases with distance from the primary tooth in each morphological class; and (3) individuals who lack at least one of their third molars (representing more irregular growth) display the highest degree of dental asymmetry (Garn et al. 1966). Notably, males had higher levels of FA than females – a result they attributed to the duplication of the X chromosome in females, providing a more consistent developmental pathway (Garn et al. 1965). This finding implied genetic determinism in odontometric asymmetry.

Suarez further investigated the topic of genetic influence on dental FA. He compared antimeric measurements of Neanderthal teeth against a sample of modern humans (Suarez 1974). Testing the probable mutation effect (PME) model, Suarez hypothesized a reduction in dental size and asymmetry through time. However, after repeated testing he found temporal increases in FA. Suarez suggested that the high level of observed dental FA was related to rising incidence of inbreeding among terminal Paleolithic Neanderthal populations. He argued that chronic consanguinity allowed a relaxation of dental canalization (Suarez 1974).

Directly addressing this assumption, anthropologists Doyle and Johnson (1977) included Suarez's Neanderthal research with additional odontometric data from two Native American groups (Eskimo and Pueblo) and the Fels' Ohio white sample, in a cross-population study.

The Neanderthal and Native American samples showed equivalent levels of dental FA, each significantly higher than the Ohio white population. Among the Eskimo and Pueblo groups, consanguinity is considered culturally taboo and is relatively rare. This

eliminates inbreeding as a causative agent. Doyle and Johnson proposed that dental FA was a result of environmental stress, most likely from resource scarcity rather than inbreeding prevalence (Suarez 1974). Their conclusion was supported by Perzigian (1997), performing a similar study in the same year. Perzigian compared three temporally and culturally distinct samples: prehistoric hunter-gathers (Indian Knoll), aboriginal maize agriculturalists (Larson site), and a modern medical-cadaver population (Hamann-Todd). He found that FA decreased through time, and was highly correlated with additional skeletal indicators of stress, such as shorter stature, Harris lines, and linear enamel hypoplasias (Perzigian 1977).

Finally, a comprehensive study spanning the 1980s by Kieser and colleagues produced a body of research directly relating environmental stability with metric and morphological dental asymmetry (Kieser 1986a; Kieser 1986b). Tests were carried out on a set of 202 dental casts sourced from the Lengua Indians, inhabitants of the marginal Chaco region of Paraguay. Comparison against a control sample of relatively well-nourished and disease-free contemporary Americans revealed higher FA in the Lengua population. Younger, more acculturated Lengua Indians with greater access to Western healthcare and nutrition had lower degrees of asymmetry than older individuals, who practiced a traditional lifestyle with fewer resources (Kieser 1986a; Kieser 1986b). Statistically powerful methods applied by Kieser, such as Euclidean map analysis and Naperian logarithmic transformations, drew a conclusive link between environmental stability and dental fluctuating asymmetry (Kieser 1990).

Dermatoglyphics

Much like dentition, dermatoglyphics may be used to measure fluctuating asymmetry. Several key qualities of dermatoglyphics render amenable to asymmetric investigation. By virtue of its position on hands and feet, dermatoglyphics exist as mirror

images available for side comparison. These patterns are postnatally permanent, unaltered by time or use. Furthermore, dermatoglyphics are relatively non-adaptive and objectively quantifiable (Newman 1970).

The most common technique quantifying dermatoglyphics is by counting the ridges on either the finger-patterns or between inter-digital regions. Adoption of the Total Ridge Count (TRC) system somewhat standardized the practice across disciplines. Unfortunately the practice sacrifices large amounts of inter-individual variation, particularly the type of variation amenable to asymmetric studies (Jantz 1987).

Preliminary work by Holt among others suggests a high heritability for dermatoglyphic traits (Holt 1968). It appears that TRC is more heritable than individual digit-counts, overall estimates (using 20 finger-ridges) approaching 0.738. Additional ridge-counts, including those of the toes and planter areas, produce much lower heritability estimates, revealing a potential environmental component (Jantz 1987).

The permanency of dermatoglyphics implies that variation is generated early in development, specifically in the prenatal environment. Babler (1978) conducted one of the principal studies in environmental selection during ontogeny. He compared the frequency of ridge patterns between a sample of spontaneously aborted fetuses (representing a population under direct environmental stress), and electively aborted fetuses (assumed to have normal developmental circumstances) (Babler 1978). The spontaneously aborted group possessed higher arch frequencies, shallower primary ridges, and greater bilateral variation. Babler concluded that the asymmetrical fetuses demonstrated an alteration, or complete arrest in developmental timing (Babler 1978). Dermatoglyphic patterns may not confer a direct selective advantage, but the canalization process in which they are created suggests rigid selective control.

Fluctuating asymmetry in the total and a-b inter-digital ridge counts (number of ridges between the base of the index and middle finger) was also investigated by Jantz and colleagues. They determined lowest asymmetry values were located near the total population mean, and highest values at the phenotypic extremes (Jantz and Webb 1982). They also confirmed earlier speculation that the right hand tends to have a higher mean ridge count than the left, and that males consistently exceed females in digital, hand, and total ridge counts (Jantz 1974). Moreover, significant population variation exists in terms of asymmetric values: African populations had the highest correlation between digits (least asymmetry), while Native Americans (Eskimos in particular) had the highest FA values (Jantz 1979; Jantz and Webb 1982). This is particularly unusual given the morphological homogeneity historically attributed to Amerindian populations (Jantz 1987).

2.2 Directional Asymmetry

Forelimb Asymmetry

Limb lateralization is one of the distinguishing features of human mechanical behavior at the population level (Steele 2000). The limbs may be considered mirror images of one another. They develop concurrently along a midline axis, and result in near symmetry in the absence of environmental perturbations (Dangerfield 1994). Lateralization in the forelimbs is commonly referred to as handedness, and characterized by preference of a particular extremity to perform skilled unimanual functions. The cerebral cortex is the portion of the brain responsible for both language and object manipulation. Control is chiefly localized on the surface area of the central sulci (von Bonin 1962). Cortex lateralization likely arose very early during hominid evolution, related to lateral arm use for tool manufacture (Dangerfield 1994; Steele 2000). Tool industry implies mastery of

asymmetrical coordination, whereby one limb is responsible for steadying an object, while another performs expert manipulation (Trinkaas et al. 1994). Therefore, the emergence of right-handedness has paralleled cerebral cortex asymmetry throughout human history (Steele and Mays 1995; von Bonin 1962).

The inclination for right-hand preference predates the historic human record. The most frequently cited statistic of human handedness is a comprehensive study by Coren and Porac (1977), reviewing over 5,000 years of human unimanual function, through drawing, painting, sculpture, historical documents, tool and weapon use. The authors determined the right hand was employed in an average of 93% of all case, regardless of historic period or geographic area. Their results have been used to characterize right-handed dominance and trace its antiquity within the *Homo sapien* species.

Anthropologists routinely attribute skeletal asymmetries in the forelimbs to behavioral handed-bias, a procedure supported by extensive evidence. Ingelmark performed the seminal work on emergent long bone length asymmetries, utilizing a series of radiographs from a German sample of juveniles and adolescents (ages 6 – 20). Ingelmark found that 97% (140 out of a total 145) of subjects demonstrated greater long bone lengths in their dominant arm. With the exception of two younger children (6 – 12 years-old group), none of the observed subjects demonstrated the opposite pattern (greater length of the non-dominant arm) (Ingelmark 1946).

More recent work on forelimb asymmetries has focused upon individuals whose occupation places elevated levels of stress and strain on their body. Professional athletes have proven invaluable subjects, because their livelihood often depends on the perfection of specific lateral movements. In a study of fifty major and minor league baseball pitchers, King and colleagues (1969) found a general loss joint integrity in the elbow, as well as

osteochondritis dissecans of the olecranon tip, and significant hypertrophy of the humeral cortical bone all concentrated within the throwing arm (King et al. 1969). Similarly, Jones et al. (1977) calculated a minimum 30% increase in humeral cortical thickness in the serving arm of professional tennis players (Jones et al. 1977).

In addition to studies among living populations, forelimbs asymmetry has been investigated in archeological skeletal material of past populations. Jones, along with colleague Ruff, continued his work in lateralization, utilizing a prehistoric sample of hunter-gatherers from coastal California (Ruff and Jones 1981). They examined the effect of age and sex on bilateral asymmetry of the humerus and tibia. Most notably, significant variation in bilateral asymmetry does exist between the sexes and age-groups. Furthermore, humeral dimensions produced greater asymmetric values than that of the tibia, and males were more asymmetrical in their humeral values (an average of 2-3 times greater). For both sexes asymmetry decreased with age. Researchers attributed results to a loss of cortical bone from the dominant side, which had been subject to greater modification in youth (Ingelmark 1946; Ruff and Jones 1981).

Concordant results have been found in the extensive skeletal data from historic archeological sites. Examples from medieval Britain include Steele and Mays' (1995) work from the Wharram Percy site of Yorkshire England, and Stirland's (1993) work in Norwich and Henry's VII's flagship, *Mary Rose*. Working at these particular sights, both research teams found the distribution of forelimb asymmetries closely approximated population-level right-handedness (Steele and Mays 1995; Stirland 1993).

While there appears to be general agreement over the existence of right-handed dominance throughout human history, degree of variation remains an unresolved issue. Schultz's early work found asymmetry to increase in the distal segments of the forelimb

(radius and ulna) when compared to the humerus, a result unsupported in many more recent studies (Lieberman et al. 2001; Ruff and Jones 1981; Steele and Mays 1995). Additionally, some authors argue that data showing left-limb preference have been underreported, or misinterpreted as ambidexterity, leading to an inflation in the magnitude of right-side preference (Steele 2000). A unique subsample was identified in the *Mary Rose* population, whose asymmetric values were unusually low, favoring left-side dimensions of muscle-attachment in the shoulder (Stirland 1993). The authors related this evidence to activity-related patterns of asymmetry. Using nautical records, they noted a high frequency of professional archers documented among the crew. Researchers hypothesized that habitually sustaining a heavy medieval longbow on the non-drawing arm (usually the left) could potentially create stress-related modification on the left side (Stirland 1993).

Clavicle Asymmetry

The clavicle is a crucial component of the upper limb—shoulder girdle complex, also subjected to lateralized operations. This early-ossifying, late fusing bone is an important location of attachment for several muscles and ligaments involved in compression, bending, and torsional pressures (Harrington et al. 1993). The geometric structure of the clavicle indicates its function. The long axis serves as a strut and all loads of the upper limbs are transmitted through the clavicle. The characteristic “S”-shape balances opposing forces. The medial two-thirds has a circular cross-sectional area, buffering compressive forces, while the lateral third is relatively flat, allowing greater muscle-attachment and range of motion (Harrington et al. 1993).

As an extension of the upper limb, the right clavicle receives heavier average loading. Historical studies on clavicle pairs report a slightly longer length for the left, and greater axial

curvature in the right (Martin 1928; Terry 1932). Various hypotheses have been posed explaining these observations, ranging from a developmental right-left gradient in nutrient supply to paired structures (von Bonin 1962), to the unequal distribution of postural weight onto the left side (Ljunggren 1979). A third hypothesis was explicitly tested by Mays et al. (1999), proposing that asymmetry results from unilateral compressive forces. Data from the Wharrem Percy archeological population generally supported this theory. The left clavicle achieves longer length in the absence of constrictive forces. The right clavicle typically displays more ligament attachments, and is more robust in terms of thickness and lateral curvature, presumably to withstand buckling stress (Mays et al. 1999).

Lower Limb Asymmetry

Less evidence has accumulated concerning bilateral asymmetry in the appendicular lower limbs. Initial studies noted discrepancies in lower limb length, but at significantly lower magnitudes than calculated for upper limbs. Garson (1879) completed one of the first systematic studies of lower limb lateralization. Limb asymmetry was observed in 71% of the skeletal sample, in 41 cases (out of 70) the left femur was longer by an average 3.8mm. In his study of anthropoid long bones, Schultz (1937) found human femora to differ on average by 2.5 mm, typically showing left predominance (compared to an average 4.5mm difference between humeri).

These preliminary observations were supported by research from several archeological samples, including archaic Californian (Ruff and Jones 1981), Pecos Pueblo (Ruff and Hayes 1983), Mesolithic Western Europe (Constandse-Westermann and Newell 1989), and historic Asian groups (Lowrance and Latimer 1957). Results denote a general pattern of slight left-dominance in terms of length, cross-sectional geometry, and mechanical

strength – particularly with respect to anterior-posterior bending forces. However, levels of asymmetry are often statistically insignificant, and the degree is inconsistent between studies.

Reduced asymmetry in the hind limbs is essentially related to the primary occupational stress placed upon the legs, bipedal locomotion. Bipedality requires equal use of the right and left sides. Biomechanical stress is loaded equilaterally in the absence of pathological conditions, the adaptive response generally produces mirrored structures in the lower limbs (Ruff and Hayes 1983). The slight robusticity observed in the left leg is closely linked to right-handedness in human populations. Unimanual functions performed by the right arm produce a high level of stress. This force is transferred in a contralateral (cross-over) pattern to the left leg, which serves as receptor, anchor, and support (Trinkaus et al. 1994). Thus, greater skeletal modification in the left lower limb may be used to predict right-handed activities. This is a valuable relationship to understand when working within bioarcheological contexts.

Articular Surface Asymmetry

In addition to increased length and cross-sectional dimensions, mechanical loading stress may potentially cause asymmetry in the surface area of articular joints. Skeletal elements that have been examined include long bone epiphyses, such as the humeral and femoral head, as well as weight-bearing structures like the sacrum. Growth in articular joints follow a trajectory that allows mechanical loads to be transferred across the connective surface without severe wear (Hamrick 1999). Joints must grow large enough to withstand large amounts of pressure, and be oriented in such a direction to efficiently withstand the stress (Hamrick 1999). If force exceeds this capacity, joint fluid lubrication and congruency are lost. This causes irreparable damage to articular cartilage and osseous components of the

epiphysis, and subsequent joint disease (Lieberman et al. 2001). Research indicates that chondrocytes, metabolic cells in the articular cartilage, are responsible for endochondral bone growth and are susceptible to mechanical loading stress (Plochocki 2004). Osteoarthritis, characteristic deterioration of joints during the degenerative period, is becoming an important area of research within skeletal biology. Increasing life-expectancies imply longer periods of use and destruction on these surfaces. Such degenerative modifications demonstrate the plasticity of joints long after the growth stage has ceased.

Plochocki (2002, 2004) has created a body of work investigating the effects of mechanical loading upon various articular joints. Utilizing an archeological sample from the Mississippian period, he examined bilateral variation in articular areas of the humerus, radius, femur and tibia. Results demonstrated greater asymmetry in the upper limbs, with a significant right skew. The lower limbs were slightly asymmetrical, but lacking a cohesive directional preference. These results support the expected pattern of right-handedness and contralateral symmetry. Ruff and Jones (1981) determined a similar pattern in the articular joints of the archaic California population, but results were not statistically significant. They did find articular asymmetry decreased with age, much like other measures of lateralization (Ruff and Jones 1981). This may be attributed to general decreases in bone surface area and density, associated with the onset of osteoporosis common in elderly individuals.

Plochocki (2002) further applied the theory of right upper-limb / left lower limb dominance to sacral morphology. The sacrum represents a “pivoting zone and primary region of weight transmission between the upper and lower body” (p. 350), and is subjected to the same forces inducing directional asymmetry in the limbs. During asymmetric activities, the sacral joint experiences greater body weight burdens and greater muscle contraction on the side opposite from the functioning upper limb. Given the frequency of

right-handedness in modern populations (90-95%) (Coren 1977), Plochocki anticipated greater skeletal modification localized on the left side of the sacrum. This hypothesis was confirmed, finding significantly larger measurements in three dimensions (posterior and lateral ala breadth, and maximum auricular height) of the sacrum (Plochocki 2002).

2.3 Ontogeny and Asymmetry

In addition to mechanical loading biases and environmental stability, inherent ontogenic factors may be a significant source of asymmetry. Von Bonin (1962) was one of the first biologists to propose a gradient in development of the cerebral elements, progressing from right to left. Applying a similar hypothesis to skeletal structures, Brown et al. (1989) suggested that oxygen tension in the blood stream played a determinative role, arriving at and supplying right osteocytes first in a path from the ventricles. Evidence for such a mechanism remains inconclusive, but derives support from early studies of prenatal and perinatal asymmetry. Schultz's (1923) dissections of over 100 pre-term fetuses found conclusive bilateral asymmetry by the fourth uterine month, and that the right arm was longer than the left in 52% of cases. He argued that these data refute explanations of asymmetry due to mechanical loading, as asymmetry observed in utero precedes loading stress (Schultz 1923). Similar results were reached by Pande and Singh (1971), demonstrating 9 out of 10 aborted fetuses had longer crown-rump length, greater muscle mass, and heavier bone weight on their right side. Correspondingly, Bareggi (1994) found longer length in all bones comprising the right arm among a sample of electively aborted fetuses aged 8-14 weeks. Both research teams interpreted their results as evidence that right-dominance is inherited, developing along gradient in the uterine environment (Bareggi et al. 1994; Pande and Singh 1971).

However, several studies produced contrary evidence. Working with an extremely large sample ($n=728$) of fetuses (gestational age 8-26 weeks), Bagnell et al. found the left humerus to be significantly longer in the majority of subjects, but found no asymmetry in the radii and ulnae (Bagnall et al. 1982). Dangerfield's (1994) work with 211 full-term Liverpool neonates found the left humerus to be slightly longer, although the forearm bones proved equal or slightly longer on the right side (Dangerfield 1994). Likewise, Steele and May's (1995) investigation on the Wharram Percy population included 14 perinatal infants and 104 juveniles. The authors found significant left-dominance in the infant subset. However, the juvenile cohorts displayed right-dominance as early as 3 – 6 years, with levels approaching the adult proportion well before puberty (7 – 12 years). They concluded that right-dominance is a product of postnatal mechanical operations, progressing with age (Steele and Mays 1995).

Further evidence situates the origin of right-dominance very early in life. A significant study by Hepper (1998) investigated lateralized behavior of 72 neonates in the first trimester (average age 10 weeks). This is the first stage at which lateral manipulations may be visualized in utero. The most commonly observed movement was a "thumb-sucking" motion, with 85% of subjects showing preference for the right arm. The authors ascribed this to muscular determination rather than an operation controlled by the brain, and concluded that early differential motor behavior likely contributes to subsequent skeletal asymmetry (Hepper et al. 1998). Their results signify the importance of osteogenic stimulus favoring dominance of the right arm.

Similar results have been found among samples of living infants and children. Butterworth and Hopkins' work with neonates and infants (aged within the first 3 months of life), found clear preference of the right hand (60 – 80%) in executing simple manual

operations (Butterworth and Hopkins 1993). A comprehensive study by Annett and Kilshaw (1983) assessed handedness through side-preference in performing a unimanual peg-moving operation. They discovered significant directional asymmetry in skill distribution. Among the youngest cohort (ages 3.5 – 5 years), 85% showed superiority in right-handed movements, representing the origin of adult lateralization frequencies (Annett and Kilshaw 1983).

Review of the literature on prenatal and infant lateralization reveals that limb dominance may oscillate during the fetal period, as results are ambiguous and often contradictory depending upon the stage of growth. Due to the nature of fetal skeletal material (often sourced from spontaneous abortions), much of the results on pre-term infants may reflect a developmentally unstable environment (Steele 2000). These data may not represent the normal trajectory of lateral development, and should only be applied to developmentally stable populations with great caution.

The osteometric data from full-term infants and the anthropometric work with juveniles better approximates stable growth patterns. A summary of this work suggests that at birth, a majority of infants possess longer length and greater musculature in the left appendage (Bagnall et al. 1982; Dangerfield 1994; Ingelmark 1946; Steele and Mays 1995). However, a pattern of right-handed dominance (and preference in unimanual tasks) originates during early development. This pattern is clearly observed by the third year and approaches adult population frequency (90%) prior to adolescence (Annett and Kilshaw 1983). Pre-existing side-bias during ontogeny is an unlikely explanation for handedness, given the plasticity of bone and tissues under habitual biomechanical pressures (Steele 2000).

2.4 Reconstructing Population History

Reconstructing the lifeways of historic and prehistoric peoples is a primary concern of bioarcheology (Larsen 1997). Bilateral asymmetry provides a vital means of understanding the specific mechanical operations and activity level of past populations. Change in the architectural structure of bones is indicative of the forces placed upon them. These mechanical pressures may arise from subsistence strategies, mobility patterns, specific gendered roles, or may suggest external environmental challenges (Bridges 1991). Intra-population comparisons of bilateral asymmetry (between subgroups) informs about the division of labor and resources between the sexes, age-groups, or social strata. Directional asymmetry studies provide a picture of human mechanical loading across geography, or transitions through time. Fluctuating asymmetry may indicate environmental flux distressing a certain group, and may distinguish a particular area or temporal period experiencing resource scarcity.

Biological/skeletal material was previously neglected in many archeological sites, but in recent decades has been recognized as vital data source for human history (Larsen 1997). The following survey is particularly applicable to the present study. The studied populations are contemporaneous with the New Lisbon Collection and/or experienced similar levels of environmental and mechanical stress. Key methods are also described for detecting and evaluating asymmetry. The literature reviewed provides several well-researched, comprehensive examples of bilateral asymmetry used to characterize past populations.

One of the first complete bioarcheological analyses in the southeastern United States was undertaken by Bridges (1991), working with remains from the Pickwick Basin Region of northwestern Alabama. The adult sample included individuals from the Archaic period, a pre-agricultural, hunting-gathering society (n=126), and the later Mississippian period,

practicing maize agriculture (n=140). The authors examined external lengths, cross-sectional properties, and bone density of the major long bones. They hypothesized greater bone size and strength for the Mississippian remains, in accordance with greater mechanical demands imposed by farming. They also anticipated a pattern of generalized asymmetry (both upper and lower limbs) for females. Ethnographic data in the American Southeast suggest the broad spectrum of new agricultural activities were attributed to women, while men continued to participate in more specific hunting activities.

These predictions were largely supported by the data. Researchers attempted to associate the observed difference in bone structure with causative, sex-specific behaviors. The primary difference between Archaic and Mississippian females was a significant increase in the left elbow measurements (distal epiphysis of the humerus and proximal dimensions of the ulna), effectively decreasing overall asymmetry. This type of change indicates a strengthening in flexion/extension abilities, and may be attributed to equal use of the right and left forearm. A likely causative operation is the corn-pounding motion, performed by traditional Native American women during maize-processing.

The transition from Archaic to Mississippian lifeways was signified in males by increasing asymmetry over the entire right arm. This change was attributed to the adoption of the bow-and-arrow hunting during the agricultural period, which requires distinct use of one arm for pulling and tensile force and the other as support. Bridges work remains a model for studying the relationship between asymmetry and function of past peoples.

Another example may be found in Angel's (1987) work on remains from the First African Baptist Church (FABC) of Philadelphia, utilized from 1823 – 1841. During the early 19th century, the free African American community in the northern United States experienced many stressors: inadequate nutrition, high exposure to infectious disease, heavily workloads,

and increasing population density. This historic sample is contemporaneous with the New Lisbon Collection, whose urban residents endured many of the same external pressures.

Angel and colleagues investigated demography and skeletal indicators of health in the FABC sample, occasionally supplemented by cemetery records. In terms of occupational analysis, researchers found high levels of asymmetry in the pectoral girdle (distal clavicle, proximal humerus and glenoid cavity of the scapula) among females. They designated a few individual female skeletons as “laundresses” – associating the use of pectoral muscles in typical washing/scrubbing motions, and correlated this frequency with their mention in cemetery records. The FABC was concluded to be a highly stressed group, given the prevalence of skeletal pathologies (porotic hyperostosis, rickets, cranial base length, traumatic incidents, etc.), asymmetry, and low life-expectancy (average age-at-death). However, socio-economic status appeared to be on the rise. This was evidenced by moderate – high stature for males, relatively low infant mortality, decreased asymmetry levels, and strong records of community support (Angel et al. 1987)

A more recent publication by Albert and Greene (1999) employs a unique sample of skeletally immature remains from Medieval Kulubnarti, Sudanese Nubia. Remains represent two distinct time periods: Early Christian (550 – 750 A.D.) and Late Christian (751 – 1450 A.D.), and range in age 11 – 31 years. The authors proposed higher levels of bilateral asymmetry in the Early Christians, reflecting the greater external stress historically documented in this population. Asymmetry was ascertained through skeletal maturation. Stage of epiphyseal union was scored for each bone, average values were computed for right and left sides, and then sides were compared for variation. The authors determined statistically significant bilateral asymmetry in the Early Christian sample, but not the Late Christians (no difference attributable to sex). Their work lends support to the use of bilateral

asymmetry in skeletal growth and maturation as an indicator of environmental stress (Albert and Greene 1999). Their technique of comparing bilateral stages of epiphyseal union is employed in the present analysis of asymmetry.

Finally, a very recent work by Sládek et al. (2007) demonstrates how asymmetry continues to provide vital insight into past behavior, especially in regions or sites where archeological material is scarce, and lifeway inferences are limited. Sládek and colleagues investigated a population of Medieval Central Europeans; dating from 2800 B.C. to 1500 B.C. Historical records indicate that a major shift occurred in subsistence patterns from late Eneolithic to the early Bronze Age. Gendered-occupational roles related to subsistence are cited as a primary motivating factor for the transition. Researchers tested this assumption by examining the long bone length and articular areas for bilateral asymmetry, but determined no significant difference between the two temporal groups.

The authors suggested a gradual shift in manipulative behavior, dispersed over several centuries, which was unobservable in the skeletal record. Furthermore, sex-specific patterns of bilateral manipulative behavior did not change through time. Males in both periods remained highly right-dominant in the upper-arm, suggesting hunting activities. Females displayed a fluctuating asymmetry pattern, with minor right-biased directional asymmetry in the humerus. The author concluded that the data support continuity in manipulative activities for both sexes. Revolution in subsistence between the Eneolithic and Bronze Age could not be attributed to a shift in gendered functions (Sládek et al. 2007). These results exemplify the ability to detect minor transitions in bilateral activity, and discern population-level continuity.

Recently, some anthropologists have warned against the use of bioarcheological skeletal data to make such sex-specific and tool-specific predictions of behavior (Steele 2000;

Stirland 1993). Critique surrounds the conclusion of a direct relationship between a single asymmetrical measurement/dimension/bone and a particular activity, given that no muscle-groups work in isolation, and that a multitude of mechanical operations utilize the same muscular area. Nevertheless, this does not invalidate using asymmetrical modifications to infer *general patterns* of activity. Researchers recommended that at least two broadly contemporaneous groups should be included in studies whenever available. Also, differences in bone-size should be standardized whenever possible, and difference due to age or sex must be accounted for. Only then may remaining asymmetrical values be related to mechanical operations, and compared between defined groups (Stirland 1993).

The use of skeletal material remains a vital component in the reconstruction of lifeways, and may be preferential in many cases. Some anthropologists support the substitution of archeological remains for modern material in certain juvenile analyses. These authors claim that past populations were more likely to suffer from infection or other fast-acting diseases, while modern groups have greater incidence of developmental disorders which have greater influence on results (Saunders and Hoppa 1993; Stirland 1993). In such cases, archeological material better approximates the normal developmental environment.

The current study applies the principles of bioarcheological investigation on asymmetrical skeletal structures. These data is supplemented by, and correlated with extensive mortuary records. Collectively analyzed, general patterns of activity and environmental stability are inferred for individuals in the New Lisbon Collection.

CHAPTER THREE: THE SAMPLE UNDER STUDY

Given that bilateral asymmetry is a product of the physical and biological environment, it is imperative to understand the context of the sample under study. When available, historic records provide additional information about socioeconomic variables, resource accessibility, and activity patterns of a given group. The New Lisbon Collection is primarily sourced from southern Portugal, representing temporal years 1806 – 1970. This time span corresponds to the earliest birth-year and latest death-year of individuals included in the study. This period was extremely important to the formation of the Portuguese identity in the modern era (Cardoso 2005).

Strategic geographic location and a long tradition of nautical travel gave Portugal a distinct advantage in exploration and colonization. During the Age of Discovery, Portugal established colonies on every continent and controlled the most productive trading routes in the world. But imperialistic prosperity was tenuous, and by the 19th century Portugal had fallen from grace. This once dominant nation suffered an oppressive government, faltering agriculture, non-existent industrial power, and a strong centralized Catholic church (Corkill 2003). During the twentieth century Portugal ranked lowest in per capita income in Western Europe, coupled with high adult and infant mortality rates. Such social statistics are often interpreted as signals of inadequate and detrimental living conditions. A number of geographic, cultural, economic, and religious variables collaborated to create an identity of stagnation and “backwardness” during these troubled times (Sandberg 1982). These key factors each suggest a stressed population, and predict responding bilateral asymmetry.

3.1 Geographical and Socio-Political Context of Portugal

Portugal is located at the western-most tip of continental Europe, occupying approximately one-sixth of the Iberian Peninsula (see Figure 1). This strategic position mediating between Europe and the Atlantic Ocean allowed Portugal an advantage in nautical exploration. Portuguese sailors discovered and maintained many significant international trading routes for centuries. Portugal rose to become the last European colonial empire, but left the nation with a heavy dependence upon imported goods (Cardoso 2005). Nautical trade was crucial because the indigenous terrain was largely infertile and incompatible with long-term agriculture. The Mediterranean Basin, with its dry climate and thin, rocky soils, made production of staple grains extremely difficult. The first Western agricultural innovations were adapted to the moist, heavy soils of northern Europe, giving these regions an early lead in mass-production and trade of grains. Southern regions, where the soil was incompatible with the new technology, could not modernize. Nations like Portugal perpetuated rudimentary cultivation techniques developed in the Roman era (Tortella 1994). Agricultural productivity in northern countries like England and France created a population surplus. Large numbers of former agriculturalists left the country-side and populated new cities in search of employment. Portugal failed to produce a comparable surplus, and did not experience a similar urban migration. With no “invisible hand” guiding industrialization, more than two-thirds of the Portuguese population practiced subsistence agriculture well into the twentieth century (Corkill 2003).



Figure 1. Map of Portugal including major cities and geographic features

The source of cultural stagnation was not limited to geographical endowment. The dominant political system was a primary factor constraining Portuguese social development. The last Portuguese monarch was overthrown prior to the turn of the twentieth century, ushering in a violent period of political instability. In the subsequent decades, Portugal experienced three major transitions in political power, finally concluding in the ascent of totalitarian dictator Antonio de Oliveira Salazar in 1926. Salazar effectively exploited Portugal's already traditional, conservative, and paternalistic heritage (Corkill 2003).

Salazar's repressive regime further challenged any attempt at modernization. He enacted restrictive monetary policies and prevented free-trade markets, effectively weakening and destabilizing the currency. Ultimately, this perpetuated the status quo. Large, privately owned land-holdings expanded in the north, while the multitude of peasant farmers practiced subsistence cultivation in the central and southern regions (Cardoso 2005). During his reign, Salazar invested in precisely the wrong products; protecting flooded markets (wheat, rye, textiles), while sacrificing the country's few competitive resources (Tortella 1994). In addition, Salazar waged several costly wars in Africa during the early 20th century in an attempt to salvage colonial interests. This eliminated a large sector of the young male population, further debilitating agriculture, and leaving women to bear the brunt of domestic duties and family provisioning (Wall et al. 2001).

The other dominating force in Portuguese life was the Catholic Church. Throughout the nation's history, Roman Catholicism has been the mandated and later unofficial religion. Portugal remains overwhelmingly Catholic, with 93 % of the population recognizing the faith (Cardoso 2005). This has had an important impact on core values, as traditional notions of authority, hierarchy, and morality stemmed from Catholic doctrine. Religious code normalized the concept of an absolute and self-perpetuating authority.

Institutional welfare became the responsibility of the church during the early twentieth century. Termed “Misericórdias”, these catholic organizations provided social and health services to the sick, poor, widowed, and orphaned. Institutions were supported by donations from wealthy patrons, responding to their “Christian duty”. However, this system of obligatory charity could not fulfill the ever-growing needs of the public and proved completely inadequate (Veiga et al. 2004)

The church also discouraged independent learning and education. By placing a higher value on oral interpretation and verbal communication, literacy was severely neglected, especially in rural areas (Reher 1998). Literacy is often considered an excellent long-term explanatory factor for economic development and prosperity (Sandberg 1982). Literacy rates in Portugal were astoundingly low throughout the 20th century, particularly when compared to other Western European nations. In the year 1900, only 22% of the Portuguese population could read, with rates reaching only 56% by 1950 (Tortella 1994). The church and government played dual roles in opposing public education. During Salazar’s term, mandatory education was reduced to three years for girls, and four for boys. It wasn’t until the late 1960s that public schooling became available to all children ages 6 – 12 (Tortella 1994).

3.2 Health and Living Conditions

Portugal’s relatively small size, semi-isolation, and permanent borders created a uniquely homogeneous population. Despite great mobility during the Age of Exploration, gene flow was primarily in the form of emigration (Reher 1998). Portugal claims no regional subdivision, significant religious, linguistic, or ethnic minorities. Although, small levels of cultural diversity exist in commercial centers of Lisbon and Porto (Cardoso 2005). This

implies that resource scarcity, illiteracy, and a repressive government control were generally shared and suffered throughout the social stratum.

The consequences of cultural depression took many forms. Living wages were exceptionally low for working class individuals. In 1950, the average 12.83 escudos (currency preceding the Euro) per day was clearly below the 42 escudos calculated as necessary for basic survival (Moreira 1950). Poverty forced many agricultural families into shear-cropping or into over-crowded urban housing. These public structures were inexpensive, but far from adequate. In a 1950 study, Moreira found that 69% of Lisbon residents had no electricity and 81% had no working septic system. The lowest social classes were pushed into peripheral slums with no public amenities (Moreira 1950).

Such conditions had inevitable debilitating effects on Portuguese health. Juvenile and infant mortality rates were among the highest recorded in Europe, comparable to Third World Nations throughout much of the twentieth century (Instituto Central de Higiene 1929). Until the 1940s, approximately one half of children died before reaching the age of 15. Infant mortality rates peaked at 200 deaths per 1000 births (Veiga et al. 2004). The main causes of childhood death were due to communicable infectious diseases, rampant in highly populated and unsanitary conditions (Gomes et al. 1999). Infant mortality was also increased by nutritional deficiencies, exacerbated by diarrhea and enteritis (Veiga et al. 2004). Mortality was similarly high in the adult population, as death-rates reached 15 death per every 1000 inhabitant in 1950 (Sobral 1990). In 1920 the life expectancy at birth was only 35.8 and 40 years for men and women respectively (Instituto Central de Higiene 1929). Mortality decreased slowly until the 1960s, when the first mass vaccination against tuberculosis was instituted, having a significant positive effect on public health (Gomes et al. 1999).

Additional measures of health, such as stature and anthropomorphic measurements, have been historically low in Portugal. Anecdotal observations have characterized the Portuguese as the shortest nationality in Europe (Padez and Johnston 1999). More recently these assumptions were tested, and appear to have biological validity. In 1983, Chamla compared changes in height throughout Western Europe. He showed that in 1880 mean stature in Portugal was only 1.8cm lower than the average stature in Holland. Over the next 100 years Portugal's mean height increased only 3.7cm, while Holland gained 15.0 cm (Chamla 1983). Addressing secular change in Portuguese height, Sobral (1990) found an increase in stature parallel to other European countries. However, changes were initiated much later and preceded much slower, even when compared to other Latin countries with a similar linguistic and cultural heritage. Similar patterns of secular change have been found in Portuguese craniometric measurements. Alterations in the length of the craniometric base is consistent with that observed in American populations, but change is observed at later dates, and over a longer period of time (Weisensee 2007).

The state of Portuguese health and economic well-being has vastly improved since the 1960s. The fall of Salazar's administration and subsequent creation of a constitutional democracy, the emergence of a strong capitalist economy, and implementation of public health policies, have all contributed to the nation's enhancement in the twenty-first century. However, the historic collection used in the present study reflects the most difficult period in Portuguese history, prior to these advances.

Skeletons in the New Lisbon Collection provide a glimpse of Portuguese health and demographic status during an important period of socioeconomic transition. Conditions discussed in this chapter indicate high levels of both developmental and mechanical stress, predicting a correspondingly high degree of bilateral asymmetry. The period of particular

interest dates from 1900 – 1930, the years immediately preceding and initiating Salazar’s reign. This era produced the greatest political and environmental instability and may be closely correlated with asymmetry.

3.3 Brief History of the Collection

The sample utilized in study was selected from skeletal remains curated at the Bocage Museum, directed by the Department of Zoology and Anthropology, and housed within the National Museum of Natural History in Lisbon, Portugal. Although the New Lisbon Collection is an assemblage of international significance, it is unfamiliar to most anthropologists outside of Portugal (Cardoso 2006).

The “New” Lisbon Collection derives its name as the second collection of this type. The previous set was amassed during the late nineteenth century by Lisbon physician, Francisco Ferraz de Macedo. In 1978 a devastating fire incinerated the National Museum, destroying the original assemblage in its entirety. Afterwards, responsibility for replacing the collection was given to Luis Lopes, a Bocage Museum technician and assistant professor of anthropology at the University of Lisbon (Cardoso 2005). Lopes founded the “new” collection in 1981, accumulating over 1550 skeleton over the next ten years. After his retirement in the early 1990s, quality of preservation and maintenance began to deteriorate. The arrival of Dr. Hugo Cardoso in 2000 began a new phase at the Bocage Museum, improving curation, and focusing on the addition of underrepresented demographic sectors such as young adults and juveniles.

Skeletons in the collection represent the greater Lisbon population and the Portuguese tradition of interment. Materials were obtained with permission by city governance, from one of five Lisbon cemeteries: Alto de São João, Benfica, Prazeres, Ajuda, and Lumiar. The

policy for urban Portuguese cemeteries is to exhume individuals from temporary graves after the legally-determined period of five years. Graves must be reused as mortuary space is limited in urban areas. Curation of the remains is then determined by living relatives or associates. Remains may be placed within small urns, and housed in blocked compartments (“ossarios”) within the cemetery. Rent of an ossario is paid by the family as an annual fee. If remains are unclaimed after the five year period, the cemetery issues a warrant for removal, and skeletal material is deposited in a storage unit awaiting a recipient. After several years in storage, neglected remains may be donated to the Bocage Museum and entered into the New Lisbon Collection. The alternative is reburial in communal mass-graves, or incineration (Cardoso 2005).

Head stones, grave plates and cemetery registers, and hospital records provide extensive biographic information for each set of remains in the collection. The suite of data is not uniform for every individual, but the majority of adult skeletons have documented name, sex, date of birth and death, place of residence, marital status, occupation, and cause of death. Data was originally collected and documented by civil registration officers in Lisbon while issuing official death certificates (Cardoso 2005).

Differential cemetery environments, time-since-death, and duration of storage have created variable taphonomic conditions. Skeletons are generally well-preserved and complete, with the exception of small bones (hand/feet phalanges) and dentition. Some soft tissue, like hair or fingernails, is occasionally present. Religious or sentimental grave goods, such as blankets, figurines and rosaries, are especially common with young adults and juveniles (for examples of mortuary goods included in the collection, see Appendix Figure A1) (Cardoso 2006).

Sampling the Collection

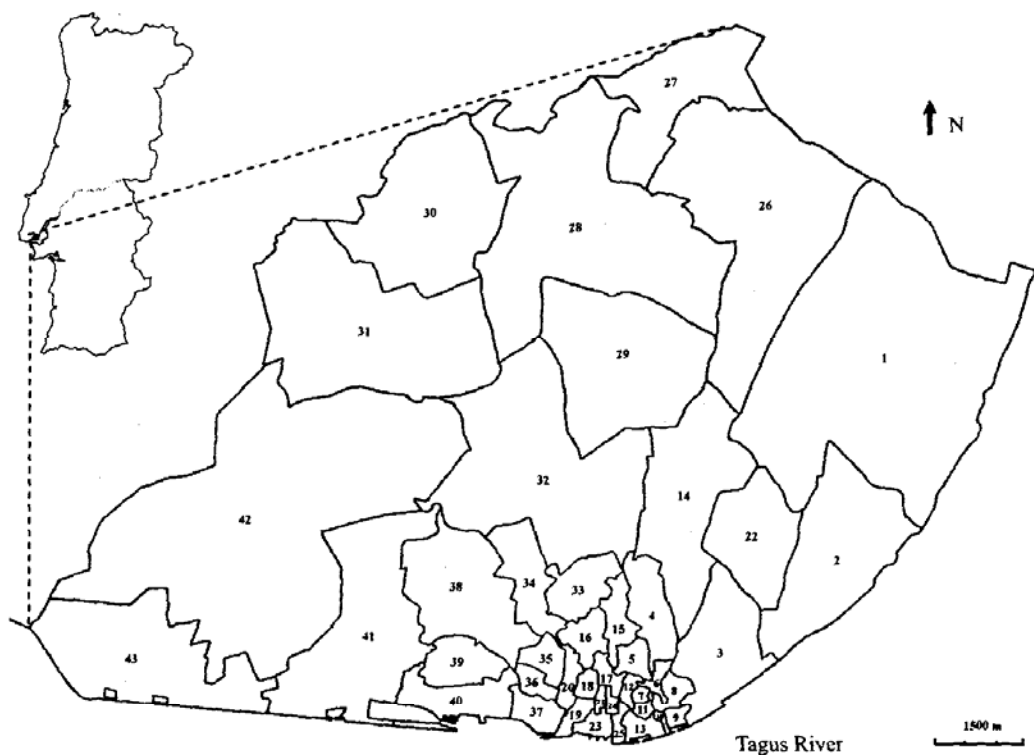
As with all archeological assemblages, this collection is a product of several biocultural, and socioeconomic filters. The result is a dubious illustration of the living population; caution must be extended before issuing statements characterizing contemporaneous Lisbonese. Biases inherent to the sampling process may be classified as either quantitative or qualitative (Cardoso 2005). Quantitative variables reflect the sheer amount of skeletal material obtained, and may be affected by burial procedures, decomposition, and preservation. Quantitative bias is particularly common with subadult material, due to several contributing factors. In many Lisbon cemeteries, the procedure from 1875 well-into the 20th century was to deposit stillbirth fetuses in communal graves. This practice was most common in the lowest socioeconomic tier. The actual number of neonatal deaths is probably much higher than the proportion represented in the New Lisbon Collection. Furthermore, cemetery workers were charged with the exhumation of remains, having no formal training in anatomy or osteology (Cardoso 2005). The small size, unrecognizable shape of juvenile bones made them a nuisance to workers, often reflected in their level of completeness.

Qualitative bias refers to the observable differences between survivors (absent in the sample) and non-survivors (sample population). These skeletons represent individuals who died prematurely or succumbed to illness, and may not be considered typical of the general populous. This is particularly significant in terms of growth, maturity, and health. This paradoxal circumstance has been termed ‘biological mortality bias’ in the bioarcheology community (Saunders and Hoppa 1993; Wood et al. 1992). The effect of mortality bias on height was demonstrated by Saunders and Hoppa (1993). Because stunted children are more likely to die than those with normal growth, the skeletal sample will tend to contain a higher

proportion of short individuals. However, some authors have argued that biological mortality bias is less influential than other methodological problems and cultural factors, and should not prevent the investigation of archeological sources. (Saunders 2000).

The city of Lisbon presents another unique cultural filter: availability of remains for museum acquisition. Each cemetery within the city is assigned a particular set of districts from which it accepts bodies. These districts (freguesias) may contain any number of neighborhoods, hospitals, or institutions (See Figure 2 for a list of freguesias represented in the New Lisbon Collection, and location within Lisbon). Since the majority of citizens died in the home throughout the 20th century, their place-of-residence usually determined the cemetery of burial. Alto de São João is the largest cemetery, traditionally associated with lower socioeconomic groups, drawing from the older city-center, industrial, and port sectors. This location is also the greatest source of skeletal material in the collection. Other cemeteries, Prazeres for example, served the out-lying metropolitan area populated by the wealthiest families, and produced the least amount of skeletal material

It was neither possible, nor necessary to include the entire collection into the sample of study. The sample was constructed in an effort to represent the population as a whole, while facilitating particular research interests. All available sub-adult skeletal material was measured (n=90) to gather the greatest amount of data from an exceptional resource. Adult remains were included based upon (1) completeness of the remains and (2) relevance to the research questions. Individuals with older birth years (1830 – 1900) are represented at a higher frequency than in the general collection to allow temporal analysis. Although the New Lisbon Collection is slightly skewed towards a female majority, the sex ratio was kept approximately equal to investigate sexual dimorphism in bilateral asymmetry.



- | | |
|---|---|
| 1- Olivais (<i>Santa Maria dos Olivais</i>) | 23- São Julião |
| 2- Beato (<i>Beato António</i>) | 24- São Nicolau |
| 3- Monte Pedral (<i>Santa Engrácia</i>) | 25- Madalena (<i>Santa Maria Madalena</i>) |
| 4- Anjos (<i>Nossa Senhora dos Anjos</i>) | 26- Charneca (<i>São Bartolomeu</i>) |
| 5- Socorro (<i>Nossa Senhora do Socorro</i>) | 27- Ameixoeira (<i>Nossa Senhora da Encarnação</i>) |
| 6- Graça (<i>Santo André</i>) | 28- Lumiar (<i>São João Baptista</i>) |
| 7- Castelo (<i>Santa Cruz do Castelo</i>) | 29- Campo Grande (<i>Santos Reis Magos</i>) |
| 8- Escolas Gerais (<i>São Vicente</i>) | 30- Carnide (<i>São Lourenço</i>) |
| 9- Santo Estevão | 31- Benfica (<i>Nossa Senhora do Amparo</i>) |
| 10- São Miguel | 32- São Sebastião da Pedreira |
| 11- Santiago | 33- Camões (<i>Coração de Jesus</i>) |
| 12- São Cristovão (<i>São Cristovão and São Lourenço</i>) | 34- São Mamede |
| 13- Sé (<i>S. João da Praça</i>) | 35- Mercês (<i>Nossa Senhora das Mercês</i>) |
| 14- Arroios (<i>São Jorge de Arroios</i>) | 36- Santa Catarina |
| 15- Pena (<i>Nossa Senhora da Pena</i>) | 37- Marquês de Pombal (<i>São Paulo</i>) |
| 16- São José | 38- Santa Isabel |
| 17- Restauradores (<i>Santa Justa</i>) | 39- Lapa (<i>Sagrado Coração de Jesus</i>) |
| 18- Sacramento (<i>Santíssimo Sacramento</i>) | 40- Santos (<i>Santos-o-Velho</i>) |
| 19- Mártires (<i>Nossa Senhora dos Mártires</i>) | 41- Alcântara (<i>São Pedro</i>) |
| 20- Encarnação (<i>Nossa Senhora da Encarnação</i>) | 42- Ajuda (<i>Nossa Senhora da Ajuda</i>) |
| 21- Conceição Nova (<i>Nossa Senhora da Conceição</i>) | 43- Belém (<i>Santa Maria de Belém</i>) |
| 22- Penha de França (<i>Nossa Senhora da Penha</i>) | |

Figure 2: Map of Lisbon administrative districts (freguesias) from 1895 – 1959
Map reproduced with permission from Cardoso (2005)

The primary factor determining inclusion into this study was preservational quality of the remains. Examination of bilateral asymmetry inherently requires both right and left structures. Therefore, only skeletons with complete and well-preserved right and left long bones were included. Individuals missing a single long bone, or portion of one long bone were eliminated to avoid difficulties of missing data in the statistical analysis. This procedure (selection by completeness) restores a level of randomness which may have been reduced by controlling sex/temporal ratios.

Demographic Structure of the Sample: Juvenile Subset

The juvenile sample documents more than a century of skeletal evidence, from 1888 (earliest birth year) to 1975 (latest year of death). The majority of individuals are documented as native Portuguese. Nationality is unlisted for a few individuals, but low-levels of immigration during this period allow the assumption of a Portuguese place of birth. The vast majority list one of the many Lisbon districts as place of residence (See Figure 2), although a number of townships in the coastal Algarve region, and the northern shipping city of Porto, were also recorded. This suggests that ethnic and anthropomorphic homogeneity characteristic of Portuguese groups may be extended to this sample.

The juvenile subset comprises 82 total individuals: 47 males and 35 females. Although data were recorded on 90 available skeletons, certain individuals were eliminated due to incomplete skeletal inventory and extreme outlying values, potentially influencing subsequent analysis. Age-at-death ranges from birth to twenty years (See Table 1). Twenty was selected as the upper limit to ensure skeletal immaturity of both sexes, given variable (and potentially altered) developmental rates between males and females. Age-at-death displays a bimodal distribution, with the majority of individuals dying very young (0 – 3

years), or near termination of puberty (16 – 20 years). This pattern is more notable in males (See Figure 3). Temporally, the number of births is greater than the number of deaths until the year 1940, when the mortality rate climbs and exceeds birth rate thereafter (See Figure 4). A post-World War II population decline is trend documented throughout Portugal, and may be noted in the birth-years in this collection (Veiga et al. 2004). Interestingly, age-at-death generally decreases through time, with high proportions of very young individuals dying well-into the twentieth century (See Figure 5). This observation may be related to the extreme depression experienced by Portugal during the first and second World Wars, as well as the late adoption of mandatory juvenile vaccination (Corkill 2003; Veiga et al. 2004).

Table 1: Number of Males and Females at Each Age Group

	0-3 yr.	4-6 yr.	7-9 yr.	10-12 yr.	13-15 yr.	16-18 yr.	19-20 yr.	Total
Male	14	5	3	4	6	10	6	47
Female	11	6	2	5	4	5	5	35

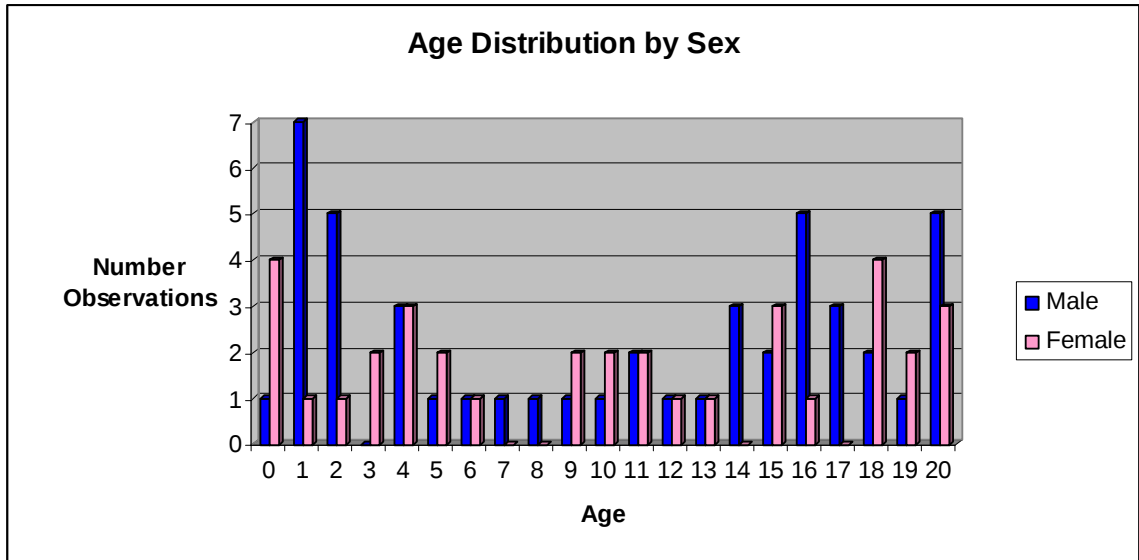


Figure 3. Age Distribution by Sex in the Juvenile Subset

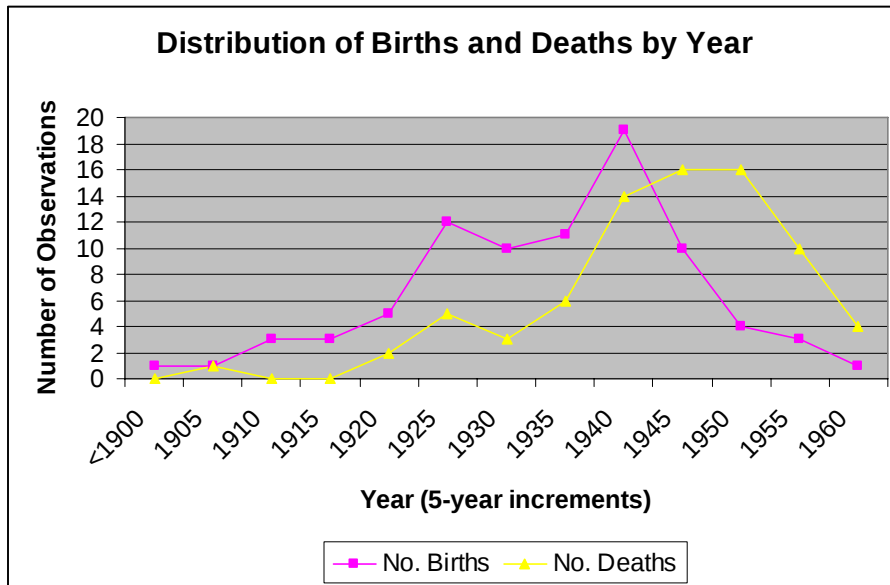


Figure 4. Distribution of births and deaths per year in the juvenile subset

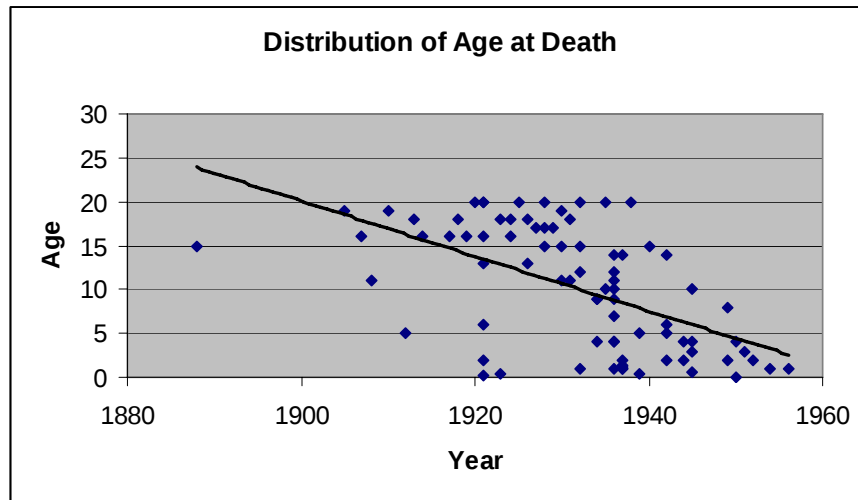


Figure 5: Distribution of Age at Death in the Juvenile Subset

Socioeconomic status was difficult to discern in the subadult sample and could not be assigned on an individual basis. Place of residence within Lisbon is an ambiguous indicator of resource availability, and occupation is rarely applicable except in the oldest subjects. However, additional data in cemetery records, such as cause-of-death, provide information on the sample as a whole. Cause of death was assigned to one of three mortality categories: infectious diseases, chronic ailments, or sudden/accidental death. Some of the most commonly noted infectious diseases were tuberculosis, meningitis, and polio. Chronic conditions included heart murmurs, juvenile cancers, and anemia. Accidental and sudden deaths were rare, but involving injury due to gunshot, occupation, falls, and poisoning. The high frequency of infectious diseases suggests relatively middle to low social position of the juvenile sample. Epidemic outbreaks are characteristic of highly populated urban areas, with poor sanitation, few resources, and lowered access to public health care (Veiga et al. 2004).

Demographic Structure of the Sample: Adult Subset

The adult sample totaled 250 individuals: 129 males, 121 females. Ages range from 21 years at death to 94 years, and birth dates span from 1806 to 1935. Number of subjects in each decade of life was made approximately equal between the sexes and through time to create a more representative cross-sectional image of each age group (See Table 2).

The majority of adults were born before the turn of the century, and expired at or around the mid-twentieth century (See Figure 6). Higher death-rates oscillated between males and females with no distinct pattern, although more male deaths may be observed in the early twentieth century (See Figure 7).

Table 2: Distribution of Age-at-Death by Sex

	21 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 80	81 - 90	91 - 94
Male	19	17	27	22	17	15	17	0
Female	23	15	11	17	16	15	20	3
Total	42	32	38	39	33	30	37	3

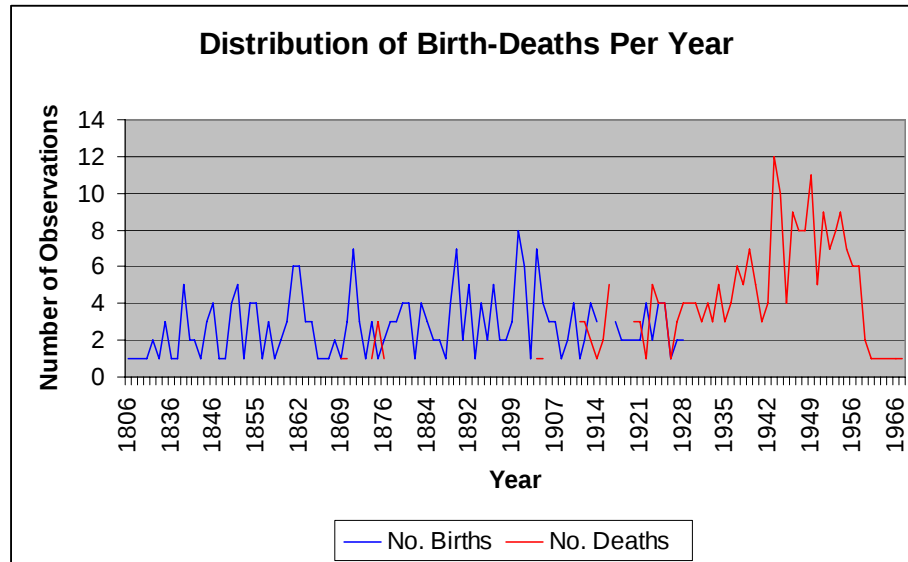


Figure 6. Distribution of births and deaths per year in the adult subset

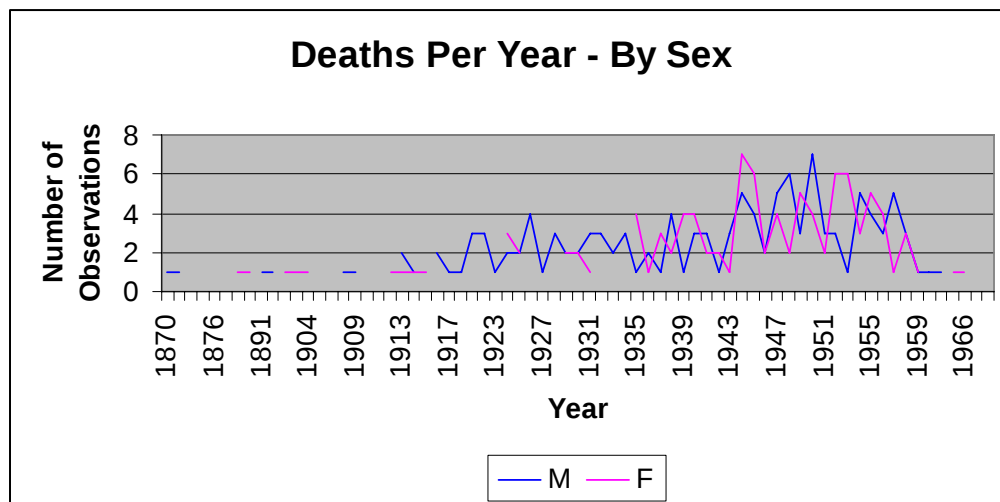


Figure 7. Year-of-death distributed by sex

Additional information collected on the adult subsample includes occupation. Due to religious and cultural conservative values, the vast majority of women (approximately 90%) were employed as home-makers (domesticas). Exceptions include a few seamstresses, shop-owners, teachers, and students (women in the youngest age demographic). Therefore, occupational differences in asymmetry were investigated only on males. Placement into one of two occupational categories was determined by activity level: high manual labor, or non-manual. Professions in each class are exemplified in Table 3 (translated into English).

Similar to the juvenile subset, cause of death was also recorded in cemetery records. Mortality was assigned to one of four groupings: cardiovascular/pulmonary, infectious, chronic, or sudden/natural. Some conditions assigned to each category are listed in Table 4.

Adults in the New Lisbon Collection generally characterize a population of mid- to low socioeconomic status. The proportion of families dependant upon a single (low) salary is consistently high, although the number of manual laborers decreases through time. High levels of urban residence, in conjunction with prevalent epidemic diseases (tuberculosis, influenza, etc) also indicate a stressed population. Conversely, the Portuguese policy of interment implies a certain level of economic affluence. Burial and maintenance was funded for at the least the minimum period, before exhumation and retrieval by the museum. Many Lisbon cemeteries offered only mass graves to the destitute sectors of society. This level of poverty is completely absent in the New Lisbon Collection, as the individuals included were able to afford at least a minimum period of burial. This should be considered prior to comparison against other collections with highly marginalized populations, such as the Terry Collection.

Table 3: Occupation Listed by Type

Manual Labor		Non-manual Labor	
Sailor or dock-worker	Bricklayer/construction	Civil servant	Professor or Student
Farmer or gardener	Motor-mechanic	Shop-keeper/owner	Postal-worker
Blacksmith	Carpenter	Printer or painter	Army-officer
Navy/Army serviceman	Factory-worker	Tailor	Land-owner

Table 4: Example Causes-of-Death by Category

Cardiovascular / Pulmonary	Infectious	Chronic	Sudden or Natural
Congestive pulmonary disease	Tuberculosis	Cancer (Stomach, lung, uterine, prostate, liver, etc)	Carbon Monoxide Poisoning
Cardiac lesions	Influenza	Stomach cancer	Gunshot
Cerebral hemorrhage	Meningitis	Diabetes	Senility
Cardiac hypertrophy	Typhoid fever	Chronic kidney inflammation	“Old-age”
Arterial hypertension	Leprosy	Hepatitis	Intoxication

CHAPTER FOUR: METHODS

Previous work in bilateral asymmetry shows that long bone length and dimension are both susceptible to stress. The progression of skeletal maturation may also be interrupted by external pressures. Consequently, metric measurements of length and dimensions of muscle attachment were analyzed for all subjects in the New Lisbon Collection. Juvenile samples were additionally scored for stage of long bone epiphyseal fusion

4.1 Adult sample

Metric Asymmetry

Postcranial long bone measurements were taken according to standards established by created by Moore-Jansen et al. (1994), published by Buikstra and Ubelaker (1994), as the standard for forensic data collection. Measurements include: maximum length of the clavicle, maximum length, head diameter, epiphyseal width, maximum and minimum diameter at midshaft of the humerus, maximum length, anterior-posterior and medial-lateral diameter at midshaft of the radius and ulna; and maximum length, head diameter, condylar width, anterior-posterior and medial-lateral diameter of the femur. Both right and left elements were measured for inclusion into the sample. A total of 250 adult skeletons were recorded, using an osteometric board and digital calipers.

Asymmetry was calculated as the difference between right and left elements of a pair, standardized by the mean of both components:

$$\text{Standardized asymmetry} = \frac{R - L}{(R + L)/2} \times 100$$

Where R is the measurement taken from the right element, and L is the measurement of the left. The above formula preserves both direction and magnitude of asymmetry, while standardizing for size, thus allowing comparison between skeletal areas and individuals. A

test of normality was run on the raw measurements and asymmetry indices, revealing a generally normal distribution in both cases, permitting the use of parametric statistics.

Reliability of measurements

Intra observer-error was tested on sixteen skeletal measurements utilized in further analysis. To assess repeatability of measurements, 20 skeletons were selected randomly from the William M. Bass Skeletal Collection. Identical digital calipers and an equivalent osteometric boards were employed on both this sample, and the New Lisbon Collection. Skeletal measurements were gathered by the researcher, and then re-taken after an interim period of a week. Repeatability was tested with a t-test of significance, as well as a correlation between the two trials. Results are shown in Table 5. No significant difference exists between the two trials for all sixteen measured areas (overall model $p = 0.3975$). The two trials are also highly correlated (r-values ranging from 0.91 to 0.99). Bilateral disparity may be considered legitimate morphological differences, rather than intra-observer error.

Table 5: Test of Metric Intra-Observer Error: Trial 1 vs. Trial 2

	Mean Difference	p-value	Standard Deviation	Pearson Correlation Coefficient
Clavicle Max Length	-0.05	0.7715	0.7591	0.9999
Humerus Max Length	0.1	0.5591	0.7539	0.9996
Humerus Epicondylar	0.1235	0.3549	0.5929	0.9932
Humerus Head	-0.1965	0.3541	0.7888	0.9806
Humerus Max Diameter	0.019	0.8274	0.4075	0.9815
Humerus Min Diameter	-0.056	0.5243	0.3662	0.9849
Radius Max Length	-0.24	0.2298	0.8261	0.9988
Radius AP Diameter	0.845	0.5905	0.5790	0.9189
Radius ML Diameter	0.0495	0.8028	0.6201	0.9277
Ulna Max Length	0.001	1.00	0.8885	0.9990
Ulna AP Diameter	0.0035	0.9410	0.2205	0.9965
Ulna ML Diameter	0.043	0.644	0.3651	0.9756
Femur Max Length	0.575	0.2738	2.2975	0.9964
Femur Condyle Max	0.05	0.8573	0.8009	0.9909
Femur Head	-0.029	0.7191	0.3575	0.9955

4.2 Juvenile subsample

Metric Asymmetry

Postcranial long bone measurements were taken according to standards published by Buikstra and Ubelaker (1994), and adapted from Fazekas and Kósa (1978). Measurements include maximum length and maximum diameter of the clavicle, humerus, radius, ulna, femur, tibia, and fibula; and maximum humeral and femoral widths. Given the wide range of long bone morphology throughout juvenile growth, epiphyses demonstrated variable stages of fusion. For subjects with unfused or open epiphyses, measurements were limited to the shaft only (generally, individuals aged eight years and younger at death). For individuals whose epiphyses were unified or primarily complete (epiphyseal scar shows >50% union), measurement was taken on the entire bone (present in individuals eight years or older at death). Ninety-two total juvenile skeletons were measured in the New Lisbon Collection, using an osteometric board and digital calipers.

Asymmetry was calculated in the same manner as the adult sample. The difference is taken between right and left elements of a pair, standardized by the mean of both components:

$$\text{Standardized asymmetry} = \frac{R - L}{(R + L)/2} \times 100$$

Where R is the measurement taken of the right element, and L is the measurement of the left.

Percentage of missing or incomplete skeletal elements was possible within the juvenile subsample, due to taphonomic and cultural factors previously discussed in chapter 3. The relatively small sample-size prevented the luxury of excluding incomplete remains from study. For samples with low levels of missingness (less than 25%), absent values were estimated with NCSS 3.0 statistical software, using a multivariate regression formula.

Samples with a high percentage of missing values were eliminated from further analysis, bringing the final juvenile sample size to 82 individuals (See chapter 3 for demographic profile).

Developmental Asymmetry

Developmental asymmetry was assessed in terms of differential stages of epiphyseal union between right and left sides. Methods follow those outlined by Albert and Greene (1999) on a Nubian subadult sample. Stage of union was examined via gross examination and scored for the following sites: medial and lateral epicondyles, proximal and distal epiphyses of the humerus; the greater trochanter, proximal, and distal epiphyses of the femur, proximal and distal epiphyses of the radius, ulna, tibia, and fibula, iliac crest, and the union between the three elements of the pelvis. The progress of fusion was scored using a four-stage system: 0 indicates no union, 1 denotes beginning union (less than half of the epiphysis is fused), 2 for nearly complete or recent union (more than half of epiphysis is fused, or union furrow remains distinct), and 3 pronounces complete union (scar of union is fading or absent). This scoring method is a modification of the McKern and Stewart (1957) method, and has been validated in numerous previous studies (Albert and Greene 1999; Albert and Maples 1995). Epiphyses exemplifying each stage may be referenced in Figure A2 in the Appendix. See Figure A3 in the Appendix for samples demonstrating differential stages of development.

The ordinal level data collected represents four increasingly advanced stages of development; higher values mean greater skeletal maturity. Magnitude of difference between the stages is inconsequential for the purposes of this study. Rather, different values between right and left sides are significant in terms of asymmetry. Epiphyseal union data were consolidated into two figures representing the average skeletal maturity of the right side, and

the average skeletal maturity of the left side. Differences between the mean epiphyseal union of the right and left sides were inferred as bilateral asymmetry.

Reliability of Measurements

Intra-observer error was tested on the four-stage method of epiphyseal closure. A sample of 20 skeletons was randomly selected from the Arikara Collection, housed at the University of Tennessee, Knoxville. The sample included both males and females, with ages ranging from 7 to 20 years at death. Seven sites of long bone epiphyseal closure were scored applying the four stage method. Samples were later rescored after a period of a week, using the same method. Due to the ordinal nature of the data, a Wilcoxon signed ranks test and a Spearman correlation coefficient were used to test for significant difference and correlation between trials (significance set at $p \leq .05$). Results are displayed in Table 6. The table shows intra-observer error to be low, demonstrating insignificant difference between the trials and a high correlation for all scored variables. Results support an objective application of Albert and Greene's (1993) four-stage method for scoring subadults in the New Lisbon Collection.

Table 6: Test of Intra-Observer Error on Subadult Development

	Mean Difference	Wilcoxon p-value	Standard Deviation	Standard Error	Spearman Rank Correlation
Medial clavicle	0	1.0	0	0	1.0
Humerus head	0.005	1.0	0.0005	0.0005	0.9901
Humerus medial epicondyle	0	1.0	0.3244428	0.072546	0.9298
Humerus lateral epicondyle	0.1	0.168218	0.3077935	0.0688247	0.9183
Humerus trochanter	0.05	0.584404	0.3940345	0.0881087	0.8343
Humerus capitulum	0.05	0.342112	0.223606	0.05	0.9530
Proximal radius	0.1	0.68218	0.3077935	0.0688247	0.9696
Distal radius	0.005	1.0	0.0005	0.0005	1.0
Proximal Ulna	0.1	0.168218	0.307793	0.0688247	0.9744
Distal Ulna	0.05	0.342112	0.223606	0.05	0.9530
Femur head	0	1.0	0.3244428	0.072546	0.9716
Distal femur	0	1.0	0	0	1.0

CHAPTER FIVE: RESULTS

Results for both the juvenile and adult subsamples were generated using statistical software packages SAS 9.3.1 and NCSS 3.0 (NCSS 2006; SAS 2005). For each individual skeleton several measurements were collected on multiple bones. This type of data set potentially produces very different results, depending upon the use of univariate versus multivariate methods. Due to the large number of variables included in this study, multivariate methods were employed whenever available. Furthermore, initial data screening revealed that a significant portion of the calculated asymmetry indices (See chapter 4: methods, subadult and adult metric asymmetry) violate assumptions of normality. When significant, the direction of departure away from normality is reported, and randomization tests were used as a means of validation (employing 10,000 Monte Carlo samples).

5.2 Juvenile sample

Is skeletal asymmetry observable in the long bones of the juvenile subset?

Data were initially screened for metric difference in length between right and left bones. Samples were counted in terms of longer side: right exceeding, left exceeding, and approximately equal (within 0.5mm considered equal). This procedure was repeated for the major long bones: humerus, radius, ulna, and femur. Raw counts are displayed in Table 7.

Table 7: Raw counts of the longer bone – Juvenile subset

Maximum Length	Right > Left	Right = Left	Right < Left
Clavicle	17	7	36
Humerus	37	9	12
Radius	37	4	9
Ulna	34	5	14
Femur	40	14	24

Results for the subadult sample are generally consistent with those produced by other studies of skeletal asymmetry. The left clavicle is longer than the right more often, while the right typically dominates the left in arm bones (humerus, radius, and ulna). The femur is more equilateral but slightly right-biased. Therefore, disparity between long bones is valid and amenable to further testing.

Which long bones contribute the greatest asymmetry and are the departures for equality significant?

Significance between right and left measurements was evaluated using a Hotelling's paired-sample T-test. The Hotellings method is preferred to a traditional univariate T-test, due to the large number of measured variables being simultaneously examined. Randomization methods of validation are necessary due to the departure from normality. Level of significance was set at $p \leq .05$, and randomization was based on 10,000 Monte Carlo samples. The overall model, including all 12 paired response variables, proved significant ($p = 0.0001$). Results on individual variable significance are displayed in Table 8.

Table 8: Hotelling's T-test of significant difference between right and left measurements – Juvenile subset

Measurement	Randomization p-value
Clavicle length	0.0265
Clavicle diameter	0.8268
Humerus Length	0.0001
Humerus Width	0.164
Humerus Diameter	0.0001
Ulna Length	0.0067
Ulna Diameter	0.1089
Radius Length	0.0234
Radius Diameter	0.0008
Femur Length	0.0482
Femur Width	0.0209
Femur Diameter	0.7293

Eight measurement pairs were considered significantly different: clavicle length, humerus length and diameter, ulna length, radius length and diameter, and femur length and diameter. A standardized asymmetry index was calculated for these eight variables. The index incorporates the right-left difference, while standardizing for size. This produces a single asymmetry value for each individual, and allows comparison between individuals or groups (See Chapter 4: Methods, for formula). The four variables with no discernable difference were not used in further analysis because disparity is not statistically recognized.

Is there a significant sexual dimorphism in juvenile asymmetry?

The eight asymmetry indices were entered into a parametric variable selection method, to determine which variables best discriminate between males and females. The variable selection procedure available in SAS 9.3.1 regrettably does not provide means for randomization methods. Sex was used as the grouping variable, significance level for entry was set at $p = 0.2$, and level for removal was set at $p = 0.15$. An optimal model was produced including five variables: humerus length, radius length, clavicle length, femur length, and ulna length.

These five variables were then used in a Hotellings 2-sample T-test (with randomization tests) to evaluate significance between males and females. Again, Hotelling's T-tests are preferred because of the multiple variables included, and randomization manages violations of normality. Significance was designated at $p \leq 0.05$, and 10,000 Monte Carlo samples were used in randomization. The overall model, using five response variables, proved statistically insignificant ($p = 0.1900$). Results on the individual variables, for both males and females are displayed in Table 9.

Table 9: Hotelling's T-test of difference in asymmetry between the sexes – Juvenile subset

Measurement	Randomization p-value	Female Mean	Male Mean	Female S.D.	Male S.D
Clavicle length	0.058	-1.0912	-0.2402	2.5837	2.6976
Humerus Length	0.0507	1.0262	0.5315	0.8182	1.4601
Ulna Length	0.65	0.9906	1.1522	1.5462	1.6315
Radius Length	0.657	3.2371	0.7494	1.1491	1.1518
Femur Length	0.4220	0.3059	0.1315	1.1498	1.1331

S.D. – Standard Deviation

It appears that asymmetry demonstrated by the juvenile males is not significantly different than the asymmetry observed in juvenile females. Two variables, clavicle length and humerus length, closely approach significant values. These skeletal areas are typically recognized as sexually dimorphic in adult populations. Results generally indicate that the sexes may be pooled in subsequent analysis on the effects of age and cause-of death on asymmetry.

Does asymmetry change with increasing age?

The relationship between asymmetry and age was first assessed with a simple linear regression. The eight significant asymmetry indices (dependant variables) were regressed against age-at-death (independent variable). Age-at-death represents complete cessation of development, and by extension, the maximum asymmetry levels achieved. Due to non-normality of the dataset, both Pearson correlation coefficient and Spearman's correlation on ranked coefficients are reported, and 1000 bootstrap iterations were used to generate confidence intervals. Results are reported in Table 10. Three variables demonstrate relatively close correlation with age – clavicle length, humerus length, and radius diameter – and were used in additional analysis.

Table 10: Correlation between age and directional asymmetry

Measurement	Pearson Correlation Coefficient	Spearman Rank Coefficient	95% Confidence Limits**	
			Lower	Upper
Clavicle L	-0.3963	-0.3993	-0.5637	-0.1751
Humerus L	0.3174	0.3352	0.2018	0.4886
Humerus D	0.0134	0.0441	-0.2106	-0.1949
Ulna L	-0.0767	-0.0884	-0.2833	0.0968
Radius L	-0.0975	-0.0636	-0.0341	0.0722
Radius D	0.2294	0.2590	0.0356	0.4354
Femur L	0.0920	0.1108	-0.1080	0.3016
Femur W	-0.1051	-0.0422	-0.3946	0.1290

** based on 1000 bootstrap iterations

The linear relationship between age and individual asymmetry values is very difficult to visualize. Therefore, individuals were grouped into twelve age-at-death cohorts. Cohorts created: 0 – 1 years, 1.1 – 3 years, 4 – 5 years, 6 – 8 years, 9 – 10 years, 11 – 13 years, 14 – 15 years, 16 – 17 years, 18 – 19 years, and 20 years-at-death, with sexes pooled. The number of individuals in each cohort is variable, depending on their representation in the sample, but was kept approximately equal. ANOVA (analysis of variance), was performed on the cohorts for each of the closely correlating variables (clavicle length, humerus length, and radius diameter). Due to non-normality, a Kruskal-Wallis One-Way ANOVA on ranked values was specifically used. This procedure calculates a mean asymmetry value for the individuals included in each cohort, and then evaluates the variation between these means.

Furthermore, an orthogonal polynomial test was applied to determine an overall trend in the mean asymmetry values for each cohort. The orthogonal polynomial applied (available in the ANOVA options within NCSS) tested for linear, quadratic, or cubic trends in the cohort data. This equation is considered appropriate even if the levels are unequally spaced or the group sample sizes are unequal, which is the case for the cohort groupings. Table 11

Table 11: Relationship between clavicle length asymmetry and age (cohorts)

Cohort	Age Group (in years)	n	Mean asymmetry index	Z-value	Standard Error	R > L	R = L	R < L
1	0 – 1	11	0.38092	1.1225	0.764100	2	0	9
2	1.1 – 3	10	1.15797	2.1327	0.801394	4	0	6
3	4 – 5	9	0.206187	1.4983	0.844744	4	1	4
4	6 – 8	4	-1.697806	-0.9041	1.267117	0	0	4
5	9 – 10	6	-0.202016	0.5520	1.034596	2	0	4
6	11 – 13	8	-0.536755	1.0158	0.895986	1	3	4
7	14 – 15	8	-1.086858	-0.5157	0.895986	1	1	6
8	16 – 17	9	-1.992812	-2.0991	0.844744	0	1	8
9	18 – 19	9	-1.857183	-1.9359	0.844744	2	0	7
10	20	8	-1.882898	-1.2971	0.895966	1	0	7

displays results from the ANOVA on clavicle length, as well as raw counts on the side producing greater bone length.

Results show that early in life, the right clavicle tends to be longer than the left. This relationship alternates after approximately age six into adulthood, where left exceeds right. Kruskal-Wallis One-Way ANOVA produced a significant value for the overall model, ($p = 0.03413$), indicating that significant difference does exist between the mean asymmetry of each age cohort. Figure 8 show change in asymmetry through the cohorts.

Positive symmetry values indicate right-dominance (demonstrated by cohorts 1 – 3), while negative indices signify a greater left measurement (demonstrated by cohorts 4 – 10). The greatest change occurs between cohort 3 (ages 4 to 5 years) and cohort 4 (ages 6 to 8 years), although results may be attributed the very small sample size in cohort 4. Results from the polynomial test determined a significant linear trend for clavicle length asymmetry ($p = 0.00161$).

Table 12 displays results from the ANOVA on humerus length, as well as raw counts of the side producing greater bone length. The left humerus tends to be longer than the right during the earliest stages of life. This pattern quickly changes with age. Right become the

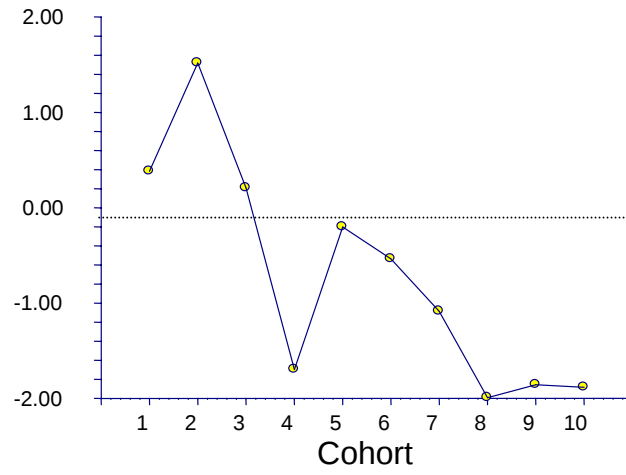


Figure 8. Mean clavicle length asymmetry for each cohort

Table 12: Relationship between humerus length asymmetry and age (cohorts)

Cohort	Age Group (in years)	n	Mean asymmetry index	Z-value	Standard Error	R > L	R = L	R < L
1	0 – 1	11	-0.253911	-2.1293	0.3674175	7	0	4
2	1.1 – 3	10	0.5788551	-1.0982	0.3853507	8	0	2
3	4 – 5	9	0.5713468	-0.7120	0.4061953	7	0	2
4	6 – 8	4	0.6180288	-0.3983	0.6092929	3	1	0
5	9 – 10	6	1.073818	0.8903	0.4974856	5	0	1
6	11 – 13	8	0.768236	0.1094	0.4308352	5	2	1
7	14 – 15	8	0.7047049	-0.5235	0.4308352	5	2	1
8	16 – 17	9	1.417374	1.6540	0.4061953	7	2	0
9	18 – 19	9	1.103238	1.0829	0.4061953	7	2	0
10	20	8	2.27338	1.4455	0.4308352	7	1	0

longer member, and the degree of disparity between the sides also increases with age. This result is probably related to handedness, as individuals increasingly engage in unimanual operations.

Kruskal-Wallis One-Way ANOVA on the humerus generated an insignificant value ($p = 0.21642$), however orthogonal polynomial results revealed a trend of increasing right-dominant asymmetry ($p = 0.00618$). Figure 9 shows the change in humerus asymmetry through the age-cohorts. Asymmetry values less than zero indicate left dominance (observable only in cohort 1), while values above zero indicate right dominance (all other cohorts 2 – 9). The greatest change occurs between cohort 1 (ages 0 to 1) and cohort 2 (ages 1.1 to 3). Humerus asymmetry generally continues to increase with, and is more notable at older ages than clavicle asymmetry, a finding corroborated by the linear trend results.

Table 13 displays results from the One-Way ANOVA on radius diameter, as well as raw counts of the side producing greater bone length. Right radius diameter is consistently greater than the left, which may be related to habitual use and handedness. Side bias does not change with age, but the magnitude of difference appears to vary.

Kruskal-Wallis One-Way ANOVA on the radius model generated an insignificant value ($p = 0.170992$), similar to humerus length. No general trend in asymmetry could be determined by the orthogonal polynomial either. Figure 10 shows the change in asymmetry through the age cohorts. Asymmetry values are uniformly greater than zero, indicating that right diameter is greater for all cohorts. Right dominance becomes slightly more pronounced with age, but does not follow a linear pattern. Disparity in the magnitude of asymmetry may warrant further investigation of a fluctuating pattern on radius diameter asymmetry.

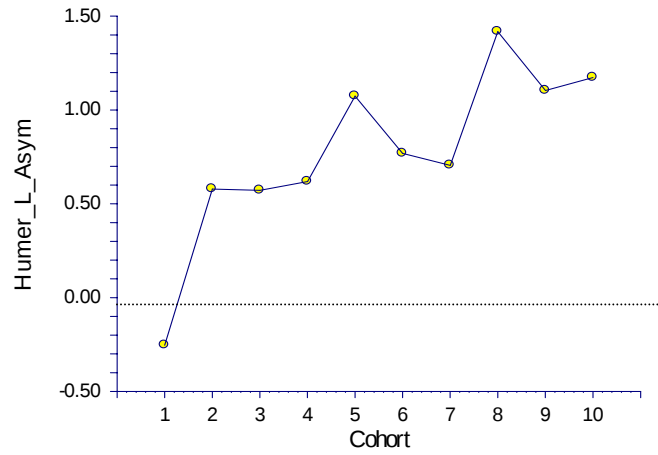


Figure 9. Mean humerus length asymmetry for each cohort

Table 13: Relationship between radius diameter asymmetry and age (cohorts)

Cohort	Age Group (in years)	n	Mean asymmetry index	Z-value	Standard Error	R > L	R = L	R < L
1	0 – 1	11	1.94878	-0.5034	1.46628	10	0	1
2	1.1 – 3	10	1.96609	-0.7723	1.53785	9	0	1
3	4 – 5	9	0.01711	-1.3648	1.62104	6	0	3
4	6 – 8	4	3.52362	0.0215	2.43156	4	0	0
5	9 – 10	6	0.36172	-1.2108	1.98536	4	0	2
6	11 – 13	8	1.59169	-0.1875	1.71937	6	0	2
7	14 – 15	8	2.73615	0.5782	1.71937	5	0	3
8	16 – 17	9	1.61099	-0.1038	1.62104	7	0	2
9	18 – 19	9	7.17561	3.1078	1.62104	9	0	0
10	20	8	3.97757	0.3594	1.71937	7	0	1

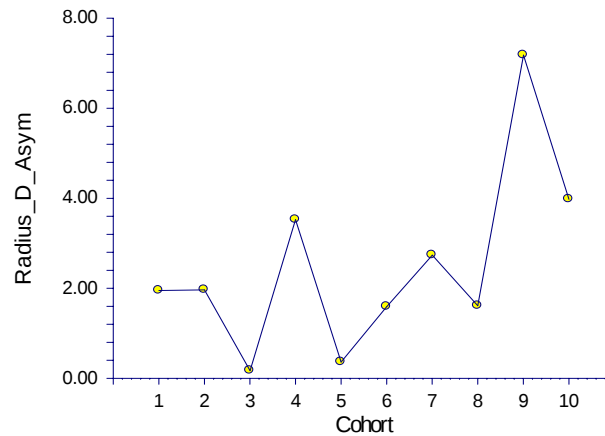


Figure 10. Mean radius diameter asymmetry for each cohort

Is there an interaction between age and sex in the juvenile cohort?

It is well documented in biological and anthropological literature that males and females follow different trajectories of development. Females begin to mature at a younger age, and achieve skeletal maturity earlier. Male maturation begins and terminates later, spanning a longer period. For this reason, the interaction between sex and age must be assessed to determine its influence on asymmetry. This interaction was examined with an ANCOVA (analysis of covariance) test. ANCOVA tests the difference between the means of one grouping variable (age-cohort), adjusted to a common value of the covariate (sex). A General Linear model was constructed with grouping variables: age (adjusted) and sex (covariate). Clavicle length, humerus length, and radius diameter were entered as response variables, as these asymmetry indices were considered influential in both sex and age analysis. Type III Sum of Squares results are reported because the test is more robust to unequal sample sizes. Significance set at $p \leq .05$.

The ANCOVA model including covariates sex and age, showed an insignificant interaction in terms of clavicle length ($p = 0.1931$). This suggests that age-related changes in clavicle asymmetry do not differ appreciably between the sexes. This relationship is displayed in Figure 11. The figure shows that males have greater clavicle asymmetry than females at all ages, except for cohorts 3 and 5. Sample size of these cohorts may play an important part in the inverse relationship observed.

The interaction between sex and age on humerus length was similarly insignificant ($p = 0.3395$). Age-related changes in humeral asymmetry also do not appear differ between the sexes. This relationship is displayed in Figure 12. Humerus asymmetry is higher in males than females, particularly in older cohorts. These results fit the expectation that males become increasingly asymmetrical as they engage in adult activities more frequently. This pattern is generally parallel in female, but to a lesser degree.

Finally, radius diameter was the only variable showing a significant relationship between sex and age ($p = 0.0309$). Males had consistently higher asymmetry values at virtually all ages. This finding may be related to greater male activity, but may also signify greater vulnerability of males during periods of environmental stress.

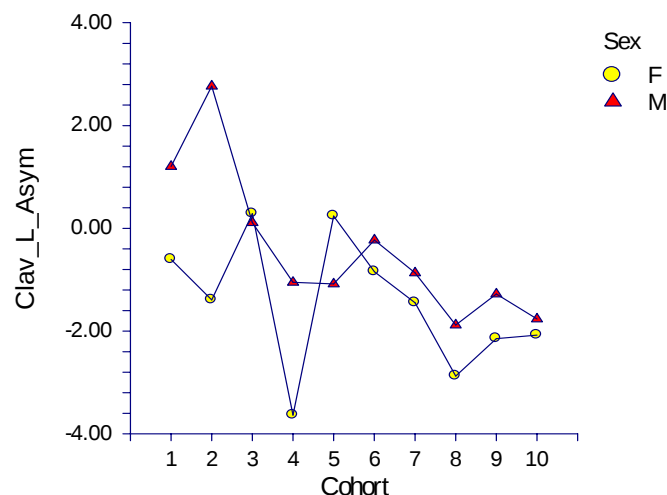


Figure 11. Clavicle asymmetry by sex and by age-cohorts

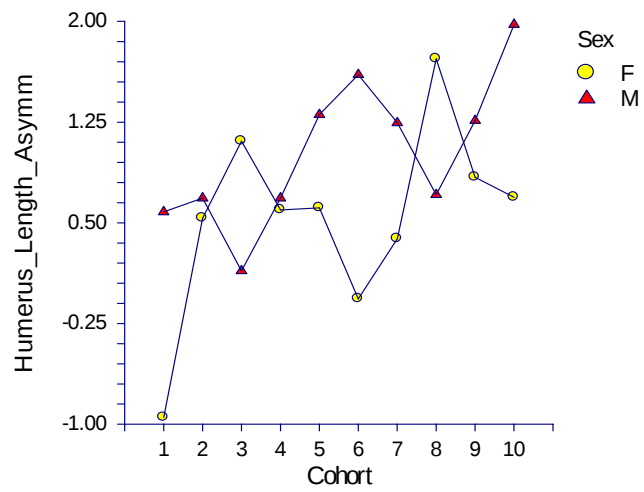


Figure 12. Humerus asymmetry by sex and by age-cohorts

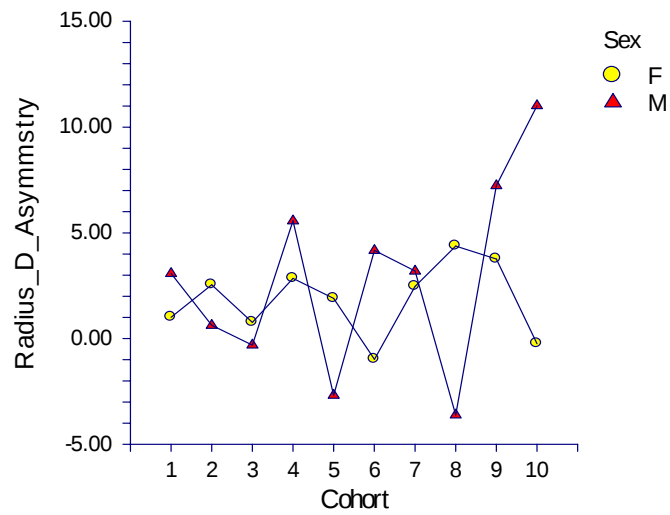


Figure 13. Radius diameter asymmetry by sex and by age-cohorts

Does asymmetry correlate with certain causes of death?

Cause of death was classified into one of three groups: chronic (n=15), infectious (n=51), sudden/accidental (n=8) (See Chapter 4: Methods, for examples in each category). Correlation between cause of death and skeletal asymmetry was analyzed with MANOVA, multivariate analysis of variance. A multivariate approach is preferred because of the multiple grouping variables (cause of death categories). Response variables included all eight significant asymmetry indices. Significance level was set at $p \leq .05$. Results from the MANOVA reveal the overall model to be insignificant ($p = 0.6773$).

However, individual analysis of variance (ANOVA) reveals clavicle length asymmetry to vary significantly between the three mortality groups ($p=0.005$). To determine the location of greatest variation (between which two groups), a Bonferroni post-hoc pairwise comparison was applied. A Bonferroni test on the means of each group (Least-Squares adjusted Means) was chosen for its ability to perform on non-normal data, and to detect a slightly smaller minimum difference between pairs. Bonferroni tests show significant difference in clavicle length asymmetry between infectious and sudden causes of death ($p = 0.03519$). Results are displayed in Table 14. This may be a function of variable sample-sizes in the mortality groups. However, the potential effects of pulmonary infectious diseases upon clavicle morphology lend support for the relationship between lower socioeconomic status and increased asymmetry. Lower status individuals are simultaneously more likely to harbor infectious diseases while engaging in intense levels of physical labor.

Table 14: Bonferroni post-hoc test on cause of death (clavicle length)

Comparison	Means Difference	Simultaneous 95% Confidence Limit		Significant?
Chronic vs. Infectious	1.7148	-0.2295	3.6592	No
Chronic vs. Sudden	1.6502	-1.3032	4.6035	No
Infectious vs. Sudden	3.3650	0.0306	6.6994	Yes

Is asymmetry correlated with additional indicators of development stress?

Skeletal development was assessed in terms of epiphyseal fusion. Juvenile samples demonstrating any stage of epiphyseal closure were used in the analysis. Total sample includes 58 individuals, with ages beginning at seven years at-death. Twelve long bone epiphyses were scored on 0 – 3 scale of fusion, according to methods by Albert and Greene (1995) (See Chapter 4: Methods). Then an average of these scores was generated for the right and left sides of each individual. Developmental asymmetry was analyzed using a Hotelling's paired-sample T-test (employing 10,000 Monte Carlo samples). Results show no significant difference between the right and left averages ($p = 0.63118$).

In addition, a single developmental score was calculated for each individual by subtracting left from the right average (right – left). This single score was regressed upon age, to assess the relationship between age and developmental asymmetry. Results show an insignificant relationship between age and epiphyseal asymmetry ($r = 0.06143$). Therefore, it was concluded that juveniles in the New Lisbon Collection do not demonstrate asymmetry in developmental epiphyseal closure.

What pattern of asymmetry may be observed in the juvenile subset?

Pattern of asymmetry is best examined on individual bones. Variables repeatedly contributing to significant asymmetry include: clavicle length, humerus length, and radius diameter, and therefore will be the variables analyzed further.

Asymmetry patterns were visualized by constructing frequency histograms and running tests of normality on the asymmetry indices for the total juvenile subset. Some variables violate normality assumptions, but the direction and magnitude of the departure play a key role in determining the overall pattern. In addition, skewness and kurtosis values

are reported as they may be considered evidence of selective pressure influencing asymmetry.

Clavicle asymmetry demonstrates a pattern consistent with both fluctuating and directional asymmetry. This dual pattern may be a function of the pooled sample, as both sexes and all age-groups were included into pattern analysis. Figure 13 displays a histogram of the frequency of clavicle asymmetry indices. The majority of observations possess an asymmetry index centered around zero (symmetry), with a slight left side bias indicated by a negative peak value. The remainder of the distribution assumes a bell-shaped curve. The relatively wide curve denotes a moderate amount of variation present in this sample. In addition, Figure 14 displays a normality probability plot on the clavicle asymmetry indices. The regression line created from the indices is very similar to a normal, linear slope. Individual values are plotted above and below this line, an oscillating pattern consistent with fluctuating asymmetry. However, negative deviation from the regression line is more commonly observed and suggests the presence of DA. Table 15 displays skewness and kurtosis values generated by the test of normality. The leptokurtic (positive) value observed for this variable typically indicates the presence of stabilizing selection and FA. Therefore, multiple data sources suggest primarily left biased directional asymmetry, with the potential for fluctuating asymmetry in a portion of the juvenile subset (perhaps youngest groups).

Table 15: Normality test on clavicle length

Variable	Skewness Test			Kurtosis Test		
	Value	Z-score	Prob. Level	Value	Z-score	Prob. Level
Clavicle length	0.73	2.67	0.0075	5.38	2.94	0.0033

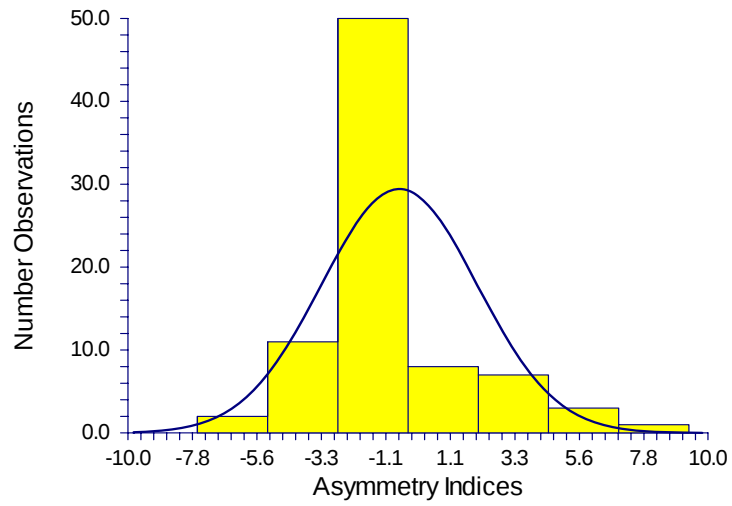


Figure 14. Histogram of the frequency of clavicle asymmetry

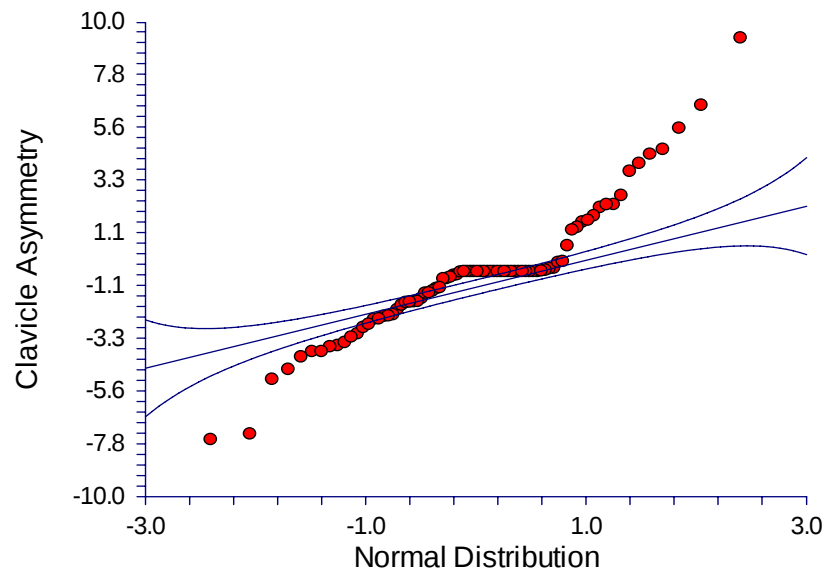


Figure 15. Normal probability plot of clavicle asymmetry

Alternatively, humerus asymmetry demonstrates a clear pattern of directional asymmetry, with bias favoring the right side. Figure 15 displays a histogram of humerus asymmetry. The frequency curve assumes a very tight/narrow distribution, with little variation away from the median. In addition, the greatest number of observations is not centered on zero as they were with clavicle asymmetry. Rather, the majority of observations, (the peak of the curve), are found slightly above zero. The tendency for positive values indicates right-bias in humeral asymmetry. The normality plot, displayed in Figure 16, produces very similar results. The regression line clearly deviates from a linear slope. While individual indices deviate both above and below the line, the vast majority of values are positive; signifying that the right member is dominant in length. In additional, skewness and kurtosis values show a significant left skew, as seen in Table 16.

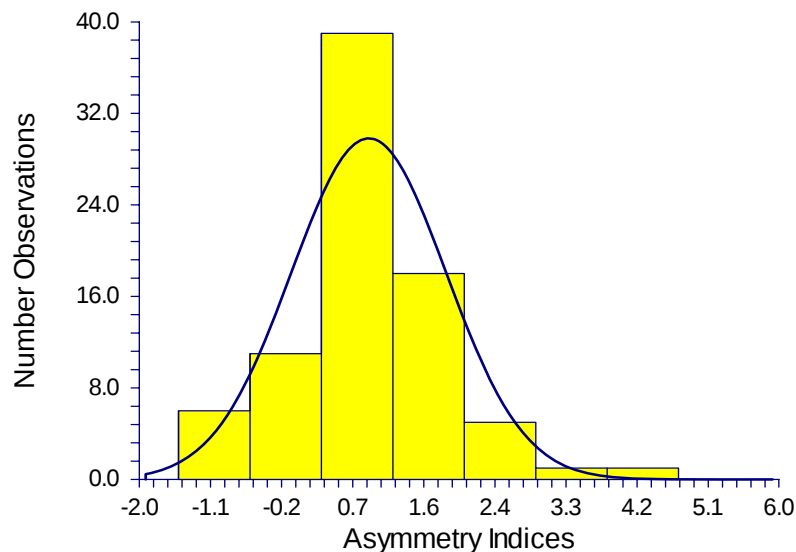


Figure 16. Histogram of the frequency of humerus asymmetry

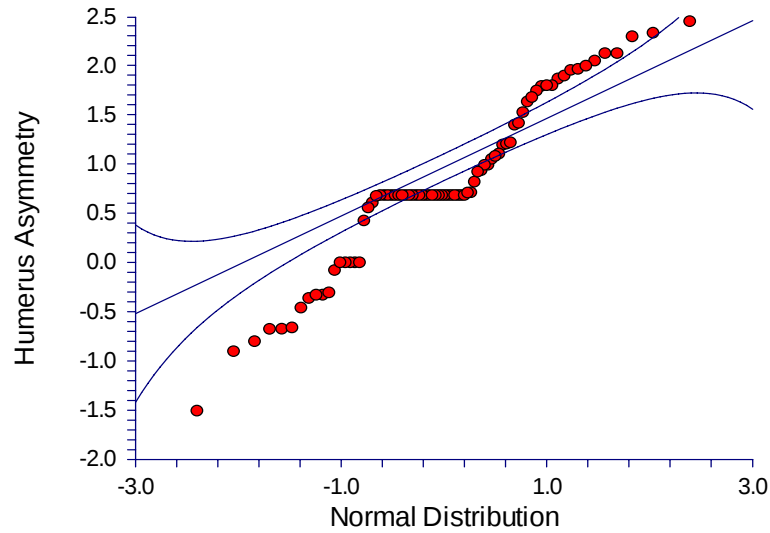


Figure 17. Normal probability plot of humerus asymmetry

Table 16: Normality test on humerus length

Variable	Skewness Test			Kurtosis Test		
	Value	Z-score	Prob. Level	Value	Z-score	Prob. Level
Humerus length	0.63	2.33	0.0197	5.62	3.07	0.0021

Alternatively, radius diameter is primarily representative of a fluctuating pattern. The histogram reveals a very wide distribution, showing an inherently large amount of variation. The peak is slightly right biased however, with the highest frequency above the zero value (See Figure 17). The normality plot shows a large distribution of individual values (See Figure 18). Most asymmetry indices fall outside of the confidence limits, but without respect to side. Characteristics of both fluctuating and directional patterns may again be a function of the pooled sample. The inclusion of both males and female, as well as all juvenile age-groups in this analysis incorporates the large amount of variation observed (statistically significant in some tests) for this group. Furthermore, the positive kurtosis value (See Table17) suggests the presence of selective forces at work.

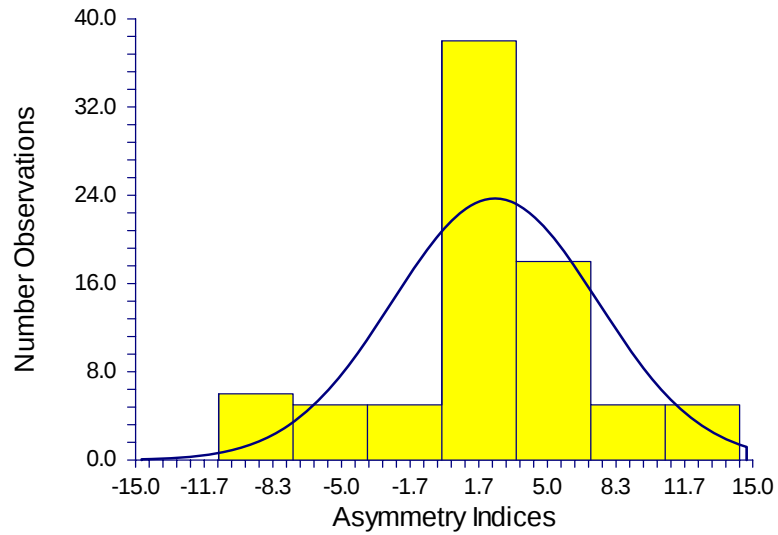


Figure 18. Histogram of the frequency of radial diameter asymmetry

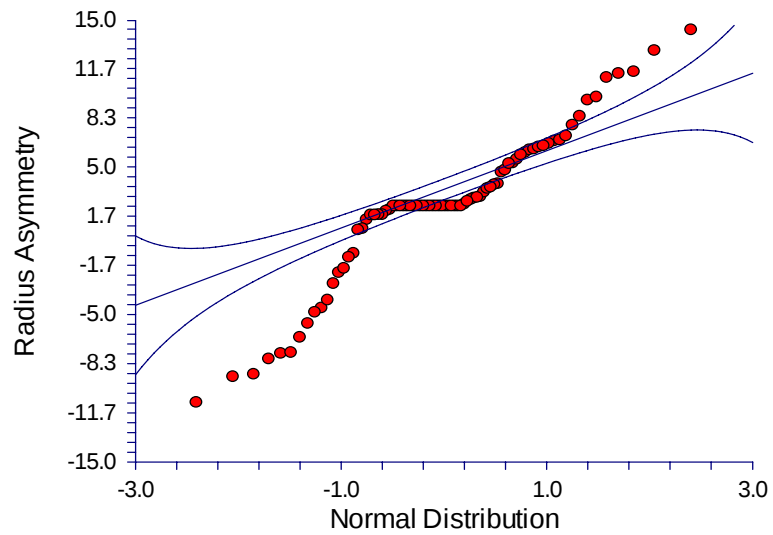


Figure 19. Normal probability plot of radial diameter asymmetry

Table 17: Normality test in radius diameter

Variable	Skewness Test			Kurtosis Test		
	Value	Z-score	Prob. Level	Value	Z-score	Prob. Level
Radius diameter	-0.46	-1.77	0.0759	3.68	1.44	0.1501

5.2 Validation of Juvenile Results

The relatively small sample size (low representation of certain demographic groups) of the juvenile subset and violations of normality serve as critiques against the use, and ability of mortuary skeletal material to accurately represent the greater Lisbon juvenile population. Thus, statistical validation methods are necessary to corroborate results.

Validation on sexual dimorphic differences in asymmetry

Validation was performed by creating a hold-out sample, including 20 males and 20 females of various ages. Hotellings 2-samples T-test was rerun ten times, on ten different the samples ($n = 40$ each trial). As before, sex was used as the grouping variable and eight asymmetry indices were used as response variables, $p \leq .05$, and results are based on 10,000 Monte Carlo samples. All trials produced insignificant values for the overall model, with p-values ranging from 0.29 to 0.87. Therefore, it may be concluded that significant different in asymmetry does not exist between juvenile males and juvenile females.

Validation of age-related differences in asymmetry

To reevaluate the relationship between age and bilateral asymmetry, age cohorts were collapsed to create new groupings. Two original cohorts were combined into a single validation group. (See Table 18 for validation group membership and size). Kruskal-Wallis One-Way ANOVA on the mean asymmetry indices was retested for significant difference between the new validation cohorts. In addition, orthogonal polynomial equations were used to determine a significant trend in the means of the five groups. As before, clavicle length, humerus length, and radius diameter were each used as response variables ($p \leq .05$).

Validation data support the results produced by the original classification: clavicle length is significantly and consistently different between the age groups, while humerus length and radius diameter are not. A significant linear trend was determined for clavicle length (increasingly left dominant), and humerus length (increasingly right dominant). Radius diameter produced an overall quadratic trend for the validation groups, a result attributable to the fluctuating nature of this variable (See Table 19).

5.3 Adult Population

Is skeletal asymmetry observable in the bones of the adult subset?

Similar to the juvenile subset, skeletal material was initially screened for metric difference in length between right and left bones. Samples were counted in terms of longer side: right exceeding, left exceeding, and approximately equal (within 0.5mm considered equal). Raw counts for the major long bones are displayed in Table 20.

Table 18: Original and validation cohort groupings

Original Cohort	Original n	Validation Cohort	Validation n
1	11	1	21
2	10		
3	9		
4	6	2	15
5	8	3	16
6	8		
7	9		
8	8	4	17
9	8	5	17
10	9		

Table 19: Results from Validation ANOVA and Orthogonal Polynomial Tests

Asymmetry Index	ANOVA validation prob. level	Orthogonal polynomial pattern?	Orthogonal polynomial prob. level
Clavicle length	0.01698	linear	0.003069
Humerus length	0.04998	linear	0.00845
Radius diameter	0.16173	quadratic	0.036263

Table 20: Raw counts of the longer bone – Adult subset

Measurement	Right > Left	Right = Left	Right < Left	Total
Clavicle max length	68	28	152	248
Humerus max length	189	43	16	248
Humerus head	120	22	106	248
Humerus epicondyle width	140	32	76	248
Radius max length	168	65	15	248
Ulna max length	170	56	22	248
Femur max length	45	115	88	248
Femur head	126	45	77	248
Femur condyle width	115	55	78	248

The ratio of right versus left length-dominance in the adult subset is generally consistent with previous studies of bilateral asymmetry. Right measurements dominated for all bones of the arm (humerus, radius, and ulna). Humerus length had the fewest deviations from this pattern; 17.3% of adults had humerii of equal length, and only 6.5% had a longer left measurement. Clavicle length shows the expected opposite relationship, as the left member was typically longer in this sample set. Only 24.7% of individuals possessed a longer right clavicle. Femur length was the most equilateral bone, a result which must be attributed to equal bipedal locomotion. The largest proportion of individuals (43.7%) had approximately equal femur length (within .05mm); however another significant percentage of samples were slightly left-biased (35.4%). Therefore, the disparity between long bones is readily observable, clearly follows a pattern characteristic of most prehistoric and historic populations, and warrants subsequent analysis.

What skeletal elements contribute significant asymmetry?

Disparity between right and left measurements was evaluated using a Hotelling's paired-sample T-test. The Hotelling's method is preferred due to randomization and permutation capabilities. Level of significance was set at $p \leq .05$, and randomization was based on 10,000 Monte Carlo samples. The overall model, including all 15 paired response

variables, proved highly significant ($p = 0.0001$). Results on individual variables are displayed in Table 21. Eleven measurement-pairs were considered significantly different from one another (variables are highlighted in Table 21).

A standardized asymmetry index was calculated for the variables. Indices were subjected to a Hotelling's one-sample T-test to evaluate significant deviation from zero. Directional asymmetry is associated with a departure from a mean of zero, in either a positive (right-dominant) or negative (left-dominant) direction. All variables showing significant right-left discrepancy also demonstrate significant directional asymmetry, right-bias was observed in all variables except for clavicle length (indicated by negative mean).

Is there a significant sexual dimorphism in adult asymmetry?

The eleven asymmetry indices were entered into a variable selection method, to determine which variables best discriminate between the sexes. Adult variable selection is the same method as that applied to the juvenile subset: parametric tests (generated in SAS) add one variable per step to build an optimally discriminating model (unfortunately lacks randomization capabilities). Sex was used as the grouping variable, significance level for entry was set at $p = 0.2$, and level for removal was set at $p = 0.15$. The optimal model produced includes six variables: Clavicle length, humerus max diameter, humerus epicondylar width, radius length, radius medial-lateral diameter, and ulna anterior-posterior diameter. These six variables were then used in a Hotellings 2-sample T-test to evaluate significance between males and females. Again, Hotelling's T-tests are preferred because of the multiple variables included, with randomization methods to resolve normality violations.

Table 21: Significant difference in Adult Subset – Side discrepancy and departure from zero

Measurement	Right – Left Significance (p-value)	Mean Asymmetry Index	Significant deviation from zero (p-value)
Clavicle length	0.0043	-1.195	0.0001
Humerus max length	0.0001	1.644	0.0001
Humerus epicondyle width	0.0012	0.968	0.0095
Humerus head	0.0638	0.335	0.0951
Humerus max diameter	0.0001	3.084	0.0001
Humerus min diameter	0.0001	2.154	0.0001
Ulna max length	0.0001	1.399	0.0005
Ulna AP diameter	0.0002	1.836	0.0009
Ulna ML diameter	0.0012	1.699	0.0006
Radius max length	0.0001	1.225	0.0001
Radius AP diameter	0.0003	1.328	0.0002
Radius ML diameter	0.0001	3.009	0.0001
Femur max length	1.00	0.417	0.9898
Femur head	0.0758	0.426	0.5854
Femur condyle width	0.0875	0.341	0.5700

*AP – Anterior-Posterior

**ML – Medial-Lateral

Humerus maximum length was not included in the model, but T-test data on this variable was included in order to make general male-female comparisons. Significance was designated at $p \leq 0.05$, with randomization based on 10,000 Monte Carlo samples. The overall model, using six response variables, proved statistically insignificant ($p = 0.8610$). Results on male and female individual variables are displayed in Table 22.

Although statistically undifferentiated, males and females tend to demonstrate specific patterns. Males typically possess larger asymmetry values, especially at indices involving bones of the shoulder (clavicle length and humerus length). Alternatively, females tend to have greater asymmetry indices in the forearm: humerus epicondylar width, radius length, and radius medial-lateral diameter. Hotelling's T-test results suggest that differences observed between the sexes are not significant, and therefore the sexes will be pooled for temporal analysis.

Table 22: Hotelling's T-test of significant difference between adult males and females

Measurement	Randomization p-value	Female Mean	Male Mean	Female S.D.	Male S.D
Clavicle length	0.7620	-1.2504	-1.4437	2.6411	2.8154
Humerus length	0.1937	1.2897	2.6526	1.1988	1.1711
Humerus epicondyle width	0.2900	1.2915	0.6676	4.2803	4.7622
Humerus max diameter	0.5020	2.9208	3.2370	3.553	3.9529
Ulna AP diameter	0.0737	1.7224	1.9411	4.4538	5.4920
Radius length	0.6610	1.2605	1.1848	1.4232	1.6579
Radius ML diameter	0.0474	3.96252	3.4479	6.0143	5.9832

Is there a significant difference in asymmetry between types of occupation?

Male occupation was classified into one of two categories: manual or non-manual, based upon the amount of required mechanical loading. (See Chapter 4: Methods, for examples of occupations in each category). Occupation further serves to indicate socioeconomic status of a population. Times of hardship and scarcity are signified by a greater portion of a population engaging in hard labor, with fewer resources to buffer the effects. Analysis by occupation was restricted to males because the vast majority of females were uniformly documented as “domesticas”, or house-wives. This role probably required an extremely wide range of activity patterns and mechanical loading stress. But it is impossible to differentiate within the female sample.

To determine if different degrees of asymmetry exist between manual and non-manual worker, Hotelling's 2-sample T-test was run on the two occupation groups. The eleven indices initially flagged as asymmetrical were used as response variables, with $p \leq .05$, and randomization based on 10,000 Monte Carlo samples. The overall model showed significant difference between the two groups ($p = 0.0259$). Results are shown in Table 23.

Table 23: Hotellings T-test for difference in asymmetry between occupational categories

Measurement	Randomization p-value	Non-manual Mean	Manual Mean	Non-manual S.D.	Manual S.D
Clavicle length	0.0287	-0.8666	-1.6507	2.9637	2.5599
Humerus length	0.0200	0.9091	1.2751	1.2982	1.1979
Humerus width	0.0815	0.6400	0.8908	4.5026	5.0698
Humerus max diameter	0.0430	2.8934	3.5763	3.7750	4.2970
Humerus min diameter	0.9730	2.3675	2.3379	3.9865	4.2182
Ulna length	0.8920	0.9219	0.9272	1.0662	1.3942
Ulna AP diameter	0.5170	1.4454	2.0980	5.48622	5.7342
Ulna ML diameter	0.6090	0.2551	0.9737	6.111	6.9497
Radius length	0.0140	0.6723	1.4454	1.8600	1.2628
Radius AP diameter	0.0296	3.96252	3.4479	6.0143	5.9832
Radius ML diameter	0.0471	3.0144	3.9401	5.9293	6.1861

Does skeletal asymmetry demonstrate a correlation with certain causes of death?

Individuals with a listed cause-of-death were placed into one of four categories: cardio-vascular/pulmonary, infectious, chronic, or age-related. (See Chapter 4: Methods, for examples in each category). Correlation between cause of death and skeletal asymmetry was analyzed with MANOVA, multivariate analysis of variance. A multivariate approach is preferred because of the multiple grouping variables (cause of death categories). Response variables included the eleven significant asymmetry indices, and significance level was set at $p \leq .05$. Results from the MANOVA reveal the overall model (including all eleven indices) to be insignificant ($p = 0.6773$). Therefore, it was concluded that cause of death for adult skeletal material does not contribute to bilateral asymmetry in the New Lisbon Collection.

Do levels of asymmetry change through time; are they associated with the significant socioeconomic transition experienced by Portugal during the last century?

In order to assess changes in asymmetry through time, the adult subsample was collapsed into a series of groups, according to date-of-birth. Each group represents a period of ten years, beginning with birth-year 1820, and terminating at birth-year 1939. Two samples were born prior to 1820, but were eliminated from temporal analysis to avoid creating a single cohort encompassing fifteen years. Number of individuals in each group is inconsistent, dependent on representation in the total New Lisbon Collection. Four response variables were selected for analysis, due to their repeated significance in previous analyses (sexual dimorphic and occupational differences) and prior literature on lateralization. Descriptive statistics on the temporal groups and response variables are listed in Table 24.

A MANOVA was used to test for significant difference in mean asymmetry value calculated for each cohort. Clavicle, humerus, radius, and ulna length were used as response variables, and determination rule was set at $p \leq 0.05$. The overall model was statistically significant, as were individual variables: clavicle length and humerus length. These results are shown in Table 25. Asymmetry tends to increase throughout the nineteenth century. The greatest increases occur with groups: 8, 9, and 10 (spanning birth years 1890 – 1919). This period coincides with determinative socioeconomic events at the beginning of the twentieth century: the Portuguese social revolution and rise of the Salazar dictatorship.

Significant differences through time were observed primarily in variables, clavicle and humerus length. Figures 20 and 21 graphically demonstrate this change in clavicle and humerus asymmetry, respectively. Notably, these measurements were also flagged as significant in males, but not females.

Table 24: Descriptive statistics on temporal groups in the adult subsample

Group	n	Years included	Years of modification	Mean Clavicle Asymmetry	Mean Humerus Length Asymmetry	Mean Radius Length Asymmetry	Mean Ulna Length Asymmetry
1	6	1820 – 1829	1820 – 1840	-3.33112	0.70860	1.34981	0.71607
2	7	1830 – 1839	1830 – 1850	-2.57909	2.04561	0.80876	0.83742
3	15	1840 – 1849	1840 – 1860	-0.53663	0.92519	1.12562	1.63091
4	24	1850 – 1859	1850 – 1870	-0.9913	1.86398	1.03234	1.31984
5	28	1860 – 1869	1860 – 1880	-0.37609	0.98406	1.15742	1.16226
6	27	1870 – 1879	1870 – 1890	-0.75998	1.53173	1.07251	1.25821
7	21	1880 – 1889	1880 – 1900	-0.41237	1.78023	1.42593	1.34694
8	34	1890 – 1899	1890 – 1910	-2.08524	1.12149	1.12918	1.25393
9	38	1900 – 1909	1900 – 1920	-1.61852	2.43152	1.60020	2.08485
10	19	1910 – 1919	1910 – 1930	-2.58868	4.69595	2.11472	3.67957
11	16	1920 – 1929	1920 – 1940	-0.62107	1.79313	1.66975	2.01325
12	11	1930 – 1939	1930 – 1950	-1.34267	1.26865	1.38115	1.06479

Table 25: MANOVA results on significant difference between temporal groups

Response Variable	D.F.	F-ratio	Prob. Level
Overall model	45	2.00	0.000162
Clavicle Length	11	1.98	0.031047
Humerus Length	11	3.73	0.000064
Radius Length	11	0.83	0.610755
Ulna Length	11	1.39	0.176987

D.F. – Degrees of Freedom

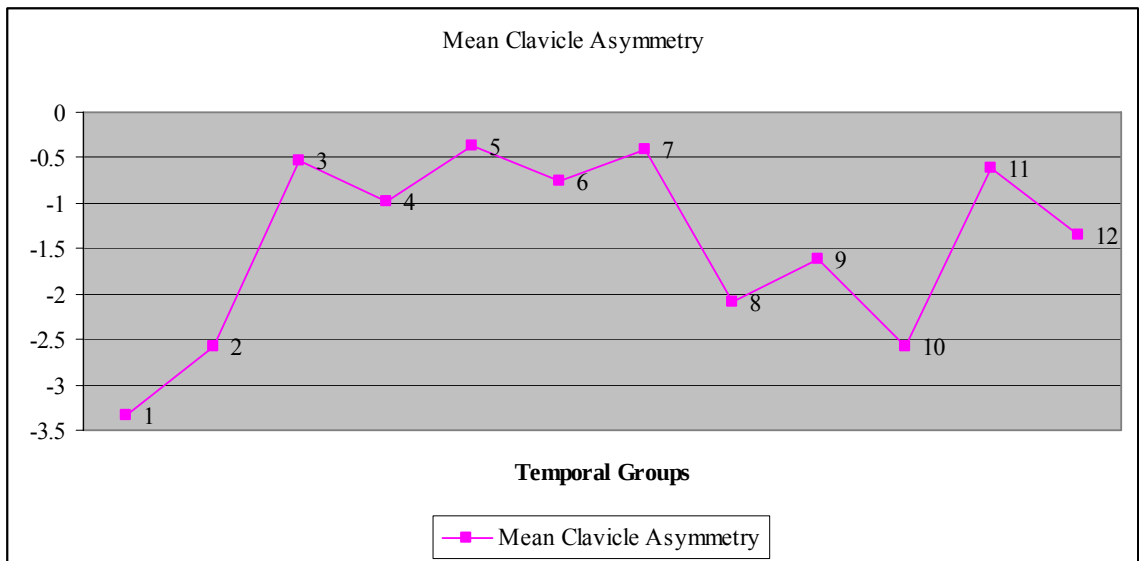


Figure 20. Change in clavicle asymmetry through temporal groups – Adult sample

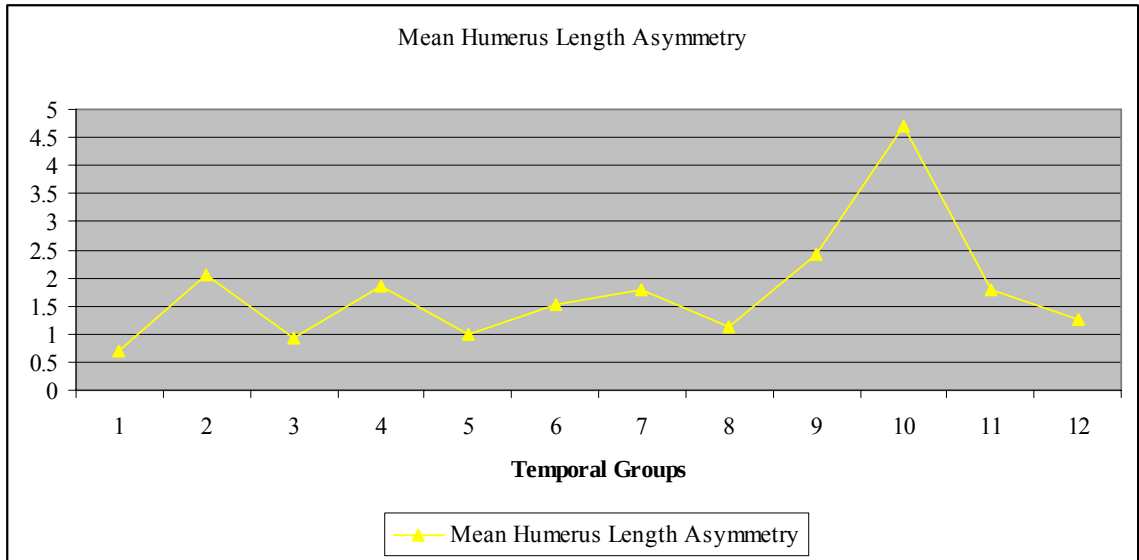


Figure 21. Change in humerus asymmetry through temporal groups – Adult sample

5.4 Validation of the Adult Results

Results from the temporal analysis of adult skeletal material were reanalyzed by collapsing original twelve groups into six groups, representative of 20-year intervals. Membership and size of validation group are displayed in Table 26. The available statistical software packages lack randomization permutation capabilities on MANOVA tests. Nonetheless, validation was performed utilizing Hotellings 2-sample T-Tests to determine significant pair-wise differences between groups. Results are displayed in Table 27.

Data confirm the results produced from the entire adult sample. Previous MANOVA determined significant difference localized with groups 8, 9, and 10. Hotellings T-tests found significant difference concentrated with group e (comprising groups 9 and 10). Tests on individual variables show clavicle and humerus length asymmetry contribute the greatest variation. Validation trials confirm the relationship between bilateral asymmetry and time (as inferred by 10-year and 20-year age groups) for samples in the New Lisbon Collection.

Table 26: Membership in adult validation groups

Original Groups	Original n	Validation Groups	Validation n
1	6	a	13
2	7		
3	15	b	39
4	24		
5	28	c	55
6	27		
7	21	d	55
8	34		
9	38	e	57
10	19		
11	16	f	27
12	11		

Table 27: Hotelling's T-test results on Adult Validation Groups:

Comparison	Overall p-value	Clavicle	Humerus	Radius	Ulna
a to b	0.0752	0.0357	0.1596	0.9651	0.0709
a to c	0.2005	0.0276	0.6368	0.9066	0.2831
a to d	0.3877	0.1257	0.9084	0.6488	0.1903
a to e	0.0433	0.0542	0.0422	0.2589	0.5079
a to f	0.2963	0.0714	0.8355	0.4561	0.3177
b to c	0.3481	0.4872	0.0926	0.8782	0.3469
b to d	0.4861	0.4988	0.1108	0.4917	0.5266
b to e	0.0027	0.0046	0.0224	0.0492	0.4807
b to f	0.2821	0.7578	0.0621	0.2197	0.7024
c to d	0.6803	0.167	0.6611	0.6589	0.7345
c to e	0.0006	0.0007	0.0387	0.0666	0.2924
c to f	0.5379	0.3796	0.3368	0.3127	0.3491
d to e	0.0291	0.0497	0.0717	0.1073	0.3293
d to f	0.8362	0.761	0.6283	0.417	0.4359
e to f	0.1997	0.0263	0.2894	0.6509	0.6579

5.5 Comparison Against Modern Skeletal Data

In order to isolate socioeconomic variables which may have contributed to bilateral asymmetry in a historic skeletal population such as the New Lisbon Collection an outlying source of skeletal material must be used for comparison. A modern American population, signified by birth dates after 1940, represents a group with moderate to high access to resources and healthcare, as well as political stability. Historically, the Portuguese population was lacking in many of these crucial factors. One inevitable result is an alteration in individuals and population-level workloads, and the development of bilateral asymmetry.

How do levels of asymmetry compare to those found in a modern population, such as twentieth-century individuals included in the Forensic DataBank?

In order to test the expectation of higher levels of asymmetry in the New Lisbon Collection, and comparative sample of modern skeletal measurements was sourced from the Forensic Databank (FDB). The Forensic Databank (maintained by colleagues at the University of Tennessee, Knoxville) contains standardized skeletal measurements on over 2500 individuals, sourced from several skeletal collections and forensic cases within the United States. To maintain a relative level of consistency, comparative samples were selected from either the William M. Bass Skeletal Collection or the University of Tennessee Forensic Collection. The comparative sample is approximately equal in size ($n=250$) and sexes ratio, spanning birth-years 1892 to 1985. Right and left measurements for major long bone length were used to calculate standardized asymmetry indices for the modern data. Mean asymmetry indices for the modern Forensic Databank (FDB) material and the New Lisbon Collection (NLC) are displayed in Table 28. Two Hotelling's 2-sample T-tests were performed to assess significant difference between the collections: FDB males versus NLC

males and FDB females versus NLC females. Sexes were divided for analysis because the magnitude of sexual dimorphism between the two samples is unknown. Hotelling's T-test results are shown in Table 29. Results shown a very clear difference in asymmetry exists between the Lisbon and the modern material. The New Lisbon Collection produced higher degree of asymmetry on all measured variables, most notably in clavicle, humerus, and ulna length. Although NLC asymmetry was also greater in ulna and femur length, levels were insignificantly higher. Figures 21 and 22 graphically show the mean asymmetry values of each sample set, divided by sex.

Table 28: Mean Asymmetry Indices: FDB vs. NLC males and females

Source: Sex	n	Mean Clavicle Asymmetry	Mean Humerus Asymmetry	Mean Radius Asymmetry	Mean Ulna Asymmetry	Mean Femur Asymmetry
FDB Males	123	-0.63670	0.50894	0.39728	0.58787	-0.13732
NLC Males	127	-1.35626	1.48877	1.30282	1.63096	-0.37125
FDB Females	122	-0.54773	0.47016	0.65396	0.9223	0.07199
NLC Females	121	-1.37513	1.63536	1.36888	1.44693	1.29771

Table 29: Hotelling's 2-sample T-test Results of Significance

Asymmetry Index	FDB vs. NLC Males	FDB vs. NLC Females
Clavicle Asymmetry	0.0120	0.0440
Humerus Asymmetry	0.0021	0.0010
Radius Asymmetry	0.0110	0.0020
Ulna Asymmetry	0.1010	0.1009
Femur Asymmetry	0.2100	0.7110
Overall Model*	0.0010	0.0010

* Overall Model results based on 10,000 Monte Carlo iterations

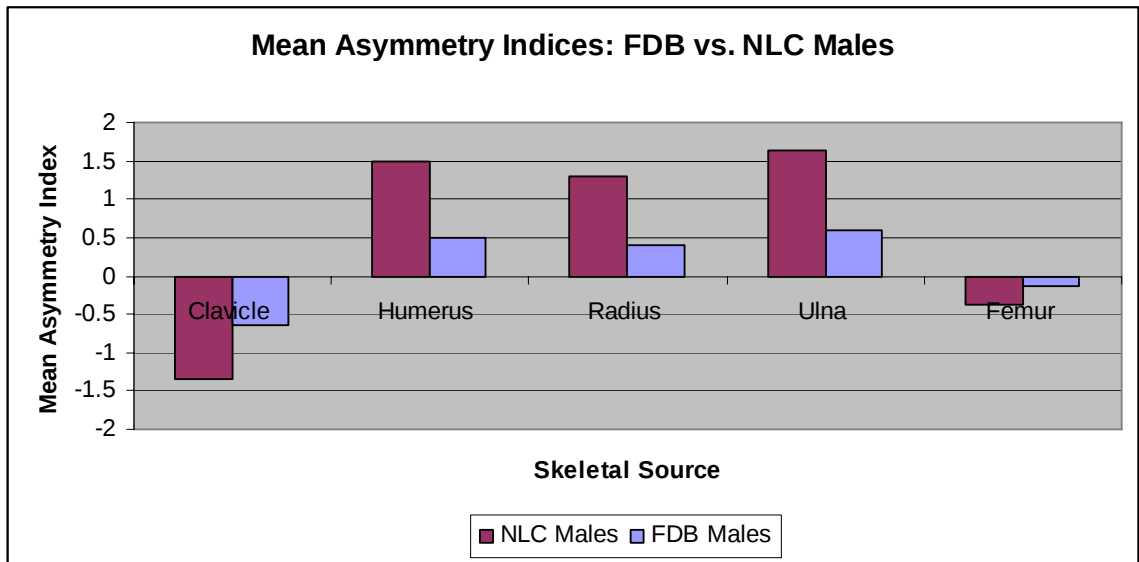


Figure 22. Mean Asymmetry Indices: Forensic Databank versus New Lisbon Collection Males

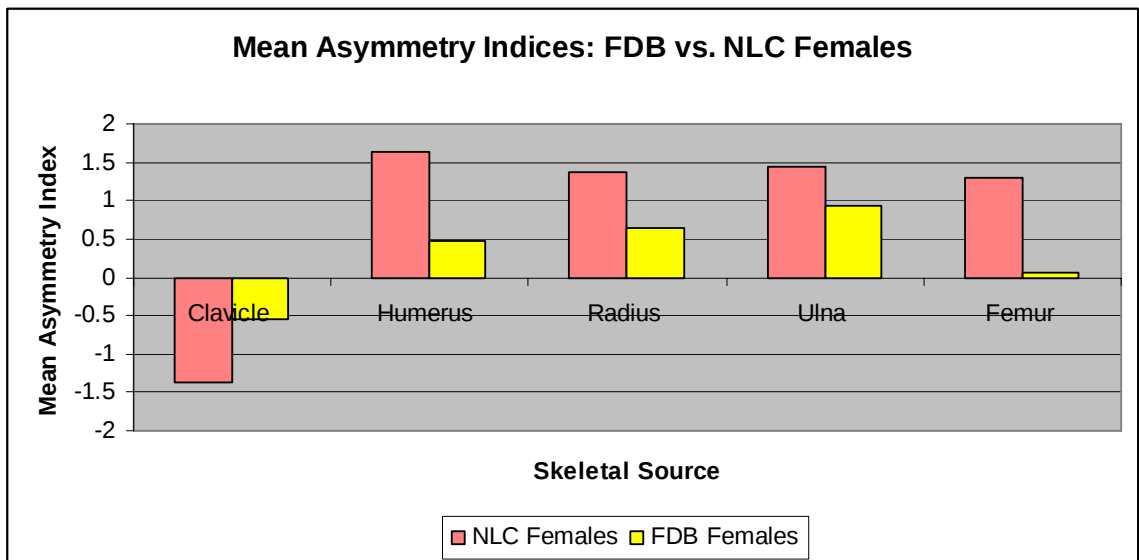


Figure 23. Mean Asymmetry Indices: Forensic Databank versus New Lisbon Collection Females

CHAPTER 6: DISCUSSION AND CONCLUSION

Analysis of the metric long bone data reveals that significant bilateral asymmetry exists in the samples of the New Lisbon Collection. The presence of both fluctuating and directional asymmetry in the juvenile subset suggests a relatively instable environment affecting subadult development, and early initiation of heavy mechanical loading. The adult subset demonstrates a primarily directional pattern, implying high levels of mechanical loading stress. This is supported by direct comparison of asymmetry levels between manual and non-manual laborers, and between individuals in the New Lisbon Collection and a modern American sample, who were presumably engaging in a much lower level of activity. Furthermore, temporal analysis reveals a period of increased physical stress, resulting in heightened asymmetry in multiple variables.

6.1 Juvenile Subsample

Significant Side Difference

Results from the juvenile subsample are generally consistent with those produced by previous studies of subadult bilateral asymmetry (Albert and Greene 1999; Butterworth and Hopkins 1993; Livshits et al. 1988). Measured variables demonstrating significant difference between right and left elements include: clavicle length, humerus length and diameter, ulna length, radius length and diameter, and femur length and width. The majority of variables are asymmetrical in terms of long bone length, which appears to be more plastic to modification than areas of width or circumference earlier in development. Several of these variables are consistent with those found to be asymmetrical in adult populations, such as clavicle, humerus, and radius measurements. Long bones which were not significantly asymmetrical

in adult groups, particularly measurements of the femur, may indicate the more variable nature of immature appendicular bones.

Sexual Dimorphism in Juvenile Asymmetry

Asymmetry indices were calculated for the eight variables demonstrating significant side difference to create a single asymmetry value for each. Step-wise variable selection procedure was employed to differentiate asymmetry between the sexes. The variable selection method ensures that only variables contributing to sexual dimorphism will be included into the model. Other potential covariates or insignificant variables are excluded to eliminate their influence in subsequent analysis. Applying the optimal 5-variable model, no significant difference could be determined between the sexes.

This result is consistent with the hypothesis posed for the juvenile subset. Immature remains are notoriously difficult to classify in terms of sex. General consensus among anthropologists is that sex is not readily determined in skeletal samples prior to puberty (Buikstra and Ubelaker 1994; Schutkowski 1993). Roughly half the samples in the New Lisbon Collection are younger than ten years of age, and samples would not be expected to exhibit maturational differences. An additional contributing factor may be the relatively low level of sexual dimorphism inherent for the population under study. Portuguese males have been historically documented as one of the shortest male populations in Western and greater Europe. In terms of long bone length, even outlying male samples may not be considerably longer than females. Lisbonese juveniles may not be sexually dimorphic because their parents were not sexually dimorphic, in raw measurements or in their asymmetry index.

In terms of fluctuating asymmetry, it appears that juvenile males and females demonstrated similar degrees of FA. Those asymmetry indices more closely assuming a

fluctuating pattern are radial diameter and clavicle length. Of these two variables, only clavicle length was selected for inclusion into the sexual dimorphism model. Males show (insignificantly) higher levels of asymmetry in both radius and clavicle asymmetry. This suggests that males and females were experiencing similar levels of developmental instability, but males were slightly more affected by these perturbations.

When analyzing sex-specific directional asymmetry, it is important to note that the majority of juveniles are very young in age and unaffected by heavy mechanical operations. Gender-specific occupational activities are presumably the source of differential stress and sexually dimorphic asymmetries. Even in a traditional society such as Portugal where adult vocations were adopted at a fairly young age, gendered activities were not as distinct in this juvenile group to produce sex-specific directional asymmetries so early in life.

Skeletal Asymmetry and Age

In terms of age, the New Lisbon Collection makes an important contribution to the anthropological literature tracking the origin and development of bilateral asymmetry, particularly in measurements of the upper limb. Debate exists concerning the earliest observance of side dominance and lateralization (Steele 2000). A number of studies suggest an ontogenic source for handedness. The ontogenic explanation proposes an inherent right-left gradient of nutrient provisioning during development, creating larger skeletal elements on the right-side early in fetal growth (von Bonin 1962). Consequently, right-side bias should be clearly observable at birth (Butterworth and Hopkins 1993). Alternatively, other research suggests that right- and left-dominance oscillates during the gestation (Bareggi et al. 1994; Steele 2000). Most neonates demonstrate left side dominance at birth, and quickly alternate to a pattern of right dominance early in childhood (Annett and Kilshaw 1983). Data from the

New Lisbon Collection are consistent with the second hypothesis. The majority of very young juveniles display longer measurements on the left side (both in length and width). After approximately three years, subadults show a clear pattern of right-dominance for most length measurements in the upper limb (radial diameter and clavicle length not applicable). Unfortunately, prenatal material was not included in the New Lisbon Collection due to Portuguese interment traditions (Cardoso 2005).

Most long bone asymmetries tend to steadily increase with age. This is particularly observed in humerus, ulna, and radius length, where the right bone becomes progressively larger. Clavicle length demonstrates the opposite pattern of left dominance. Results from the orthogonal polynomial equations show a general linear trend of increasing asymmetry for these variables. With maturity comes greater participation in occupational activities. Many young adults were expected to contribute to the family income upon completion of formal education, which required only three to four years (females and males, respectively) (Cardoso 2005). Forms of apprenticeship and work included agricultural farming, fishing for subsistence and profit, enlisting in maritime expeditions, or performing the standard period military service required by Salazar's administration (Kaplan 1998).

Investigation of age-sex variable interaction proved insignificant, implying that sex of a sample did not affect the relationship between age and asymmetry. This result may have several causes. The small size of some cohorts ($n=7$) may have obscured sex-related differences. Biological mortality bias inherent in all cemetery samples may additionally mask the effects of other influential variables (Saunders and Hoppa 1993). Normal, healthy Portuguese children may very well differ by age and sex – but the healthy juveniles who survived to adulthood are not those included in mortuary samples such as the New Lisbon Collection (Wood et al. 1992). Yet the low level of sexually dimorphic asymmetry observed

in the adult subsample corroborates the insignificant age-sex interaction in the juvenile subsample.

Juvenile Asymmetry and Mortality

Multivariate analysis of variance (MANOVA) was used to test for differences in average asymmetry between three types of mortality: chronic conditions, infectious diseases, and sudden/accidental death. Of the twelve variables included, clavicle length was the only significant asymmetry index. Pairwise analysis of the groups revealed that the source of greatest variation was the comparison of infectious to sudden/accidental causes of death. Results may be a product of sample size. Sudden death is the smallest category, with far fewer samples than other mortality groups. However, the link between clavicle asymmetry and the prevalence of infectious diseases may be meaningful.

Throughout the nineteenth and twentieth century, tuberculosis was the leading infectious cause of death in Portugal. TB was virtually eradicated in most Western countries by implementing public immunization programs for all school-age children. But in Portugal tuberculosis remained highly lethal, especially in very young and elderly individuals. Furthermore, in 1945 the childhood mortality rate was still 142.1 deaths per 1000 births, primarily due to communicable diseases and nutritional deficiency (Santos and Roberts 2001). High prevalence of infectious disease is closely linked to socioeconomic position, and is a crucial indicator of relatively low status of samples in the New Lisbon Collection. Skeletal evidence of TB in the New Lisbon Collection is common, particularly in older individuals who may have harbored the illness for a long period of time before succumbing. Given extended infirmity, tuberculosis will progress to the secondary and tertiary stages, infecting the skeletal structures which encompass the lungs and pulmonary tract (Ortner

2002). The ribs, sacrum, and portions of the upper limbs such as the distal end of the humerus, are the skeletal areas most commonly exploited by tuberculosis (Santos and Roberts 2001). See Figure A4 in the Appendix for skeletal evidence of tuberculosis afflicting individuals in the New Lisbon Collection.

Although the mid-torso is the region primarily affected by tuberculosis, the clavicle has also shown tubercular modification in archeological populations. More importantly, the archeological samples demonstrating evidence of tuberculosis were immature (Ortner 2002). This pattern may be related to the long period of clavicle development. This bone is one of the first to ossify but last to complete fusion, necessitating constant metabolic maintenance throughout development (Mays et al. 1999). In addition, the clavicle is closely positioned to structures in pulmonary system (located inches from the first rib), sharing a blood supply. Tuberculosis could ultimately be responsible for heightened levels of clavicle asymmetry in the juvenile subset. By extension, discrepancy between infectious and sudden causes of death could be related to the prevalence of tuberculosis in this skeletal sample.

Pattern of Juvenile Asymmetry

The juvenile subsample of the New Lisbon Collection primarily demonstrated the onset of directional asymmetry, with potential evidence of fluctuating asymmetry on at least one variable. Pattern of asymmetry was visually and statistically assessed through frequency histograms and tests of normality on three significant asymmetry indices: humerus length, clavicle length, and radius diameter.

Humerus length asymmetry clearly demonstrates a directional pattern. The frequency histogram shows a narrow bell-shaped curve, with the majority of asymmetries centered around a positive value of 1.3, indicating a clear and consistent right-side bias (See Figure 16

in Chapter 5). Graph of the residuals (See Figure 17) produced by the normality test confirms the pattern of positive asymmetry values and right-side dominance. The direct positive relationship between humerus asymmetry and age further supports a mechanical explanation for humerus asymmetry. Parallel increases in age and asymmetry signify the performance of adult occupational activities.

Fluctuating asymmetry is exemplified by radius indices, and to a lesser degree clavicle length asymmetry. Frequency histograms of radial diameter shows a very wide bell-shaped curve centered on the zero-point. The clavicle histogram curve is close to zero, but slightly skewed to the left which shows a minor left side bias. Tests of normality for both variables reveal positive kurtosis values for radius and clavicle, 1.44 and 2.94 respectively. Leptokurtosis in population-level investigations of asymmetry have been linked to stabilizing selection forces (Palmer and Strobeck 1986). Extremely asymmetrical organisms (right or left side-bias) represent extreme developmental instability, prove to be less fit and are consequently culled from a population (Leung and Forbes 1997; Palmer and Strobeck 1986). This form of asymmetry is heavily dependent upon stability of the developmental environmental. In the absence of external mechanical stress, asymmetry exclusively reflects the inability of the body to grow equilaterally (Dangerfield 1994). The result is a frequency centered upon zero (symmetry), but a high degree of variability existing on either side. Such is the pattern observed in the radial diameter indices.

Clavicle length asymmetry produces a complex pattern of both fluctuating and directional asymmetry. This variable appears to assume a fluctuating pattern earlier in life, when developmental stability is the primary determinative force and prior to the onset of major mechanical stressors. Later in life the clavicle serves as part of the shoulder girdle

complex, bearing and redistributing weight. Clavicle asymmetry alternates between right and left-bias during early childhood, becoming increasingly left-biased with age.

Radius diameter shows the most recognizable fluctuating pattern. Within this sample measurements of diameter do not appear to be heavily modified by loading stress, and therefore asymmetry may be more reflective of the external developmental environment. Mean values show high variability between the cohorts, especially at the youngest stages. Dissimilarity tends to decrease with age, and variation between the cohorts is correspondingly reduced. Presumably, as individuals grow and age their development becomes steadier. A variable closely related to environmental and developmental stability should have an inverse relationship between variation and age (Trivers et al. 1999).

There are additional skeletal markers of environmental instability in the New Lisbon Collection. The high rate of infant and early childhood mortality historically describes a population of third world status (Veiga et al. 2004). Within the juvenile subset, infectious causes of death are the most common. As previously discussed, high prevalence of contagions are often caused by crowding, poor sanitation, and low access to preventative and acute healthcare.

In addition to contagious disease, there is also evidence of malnutrition found in the New Lisbon Collection. Nutritional deficiency is often observed in skeletal samples in the form of rickets or osteomalacia. Rickets is characterized by a lack of vitamin D in juvenile skeletons. Vitamin D is responsible for the absorption of calcium and phosphorus in the bone matrix, and is usually obtained through ultraviolet rays or a dairy-rich diet. Without Vitamin D, bones are incompletely mineralized and more plastic to loading. The effect of rickets on a growing child's skeleton is a bending deformity of weight-bearing long bones. Pathology may heal in adulthood, but residual curvature is often lasting and discernable in skeletal

material. See Figure A5 in the Appendix for examples of healed rickets in the New Lisbon Collection. The adult form of rickets is referred to as osteomalacia. Also caused by nutrient deficiency (Vitamin D as well as protein and fat), osteomalacia is diagnosed by the bending and collapse of additional weight-bearing elements: the sternum, vertebrae, sacrum, and os coxae. Osteomalacia is not present in long bones, as they are fully formed in adult skeletal material. See Figure A6 in the Appendix for potential evidence of osteomalacia.

While adult individuals pictured in the Appendix obviously survived these periods of nutritional scarcity, individuals in the juvenile subset may not have been as lucky, ultimately contributing to their mortality. Furthermore, radial fluctuating asymmetry was observed at higher levels in the juvenile males (though insignificantly higher). Males are categorically more susceptible to violations of developmental stability. In periods of environmental scarcity, males are more likely to show anthropometric modification like stunting in height, wasting of body fat and musculature, and higher levels of dental asymmetry (Bogin 1999; Garn et al. 1965). Even in the twenty-first century, a study on height of Portuguese children found males to be at least one standard deviation smaller than their female counterparts in nearly all age-groups (Varela-Silva and Bogin 2003). Furthermore, males appear to be more expendable at the population level. Juvenile males often have higher mortality rates (Veiga et al. 2004), a pattern which extends into adulthood in the form of lower average life span for men in most populations (Bogin 1999). Therefore, relatively higher fluctuating asymmetry in New Lisbon juvenile males further indicates the presence of environmental scarcity and/or instability.

6.2 Adult Subsample

Significant Side Difference

Results from the adult subset of the New Lisbon Collection confirm the previous literature that arm bones are far more asymmetrical in terms of length and width than bones of the legs. A limited number of archeological samples have demonstrated considerable leg length asymmetries, a result that investigators attributed to the use of the left leg as an anchor, supporting heavy forces produced by archery or bow-and-arrow hunting (Fresia et al. 1990; Ruff and Hayes 1983; Steele and Mays 1995). Adults in the New Lisbon Collection appear to mimic most modern populations for whom bipedal locomotion is the primary stress placed upon the legs.

The majority of right-left discrepancies were found in measurements of length. A few dimensional areas (width and diameter) were found to be asymmetrical, but the indices were dissimilar to those discussed in the previous literature on bilateral asymmetry. For example, Bridges (1991) found Mississippian females to be highly asymmetrical in radial width, while New Lisbon women were approximately equilateral at this measurement, but more asymmetrical in humerus, radius, and ulna length. This implies that Lisbon individuals were engaging in activities different than other archeological populations, resulting in an alternate suite of asymmetries. Moreover, the adult subset shows a more generalized pattern of asymmetry, which may be observed over several measurements on multiple bones. This suggests that individuals were experiencing various forms of mechanical loading, and that the presence of asymmetry cannot be attributed to a single operation or activity such as maize-grinding or washing laundry.

Pattern of Adult Asymmetry

The data support a primarily directional pattern of bilateral asymmetry (BA). That is, asymmetry is clearly and consistently biased towards a single, predictable side. The right humerus, radius, and ulna are larger in all measurements, especially in terms of length. This may be attributed to preferential use of the right arm for mechanical operations like lifting, pulling, and carrying. Alternatively, the clavicle shows a similar pattern of dominance in the left bone, a result which also may be related to use of the right arm. Serving as anchor and strut to the right limb, the right clavicle bears a large portion of mechanical stress (Terry 1932). Studies have shown that the right clavicle endures modification in width and cortical bone thickness, in order to prevent failure and fracture along the axis. In the absence of additional stress, the left clavicle does not receive any extraneous modification, rather developing in a normal trajectory to reach its intended size and shape (Mays et al. 1999). Therefore, the right clavicle is sturdier and more robust in order to withstand additional stress produced by the right arm, while the left clavicle represents normal growth and development.

Fluctuating asymmetry (FA) also potentially exists among the adult in the New Lisbon Collection, but its presence is probably masked by the high degree of lateralization observed in the long bones. Effects of FA may be extracted from asymmetry indices according to methods by Palmer and Strobeck (1986), applied by Trivers et. al. (1999). Because directional asymmetry was so highly discernable and amenable to a wide variety of statistical analyses, investigation of FA was beyond the scope of the present work. However, given the great potential for early developmental as well as mechanical stress, the presence of FA should and will be investigated in future research.

The majority of variables are asymmetrical in terms of length. This implies that observed bone modification occurred in late adolescence and very early adulthood, prior to

the fusing of long bone epiphyses (terminating linear growth). This period corresponds to ages 10 to 25 years (the age by which most males and females complete long bone epiphyseal closure). This is consistent with data from the juveniles, as humerus length demonstrated the highest directional asymmetry in the older cohorts. Habitual manual operations producing asymmetry began at a young age and likely continued throughout adult life.

Supplementary evidence in the form of historical records relates a high level of mechanical stress among nineteenth and early twentieth-century Portuguese. Mortuary documents on occupation list high proportions of manual laborers. Although Lisbon is Portugal's most urbanized center, citizens participated in subsistence level workloads. Typical vocations included: mariner, shipping-dock worker, agriculturalist, fishermen, soldier, and construction worker. Each of these activities would require an intensive level of physical labor and informal apprenticeship at a young age.

Skeletal pathologies in the New Lisbon Collection further support a mechanical explanation for observed asymmetry. The adult subset demonstrates a wide range of skeletal degeneration at various joints and points of muscle-tendon attachment. Although degenerative modification is more common in older individuals, it may commence at any age due to chronic stress caused by the wear and tear of habitual usage (Dangerfield 1994). Deterioration in relatively young and middle-aged skeletal samples is frequently observed in the New Lisbon Collection.

Osteoarthritis (OA), or Degenerative Joint Disease (DJD), is the most common joint affliction in both modern populations and in archeological groups (Ortner 2002). Osteoarthritis begins with a loss of cartilage interfacing the bones in a joint, followed by a reaction of the exposed bone on this surface and at the joint margins. Characteristic reactions include: the formation of bony spurs (osteophytes) along joint edges, the creation of lamellar

bone bridging the synovial gap (created by cartilage deterioration), fusion of the bones in the joint, as well as pitting and porosity (Rogers and Waldron 1995). Eburnation, the polishing of joints surfaces caused by bone-on-bone friction in the absence of cartilage, is also associated with osteoarthritis. In order to diagnosis OA in a skeleton, eburnation or at least two other characteristic indicators must be present (Rogers and Waldron 1995). See Figure A7 in the Appendix for examples of osteoarthritis in the New Lisbon Collection.

Along with osteoarthritis, skeletal fractures are also indicative of high levels of mechanical loading stress (Stirland 1993). In modern groups, a high percentage of fractures are incurred indulging in sports activities or automobile accidents. But incidence of fracture in Portuguese samples is more likely due to work-related accidents. The most common type of healed fracture observed in the collection involves the medial portion of long bones, or areas associated with joints (such as the neck of the femur). See Figure A8 in the appendix for examples of fracture present in the New Lisbon Collection.

These suppositions were statistically verified by direct comparison between manual and nonmanual laborers. As hypothesized, the individuals who were employed in manual vocations had significantly higher asymmetry at all measured variables. While beyond the scope of the present work, it would be interesting to analyze differences in osteoarthritis and fractures between the occupational groups, as well as a correlation between frequency of fractures and level of long bone asymmetry.

Sexual Dimorphism in Adult Asymmetry

As with the juvenile subset, variable selection methods were employed to generate an optimal classification model including six variables for adults. The model revealed insignificant difference between the sexes in all but one variable. Results oppose the

standard hypothesis. Males were expected to demonstrate higher asymmetry due to greater participation unimanual activities, especially considering typical strenuous male professions.

Most female occupations are listed as “domestica” in the historical records, which conveys the role of a housewife but does not describe the range of activities or the workload required. It is very possible that the female workload was relatively high in historic Lisbon, particularly during the dictatorship. Ethnographic and historic records hold Portuguese women responsible for all domestic and family affairs (Cardoso 2005). This could entail any number of activities from tending fields, working on the docks with their spouse, working as a seamstress or laundress, or toiling in textile factories. Even simple domestic duties like cooking and washing were made more difficult by the lack of utilities in Lisbon, as virtually no home had private plumbing even by 1950, and water had to be carried for miles from public facilities (Moreira 1950). Reher (1998) noted in his analysis of Portuguese life that women provided for the family during periods of hardship or when the patriarch was seasonally employed abroad. Therefore, the correct expectation may be relatively high levels of bilateral asymmetry for both sex-groups when compared to other populations, but without significant difference from one another.

Of the variables where males and females do differ, these may be attributed to more frequent use of particular musculature systems. The male versus female averages for each variable are displayed in Figure 23. Males tend to have higher asymmetry in indices of the upper arm: clavicle and humerus (length and maximum diameter), while females have slightly higher asymmetry in the forearm: radius and ulna. This suggests that males may have been using the shoulder girdle more heavily in actions involving lifting, pulling, and carrying weight. Alternatively, females may have performed more duties requiring dexterity of the forearms and wrists, with pressure applied to the elbow joint. Anthropologists

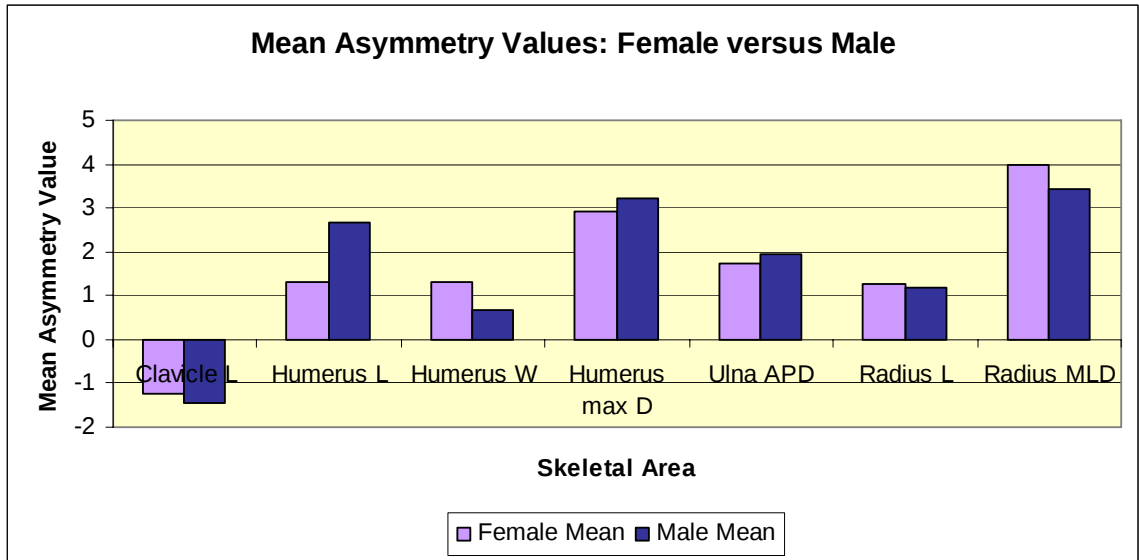


Figure 24. Mean Asymmetry Value for New Lisbon Collection Males and Females at each Asymmetry Index

generally discourage suggestion of precise actions and motions creating bilateral asymmetries, however, the New Lisbon Collection provides a clear link between documented level of activity and resulting degree of asymmetry.

Asymmetry through Time

The period used in temporal analysis spans years 1806 to 1945, represented by samples' year of birth. A multivariate analysis of variance (MANOVA) was used to analyze difference in the mean asymmetry value of each group (average of that ten year period of time) in four indices: clavicle, humerus, radius, and ulna length. The pattern produced by the twelve decade-groups shows a notable increase in humerus and clavicle asymmetry occurring in groups 8, 9, and 10. Randomization on the twelve groups was not possible due to a lack of statistical software with permutation MANOVA capabilities. Yet other available validation methods confirm significant difference localized in groups 8 – 10. These groups correspond to birthdates 1890 to 1919.

Because bilateral asymmetry in the adult population fits a directional model of mechanical stress, the period of interest is actually when the greatest mechanical loading would have occurred. For length asymmetry, the crucial period of linear long bone growth is approximately the first twenty years of life. Therefore, the period of time associated with the greatest mechanical stress spans 1890 to 1940. This coincides with an era of significant socioeconomic and political transition in Portugal.

At the end of the nineteenth century, the last great pandemic episodes (water born contagions: cholera, yellow fever, etc) took a tremendous toll in Portugal, eliminating a significant portion of the national population (Gomes et al. 1999). Precise mortality rates are unclear as census records are scarce and unpublished from the era. It is possible that contagious disease had a lesser effect in urban areas like Lisbon, but this hypothesis remains unsubstantiated by the available data. For most moderately modernized countries, epidemic diseases create a waterfall of detrimental effects, indirectly affecting most members in a population. Pandemic disease likely disturbed traditional Portuguese socioeconomic structure, which was already tenuous at best.

In periods of serious illness, adolescents and young adults would be the first family members commandeered to assist their parents (Kaplan 1998). While mothers were preoccupied caring for ailing relatives (children and the elderly), older daughters were needed to perform many of the intense household duties like transporting water, cooking, cleaning, and textile working. And if the mother herself were afflicted, the oldest daughter would accept all domestic responsibilities (Reher 1998). Similarly, if the father became ill, older sons would assume the role of patriarch, performing their father's job or finding additional employment. Most occupations available to young men at the turn of the century required hard labor from unskilled workers, requiring only "*um atrás, jovem y forte*" (a

young and strong back) (Kaplan 1998). Even if parents were fully capable, working offspring could have provided the extra income needed to pay for doctors and medication.

The Portuguese political system also experienced several key events at the turn of the century which held great consequence for its young citizens. The Portuguese monarchy had been in a severe state of decline and lacking strong leadership for several decades, beginning approximately 1880 (Kaplan 1998). The regicide of King Manuel I in the year 1910 ended Portugal's monarchical system of government and threw the country into a period of turmoil. The subsequent Republican revolution was extremely volatile, as various factions violently fought for control. In approximately fifteen years, Portugal endured more than 45 different governments. Stability was finally achieved in the form of a "*pronunciamento*", a military coup by António de Oliveira Salazar in 1926 (Kaplan 1998). Although Salazar's administration promised solidarity, it ultimately brought the Portuguese people decades of cultural, social, and economic depression, robbing them of their formerly-proud identity (Cardoso 2005).

Presumably this transitional period, encompassing the monarchical fall and rise of the new dictatorship, represents the time of greatest stress. The "*escudo*" (former Portuguese currency) diminished in value while becoming increasingly more difficult to earn. Individuals had to work harder and longer hours in an attempt to maintain the same standard of living (Corkill 2003). Preventing this were several governmental policies imposed by Salazar. Large tracts of arable land are rare in Portugal. Consequently, productive private farms were taken under government ownership, forcing many families into subsistence farming (Corkill 2003). A standard tour of military service was required by a generation of young Portuguese men to fight Salazar's disputes in Africa (a final attempt to salvage Portugal's colonial power). Similar to Roosevelt's New Deal in America, Salazar

also created several public works projects, extending and repairing the infrastructure of the country. However in contrast to Roosevelt's plan, labor was mandatory and provided meager compensation to the workers (Kaplan 1998). Regardless, many chose such high labor occupations because it provided job security or assured government-issue housing, although it was typically inadequate and over crowded (Kaplan 1998).

In addition to higher workloads, fewer resources were available to buffer the detrimental effects. Food resources decreased in availability and nutritional quality. The continued use of public water facilities, in conjunction with over-population in the city center of Lisbon, promoted the spread of infectious diseases (Gomes et al. 1999; Santos and Roberts 2001). Although sickness and injury were commonplace, treatment through public or private healthcare was scarce. Preventative care, such as immunizations, was not readily available until the mid 1960s (Gomes et al. 1999). The primary system of acute care was funded by religious alms, an arrangement which proved completely inadequate (Veiga et al. 2004). The implication is that many individuals continued to work while sick and/or injured because they could neither afford the proper healthcare nor the missed wages. Such a system predicts exactly the type of skeletal pathologies commonly observed in the New Lisbon Collection: improperly healed fractures, early skeletal degeneration, and evidence of infectious diseases.

Throughout most of the nineteenth century (represented by the first seven temporal groups used in this study), minimal fluctuations in the degree of directional asymmetry are observed. Beginning with birthdates 1890, significantly increased asymmetry is noted in two of the investigated variables: clavicle and humerus length. Average asymmetry levels remain high until birthdates 1920 (group 11), when asymmetry subsequently begins to decrease. Notably, these were also indices in which the males had slightly higher average asymmetry, significantly so in humerus length. This suggests that over time, males experienced greater

change in their activity and asymmetry patterns than females. This finding is supported by cemetery records, as the number of manual-laborers also decreases through time. For females, domestic employment is generally constant throughout each decade. Unfortunately there is no comparable means to investigate the transition in female domestic activities over time.

Comparison against Modern Skeletal Material

Hotelling's T-test was used to assess difference in asymmetry between samples in the New Lisbon Collection (NLC) and samples included in the modern Forensic Databank (FDB). While sex-related difference in asymmetry is minimal in the New Lisbon Collection, sexually dimorphic asymmetry is unknown for the FDB. Therefore, sexes were separated for comparison. Tests revealed significant difference in three out of the five tested indices, with Lisbon samples demonstrating higher asymmetry in every variable. In fact, the average Lisbon female value was higher than the mean FDB male at each index.

Results cannot be attributed to age, as the average age-at-death is comparable between the samples. In the absence of major demographic differences (age, sex) the primary cause of variation in directional asymmetry tends to be differential degrees of mechanical stress. Presumably, individuals in the NLC were engaging in a higher proportion of unimanual activities requiring more intensive labor. Occupation is undocumented for FDB samples so habitual actions are unknown. However, most samples are sourced from the Eastern United States, born within the twentieth century. Basic access to resources and healthcare may be confidently assumed. While the two samples are generally contemporaneous, the average date of birth in the FDB is several decades later than that of the NLC. The NLC samples represent a more depressed population from a slightly older

period. Resulting higher levels of asymmetry in the NLC than for the FDB is therefore not surprising.

6.3 Suggestions for Future Research

Fluctuating Asymmetry

Investigation of fluctuating asymmetry (FA) in long bones of the adult subsample was regrettably neglected in the present work due to time constraints and research focus. However, FA is readily calculated from adult long bone measurements using methods by Palmer and Strobeck (1986) and applied by Trivers et al. (1999). The mean difference for each character is subtracted from individual side difference (right – left). The remaining value represents FA (Palmer and Strobeck 1986). Levels of FA could then be correlated with observed levels of directional asymmetry in the adult subset to test for similar pattern trends through time.

In the juvenile subset, further investigation of individual socioeconomic status of samples would provide a closer relationship between environmental stability and resulting fluctuating asymmetry. Following the methods of Cardoso (2005), additional historical records describing place of birth, father's occupation, and average household income could be used to categorize juvenile samples into several hierarchical groups of socioeconomic status. Groups could then be compared in terms of their pattern and degree of asymmetry. Access to government records and lack of Portuguese literacy prevented a more comprehensive investigation in the current research.

It would also be productive to investigate additional indicators of developmental stress, and correlate these markers with levels of fluctuating asymmetry. Potential skeletal indicators include: Harris lines, linear enamel hypoplasias, cribra orbitalia, and delayed

growth (shorter stature) in the juvenile subset (Ortner 2002). Dentition could also be analyzed in terms of FA, following the methods of Keiser (1990) by comparing dimensions (bucco-lingual and mesio-distal) of the second molar for asymmetry. The amount of time allotted to data collection prevented further analysis, but the expectation holds that degree of FA will demonstrate a high correlation with additional evidence of resource scarcity.

Finally, the role of genetic determinism in fluctuating asymmetry could potentially be assessed in the New Lisbon Collection. If original government, religious, or family records were used to reconstruct family lineages (linking adult samples to juvenile samples or at least listing the number of offspring) degree of asymmetry could be compared between parent and offspring. Previous studies have shown a high correlation between maternal fluctuating asymmetry and that of her child (Livshits and Kobylansky 1984). Most studies of this type have been performed using radiographs (producing an incomplete picture of true skeletal structure), or on aborted fetuses (representing highly altered developmental pathways) (Livshits et al. 1988; Livshits and Kobylansky 1989). The New Lisbon Collection could provide a skeletal sample to evaluate level of heritability in fluctuating asymmetry – but only if familial relationships and/or fecundity could be determined.

Directional Asymmetry

Long bone length and external measurements were utilized in this study due to availability of the necessary measuring equipment and ease of transport. However, it may be more productive to analyze bones in terms of shape and three-dimensional morphology, requiring the use of CT-scanning technology. In response to loading stress, skeletons in the New Lisbon Collection may have been modified in terms of three-dimensional shape: thickening and buttressing of compact bone, in addition to absolute

length. The cause of three-dimensional alterations is similar to that which produces length asymmetry: redistribution of weight, prevention of avulsion, and buffering against mechanical stress. This type of asymmetrical change may be observed in elements such as the femur, which were not considered significantly asymmetrical in this study.

Dimensional measurements of the femur have demonstrated DA in previous studies on archeological population, using CT-scanning methods (Ruff and Hayes 1983; Ruff and Jones 1981).

Temporal changes in directional asymmetry may also be correlated with other indicators of skeletal change in the Lisbon populations, such as secular increases in statures and craniometric morphology. Multiple corresponding indicators of change would underscore significant periods in Portuguese history instigating skeletal modification. Such a result would support the use of bilateral asymmetry to interpret general patterns of socioeconomic transition at the population level.

Finally, levels of directional asymmetry in the New Lisbon Collection should be compared against samples from other Portuguese collections, such as the Coimbra Identified Skeletal Collection (CISC) housed at the University of Coimbra. Samples in the Coimbra Collection are from roughly contemporaneous with the New Lisbon Collection (birthdates spanning mid-nineteenth to mid-twentieth century), but primarily sourced from agricultural areas in central and northern Portugal. These samples may demonstrate a different pattern of mechanical loading stress, and may possess lower, higher, or equivalent levels of asymmetry. Comparison would provide a more comprehensive picture of asymmetry throughout the Portuguese nation and determine if

the effects of mechanical stress were generalized throughout Portugal or concentrated within Lisbon.

6.4 Conclusion

Investigation of bilateral asymmetry in the long bones of skeletons included in the New Lisbon Collection yields the following conclusions –

For the Juvenile subset:

1. Significant asymmetry exists in the juvenile subset, particularly in measurements of clavicle, humerus, radius, and ulna length.
2. No difference in level of asymmetry could be determined between the sexes, indicating that juveniles were uniformly responding to environmental and mechanical stress.
3. Moderate difference in clavicle asymmetry was found between infectious and sudden causes-of-death, which may be related the prevalence of tuberculosis in this population and its effect on clavicle morphology.
4. Clavicle and humerus length asymmetry have the closest correlation with juvenile age, increasing with maturity. Asymmetries progressively assume the adult pattern of right-dominance in the humerus, and left-dominance in the clavicle.
5. Juvenile samples demonstrate primarily the initiation of directional asymmetry patterns. Skeletal areas experiencing greater loading, such as clavicle and humerus length, show high lateralization related to mechanical loading stress. A variable with less direct causation, like radius diameter, appears to be more closely related to environmental stability and represent the best example of fluctuating asymmetry in this sample.

For the Adult subset:

1. Significant asymmetry exists in the adult subset; patterns correspond to those observed in other archeological and modern populations. The upper limbs are characterized by right-side dominance, while the clavicle alone shows left bias. Lower extremities fail to demonstrate significant asymmetry.
2. Asymmetry in the adult sample assumes a highly directional pattern, potentially masking moderate fluctuating asymmetry. Skeletal modification is primarily caused by mechanical loading stress, as inferred via known occupation.
3. Sexual dimorphism in asymmetry could not be determined in the adult subsample, a result attributed to relatively equal levels of habitual mechanical loading.
4. Individuals employed as manual laborers possess significantly higher asymmetry than non-manual workers at all measured variables.
5. Two variables, humerus and clavicle length, vary in degree of asymmetry through time. The period contributing the greatest change spans years 1890 to 1930, corresponding to an era of significant political transition and socioeconomic depression in Portugal.
6. Samples in the New Lisbon Collection are far more asymmetrical than modern skeletal material included in the Forensic Databank, presumably due to higher activity patterns and greater mechanical stress incurred in adolescence and early adulthood.

Bilateral asymmetry in the New Lisbon Collection generally reflects a population experiencing moderate to high levels of environmental and mechanical stress. Historical

documentation corresponding to the period from which skeletal material was received supports the existence of significant social and political instability within the Lisbon environment. Greater nutritional and mechanical loading stress was placed upon individuals during crucial life stages of growth and development and adult livelihood, consequently creating higher lateralization in the long bone morphology. Furthermore, levels of bilateral asymmetry in the Lisbon sample are significantly higher than that of modern American skeletal material, presumably due to differential access to resources and activity patterns.

Results from the New Lisbon Collection demonstrate a direct relationship between activity levels and resulting forms of bilateral asymmetry. Extensive mortuary records correlate each the skeletal sample with known occupation and the external stressor inducing bone modification. This relationship may be extended to other studies within anthropology.

Bioarcheologists and paleoanthropologists seldom possess comparable documentation associated with their research, and their conclusions must exclusively drive from skeletal and material evidence. Results from the New Lisbon Collection validate hypotheses of increased mechanical and/or environmental stress, when high levels of bilateral asymmetry are observed within a sample population. Presumably, prehistoric populations and pre-modern ancestral groups would have experienced levels of stress greater than the New Lisbon Collection, so that significant bilateral asymmetry may be predicted for these groups. The present research provides a model for reconstructing the activity levels and environmental context of past populations, for whom no additional documentation exists.

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APPENDIX

Figures A1. Examples of religious and sentimental items included with the burials, eventually accessioned with the remains in the New Lisbon Collection.



(a) Crucifix icon



(b) Articles associated with an infant



(c) Cross pendant from a rosary

Figure A2. Skeletal elements and epiphyses exemplifying the Four-Stages of Fusion Method. Stage 0 – no fusion (epiphysis completely unattached); Stage 1 – partial or beginning fusion (furrow is less than half-fused); mostly complete or recently fusion (epiphysis is more than half fused or fusion scar remains conspicuous); Stage 3 – complete or terminal fusion.

Stage 0.



Stage 1.





Stage 2.



Stage 3.

Figure A3. Example epiphyses demonstrating developmental bilateral asymmetry. (a) right and left humeral heads (b) right and left distal radii (c) right and left distal ulna



(a) Right and left humeral heads in differential stages of development. The right element was scored at a stage 1, while the left side was more delayed at a stage 0.



(b) Right and left distal epiphyses of the radius in differential developmental stages. The right element is more advanced, scored as a stage 1. The left element, showing no fusion, was recorded as a stage 0.



(C) Right and left distal epiphyses of the ulna in differential developmental stages. The right element is more advanced, and was scored accordingly as a stage 2. The left element, showing no fusion was recorded as a stage 0.

Figure A4. Skeletal evidence of tuberculosis in the New Lisbon Collection.



(a) Clavicle pair with significant asymmetry. The right (above) is notably shorter than the left (below). Cause-of-death was listed as “Potts disease”, an archaic term for tuberculosis.



(b) Vertebral column affected by tuberculosis. Note that fusion occurs at the thoracic rather than typical lumbar region. Minor collapse is also present in vertebral bodies although difficult to discern in the figure.



(c) Ribs showing advanced stages of tubercular modification



(d) Close-up of tubercular rib

Figure A5. Skeletal evidence of healed childhood rickets



(a) Characteristic lateral bowing of tibiae



(b) Left tibia (see above) and associated fibula showing extensive curvature

Figure A6. Possible evidence of osteomalacia



Collapse of the sacrum is characteristic of the osteomalacia. Weight-bearing regions commonly affected in adult population include bones of the pelvic girdle. Causation due to osteomalacia is supposed, as cause of death in this sample was undocumented.

A7. Evidence of Osteoarthritis



(a) Formation of osteophytes, “lipping” along the joint margins of the knee. (distal femur above, proximal tibia below)



(b) Osteoarthritis of the hip joint. Osteophytic lipping has formed along the acetabulum of the os coxa and femoral head. Porosity and mild eburnation are also observed on femoral head.



(c) Extensive eburnation has developed on the distal end of the humerus (capitulum and trochlea)



(d) Sacro-iliac fusion of the right os coxa and sacrum.

A8. Evidence of fracture



(a) Improperly healed fracture located at proximal end of femur. Extensive infection (osteomyelitis) and subsequent modification has occurred.



(b) Fracture involving distal end of both tibia and fibula.



(c) Healed fracture at medial portion of right clavicle. Note, right clavicle typically involved in greater weight-bearing as an extension of the upper right arm.



(d) Irregularly healed break in the posterior portion of a right rib.

VITA

Shannon Elizabeth May was born in Houston, Texas on March 1, 1983. After moving to Austin, Texas with her family, she graduated from James Bowie High School in 2001. Shannon attended Texas State University, graduating summa cum laude in 2005 with a Bachelors of Arts in anthropology. In the fall of 2005 she began her graduate career at the University of Tennessee, Knoxville. She received a Masters of Arts in anthropology, with a minor in statistics in December of 2007. Shannon is currently pursuing her doctorate in anthropology at the University of Tennessee, with a focus in skeletal biology and forensic anthropology.