9-22-2014 12:00 AM

A Statistical Investigation of Nonmetric Vertebral Traits with a Skeletal Population Sample from the Dakhleh Oasis, Egypt

Tiffany A. Sarfo, *The University of Western Ontario*

Supervisor: Eldon Molto, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Arts degree in Anthropology

A Statistical Investigation of Nonmetric Vertebral Traits with a Skeletal Population Sample from the Dakhleh Oasis, Egypt

(Thesis Format: Monograph)

by

Tiffany Sarfo

Graduate Program in Anthropology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Masters of Arts

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

Abstract

This paleogenetic study utilizes 17 nonmetric epigenetic vertebral traits to determine their suitability for studying past genetic relationships. The samples utilized were from Egypt's Dakhleh Oasis. Though infracranial nonmetric traits have a limited role in the study of past population genetics, this study has shown their value for elucidating past genetic patterns for intragroup analysis. The key to their utilization is to test the epigenetic factors (e.g., age, sex, symmetry and intertrait correlations) which were done using a number of statistical tests including Phi coefficient, G-test and the Odds ratio. This study utilized a novel set of spatial statistics to examine within-group genetic dynamics of the Kellis 2 cemetery. Five traits support previous research that demonstrated this cemetery was organized along patrilocal and patrilineal lines. This thesis has demonstrated the genetic value of vertebral epigenetic traits and argues for their continued use in paleogenetic research.

Keywords

Epigenetic trait, nonmetric trait, intertrait correlations, genetic, phi coefficient, odds ratio, spatial analysis, kinship analysis, Dakhleh Oasis

Acknowledgements

I would like to extend my thanks to all those who have supported and encouraged me throughout this thesis project:

My supervisor, Dr. Eldon Molto for your guidance, support and your invaluable insight which has been integral for the completion of this thesis.

To James Keron, thank you for your time and patience as you have been essential for the spatial statistical portion and maps in this thesis.

I also would like to thank my examination committee, Dr. Ian Colquhoun, Dr. Alexis Dolphin and Dr. Dan Shrubsole for taking the time to read this thesis and providing me with insightful feedback.

To my friends, thank you for all the laughs, moral support and friendship throughout this journey as well as to those few friends who graciously opened their homes to me throughout this year.

And finally, to my family, thank you for the prayers and moral support, and for the push I needed to pursue my Master's.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1

Statement of Problem

1.1 Introduction

Epigenetic traits of the human skeleton, particularly of the skull, have had a long history in the study of paleogenetics. The last century, in particular, witnessed both the rise and fall of the use of epigenetic nonmetric traits in paleopopulation research. Part of the fall was attributed to rise of molecular anthropology in the 1980s and 1990s, whereby actual DNA could be analyzed to determine genetic relationships. This was coupled with problems in the research designs of the traditional morphologically-based paleopopulation genetics and the misunderstanding of the expression of epigenetic traits in the development of morphological variation. The pioneering article by Berry and Berry (1967) posited that nonmetric epigenetic cranial traits were highly genetic, were independent of each other, were independent of age and sex, and could be easily scored and standardized. Critical evaluation of these assumptions in the latter decades of the 20th century resulted in many challenges, particularly in the development of proper traitlists (Ossenberg 1976). For example, of the original 30 traits overviewed by Berry and Berry, only 7 can be confidently used to study paleopopulation genetics (Molto personal communication 2014). Yet, the vast majority of researchers in the period between the 1970s and 1990s utilized the Berry and Berry traitlist with conflicting results arising as a consequence. Researchers who examined the assumptions generally found that many nonmetric traits were not independent of age, sex and symmetry (Ossenberg 1969, Suchey 1975, Molto 1985), and above all were not easily scored and standardized (Molto 1983). The latter is a fundamental requirement of the scientific method.

While, the majority of nonmetric trait studies focused on the traits of the skull for several reasons (i.e., many traits in a single unit, many museum collections had only skulls etc.), few researchers used infracranial traits. Most notably, Saunders (1978) demonstrated their potential for addressing paleogenetic relationships, as did Barnes (1994) more recently for axial skeleton variants. However, the same problems that

limited the use and value of cranial traits affected the infracranial data. For both cranial and infracranial traits, there were no clear descriptions, precise photography or diagrams, and no standard database. Even the attempt by Buikstra and Ubelaker (1994) to produce a standard is open to criticism as the traits are poorly described and the diagrams are imprecise. In fact, they had a total of 33 traits – 28 from the skull and 3 from the infracranial skeleton, many of which were cranial traits from the Berry and Berry trait list. Moreover, close examination of the cranial traits shows several of dubious values for scoring reliability (e.g., mastoid foramen, infraorbital foramen, flexure of the superior venous sinus) and/or genetic meaning (e.g., auditory exostoses, mandibular torus). They described only three infracranial traits, all from the cervical spine (posterior and lateral bridging of the atlas, and divided foramen transversarium of C7). As Buikstra and Ubelaker's (1994) publication is currently the most commonly reported source for nonmetric traits, the dearth of information on the infracranial skeleton is noteworthy.

While researchers in the past argued, quite correctly, that the prevalence of nonmetric traits at the population level (p/n = %) effectively reflected genomic differences, the fact that the heritability of nonmetric traits was quite variable is a major criticism for their use in paleogenetic research (see Sjøvold 1984). Today, with the advent of molecular genomics, the role of epigenetic factors (e.g., nutrition) in the genesis of both normal and disease processes has taken on a new meaning. It is probably quite correct to suggest that we are witnessing a paradigm shift in genetic research, as epigenetic factors are known to be critical in the development of normal and abnormal variation and diseases like cancer. The implications of epigenetics for nonmetric traits, particularly the rarer ones, can be used to predict which individuals would be most closely related which then can be used as markers to target skeletons for molecular investigation.

With the above as a brief background, this thesis focuses on the use of infracranial nonmetric traits of the vertebral column to investigate intragroup paleogenetic relationships in a population sample from the Dakhleh Oasis in the middle of the Egyptian Sahara desert. *The Null Hypothesis (H_o) is that these data are not applicable to elucidating past population genetics*, a hypothesis that is designed from previous

nonmetric cranial and infracranial paleogenetic research on the Oasis samples. More specifically, I examine the following questions:

1. Can infracranial nonmetric traits be used to effectively study genetic affinities of past populations?
2. Are the vertebral traits independent of significant epigenetic influences of sex, age, or symmetry?
3. Are vertebral traits statistically independent of each other, and if not, are there specific developmental patterns (e.g., caudal or cranial shifting) that can explain these findings?

The thesis has five additional chapters. Chapter 2 overviews nonmetric traits in a historical context, focusing on past and current research in bioarchaeology. Chapter 3 reviews the growth and development of the vertebral column and describes, in detail, the 17 nonmetric traits that will be used in this thesis. The chapter includes a discussion of the various factors that can influence development of the spine. Chapter 4 describes the archaeological site and the population samples. An overview of the methodological and statistical approaches for the examination of the epigenetic factors is also provided (age, sex, symmetry, intertrait interaction). Chapter 5 provides the results of all statistical tests with emphases on significant intertrait correlations and the patterns of spatial analysis for one of the cemeteries. The final chapter, discusses the significance of the results in terms of how the interpretations improve our understanding of human biology of the Oasis population(s) and provides future directions for nonmetric epigenetic traits in bioarchaeological research.

Chapter 2

Background

2.1 Introduction

This chapter briefly reviews the historical background of nonmetric traits, and examines relevant research that has further developed our understanding of nonmetric variants. Emphases on studies regarding infracranial nonmetric traits as well as the role of epigenetic influences are covered. This chapter concludes with the restatement of the research questions being addressed in this thesis.

2.2 Nonmetric Traits

Nonmetric traits, also called discrete, epigenetic, discontinuous, and quasi-continuous traits, can be readily recorded on skeletal material (Buikstra and Ubelaker 1994, Saunders and Rainey 2008). These traits are not generally measured; they are visually scored. Most traits can be categorized into five groups; hyperstotic, hypostotic, foraminal, fusion and other (e.g., variations in shape). Hyperstotic traits represent ossification into structures that are normally soft tissue or ligamental, while hypostotic traits represent some form of ossification regression. Foraminal traits are characterized by variations in the number and location of foramina while fusion encompasses sutures, ossicles and small bones which can appear on suture lines and are commonly seen on the skull (Richtsmeier and McGrath 1986, Buikstra and Ubelaker 1994). Variations in shape can reflect differences in growth patterns such as the fronto-temporal articulation on the skull. In most cases, nonmetric variants have no or limited effect on the function of the skeletal-soft tissue elements although there can be clinical symptoms or genetically-based conditions linked to the presence of a trait (Saunders and Rainey 2008).

Nonmetric traits have a number of advantages over metric traits, particularly, for use in paleogenetic research in that they can be scored on fragmentary, incomplete and even poorly preserved bone – the predominant condition in archaeological assemblages (Buikstra and Ubelaker 1994). Although most traits are usually scored dichotomously,

certain traits are scored gradationally, although statistically they still rely on the dichotomous scoring system. Most traits at the population level (i.e. prevalence) are hypothesized to be under genetic control, which has made them suitable markers of biological relationships between and within populations.

2.3 Historical Background

Biological anthropology has focused on the study of human origins and human variations in antiquity with formal writings first appearing in the Enlightenment period of the eighteenth century (Little and Sussman 2010). Friedrich Blumenbach, the father of biological anthropology, focused on human cranial variations within and among populations. While biological anthropology was not considered a discipline during Blumenbach's time, his research, which included scoring skeletal variants, would be the starting point for the discipline and for intensive research on the human species (Birx 2010). During the eighteenth century, race was the central focus in the study of human variation and remained a dominant theme for over a century. Today, with the advent of genomics the question of race is now resolved, it is clear humans do not exhibit biological races (Long and Kittles 2009).

The importance of nonmetric traits in skeletal biology, as noted in chapter one, has had somewhat of an acrimonious course, particularly in regards to infracranial variations. No doubt the emergence of evolutionary theory in the mid-19th century and the genetic revolution of the early to mid-20th century elevated their potential. In keeping with the early development of biological anthropology, nonmetric trait variations were used to justify racial classification, particularly from the skull. A hierarchical programming of cranial traits emerged whereby the search for traits commonly found among 'lower life forms or animals' became the main objective to support the idea that certain population groups were superior to others (Saunders 1978, Armelagos and van Gerven 2003). With time, it became clear that the logic behind racial hierarchies was without scientific merit and soon reliance on morphological traits fell into disuse.

The re-emergence of research on discrete traits in the mid-20th century coincided with the emergence of the New Physical Anthropology (Washburn 1951), which

emphasized genetic explanations behind human variations, and tried to take the field in a new direction - that of avoiding racial typology. With monogenetic traits being emphasized (e.g., blood groups), it was argued that nonmetric skeletal traits where similar to blood types and were much better suited to the study of microevolutionary changes than the multifactorial craniometric traits. This hypothesis has since been rejected in recent decades (Molto 1983).

As noted, the majority of research focused on cranial traits, with very few researchers examining infracranial skeletal traits- a trend that would continue well into the 21st century. One primary reason for this emphasis is that many of the skeletal collections in the world's museums only contain crania. The pioneering research of Berry and Berry (1967) mentioned in chapter one, in fact, utilized population cranial samples from several museums (e.g., British Museum of Natural History) representing broad populations to define the role of discrete traits in biological anthropology. Unfortunately, as noted previously, their trait list was not suited to the problem of determining relationships in past populations, and their trait list became an Achilles' heel for the science (Molto 1983).

Scott (1893) was one of the first individuals to utilize infracranial traits using samples that represented the Maori and Moriori while LeDouble (1912) was the key resource in trait identification. LeDouble, a French anatomist, in addition to working on the cranium and face, also did extensive work on the vertebral column. Le Double noted every possible variation that could be found on the vertebral column although he did not account for age, sex and side.

The seminal work by Earnest Hooton et al. (1930) on the Pecos Pueblo population in New Mexico was very important for the development of the use of nonmetric traits. Approximately 102 nonmetric traits known as 'Harvard forms' found on the cranium and the infracranial skeleton were recorded; the work of Hooton et al. (1930) was among the earliest to examine the influence of side, age and sex on prevalence and expression. Their work continues to be a key resource for forensic anthropologists when assessing ancestry (Hefner 2009). Up until the 1950s, there were few publications that looked at particular individual traits on the infracranial skeleton (Kiyono and Miyamoto 1926, Hrdliçka 1932,

1934, Snow 1948). Over twenty years later, Saunders' (1978) work on nonmetric traits in the infracranial skeleton would set the standard for using infracranial traits in bioarchaeology research.

2.4 Genetics

In 1951, Washburn promoted the "New Physical Anthropology", advocating for the use of the experimental method in anthropological research. In genetic studies, this research was pioneered using studies of *Mus musculus* (the house mouse). Major research by Grüneberg (1954) entitled, "Genetical Studies on the Skeleton of the Mouse" pioneered experimental research on nonmetric traits. The experiments involved studying nonmetric trait development in inbred lines of mice with the goal of understanding and determining the mode of inheritance. Grüneberg quickly discovered that there was no simple genetic interpretation that could be made. The traits he studied did not follow a simple Mendelian mode of inheritance as correlations found between parents and the offspring did not result in the expected outcomes. He explained this incongruence as expression of the extreme ends of a continuous distribution.

A model for a genetic control of variants was then proposed by Grüneberg where he discovered among the inbred strains that individuals with a common trait were genetically alike to each other while those without the trait were also genetically alike to each other. He attributed this to single gene mutations as a potential inducer for the formation of the trait. He proposed that there was a physiological threshold that determined the presence of the trait; individuals who pass the threshold, will see the trait manifest.

Using the absence of the third molar (a trait) as an example, Grüneberg coined the term quasi-continuous. He describes these types of traits as having a continuous distribution that is rendered discontinuous by a limiting threshold. Any given trait has a continuous distribution found within the genome which can be influenced by genetic and environmental factors. A discontinuous threshold is imposed on the distribution which marks whether or not a trait is manifested in the individual. In the case of the absence of the third molar, a link was made between the trait and the size of the tooth germ. The

absence of the third molar which is a discontinuous trait from an underlying continuous distribution is affected by the size of the tooth germ which is influenced by both genetic factors of the individual and the mother. Any size variations can be attributed to both environmental factors such as maternal environment and genetic influences as multiple genes with small additive effects are deemed factors.

Grüneberg then set out a number of parameters describing the properties of a quasi-continuous trait. For quasi-continuous traits, there is a correlation between penetrance and expressivity. The further shifted away the traits are from the critical level of the continuous distribution, the higher the percentage of individuals with the trait and the greater the expression will be on affected individuals. Grüneberg also emphasized the fact that quasi-continuous traits are sensitive to environmental factors which can occur *in vivo* or post-birth, and that effects of multiple genes are additive, although quasi-continuous traits can sometimes be strongly influenced by single genes.

Since the work of Grüneberg, there have been further mice and rodent studies. Searle (1954a) noted genetic differences were the same within and between mouse strains. Collective work by Deol, Grüneberg, Searle and Truslove (1957) found that the changes they saw among seven mouse strains over fifteen generations must be genetic in nature as the changes could not be attributed to a dietary cause.

In 1967, Berry and Berry published "Epigenetic Variation in the Human Cranium", a paper that was groundbreaking for the study of nonmetric traits and was heavily influenced by Grüneberg's work. Berry and Berry focused on their genetic properties, citing that nonmetric traits (what they called "epigenetic") were genetically determined based on the fact that: (1) family studies have shown that such traits are inherited; (2) the frequency of a particular trait is constant in a given population; and (3) the quasi-continuous traits described by Grüneberg are inherited entities. With the use of crania from eight different populations and examining 30 traits, Berry and Berry concluded that nonmetric variants were superior to morphological measurements for determining biological relationships as these variants lacked associations with sex, age, and correlations with each other, and they were easily scored; this made these traits ideal for statistical analysis for biodistance studies. However, there are a number of problems

with the claims made by Berry and Berry. Age dependency was inadequately tested in their study, the role of environmental or non-genetic effects on nonmetric variants were downplayed and the methodology used to examine sex differences was flawed as different population groups were amalgamated together and then separated by sex.

Despite these shortcomings, the work of Berry and Berry heavily influenced many publications on nonmetric variants during the 1970s and 1980s as many researchers began to primarily focus on cranial traits with only a few studies on infracranial traits (Finnegan 1973, 1978, Gaherty 1973, Riggs and Perzigian 1977). Unfortunately, the conclusions reached by Berry and Berry led many researchers (Kellock and Parsons 1970, Knip 1970, Rightmire 1972) to assume that traits were free from any significant sex, age or intertrait associations causing methodological issues to appear in many papers (Saunders 1978). While many followed the methodology set out by Berry and Berry, the 1970s and 1980s also saw criticism (Ossenberg 1969, Corruccini 1974, Saunders 1978, Molto 1983) of Berry and Berry's assertions that variants lack age, sex, and intertrait correlations, and a number of individuals set out to look at the non-genetic factors that could affect the manifestation of nonmetric traits.

2.5 Epigenetic Factors

The term 'epigenetic', when first introduced during the time of Berry and Berry's paper, was meant as a term to focus on the modification during development and the non-Mendelian nature of inheritance that nonmetric variants appear to display. As subsequent research shed light on the properties of variants, the definition of epigenetic came to mean external factors that have some form of effect on development. Epigenetic factors, which are thought to stem from embryonic inductions involving tissues or cells from other organs or anatomical systems and appear to have large effects on trait expression, have yet to be resolved or completely understood (Tyrell 2000).

Trait development and trait heritability are key factors for analyzing nonmetric traits but are hard to delineate. For the majority of traits, embryology remains to be poorly understood. Since traits differ from each other with regard to the degree of genetic determination, understanding trait heritability by examining the proportion of the

variance that is attributed to multiple gene effects becomes important (Suzanne 1975, Prowse 1994). Estimates of heritability can only be applied to the population from which it was derived, as trait manifestation differs across populations (Clark 1952). Though there are limited studies for both human and animal models on the heritability of traits, work by Cheverud and Buikstra (1981) determined that the degree of variability was higher in hypostotic and hyperstotic traits in comparison to foramina traits, and heritability is higher in nonmetric traits versus metric traits. Hauser and De Stefano's (1989) work on humans has also shown that expression of a number of traits has a high genetic portion. However, the heritability of nonmetric traits has also shown to be quite variable, which has been a major cause for criticism against the use of nonmetric traits (Sjøvold 1984).

Because trait development and trait heritability are complex, this has, in part, compromised research in this area, posing some issues when studying traits for population analyses. Other factors such as age dependency, sex differences, side expression, and intertrait correlation, which are in part rooted in understanding development and heritability of traits, have been better addressed in the literature but their influences on nonmetric traits are not completely understood (Tyrell 2000).

Like Grüneberg, who did extensive work on the mouse looking at trait variation, there were other investigators during the early 1950s who also contributed work to the study of epigenetic traits using mice. Searle, in addition to his genetic work, wrote a series of articles focusing on non-genetic factors affecting trait appearance. Searle (1954b) looked at the effects of diet change on mouse lines where he observed that changes in diet affected a trait by moving the threshold on the continuous distribution. The change in diet affected the change in body size, which is mediated through maternal physiology. These changes then affect trait frequencies. Similar results were also found by Deol and Truslove (1957) as they also looked at diets and their connection with maternal effects.

A particular focus, during the 1970s and 1980s, on age effects, sex differences, side expression and intertrait correlation began to become more prominent as they contrasted many of the assumptions by Berry and Berry on the minimal effects these

factors had on nonmetric data. Ossenberg (1969), Corruccini (1974), Korey (1980), Saunders (1978) and Molto (1985) during this time looked at other factors that could influence trait appearance.

2.5.1 Age Correlations

Nonmetric traits are believed to be the final points of genetically controlled development processes which are affected by environmental factors (Saunders 1989). Traits are usually characterized by a lack of fusion, and therefore not considered a "variant" until the age of normal development has passed which follows a general age pattern (Saunders and Rainey 2008). Since nonmetric variants are better discriminators of age among adults compared to metric traits, determining age effects among nonmetric variants is important (Corruccini 1974, Carpenter 1976 in Winder 1981). Akabori's (1933) work on cranial nonmetric traits was one of the first studies to show modification of traits through time. This was substantiated by Saunders and Popovich (1978) as they showed the effects of age on trait expressivity. Ossenberg (1969), who took an embryological/developmental approach when studying cranial traits, noted that hypostotic traits decreased with age while hyperstotic traits increased with age as these were found to be more strongly expressed in older individuals, but this expression seemed to be population specific. Ossenberg (1969) also noted that there were traits that were age-stable such as variation in the number of presacral vertebrae. Traits such as spina bifida occulta, ossified apical ligament and atlas bridging are found to be age-stable in post-adolescence (Saunders 1989).

Those who have examined age association have come to different conclusions regarding the degree of age effects found on traits and this is primarily due to different sample size, different statistical analyses that have been employed which are not comparable, and the use of different traits in these analyses. Finnegan (1978) looked at age correlations of the infracranial skeleton and noted that age does not affect distance studies. However, Korey (1970, 1980) suggested that age association can be a very present concern in the study of nonmetric traits (in Winder 1981). Buikstra (1976) suggested the removal of traits that were found to have an age association unless corrective measures (e.g., use of only post-adolescent individuals, and combining partial

and complete ossification expression of the traits) have been applied. Saunders' (1978) work on the infracranial skeleton revealed age changes can affect distance results, leading to her recommendation for the elimination of traits that present a strong age association from distance studies even in cases where the sample only consists of adults.

2.5.2 Sex Correlations

Many studies have demonstrated that the prevalence and expression of nonmetric traits can be influenced by sex. From a genetic standpoint, because males have an X and Y chromosome and females have two X chromosomes, sex-linked genes may influence the threshold potential of some nonmetric traits (Saunders and Rainey 2008). During the developmental stage, the production of hormones- particularly the sex hormones estradiol and testosterone- contribute to sex differences, which may influence trait development. Differences usually manifested through size differences and differential bone disposition could explain why males, who are on average larger and have more robust bones, show a higher frequency of hyperstotic traits while females tend to smaller and have more hypostotic traits. The sex bias that is environmentally influenced can be largely due to cultural gender roles, which can create different environments for males and females. Cultural influence can either heighten or diminish the sex difference in trait frequencies (Saunders and Rainey 2008).

As noted, sex differences have been examined in a number of populations for a good portion of the 20th century (Hooton 1930, Akabori 1933, Sublette 1966, Anderson 1968, Cybulski 1972) as we began to understand the genetic aspects of nonmetric traits. However, there is debate as to how much influence sex differences have on nonmetric trait expression. It has been suggested that the sex influences on trait frequencies may be random but they can be influenced by cultural biases (e.g., residence practices). Others suggest that sex differences are due to sexual dimorphism, which is expected, and can greatly affect biological distance studies (Saunders 1989, Brown 2013). An association has been uncovered between the higher prevalence of males with hyperstotic traits and the higher prevalence of females with hypostotic traits (Saunders 1978, Molto 1985), while there appears to be no sex-related patterns for foraminal traits (Saunders 1989). This is consistent with the potential influence of sexual dimorphism on trait frequencies

(Grüneberg 1954). As sexual dimorphism varies between populations across time and space, the prevalence of traits will vary accordingly.

Not only are there conflicting views on how sex differences influence nonmetric traits, there are contrasting views as to how to handle sex influences. Finnegan (1972) asserts that traits that exhibit sex association should be omitted from population research and the proportions of each sex should be equal, while Anderson (1968) and Corruccini (1974) contend that the sexes should be analyzed separately and not pooled together. On the other hand, Gaherty (1970) suggests traits that do not exhibit any sex correlations should be pooled together and for traits that show sex bias, omit one of the sexes. An additional factor that is important when looking at sex differences is the context of the skeletal material (e.g., fragmentary versus complete). Variables such as geography, time and sample size are critical when deciding which approach to use when dealing with sex correlations.

2.5.3 Side Correlations

Symmetry variation in the prevalence of nonmetric trait is a commonality across paleogenetic studies (Molto 1985). Searle (1954), using mice, noted that there was a tendency for the right side to show asymmetry that he attributed to variable vascular asymmetry. Ossenberg (1969), who also noted asymmetries on human crania, attributed these to some unknown form of physiological asymmetry, and that this may not be a random occurrence as hypostotic traits appeared more on the right side and hyperstotic traits appeared more often on the left side. These findings are consistent with those reported by Saunders (1978) and Winder (1981) for the infracranial skeleton. Ossenberg (1981) later postulated that if traits were under genetic control then it is likely that the bilateral appearance of a trait is representative of increased genetic signal. Prior to Ossenberg's research, Trinkaus (1978) suggested that if variants were just under genetic control there should be equal bilateral expression and unilateral appearance of traits could demonstrate the influence of environmental factors such as nutrition, climate and bio-mechanical stress during growth and development. Further research by Korey (1980), Saunders (1978) and Winder (1981) observed that with developmental age, prevalence of

bilateral traits increases, leading to the conclusion that unilateral appearance is attributed to random environmental disruptions taking place during development (Saunders 1989).

Scoring traits with side differences has been greatly contested; Ossenberg (1981) argued that if the assumption that traits are under genetic control is true, bilateral traits should be scored separately (side count) to heighten the strength of the genetic meaning. This supports Berry and Berry (1967) who favoured the side count for bilateral traits (one trait = two counts for right and left). This approach is particularly relevant when samples are highly fragmented. Korey (1970) has conversely suggested that trait prevalence should be scored as a function of the individual as counting bilateral traits twice introduces redundant information which can be problematic for distance studies. McGrath et al.'s (1984) research on closely related rhesus macaques revealed asymmetry was low, indicating that genetics was not an influence on asymmetric expression but they also noted that genetic correlations between sides was high; the genetic make-up for each side were the same. Ultimately McGrath et al. (1984) supported using the individual to determine trait prevalence. This scoring technique is believed to be best used when samples are well preserved as this helps reduce redundant information.

2.6 Intertrait Correlations

Grüneberg's (1954) genetic work on the mouse found intertrait correlations among skeletal variants to be quite low, and random, which was later corroborated by a number of other studies (Berry and Berry 1967, Suchey 1975). Because studies supported the fact that intertrait correlations are not common, subsequent researchers have rarely attempted to look into intertrait correlations. However, this changed when Sjøvold (1977) showed that sample sizes significantly influence trait correlations. Sample sizes that were in the hundreds would uncover both genetic and environmental correlations which could reveal meaningful information on trait expression and prevalence. Sjøvold (1977) suggested the use of the phi coefficient for statistical testing as this statistic provides the degree of association. In addition to Sjøvold (1977), a number of researchers found significant results among their data sets (Ossenberg 1969, Korey 1970, Buikstra 1976). Among these researchers, patterns were uncovered explaining some of the significant correlations that were being observed which were biological in nature: (1) association among traits can be

due to a common embryological origin (Molto 1985); (2) association can be found among traits that are hyperstotic or hypostotic, as these traits can be influenced by environmental and/or developmental factors (Ossenberg 1969, Korey 1970); and (3) association can result from traits expressing alternate versions of the same underlying variable (Ossenberg 1976, Molto 1985).

Saunders (1978), one of very few researchers who researched intertrait correlations of the infracranial skeleton, found statistically significant correlations. However, after examining traits that were similar to each other and removing any redundancies in trait observation, she noted that there were low levels of intercorrelation among the traits. Edwards (2005) also found no significant correlations between six atlas variants using a sample from the Dakhleh Oasis.

While there may be speculation as to the prevalence of intertrait correlations in populations, it is clear that correlations found to be significant can skew results for biodistance studies (Molto 1985). It then becomes important to review all significant correlations and it has been suggested that traits that appear to be correlated should be removed from analysis (Molto 1985).

2.7 Biodistance Studies

The discovery of the genetic properties of nonmetric traits and their potential in biodistance studies has caused both excitement and contestation in bioarchaeological research. Biodistance analyses uses phenotypic data to estimate genetic similarities among populations with the goal being to reconstruct population origins and migration patterns, with the underlying premise that populations who exchange mates are more likely to be phenotypically similar over time (Buikstra et al. 1990, Stojanowski and Schillaci 2006). Following this premise, biodistance analysis have a few primary assumptions: (1) samples are an accurate representation of the population; (2) changes in allele frequencies result in changes in skeletal traits that can be measurable; (3) environmental effects are limited or randomly distributed among the population; and (4) inheritance of phenotypic variation is additive and there is strong resemblance among relatives (Stojanowski and Schillaci 2006). The degree to which these underlying

assumptions are met is based on two factors, sample size and trait selection (Ubelaker 1999). Ubelaker (1999) suggested at least 100 unbiased adult individuals for each population be used for comparison. Cranial and dental traits are most commonly used while infracranial traits have rarely been used. The view that infracranial traits are primarily functional and are not useful for genetic comparison is, in part, influencing this trend (Saunders 1978) as infracranial traits are subjected to selective mechanisms or lack inter-individual variability, though this is not true for many traits particularly of the vertebral column.

Laughlin and Jorgensen (1956) were two of the first researchers to examine nonmetric human skeletal data to look at biodistance. They believed that the inclusion of nonmetric traits in studies of biological distance between populations would be appropriate and they were able to substantiate this claim with their study on Greenlandic Eskimos using a variation of Penrose's size and shape statistic; demonstrating with nonmetric variants that the two populations were isolated. However, Berry and Berry used the mean measure of divergence developed by C.A.B Smith to calculate the divergence between two populations based on nonmetric cranial variants. This statistic with modification (e.g., different angular transformation) is still in vogue as it has become an increasingly popular method for biodistance; using genetically influenced markers with quantified expression of separation between populations (Saunders 1989, Stojanowski and Schillaci 2006).

2.7.1 Kinship and Cemetery Structure Studies

There has been a large focus on inter-population studies, and within population studies (e.g., in a cemetery) to make inferences regarding kinship as well as the cemetery structure have been attempted. Similar to the underlying premise of biodistance, kinship follows the assumption that members of a family are more phenotypically similar to each other because family members share common alleles. The goal for kinship studies therefore is the identification of family groups based on the presence of shared rare traits, which can be done in part with the structure of individuals in the cemetery. Cemetery structure also has goals to identify social or political groups. It is important to note that only in rare instances do bioarchaeological kinship analyses identify the exact

genealogical nature of these family relationships. The use of DNA and/or historical information can greatly assist in identifying genealogical relationships.

The genesis of kinship studies developed in the 1970s with the work of Sublet and Lane (1972) as they set out to "examine the application of nonmetric osteological data to discern residence patterns within a particular population" (p 187). Numerous studies examining kinship using both metric and nonmetric data began to appear while a limited number of these papers made use of infracranial traits (Case et al. 1998, Regan et al. 1999). The use of discrete traits for infracranial study became more prominent during the 1990s (see Alt and Valt 1991, 1992, 1995, 1998). They stressed that traits needed to be rare, heritable, genetically independent, independent of age and sex as well as easily observable. Focus on the use of rare or genetically anomalous traits for identifying closely related individuals, makes infracranial anomalies such as sacralization of L5, and spina bifida occulta a good choice for study (Stojanowski and Schillaci 2006).

2.8 Current Nonmetric Research

Epigenetic traits have therefore been shown to be useful for discerning both inter and intrapopulation genetic relationships (Saunders and Rainey 2008). While the majority of studies still use cranial traits, a few studies have shown the value of infracranial traits (Saunders 1978, Donlon 2000). In addition to population relationships, different avenues are being explored to better understand the potential for nonmetric traits. Traits are now being investigated with radiography (Bouille 2001) and technologies such as 3D imaging, to better understand how and why a trait is expressed. As noted in the introduction, epigenetics is now becoming more useful in fields of molecular biology and disease research. The experimental and theoretical components make epigenetic traits valuable for looking at complex genetics and disease processes (Petronis 2010). Although such studies are in the beginning stages, the role of epigenetic traits in paleogenetic research is now increasing and with ancient DNA research, the potential for determining genetic relationships in the past has never been greater.

2.9 Research Questions

The lack of standardization and the assumption that infracranial traits are functionally influenced has led to a paucity of research in this area, hampering the use of infracranial traits in nonmetric studies although early work has shown its potential. This thesis attempts to fill the gap in nonmetric literature by taking a particular focus on vertebral traits in the Dakhleh population. The central focus here is whether infracranial traits can be effectively used to reveal genetic affinities of a past population. If infracranial traits are just as effective as cranial traits, better attention needs to be paid to the potential information that these traits can provide. Also, do sex, age, symmetry and intertrait correlations appear at a statistically significant level in the Dakhleh Oasis? If so, what implications does this have for the use of vertebral traits for the study of this population and for nonmetric traits in general? Finally, can the study of vertebral traits reveal an intracemetery pattern as it has been shown for cranial traits (Molto 2002)? Answering these questions will be key in leading future directions for the study of epigenetic traits.

Chapter 3

Growth and Development and Trait Description

3.1 Introduction

This chapter briefly reviews the growth and development of the vertebral column from conception to adulthood for each vertebral region. This is followed by descriptions of all the nonmetric traits used in this thesis. The chapter then describes the genetic and environmental influences that affect growth and development of the vertebral column and possible factors involved in the genesis of variants used in this research. The chapter concludes with a brief look at Barnes' (1994) hypothesis and its possible connection to the genesis of vertebral nonmetric traits.

Growth involves increases in size and functional complexity (Scheuer and Black 2000). Because growth can differ among populations, between different sexes and among individuals of the same population, growth patterns are visible responses of adaptation through natural selection, differential reproductive success, and genetic and environmental pressures (Barnes 1994).

During development, delays in reaching critical genetically-determined threshold events can distort the normal appearance of the vertebral column. Understanding and detecting developmental defect patterns, which can influence nonmetric trait genesis, is important for the interpretations of these defects, to better understand biological affinities, and to identify cultural and environmental influences (Barnes 1994). Individuals with major congenital defects are rarely found in ancient skeletal populations as they likely would have died early in infancy. Minor defects, however, would have limited effects on survivorship resulting in the prevalence of traits in all skeletons. This creates a bias in the archaeological record as to the types of defects found in a population (Barnes 1994). While most defects and variants occur early in embryonic life, affecting the normal development of the neural tube, notochord, and paraxial mesoderm, a large number of defects are not noticed until complete ossification or some trauma has induced symptoms (Barnes 1994). In order to better understand the context in which the

nonmetric traits studied in this thesis are expressed, a brief review of embryology and development of the vertebral column is necessary.

3.2 Development of the Vertebral Column

Bones develop from two pathways, endochondral cartilagous ossification and intermembranous ossification. In endochondral development, there is a cartilaginous precursor that forms, preceding ossification. This pathway occurs in the infracranial skeleton including the vertebral column. Approximately two weeks after conception, there are cells from the primitive streak - a dense band of perimordial cells which differentiate and migrate, eventually becoming a wide range of body components. One subset of these perimordial cells grows from the knob (Hensen's node) to form a column of cells known as the notochord *(chordra dorsalis).* It is the notochord that becomes the framework where the blastemal vertebral column is preformed from the paraxial mesoderm- columns of mesenchymal tissue that will later become somites. Remnants of the notochord tissue become the apical and alar ligaments for the axis and the nucleus pulpous in the intervertebral disks. Formation of the neural plate occurs just above the knob which will become a groove and eventually the neural tube. Closure of the neural tube takes place early in embryogenesis when there is adequate vascular circulation, amniotic fluid and cerebrospinal fluid. Many agents are known to interfere with normal closure and can lead to a number of neural tube defects such as spina bifida occulta (Barnes 1994, Schoenwolf et al. 2009).

Somite structures derived from the paraxial mesoderm, contain the precursors for the axial skeleton. When each somite forms, they separate into different subdivisions. The ventromedial portion of the somite undergoes an epithelial-to-mesenchymal transformation. These cells, in addition to the somitocele cells (core cells), form the sclerotome around week four of intra-uterine life. It is the sclerotome that will develop into vertebrae (Schoenwolf et al. 2009). Some of the cells that form the sclerotome migrate to the notochord and the neural tube. The ventral portion of the sclerotome that migrates to the notochord will form the rudimentary vertebral body. The dorsal area of the sclerotome will migrate to the neural tube which will form the precursors to the vertebral neural arches and the vertebral spine while the sclerotome found laterally to the

dorsal area will form the transverse processes (Scheuer and Black 2004, Schoenwolf et al. 2009).

A sclerotome is differentiated cranially and caudally as it divides in half with each portion expressing different genes and densities. The caudal half of the sclerotome is quite dense compared to the cranial half. It is believed that the neural arches, the costal elements and pedicles develop from the caudal portion of the sclerotome. Based on the theory of resegmentation, it is believed that the caudal portion of one sclerotome fuses with the cranial portion of another sclerotome to create the intersegemental structure which will eventually create the vertebral body. Sclerotome resegmentation also results in intersegemental arteries passing over the vertebral body instead of passing through the sclerotomes (Schoenwolf et al., 2009).

3.3 Development of Vertebral Elements

The development of each vertebral element (cervical, thoracic, lumbar, sacrum and coccyx) undergoes a tightly regulated sequence of events that are described herein.

3.3.1 Atlas Vertebra

The first cervical vertebra ossifies from three primary centres – one at each of the lateral masses and posterior of the articular pillar at week seven of prenatal life. In approximately 2% of cases, in the second year of life, another ossification center appears which forms the posterior tubercle. At birth, the atlas is represented by two bony lateral masses which contain the superior and inferior articular masses in addition to a small portion of the posterior arch and the posterior bar of the transverse process (Scheuer and Black 2004). The posterior arches have well-formed ossifications centres that extends to the facets that are not ossified at this point. Both the anterior portion and some of the posterior arch associated with the spinous process are still cartilaginous (Ogden 1983). In the first year following birth, the atlas is increasing its overall size and the amount of cartilage has decreased as more ossification of the posterior elements continues. By age three to four, the transverse process that was represented by the short posterior bar has now fused with the anterior bar completing the foramen transversarium. Also, the foramina are almost near completion by this time. The absence /presence plus the form of

the foramina relies on the formation of the vertebral vessels. The fusion of the posterior arch occurs in the fourth and fifth year although it is not unusual to have it open into adulthood. The anterior (neurocentral) junctions may not close until the fifth or sixth year. The atlas reaches close to its final adult size by age six after which there is a bit of growth and increase in width (Scheuer and Black 2004).

2.3.2 Axis Vertebra

The second cervical vertebra, the **axis (**also called epistropheus), ossifies from five primary centres – one for the true centrum of the axis, one for each half of the neural arch and one for each half of the body of the dens. Centres of ossification appear in the neural arch before the centra during week seven to eight of prenatal life. Ossification then develops in the laminae and the neurocentral junction. The centrum of the axis begins ossification between the 4^{th} and 5^{th} month while ossification centres appear in the odontoid process allowing the intradental synchondrosis to fuse by birth and possibly as early as the seventh to eighth month of prenatal life. Neural components can be identified by 4-5 months while the centres for the centrum and dens are recognizable toward the end of prenatal life to birth. At birth, the vertebral body articulates with the vertebral arch and neurocentral joints (Evangelopoulos 2013). An intradental sulcus that is found on the dens (posteriorly) persists until age three to four. Around that time, the intradental sulcus has filled in; the posterior synchondrosis that is between the neural arches also begins to fuse in addition to the dens laterally fusing to the neural arches of the dentroneural synchondrosis. At this time, the transverse processes with the foramina transversaria are near completion. The dentalocentral junction and the paired neurocentral junctions fuse by age four to six and all lines of fusion disappear by nine to 10 years of age. The ossiculum terminale which is a small nodule at around age two appears in the chondrum terminale and fills the apical cleft and fuses with the apex of the dens by age 12 (Scheuer and Black 2004).

3.3.3 Cervical Vertebrae

The **third to seventh cervical vertebrae,** like the atlas**,** ossify from three primary ossification centres and follow the general ossification pattern of any typical vertebra.

The neural arches are first to begin ossification, appearing by the second month of prenatal life, which is characterized by a developing foramen transversarium. The centra begins a bit later, appearing in C7 by the beginning of the third month in utero extending cranially to C3 which begins no later than the fourth month. At birth each cervical vertebra has three bony components, a centrum and two lateral masses separated by cartilage. All cervical laminae unite posteriorly within the second year and by this time each cervical vertebra is characterized by two bony elements. During the third and fourth year of life, neurocentral fusion takes place along with the completion of foramen transversarium. At this point, the vertebra is close to adult morphology.

The typical cervical vertebra has six epiphyses, while the atlas reportedly shows epiphyses at the tips of the transverse processes and the axis typically has five epiphyses or six if the ossiculum terminale is included. Annular rings begin to fuse to the vertebral body starting with the upper cervical vertebrae and continue in the caudal direction to C7. Annular union begins during the end of puberty (age 17 to 19) and union is usually complete by 25 years. Vertebra found in higher cranial positions are at a more advance stage of maturation than caudally placed vertebra at any age (Scheuer and Black 2004).

3.3.4 Thoracic Vertebrae

Thoracic vertebrae also have primary centres within the three primary elements. Primary centres in the neural arches and centra appear by the third prenatal month. Centres for the neural arches first appear in the first and second thoracic vertebra in week eight and by week 10, an ossification centre can be found in each half of a thoracic segment. Ossification centre first appear in the upper thoracic vertebra and in a caudal direction works its way to the mid and lower thoracic levels. Between week eight and nine of prenatal life, the costal element begins ossification. By birth, each vertebra is presented in three bony masses - an anteriorly placed centrum with neural arches found posteriorly. Fusion of the laminae often occurs within the first and second year of life with union usually beginning in the lower thoracic segments. In addition, neurocentral fusion begins in the lower thoracic region in year three to four with completion by year five to year six. By age six, there is one bony structure representing the vertebra (Scheuer and Black 2004).

3.3.5 Lumbar Vertebrae

Like the cervical and thoracic vertebrae, the **lumbar vertebrae** also follow the general ossification pattern. Ossification commences in the centra starting at the upper lumbar vertebrae by 9 to 10 weeks and reaches L5 by the third prenatal month. In the neural arches, ossification begins at 11 weeks in the upper vertebra and reaches the L5 by the fourth prenatal month. Just like the other presacral elements excluding the axis, there are three bony elements at birth; a centrum positioned anteriorly and two neural arches posterior. The synovial articular rests in its vertical position at around age 1, when the child begins walking. The transverse process starts to develop and is visually detectable by the end of the first year to the beginning of the second year. The laminate unites in L1-L4 by the end of the first year of postnatal life, and in the fifth year, the laminae of L5 sometimes remains unfused resulting in spina bifida occulta. The neurocental fusion of the L5 usually begins during the second to third year and fusion is complete by the fourth year. The seven ossification centres in lumbar vertebra first appear in the mammillary processes and are followed by the transverse and spinous process. They appear in the L5 first and work up cranially to appear in the L1 last. This is also true for ring epiphyses which begin at L5 and fuses last in L1 (Scheuer and Black 2004).

Generally the lumbar vertebra contains five secondary ossification centres. These centres are located at the tips of the transverse and spinous process and annular rings (Scheuer and Black 2004; Evangelopoulos 2013). Annular rings are found on the periphery of both the interior and superior surfaces of the vertebral bodies which typically begins at puberty (12-16 years) and fuse by the end of puberty (18 years) and certainly by age 24. Annular epiphyses which can take on a horse-shoe or ring appearance can be detected as early as two to six and a half years and begins ossification by age 13. It has been shown that annular rings fuse to the vertebral body after it has completed growth which is during the later pubertal period (Scheuer and Black 2004).

3.3.6 Sacrum

The **sacrum** has a more complicated development relative to the other vertebra as it develops from 21 primary ossification centres. The complicated development has resulted in reporting problems in the literature (Schwartz 1995). Each of the sacral elements has the usual three centre of ossification and the first to third and sometimes fourth sacral segment incorporate costal elements. While a majority of estimates cite that ossification centre appear around the second to third month of prenatal life in S1 and S2 (Clemente 1984, Netter 1987, Scheuer and Black 2004). Fazekas and Kosa (1978) support six months for the appearance of ossification centre. In the fourth month, ossification centres appear in S3-S4 and the neural arches of S1-S3. By the fifth month, ossification of the centrum in S5 occurs in addition to the neural arches of S4 –S5. Pairs of costal elements appear between the sixth and eighth prenatal month which appears to be the consensus. Each element of the sacrum is identifiable in isolation after the first year of birth. The neural arches unite with the costal element at age two to five years before it unites with the centrum by two to six years of age. Age six marks where all primary centre have fused with each sacral segment with the exception of the spinous processes posteriorly. Between ages seven to 15, the laminae fuse with each sacral segment which remains separate until puberty, the sacral hiatus (Schwartz 1995). The costal elements of the primary sacral centres begin to fuse with each other at age 12 starting at the lowest sacral elements and moving in the cranial direction where the S1 and S2 are last to begin fusion between 25 to 30 years of age (Schwartz 1995 Scheuer and Black 2004). At the same time, the annular epiphyses have formed and are also beginning fusion in a caudocranial direction. The epiphysis of the sacro-iliac joint which will form a thin sheet of bone that covers the articular surface appears around 15 -16 years and fuses by 18+ years (Scheuer and Black 2004).

The general rule for the sacrum is that, if there is a hiatus between the sacral bodies, the individual is not younger than 20 years. The gap between S1 and S2 usually fuses by the early 30's, although approximately three percent of individuals have S1 and S2 unfused through adulthood in the Dakhleh sample.

3.3.7 Coccyx

Very little information about the ossification and development of the **coccyx** is available and it appears that there are some discrepancies between researchers. The coccyx is composed of three to five segments but it appears that each coccygeal segment arises from one ossification centre where separate centres may appear in the cornua, the superior projecting processes of the first segment. The ossification of the first segment appears around the end of prenatal life to the first year of life which is also the same for the cornua. The centre for the second body appears between three to six years of age, the third body, around 10 years of age while the fourth body around puberty. It is around puberty the coccyx begins to take on its characteristic recognizable adult form. There does not appear to be constant epiphyseal structures for the coccyx although annular rings may appear when the coccyx becomes fused into the sacrum. Schwartz (1995) reports that the third segment ossifies between the ages of 10 to 15 while the fourth segment ossifies by 14-20 years of age. Fusions of the coccygeal segments are variable but there is some degree of fusion by 25-30 years in the cranial direction and fusion of the first and second segments not until the fourth decade of life (Schwartz 1995, Scheuer and Black 2004).

3.4 Age Estimation and Sex Determination

The use of secondary centre of ossification (epiphyses) are particularly beneficial in differentiating vertebra that are of adolescent age (12-17) and of adult age (18+) as these years are crucial for the fusing of many epiphyses found on the vertebrae. While epiphyses and annular rings may not be able to pinpoint a specific age at death, they provide information during the time around puberty, and are highly sex-specific, especially annular rings as it appears there is a higher frequency of early calcification of these rings found in females (Bick and Copel 1950, Scheuer and Black 2004). As expected, the union of the epiphyses begins earlier in females than in males and individuals of the same sex can show varying times of union; because of this, there are fewer age indicators for the infracranial skeleton especially the vertebral column of the adult and subadult individual (White et al., 2012). There are a few morphological sex differences for the vertebral column. Boyd and Trevor (1953) report that the atlas is distinctively larger in the males than in females, while Flander (1978) notes the sacrum is

longer and narrower in the male than the female. According to Brothwell (1981), the auricular surfaces of females are limited to the first and second sacral segment while in males it extends to the middle of the third sacral segment. Age estimation and sex determination should not be done based solely on vertebral morphology but should be used in conjunction to other well-known and tested age and sex methods such as dental eruption and pelvis analysis using the Phenice method. In cases where vertebrae are found in isolation, age estimation and sex determination cannot be determined without other aging and sexing methods. As noted above, the fusion of S1 and S2 can be used to age individuals into broad categories under and over 30 years.

3.5 Nonmetric Trait Description

The following are descriptions of the 17 nonmetric traits that will be analyzed in this project. These traits are organized by bone type – traits that appear on the atlas, the axis, cervical vertebrae, lumbar vertebrae and the sacrum. Photographs of the traits can be found in Appendix A.

First Cervical Vertebra - Atlas

Divided Superior Facet (Figure A.1) - Facets of the superior articular surface are supported by the lateral masses and articulates with the condyles of the occipital. When present, the facets are separated by a ridge or groove resulting in the bipartition of the superior facet into two discrete facets on either one or both facets, unilateral and bilateral respectively (Finnegan 1978). The facets may vary largely by size, shape and depth. This hypostotic trait is believed to be of an embryological origin - a manifestation of the occipital vertebra at the base of the crania which is a remnant of the anterior portion of the neural arch of the proatlas (von Torklus and Gehle 1972, Saunders 1978). However Singh (1965) posits the variability of the facets may be an evolutionary development towards restricting movements of the alanto-occipital joint.

Lateral Bridge (Figure A.2) **–** First described as posterior glenoid variant by MacAlister (1893, 1896), this hyperstotic trait is a partial spur or a complete bridge of bone that forms from the superior articular process or the lateral mass of the atlas to the posterior root of the transverse process allowing a vertebral artery to pass. The presence of a

complete bridge results in the formation of the retroarticular canal (Finnegan 1978). This variation is reported to occur between 1.8 and 3.8 % of the population (Kavaklu et al. 2004). There is debate regarding the origin of the lateral bridge. It is hypothesized that the lateral bridge is a rudimentary transverse process of the proatlas sharing formation with the para-condylic and epitransverse process (von Hayek 1927) while others have suggested the bridge arises from the cranial half of the atlas and represents late ossification of its ligamentous bridge over the vertebral artery (Barge 1918, Ludwig 1953). Other studies have challenged the latter as their development appears early in life (Selby et al. 1995, Saunders et al. 1976).

Posterior Bridge (Figure A.3) **–** This congenital variant is characterized by a bridge of bone which arches over the sulcus of the vertebral artery and first cervical nerve behind the superior articular facets. The bridge forms a foramen in which the vertebral artery and the suboccipital nerve passes. This hyperstotic trait can occur unilaterally or bilaterally and is found either complete or incomplete though this variant is often seen bilaterally and frequently forms an incomplete bridge. The posterior bridge, also known as ponticulus posticus, lies in the same plane as the alantoccipital ligament which led to the belief that the ponticulus posticus occurred as a result of ossification of the alantocciptial membrane. Since distinct ossification centre and well-organized bone has been found, this variant is now considered to be a rudimentary primitive structure (Wight et al. 1999). This trait is known to be controlled by genetic factors and the incomplete form is more common in females (Dugdale 1981, Barnes 1994, Wight et al. 1999). This trait varies in population prevalence from 5% to 35%.

Posterior Arch Foramen (Figure A.4) **-** The vertebral artery is accommodated by grooves that pass through the anterior superior surface of the poster arch and under the superior articular facets. Occasionally this groove becomes a foramen or bony spur that passes posteriorly from the lateral side of the posterior neural arch to accommodate vein(s) and/or artery. This foraminal trait also commonly known as retroarticular bridge presents itself either unilaterally or bilaterally (Saunders 1978; Edwards 2005).

Posterior Cleft of C1 (Figure A.5) **-** This rare hypostotic trait is a developmental deficiency of the posterior neural arch resulting in a cleft; a bifid atlas. Ossification of the

posterior arch usually proceeds from two centres of the lateral masses normally fusing by age three to five. In rare cases, a third ossification center is found in the region of the posterior tubercle and unites secondarily with the lateral processes of the arch. The posterior cleft can range in expression from moderate defects - a tiny hiatus or a sizable gap leaving the spinal cord unprotected (median, unilateral and bilateral clefts) to complete lack of ossification (Schulze and Buurman 1980, Edwards 2005). The appearance of these clefts is attributed to defective or absent development of cartilaginous preformation of the arch. Cleft vertebrae are the result of a non-union of bony elements; usually not indicative a serious congenital defect. Clefting of the posterior arch is estimated to occur in approximately 5% of adults (Barnes 1994).

Axis

Ossified Apical Ligament (Figure A.6) –The apical ligament, a small collection of elastic fibres surrounding a notochordal remnant that connects the odontoid process to the basion becomes ossified in a bony tubercle-like fashion. The apical ligament is derived from the centrum of the proatlas; the fourth occipital sclerotome (Saunders 1978, Tubbes et al. 2000). Ossification of the dens starts at the base of the axis and proceeds cranially in two rays or dental processes with fusion ranging from birth to six years. Scored as present or absent, this hyperstotic trait does not appear to be have strong age or sex influences, prompting a strong inheritable factor for this trait (von Torkus and Gehle 1972, Saunders 1978).

Cervical Vertebrae

Incomplete Foramen Transversarium (Figure A.7) **–** The foramen transversaria which transmits the vertebral artery, veins and sympathetic plexus accompanied by the vessels is formed by a vestigial costal element fused to the vertebral body as the transverse process of the vertebra is normally fully ossified during early childhood (Taitz et al. 1978, Edwards 2005). The absence of the costal element forming the transversarium foramen can be seen unilaterally or bilaterally. This trait presents itself on all the cervical vertebrae but will only be studied on C1, C2, C6 and C7 for this project. Commonly found on the atlas and axis, both the anterior and posterior tubercles of the transverse

process may not unite through the costotransverse bar (Saunders 1978). Taitz et al., (1978) also note that the foramen transversarium of the axis differs from the other cervical vertebra as the foramen of the axis is an angulated canal with an inferior and lateral opening while other cervical vertebra have a simple short foramen.

Divided Transversarium Foramen (Figure A.8) – Commonly seen in the literature as double foramen transversarium or accessory transverse foramen, this trait is like the incomplete transversarium foramen where the trait is characterized by a foramina on the transverse process where arteries, veins and nerves are transmitted but differs by the fact there is an additional foramen which is usually smaller to the primary foramen. This trait will be examined on the sixth and seventh vertebrae although it is reportedly found on C3 to C7. It appears that this trait appears more commonly on C6 than C7 and unilateral expression is thought to occur more often than bilateral expression. This trait is believed to have an incidence range of 1.5 to 5% (Taitz et al. 1978, Das et al. 2005, Chandravadiya et al. 2013). Duplication of a vessel or an artery, more specifically, a failure of a controlled regression of two arteries and a segment of the primitive dorsal aorta its thought to cause the appearance of this trait (Sim et al. 2001 Murlimanju et al. 2011).

Lumbar Vertebrae

Spondylolysis L4/L5 (Figure A.9) **-** This variant is represented by a local osseous defect of the pars interarticularis[1] which tends to create a unilateral or bilateral cleft within the neural arch (Fredrickson et al. 1984). Spondylolysis primarily involves the L5 (95% of cases) and commonly occurs bilaterally (Grogan et al. 1982, Teplick 1986). In the case of bilateral expression, spondylolysis may result in spondylolisthesis where the vertebral body together with the transverse process, pedicles, and upper articular facets may separate from the lamine, the spinous process and the inferior articular facets allowing slippage or movement of the vertebra. Spondylolysis is often linked with the congenital malformation of the adjacent facets in addition to enlarged superior articular facets (van Roy et al. 2006). Hereditary predisposition to the pars defects combined with a stress factor is believed to be the likely pathogenesis (Troup 1976). Spondylolysis is also noted

[1] The bony mass between the superior and inferior articular processes of the facet joints at the junction of the pedicle and lamina

to be strongly associated with spina bifida occulta (Fredrickson et al. 1984). While the prevalence of spondylolysis is estimated to about 6-8% of the population, it can reach as high as 63% in individuals engaging in certain sporting activities which has led to the hypothesis that its etiology is stress related (Merbs 1989, Leone et al. 2010). Spondylolysis of the fourth lumbar vertebra is not as commonly seen as the fifth.

Sacralization of L5 (Figure A.10) **–** Sacralization of the L5 vertebra is characterized by the fifth lumbar vertebra becoming incorporated into sacrum. Incomplete sacralization is indicated by accessory articulations between the transverse process of the L5 and the ala of the sacrum while complete unilateral or bilateral fusion between the L5 and the S1 transverse elements indicate complete sacralization (Mahato 2010a). The vertebral body of the lowest lumbar segment takes on a "wedging" appearance where there is a decrease height between the sacralized vertebral body and S1 (Konin and Walz 2010).

Thoracic/Lumbar Vertebrae

***Extra Vertebrae* –** Numerical variation of the presacral vertebrae and at times sacral vertebrae mostly derive from segmental border shifts where vertebrae at transition borders assume the characteristics of the region below or above the border. Occasionally, numerical variations are due to the abnormal number of somites. Extra vertebral segments can generally be identified at the borders between different types of vertebrae. Most extra vertebral vertebrae appear at the thoracolumbar or lumbosacral borders. Occasionally an extra vertebra may appear at the sacrocaudal border while extra segments at the cervicothoracic border are rare (Barnes, 1994). It is difficult to determine the exact vertebrae count especially with prehistoric remains as incompleteness of skeletal remains makes it impossible to determine the exact number of vertebrae.

Sacrum

Spina Bifida Occulta – S1 (Figure A.11) – Spina bifida occulta (SBO), which is a form of spinal dysraphism, represents an abnormal neurulation characterized by an incomplete dorsal midline closure of the osseus tissues of the developing embryo affecting primary embryonic layers (James and Lassman 1972, 1981). This type of spina bifida known as occulta is milder, and often non-symptomatic due to the tough fibrous band that usually

takes the place of the missing bone. SBO appearance can vary from a small failure of fusion, a notch in a spinous process, to an absence of bone between the pedicles commonly occurring symmetrically. Spina bifida of the S1 usually results in a bifid spinous process. When the entire sacrum is open, the posterior laminae of all sacral vertebrae are unfused with the spinal canal wider than normal (Saunders 1978, Barnes 1994, Senoglu et al. 2008). SBO is more common in S1 although it can be found in any vertebra. The presence of the clefts seems to be dependent to both environmental and genetic factors in addition to the variability in the timing of fusion of the vertebra.

3.6 Genetic and Environmental Influences

Growth and development of the vertebral column is affected by several factors which include, but are not limited to, genetics, nutrition, hormones and mechanical forces (LeVeau and Bernhardt 1894). The importance of each factor will vary across time and geographic location. While most defects have a multifactorial etiology it is believed that 90% of developmental defects have an unknown underlying genetic influence with a third having monogenetic causes (Barnes 1994, Roberts and Manchester 2005). For most traits, the exact etiology is unknown.

As shown, the atlas has quite a complex embryological origin as somites from the basiocciput is involved in the final formation of the C1. In addition, somites from the first vertebra are involved in the formation of the dens of second vertebra. The complexity of the formation process may explain the high prevalence of congenital anomalies affecting this cervical vertebra (Ogden 1983)

Defects of the neural tube, particularly spina bifida occulta, have a wide prevalence range in the population (Saunders 1978). It appears that there are both genetic and environmental influences causing neural tube defects (Barnes 1994). Maternal nutritional deficits of zinc, folic acid and selenium, which help regulate cellular growth, in addition to an inherited faulty folate metabolism in mothers, are known to influence neural tube development (Yates et al. 1987, Barnes 1994). Egypt is particularly known to have zinc-poor soil which can exacerbate the prevalence of individuals with spina bifida when coupled with a defective folate metabolism (i.e. folic acid). Defects that occur early

in the development of the neural tube can result in the non-closure of the arches. When the inductive signal from the neural tube is absent or insufficient to initiate the appropriate development of the neural arches, spina bifida can result involving the failure of the laminae of one or more neural arches to fuse in the midline.

Defects that are of a paraxial mesoderm origin also have strong genetic influences indicated by studies on monozygotic twins, pedigree studies and laboratory mice. Defects of this category can result from various genetic and epigenetic factors with a belief that temporal delays with alterations of structural or enzymatic proteins play a role. Cleft vertebra, sacralization of the L5 and numerical variation of vertebral segments seem to be commonly affected (Barnes 1994).

There are also defects that have a genetic component confounded with some form of mechanical stress or trauma that appears to be quite common among traits of the vertebral column, especially spondylolysis. Spondylolysis of L5 is known to increase with age and is more common in males again supporting a role for mechanical stress in its etiology. Traits dealing with excess foramina or changes to the foramina as seen in cervical vertebrae are also thought to have both genetic and mechanical effects as the course of the vertebral artery and the tortuosity of the vertebral artery could cause additional foramina (Kaya et al., 2011).

3.7 Cranial and Caudal Shifting

The phenomenon of cranial and caudal shifting of the vertebral column, which appears in all mammal species, has been studied on numerous different human populations (Shore, 1930, Allbrook 1955, Bornstein and Peterson 1966, Merbs 1975). Cranial shifting affects the vertebral segment above the designated border as it takes on the characteristic of the vertebral segment that has joined below it and caudal shifting affects the vertebral segment below the designated border as it takes on the characteristic of the vertebral segment that has joined above it. These shifts can display a variety of expressions (Barnes 1994). Shifting patterns can differ across different populations, within the same individual, and shifting at the different borders can occur in different directions which is possible as precursors at the different parts of the vertebral column develop at different

times. Generally, caudal shifting occurs more often in humans than cranial shifting. In cases where cranial shifting does occur, this pattern is found more among females than males which has alluded to genetic factors playing a role (Merbs 1974, Barnes 1994).

3.7.1 Genetic Influences

Kühne (1934, 1936) was one of the first individuals who looked at the genetic aspect of vertebral variation with his studies on twins and pedigrees. He developed a genetic model that explained that vertebral shifting was under the control of two alleles – a dominant allele for cranial shifting and a recessive allele for caudal shifting. While subsequent research criticizes Kühne's model (Merbs 1974), Kühne's work in identifying a significant cause for cranial and caudal shifting at certain regional borders is still supported (Barnes 1994).

The reason for these shifts during morphogenesis is not clear, but a likely trigger is thought to be a delay in the formation of the vertebral developmental unit that borders two regions. It appears that the neural arches are primarily affected which indicates that the denser portion of the sclerotome is responsible for the delay (Barnes 1994). Also, the reason for the higher prevalence of shifting in humans relates to our bipedaility.

Genetic studies conducted on mice have identified a series of Hox genes that play a significant role in the segmentation of the vertebral column. Hox gene expression is regulated in the presomitic mesoderm by a segmentation clock that is regulated by *Wnt, Notch, Fgf* and retinoid signaling. Changes to the segmentation clock are marked by transformations of the vertebrae. Loss-of-function mutation of the Hox8 or Hox10 genes leads to cranialization while the gain-of-function of Hoxa10, caudalizes the vertebra. Timing of the mutation is also important; misexpression of Hoxa10 in the presomitic mesoderm results in vertebral changes but if this misexpression takes place after somitigenesis, this leads to only minor rib abnormalities. Retinoic acid as mentioned regulates Hox expression partly by inducing another gene, *Caudal.* The loss-of-function of two or more members of the retinoic acid receptor leads to cranialization; caudalization of vertebral segment occurs when there is excess retinoic expression (Schoenwolf et al. 2009). There are many other genes, enzymes and pathways known to

influence caudal and cranial shifting, however, due to the scope and limits of this thesis, additional pathways and genes will not be further discussed.

The genetic influence that these traits appear to have seems to be great, and likely the reasoning behind Barnes' assertion that the development of some nonmetric traits is influenced by the cranial or caudal development of the vertebral column. Because of the genetic influence behind cranial and caudal shifting, it is expected that traits that experience caudal or cranial shifting should be correlated. This will then herein lead to the examination of two variants, ossified apical ligament and sacralization of L5 which, according to Barnes is the result of cranial shifting at the vertebral borders.

Chapter 4

Materials and Methods

4.1 The Dakhleh Oasis Project

The Dakhleh Oasis Project (DOP) is a long-term regional study in Egypt which examines the relationship between the environment and human activities over several millennia from the upper Paleolithic to the late Roman Period (50,000 B.C. - 550 A..D.) (Mills 2010). The DOP is a multidisciplinary program that includes researchers from geography, geology, paleobotany, history, biological anthropology, archaeology and linguistics (Mills 2010). This thesis focuses on the bioarchaeology component, which is a subarea of biological anthropology. The infracranial epigenetic data used in this thesis were collected in field seasons spanning two decades (1986-2007) by Dr. Molto, the director of the bioarcheaological portion of the DOP (Molto 2001, Brown 2013).

4.2 The Dakhleh Oasis

Dakhleh is one of five major Oases in Egypt's western desert (Figure 4.1). It is located approximately 800 km south-southwest of Cairo measuring 100 km east to west and 25 km long from north to south. It has a land area of 2,000 – 3,000 km^2 (Fairgrave and Molto 2000). The present climate is extremely arid with annual rainfall of 0.3 mm (Wheeler et al. 2011). Research has shown that conditions in the Oasis throughout the Pharaonic and Roman times were virtually identical to the modern climate. The arid conditions have resulted in excellent preservation of both material culture and skeletal remains (Molto 2001). Human occupation over the millennia has been possible because the Oasis has one of the largest aquifers in the world, which has supported the development of an agriculturally-based subsistence. Irrigation-based technologies such as the animal-driven waterwheel also likely contributed to growth in Dakhleh, supporting increase frequency in crops and an increase in usable area for agriculture. The Oases of the Western desert is known to be rich in salt, and a number of goods such as olives and dates which were likely used to support foreign trade along the Nile during the Roman period (Bagnall 1993). The Roman influence also supported the creation of temples and

villages, as well as changes in ideologies in the Oases. Various literary texts mainly written in Greek and Copic have been found, supporting a shift towards Christianity which is reflected in burial practices (Gardner et al. 1996). It is believed under Roman rule, there was a marked improvement in health in the Dakhleh Oasis (Wheeler 2012).

The majority of the ancient settlements are localized in two distinct zones; Mahoub to Ismant (ancient Kellis), in the west-central portion and the villages of Balat, Bashendi and Teneida found in the eastern section (Haddow 2012). Major cemeteries at Ain Tirghi (AT) and Kellis (K1 and K2) have been excavated by the bioarchaeology team and provide the data for this thesis (Figure 4.2).

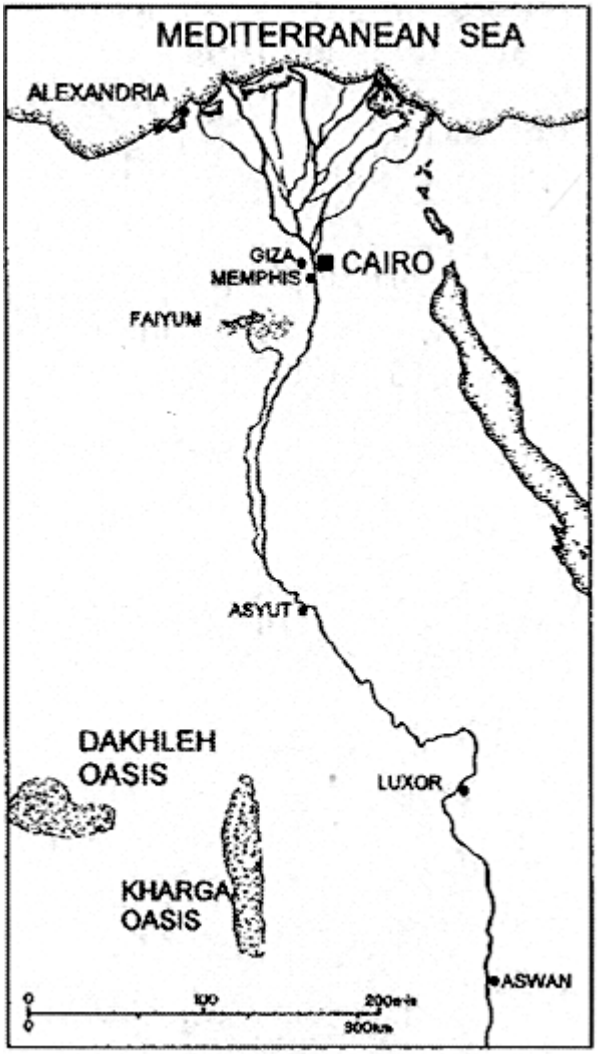

Figure 4.1 The Oasis in Egypt (Molto 2002)

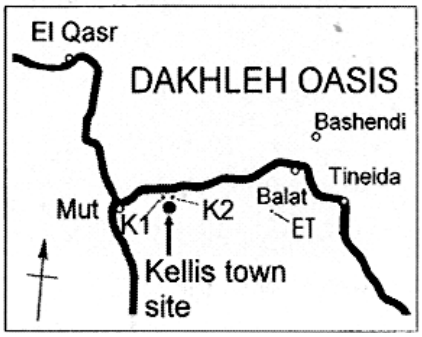

Figure 4.2 Location of the Ain Tirghi and Kellis emeteries (Molto 2002)

4.2.1 Ain Tirghi

This large cemetery is located approximately 8 kilometers southwest of the village of Balat in the eastern portion the Oasis. Designated 31/435-D5-2, this site occupies several curving mounds of red clay (Frey 1986). During the initial survey (1982-1983 field season), the mounds were covered by a thin layer of sand in the depressions, often with bone and pottery fragments. These depressions were the result of exploratory digging that took place over time for a variety of purposes (e.g., tomb construction, locating tombs for reuse and plundering). Estimates for the number of tombs at this cemetery based on surface surveys have been difficult to determine due to the fact that no known settlement is associated with the cemetery although it is thought to be hidden under modern fields. It is estimated that there are over 100 tombs (Frey 1986, Molto 2002).

Artifacts recovered from the cemetery have mixed historical contexts due to extensive plundering. The key cultural materials found in context have been dated to the Third Intermediate Period, and Late Periods (Frey 1986, Molto 2001). Most of the tombs have been looted and the burials and the skeletal remains were commingled. Three tombs, 31, 34 and 52 have many intact skeletons. Two skeletons from Tomb 31 with good context had AMS radiocarbon dates that are affiliated with the Third Intermediate Period circa 100-800 B.C. (Molto 2001). The tombs at Ain Tirghi were cut into the gebel with individuals placed randomly to maximize space. When more space was needed, earlier burials were pushed aside or heaped against walls. The bodies at Ain Tirghi were prepared in a number of ways; some were placed in wooden coffins, fewer in ceramic coffins, and the majority wrapped in linen (Molto 2001). No anthropogenic

mummification practiced. From epigenetic trait analysis, it has been hypothesized that the Ain Tirghi tombs were family crypts (Molto 1987).

4.2.2 Kellis Town site

The town of Kellis was an important political and economic centre in the Oasis from the Ptolemic times (332 - 30 B.C.) to the fourth century A.D. It was abandoned circa 450 A.D., possibly due to desertification and/or diminished water supplies. At its zenith in the mid-4th century A.D., it is likely housed two to three thousand people. The ruins of Kellis are evident on today's landscape, a vista that was a welcome sight to many caravans on the trade route from the Nile to all the Oases (Wheeler et al. 2011).

Within the village town site, several family cemeteries were constructed (Molto 2003). Ground plans of the site show up to 20 monumental tombs, most notably North Tomb 1 and North Tomb 2. From the few traces of the original Pharaonic decoration, a date around early 1st century CE has been assigned to the paintings on the walls, whereas most of the ceramics found in the tomb date to the fourth century A.D. (Monash University 2012). The skeletons excavated from these tombs and studied herein date to the Roman period and are contemporaneous with Kellis 2.

4.2.3 Kellis Cemeteries

Two large cemeteries outside of the village contained human remains from the Kellis population. Kellis 1 (K1) is located just north-west of the village and Kellis 2 (K2) which is the approximately 1 kilometer east of K1 is just north of the village (Birrell 1999). Kellis 1, dates to the Ptolemaic and early Roman periods, while Kellis 2 dates to the Romano-Christian period via AMS radiocarbon dates.

4.2.3.1 Kellis 1 (31/420-C5-1 designation by DOP)

Kellis 1 (K1) contains a large number of small chamber tombs which are categorized into two groups, those cut into the red Nubian clay and those dug into the clay of the higher sandstone terrace. The majority of the crypts were of the former variety. As noted, they date to Ptolemaic and early Roman periods circa 332 to 30 B.C. based on grave goods and radiocarbon dating. Most of the remains were wrapped in linens if they were not

placed in coffins. The K1 cemetery is thought to be associated with a pagan population due to the presence of elaborate grave goods and the absence of the Christian-type interments, where primary interments were positioned in an east-west orientation with their heads to the west (Molto 2001). Newer additions to the burial chambers were placed on top of previous inhumations. Disarticulated and disturbed human remains were spread around the rear and sides of the chamber, thought to be done to create more room for later subsequent burials (Birrell 1999). The latest inhumations of burials cut into the Nubian clay had generally well-preserved mummified individuals while the inhumations found in the tombs, dug into the clay of the sandstone, were skeletons with disturbed mummified remains located at the rear of the chamber. By the 1992-1993 field season, 470 individuals from 37 tombs had been excavated (Molto 2001, Brown 2013). The graves from Kellis 1 included in this thesis project are from tombs 3D, 3H, 3K and N1.

4.2.3.2 Kellis 2 (31/420-C5-2 designation by DOP)

In the early Roman period, when Egypt was Christianized, the people of Kellis switched their burial mode to a new cemetery called Kellis 2 (K2). K2, approximately 1 km east of K1, is densely filled with pit graves cut into red Nubian clay. Generally, the pits contain single inhumations where bodies were placed in a supine position with the head towards the west. Pits vary in length, width and depth based on the body interred. The vast majority of burials had a single inhumations placed on the hard clay at the bottom of the pit and no coffins were used (Birrell 1999, Wheeler et al. 2007). Hands were placed over the pubic region or beside the thighs with the former found primarily among female burials. Bodies of fetuses, young children and adults were wrapped with linens and only a few graves contained artifacts (Bowen 2003). Ceramic evidence, in addition to AMS radiocarbon dating, indicates this cemetery was in use during the Romano-Christian period from 50 to 450 A.D., reflecting the shift from pagan burials at K1, to Christian burials (Stewart et al. 2003, Brown 2013). To date, over 700 K2 skeletons have been excavated (Wheeler 2012). Like K1, bone condition is excellent, but the burial representation at Kellis 2 is considerably better because of the single burial custom, and the paucity of artifacts partly led to less looting.

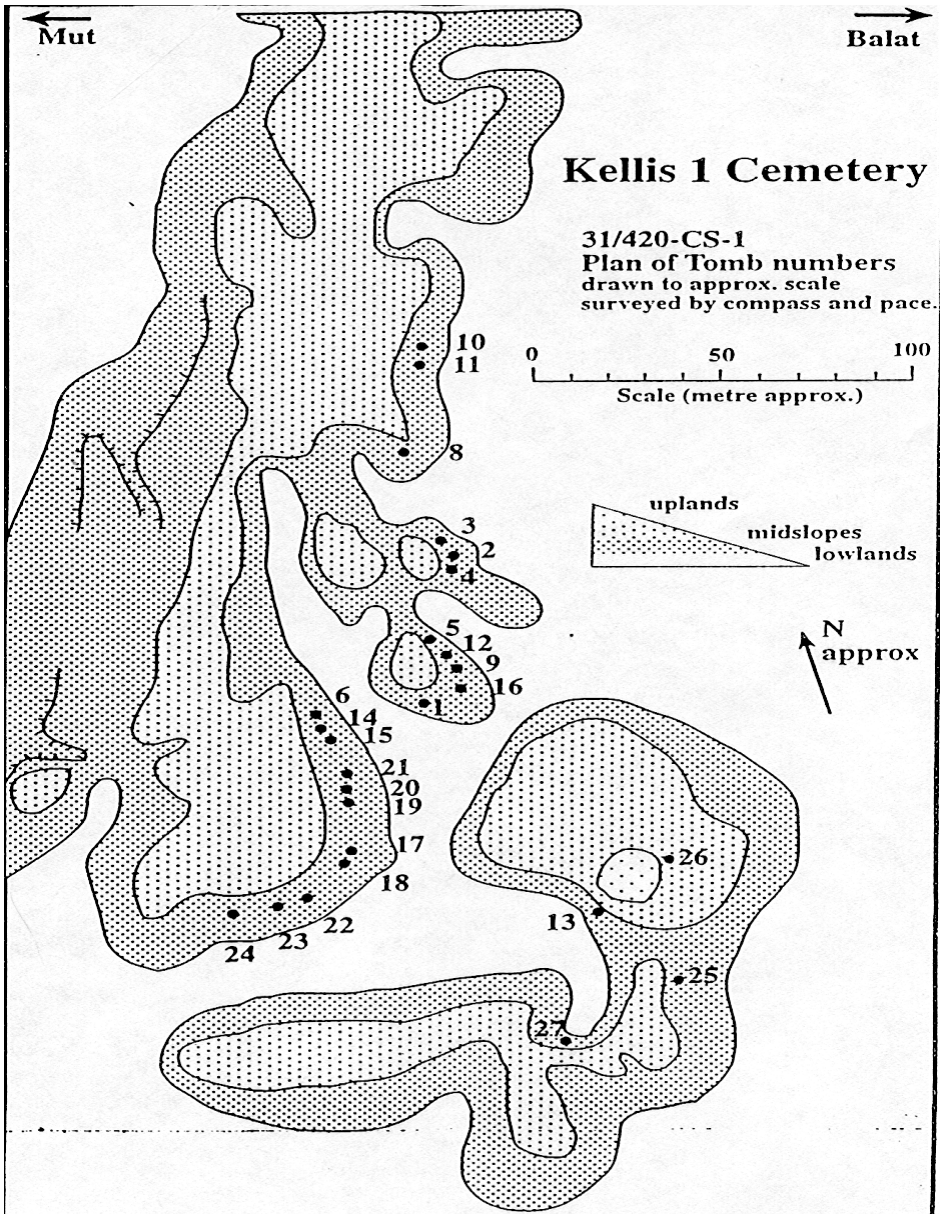

Figure 4.3 Cemetary plan of Kellis 1 (Molto 2001)

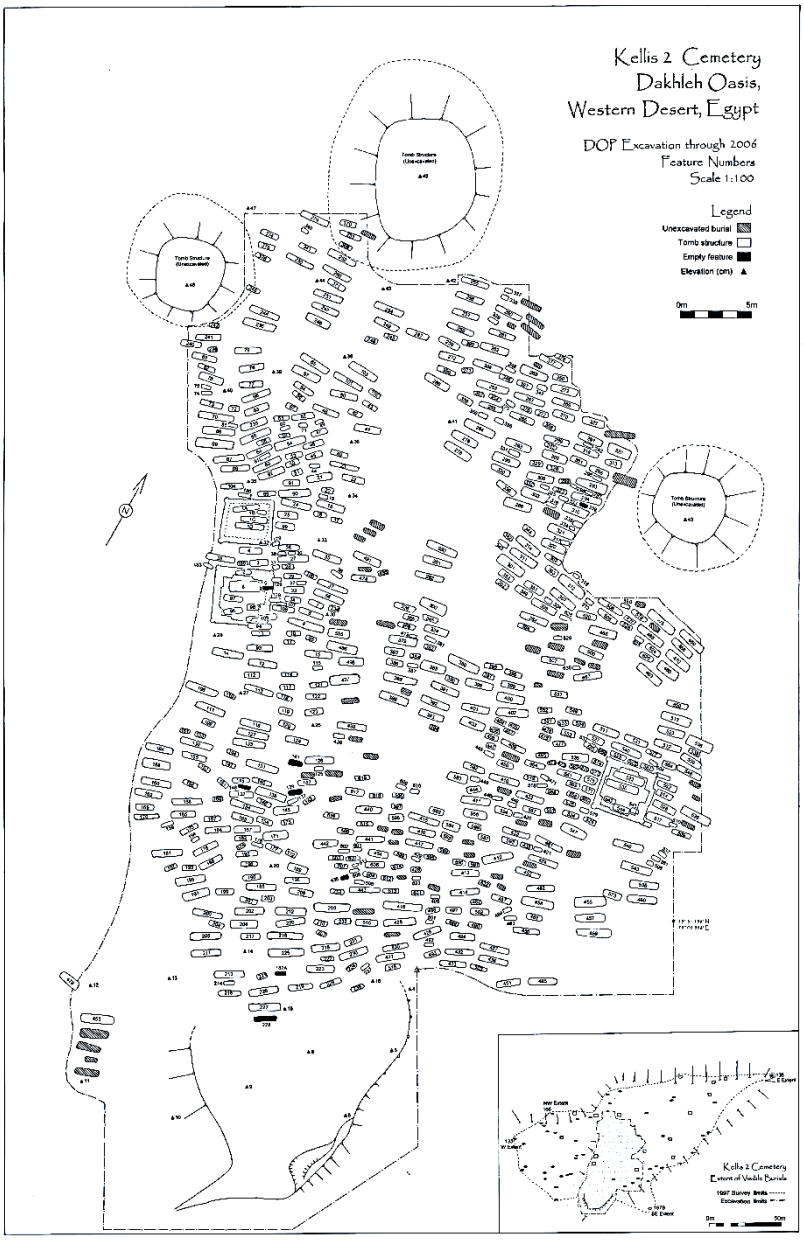

Figure 4.4 Cemetery plan of excavated burials at Kellis 2 (Courtesy of JE Molto)

4.3 Sample

The thesis sample consists of 310 complete spines from the Kellis and Ain Tirghi cemeteries. From the 310 complete spines, 174 are females, 129 are males and 7 are of unknown sex and age. These seven individuals were culled resulting in a final sample of 303 divided into two age groups: 20-35 and 36+. There were 154 in the former age group and 149 in the latter. In terms of site breakdown, 213 of the spines were from Kellis 2, 75

from Ain Tirghi, 11 from the Kellis town site and 4 were from Kellis 1. There were less than 10 adolescent complete spines in the samples so this small sample size precluded their use in this study.

4.3.1 Age Estimation and Sex Determination

Sex and age estimations were done in the field by Dr. Molto in conjunction with the DOP bioarchaeology team members. Sex determination was based primarily on the 'Phenice Method' (Phenice 1969). When the os pubis was not available, sex was based on standard robusticy characteristics of the infracranial bones and skull (Buikstra and Ubelaker 1994). Adult age of death was determined using the pubic symphysis (Brooks and Suchey 1990), rib morphogenesis (Iscan et al., 1984) and dental attrition. As noted sex estimation and age determination were done in the field; blind studies were conducted to test for intraobserver and interobserver error. High degrees of concordance were found among the researchers. Thus, the estimates are reasonably unbiased and are accurate profiles of each individual (Brown 2013, Molto personal communication).

4.4 Scoring Traits

Dr. Molto collected and recorded data on 38 metric traits and 76 nonmetric traits (50 cranial traits, 21 infracranial traits and 5 dental traits) on the skeleton (Molto 2001). From the 21 infracranial traits that were noted and scored, 17 of these traits will be used for the purposes of this thesis as described in Chapter 3. Observer reliability was tested for all the all vertebral traits. Each trait was scored using five different options; absent (0), partially expressed or expressed unilaterally on the right side (1), expressed unilaterally on the left side (2), fully expressed or complete bilateral expression (9), or unobservable (x). As statistical testing will be conducted in a bivariate form (more details to proceed in the statistical section of this chapter), all traits that scored a (1), (2), or (9) will be amalgamated together into one group denoted as (9) to include all traits that show any form of expression of the trait. All traits are ultimately scored as present (9) or absent (0).

4.5 Statistical Analysis

A number of statistical analyses are used herein to address the research questions and hypotheses stated in Chapter 1 and 2.

4.5.1 G-Test

The G-test, a log-likelihood test, has two functions: it tests for goodness of fit (comparing frequencies to theoretical expectancies), as well as, a test for independence (comparing frequencies of one variable for different variables of another variable) like the chi-square (McDonald 2009). The G-test provides a finer result in comparison to the popular chi-square and it has an additive dimension in that it can be used to test the independence of traits of more elaborate data (Sokal and Rohlf 1981). The null hypothesis is that proportions of individuals expressing a nonmetric trait will be independent of sex and age and side expression. The G-test is calculated as follows:

$$2 \sum O_i \cdot ln\, O_i /E_i$$

where the observed frequencies are used to calculate the expected frequencies. In cases where there are any cells that contain a value less than 10, the Yates' continuing correction will be applied as this correction is used to prevent overestimation of statistical significance when the data are small.

The G-test and its associated p-value will be used to calculate sex differences (male and female), age differences (20-35 and 36+) for each of the 17 traits and side expression (left and right) for 10 traits as not all traits showed left right expression. P-values will be tested at the 0.05 level. The G-test value will be calculated using a formula executed in Excel 2010. The p-value alongside Yates correction (when applicable) will be calculated using an online calculator called G-test calculator (i.e., http://elem.com/~btilly/effective-ab-testing/g-test-calculator.html).

4.5.2 Phi Coefficient

Chi-square has been the traditional test used to analyze epigenetic parameters (age, sex, side, symmetry and intertrait correlation). However, it has many drawbacks particularly for use in studying intertrait correlations (Sjøvold 1977). The chi-square test cannot tell us how variables are related; it only measures the differences between the expected and observed values and more importantly, the chi-square cannot describe the strength of the relationship (association) between or among variables. While it can be determined that a relationship is significant statistically, whether this relationship is strong or weak cannot be established (Shennan, 1997). In order to gain information regarding the strength of any intertrait correlations, Sjovold (1977) and Molto (1980) recommend the use of the phi coefficient for determining trait interaction. The phi coefficient is not as sensitive to sample size as the chi square is. In addition, the phi coefficient is equivalent, mathematically, to the Pearson r, which is used on metric variants, therefore, using the phi coefficient allows for better comparison between metric and nonmetric data (Molto 1980). The phi coefficient measures the amount of association between two categorical variables. The main weakness of Phi is that it is limited to bivariate testing. In order to compute data using a 2 x 2 matrix for bivariate analysis, full expressions originally denoted as "9" will combine with the partial/incomplete expression already labeled as "1" or "2" in order to meet this criteria. Value cells which contain 'x' will not be included in intertrait analysis.

Table 4.1. Sample 2 x 2 contingency table

		Trait A		
Trait B		Present	Absent	Total
	Present	a	b	a + b
	Absent	c	d	c + d
	Total	a + c	b + d	a + b + c + d

The phi with a 2 x 2 contingency table is calculated using the following formula,

$$\varphi = \frac{ad - bc}{\sqrt{(a+b)(a+c)(b+d)(c+d)}}$$

where a result of 0 indicates that there is no association between the two variables. A score of -1 or +1 means that there is a perfect negative or positive association (correlation) between the two traits respectively. In other words, a value of -1 means the two variables are completely independent of each other; they never occur together while a value of +1 means that the studied variables are completely correlated; they always occur together.

The phi coefficient will be used on all trait pairs of the 17 traits studied. One hundred thirty six pairwise comparisons for both males and females will be analyzed separately and then the sexes will be combined to represent the population. In addition, left and right side expressions of the traits using 182 pairwise comparisons will be tested for both the male and female population separately and again will be combined for the entire population. Tests were run to determine correlations at the 0.05 confidence level. Any significant correlations were analyzed for possible sex, age or side differences, and possible biological etiology for correlation.

In addition to using phi coefficient for intertrait correlating, phi coefficient will be used to look at bilateral occurrence of traits that are scored by unilateral/bilateral or left/right side expression. Phi coefficients and their associated p-values will be calculated using SPSS Statistics for IBM Version 19.0.0

4.5.3 Index of Bilaterality

Most nonmetric traits occur more often unilaterality than bilaterally. Also most traits have prevalence lower than 30%, which means the absence is $\geq$ 70%. When traditional statistical testing is conducted the common absence cell is usually very large and distorts statistical results leading to Type 1 errors. In symmetry, most studies report significant results because of this artifact. Molto (1985) proposed a new statistic, the bilaterality index which tells which traits have greater bilateral tendencies. It is calculated as follows:

$$\frac{\textbf{fequency of bilateral occurrence}}{\textbf{frequency of bilateral occurrence} + \textbf{frequency of unilateral occurence}}$$

An index value greater than 50 indicates that the trait tends towards bilaterality, while an index value less than 50 indicates that the trait tends towards unilaterality. The index can be further tested using the odds ratio.

4.5.4 Odds Ratio

Like the phi coefficient, the odds ratio is another measure of association statistic although it is more commonly used in clinical research as an approximation to relative risk (Daya 2000, Enticott et al. 2012). Odds ratio is of great use as it provides an estimate with a confidence interval of the relationship between two binary variables (Bland and Altman 2000). For this thesis, the odds ratio will be used to determine how many times it is more likely for two traits to appear together rather than appear separately. An odds ratio value of 1 signifies that there is no association between the two traits, the traits are independent. An odds ratio greater than one indicates that there is some type of association between the two traits; the larger the value, the stronger the association between the traits. Waldron (2009) specifies that an odds ratio greater than 2 is usually a strong enough association to be reviewed for further investigation. An odds ratio that is less than 1 signifies a negative association between the two traits. Using the 2 x 2 table (**Table 4.1**), the odds ratio can be calculated using the formula,

$$\frac{a/c}{b/d}$$

which can be further simplified to:

$$\frac{ad}{bc}$$

Where a = individuals who possess trait A and B,
b= individuals who possess trait A and not trait B
c= individuals who possess trait B and not trait A
d= individuals who do not possess trait A and B

After the odds ratio has been determined, the confidence interval at 95% is used to determine the precision of the odds ratio, although in practice it is used as a substitute of presence for statistical significance (Szumilas 2010), can be calculated using the following formula:

$$Ln\ (OR) \pm 1.96\ x\ \sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$$

where adding and subtracting the confidence coefficient by the standard error to the natural logarithm of the odds ratio point estimate produces the upper and lower limits respectively.

If the 95% confidence interval excludes 1 (the null value) then the correlation is confidently thought to be significant. If the 95% confidence interval does contain 1, then it cannot be determined decidedly that the correlation is not significant. However, in cases where the odd ratio point estimate is high and the confidence interval does include 1, such cases should be examined more closely (i.e., look at the phi coefficient to determine if the correlation appears to be significant).

The odds ratio will be used for all trait pairs of the 17 traits studied. One hundred thirty six pairwise comparisons for both males and females will be analyzed separately and then the sexes will be combined to represent the population. In addition, left and right side expressions of the traits using 182 pairwise comparisons will be tested against the male and female population separately and again will be combined for the entire population. Any significant correlations will be analyzed for possible sex, age or side differences, and possible biological etiology for correlation.

The use of odds ratio to test intertrait correlation in the literature is quite rare; it appears there is only one study using the odds ratio on nonmetric data of the cranium (Brown 2013). Odds ratio and their associated confidence intervals at 95% will be calculated using SPSS Statistics for IBM Version 19.0.0.

4.6. Spatial Statistical Analysis

The data used for this thesis not only provides details on nonmetric data on an Egyptian population, but also offers specified location coordinates and these spatial locations could potentially reveal important information about the relationship of these traits to their position. For this reason, spatial statistical analysis will be performed. Maps illustrating the number of occurrences of each of the 17 traits by *joining the trait spreadsheet to the project's GIS site map.* The (X,Y) coordinates were extracted from the GIS into an Excel file and combined with a spreadsheet containing data on trait presence and absence. Following the formation of the maps, statistical routines that fall under the category of Point Pattern Analysis were conducted using a set of R routines (Keron 2014). All routines are distance-based as various statistics will calculate the distances between events.

4.6.1 Proximity Probability Analysis

This technique developed by Keron (2014) works to count the pairs of graves of a specified nonmetric trait within a specified radius. The routine goes through the list of graves with the trait in question one by one, counting the number of other graves that can be found in the specified distance established. Any given pair that is found is only counted once. The probability of this final count found in a random distribution is calculated by using a Monte Carlo routine, which works as it locates *n* amount of events randomly in the study area; the results of the randomized pattern can be used to calculate an empirical expected frequency distribution and determine how unusual an observed pattern may be (O'Sullivan and Unwin, 2003). The Monte Carlo routine is run on the graves which exhibit a presence or absence of the trait. The Monte Carlo routine randomly selects graves that match the count of graves that exhibit the trait using 99 randomizations (Keron 2014). The proximity probability analysis will run on all 17 nonmetric traits using individuals from the Kellis 2 site, and will be executed in R statistical program for Microsoft Version 3.0.2 (2013).

4.6.2 Cross Proximity

The cross proximity is very similar to the proximity probability and was also developed by Keron (2014), but it considers the possibility of co-occurrence of two sexes. Like the proximity probability, it counts the pairs of graves within an established distance. However, unlike the proximity probability, which provides a global statistic (one single numeric statistic), four statistics are produced. The first statistic counts the number of pairs only using the male data. The second statistic counts the number of pairs of males and females who exhibit the trait starting with the males. The third statistic counts the number of pairs but instead uses female data while the final statistic looks at the number of pairs of females and males starting with females. The counts for the second and fourth statistic are the actual cross comparison. In addition, the second and fourth statistic will be the same but the probabilities may differ as probabilities are calculated by randomly selecting samples without replacement using a Monte Carlo routine at 99 randomizations. The cross proximity probability analysis will run on all 17 nonmetric traits using individuals from the Kellis 2 site, and will be computed in R statistical program for Microsoft Version 3.0.2 (2013).

4.6.3 Cross Nearest Neighbour

The nearest neighbour (NN) is a ratio that is defined by the average over all the points between a point and the nearest other point divided by average distance to be expected if the same points were randomly distributed in the same area. A random distribution would yield a value around 1 whereas clustering would equate to a value range of 0 to less than 1 and a more even distribution would yield values greater than one with upper limits of about 2.15 (Kintigh 1990). There are some issues that arise when using nearest neighbour such as the boundary problem where the distribution of the objects in question exceeds the edge of the study area. This is problematic as it can distort the NN statistic; points that are close to the edge are computed to points that fall within the study area while there may be points that are closer but lay outside the boundary. The nearest neighbour is also subjected to the size of the boundary of the study area (Keron, 2014).

The nearest neighbour that is used in this thesis was developed by James Keron (2014); known as the cross nearest neighbour, it is a variant of a statistic used in archaeology called "between type nearest neighbour" (Kintigh 1990). Using Keron's statistical cross nearest neighbour, the average nearest neighbour is calculated normally in the traditional fashion but then it is evaluated where grave location and sex are held constant while the trait is randomly distributed over them which provide a value labeled as Rand AvgNN. This is different from the traditional Nearest Neighbour statistic, which evaluates the expected distribution against complete spatial randomness (CSR). As O'Sullivan and Unwin note (2003), CSR is the least likely situation to occur in human activity and it is an acceptable strategy to use any other model which one wishes to evaluate. In this case, we hold grave location and sex fixed and then randomly distributes the trait over them. One more value is then computed, NNRatio which is analogous to the traditional NN Statistical where the actual distance is divided by the randomized distance using a Monte Carlo technique using 99 randomizations. Values below 1 indicate clustering, values over 1 describes an even distribution while values near or at 1 marks a random distribution. The cross nearest neighbour routine will run on all 17 nonmetric traits using individuals from the Kellis 2 site, and will be executed in R statistical program for Microsoft Version 3.0.2 (2013).

4.6.4 Hodder and Okell's A Statistic

Hodder and Okell's spatial association, but more commonly seen as Hodder and Okell's A (HOA) statistic, is a statistic used to address some of the problems associated with nearest neighbour as it measures the spatial association incorporating the nearest neighbour distances and the distances from one point to every other point found in the distribution (Kintigh 1990). An advantage of HOA is that it is not affected by the boundary problem as all the points in the distribution are used. HOA works to measure the degree of segregation between traits that are absent and present by taking the average distance between all points that are present, multiplying it by the average distance between all points that are absent and dividing it by the square of the average distance between all the distance that are present and absent. A value of 1 indicates clustering while a value considerably less than 1 indicates segregation. A value greater than one is

considered to be empirically rare in archaeology (Kintigh 1990, Keron 2014). A Monte Carlo will then be run to provide an approximation of statistical significance using 99 randomizations. Hodder and Okell's A statistic with the associated probability will run on all 17 nonmetric traits using individuals from the Kellis 2 and will be executed in R statistical program for Microsoft Version 3.0.2 (2013).

4.7 Conclusion

This chapter has overviewed the samples used in the thesis and the statistical methods used to test they hypotheses. Despite the data having not been collected by me, I did considerable training on the issues surrounding the scoring and epigenetic analysis of the traits and the archaeological context of the Dakhleh burials in order to precisely define the methodological approach outlined.

Chapter 5

Results

5.1 Introduction

This chapter reviews the results of the statistical analyses conducted on Dakhleh Oasis infracranial nonmetric data. The initial hypothesis addresses the influence of age, sex, symmetry and intertrait correlation on the expression of the infracranial variants. The null hypothesis (H_o) is that the prevalence of these traits in the Dakhleh population sample are independent of these epigenetic factors, which would increase the validity of use of these traits in paleogenetic research. In addition, a second null hypothesis addresses the developmental model of Barnes (1994) in terms of border shifting impacting the prevalence and expression of these traits. A final hypothesis concerns the spatial distribution of these traits in the K2 cemetery which states that these traits are not useful for determining familial relationships. By convention, the acceptance or rejection of the hypotheses is based on the 95% to 90% confidence level for the statistical tests outlined in Chapter 4.

5.2 Age, Sex and Side Analysis

These analyses, which are based on the combination of the g-test, odds ratio and phi coefficient are done to determine the influence of these factors on trait expression, and to determine which traits are more likely to be influenced. It is important to acknowledge the fact that positive significant statistical associations can occur by chance. For this reason, traits that are found to be significant at 0.05 require an explanation that will be presented in Chapter 6.

The results of the g-test and odds ratio for the male and female data are shown in Table 5.1. Of the 17 infracranial traits, two traits, the posterior bridge (0.03) and spondylolysis L5 (0.02) show significant sex difference, in both cases being higher in males. The lateral bridge which closely approaches significance (7.459, 0.06) is also found more commonly among males. The results for the odds ratio are similar. Three traits, the lateral bridge, posterior bridge, and spondylolysis of L5 show significant sex

differences. These male associations make sense as the atlas traits are hyperstotic and the spondylolysis is thought to have an etiology that is stress related (e.g., males involved in heavy labour work) (Merbs 1989).

Table 5.1. G-Test (P<0.05) and odds ratio analysis of different nonmetric traits comparing females and males

Trait	G Test	P-Value	Odds Ratio	Confidence Interval
Sup Fac	0.284	0.59	1.180	0.643, 2.166
Lat Bri	7.459*a*	0.06	*7.684*	1.647, 35.844
Pos Bri	8.669	0.03	*2.560*	1.359, 4.824
Pos Arf	0.027	0.87	0.953	0.535, 1.696
IncFT C1	2.022	0.16	0.572	0.260, 1.259
C1 Cleft	0.000*a*	1.00	1.044	0.352, 3.101
IncFT C2	1.021	0.31	1.379	0.740, 2.569
OsAp Lig	0.073	0.79	0.922	0.511, 1.663
DivFT C6	0.056	0.81	1.063	0.639, 1.769
DivFT C7	0.306	0.58	1.198	0.632, 2.270
IncFT C6	0.000*a*	1.00	1.363	0.270, 6.890
IncFT C7	0.074*a*	0.78	1.279	0.501, 3.268
SpoL4	2.260*a*	0.13	*4.169*	0.827, 21.020
SpoL5	5.146*a*	0.02	*3.117*	1.230, 7.895
Sac L5	0.901*a*	0.34	0.619	0.270, 1.419
SBO	1.975*a*	0.16	1.582	0.834, 3.000
Vert	1.210*a*	0.27	0.333	0.068, 1.636

Note: G-test values followed by *a* have been corrected using Yates correction.

Table 5.2 shows the g-test and odds ratio values testing age difference between young adults (20-35) and older adults (36+). The results of the g-test show three traits with significant age differences: c1 cleft and spina bifida occulta are found to be age dependent, having statistically higher prevalence in early adulthood while the ossified apical ligament is significantly more statistically prevalent in the older groups. Spondylolysis of L4, approaches significance (3.458, 0.06), being higher in the older age cohort. The first two traits are hypostotic and these traits are generally higher in the younger cohorts, while the L4-spondylolysis data though making sense from the etiological standpoint is at variance to the data of L5 spondylolysis which also shares the same etiological background. However, when the odds ratio is used to test the traits, the results are slightly different. The ossified apical ligament was the only trait that was found to be statistically significant where the confidence interval did not include one and spondylolysis of L4 has a high odds ratio point estimate.

Table 5.2. G-test (P<0.05) and odds ratio analysis of different nonmetric traits comparing age ranges from 20-35 and 36+ years

Trait	G stat	P-Value	Odds Ratio	Confidence Interval
Sup Fac	1.356	0.24	1.432	0.780, 2.628
Lat Bri	0.000	1.00	0.992	0.311, 3.162
Pos Bri	0.361	0.55	1.209	0.650, 2.249
Pos Arf	0.379	0.54	1.195	0.677, 2.108
IncFT C1	0.287	0.59	0.818	0.393, 1.706
C1 Cleft	3.864*a*	0.05	0.255	0.070, 0.938
IncFT C2	0.100	0.75	0.905	0.487, 1.682
OsAp Lig	33.141	0.00	*6.568*	*3.215, 13.416*
DivFT C6	2.403	0.12	0.671	0.404, 1.113
DivFT C7	0.066	0.80	0.902	0.487, 1.739
IncFT C6	0.133*a*	0.72	1.951	0.351, 10.852
IncFT C7	0.150*a*	0.70	1.351	0.524, 3.482
SpoL4	3.458*a*	0.06	7.304	0.887, 60.143
SpoL5	0.142	0.71	0.846	0.353, 2.024
Sac L5	0.000	1.00	1.023	0.469, 2.230
SBO	6.567	0.01	0.422	0.214, 0.834
Vert	1.809*a*	0.18	3.560	0.724, 17.492

Note: G-test values followed by *a* have been corrected using Yates correction

Table 5.3 shows the results of symmetry (right versus left) testing using the g-test. Of the 10 traits that were scored by side difference (bilaterality), none of the traits showed a significant side difference at the 0.05 level. Two traits, incomplete foramen transversarium of C2 (2.861, 0.09) and divided foramen transversarium of C6 (3.271, 0.07) are approaching significance with both traits appearing on the right side more often.

Table 5.3 G-test (P<0.05) analysis of symmetry for 10 bilateral nonmetric traits

Trait	G stat	P-Value
Sup Fac	0.538	0.46
Lat Bri	0.000*a*	1.00
Pos Bri	0.008	0.99
Pos Arf	0.554	0.46
IncFT C1	0.162	0.69
IncFT C2	2.861	0.09
DivFT C6	3.271	0.07
DivFT C7	2.526	0.11
IncFT C6	0.580*a*	0.45
IncFT C7	1.880	0.17

Note: G-test values followed by *a* have been corrected using Yates correction

Unlike testing for age, sex and side differences, the use of the g-test is not sufficient to test for the homogeneity of trait prevalence (Molto 1980 in Edwards 2005). The use of phi coefficient can adequately address this problem. The phi coefficient and odds ratio results for the ten traits are shown in Table 5.4 found below.

Table 5.4. Phi coefficient (P<0.05), odds ratio and index analysis of bilateral expression of different nonmetric traits

Trait	Phi- coefficient	P-value	Bilaterality Index	Odds Ratio	Confidence Interval
Sup Fac	0.737	0.00	64.3	1.80	0.930, 3.484
Lat Bri	0.383	0.00	25.0	0.33	0.071, 1.527
Pos Bri	0.568	0.00	46.0	0.85	0.430, 1.679
Pos Arf	0.434	0.00	35.9	0.56	0.302, 1.038
IncFT C1	0.502	0.00	37.5	0.60	0.252, 1.428
IncFT C2	0.412	0.00	31.4	0.46	0.227, 0.933
DivFT C6	0.473	0.00	53.5	1.15	0.768, 1.722
DivFT C7	0.500	0.00	38.8	0.63	0.292, 1.359
IncFT C6	0.308	0.00	16.7	0.25	0.022, 2.831
IncFT C7	0.530	0.00	36.8	0.58	0.188, 1.793

There is a significant association found with all the traits that were scored by left and right side prevalence; bilateral traits are highly correlated. Based on the bilateral index where a value greater than 50 tends towards bilateral appearance, divided superior facet tends to appear in a bilateral fashion (64.3) and divided foramen transversarium of C6 tends slightly towards bilaterality (53.5). The rest of the traits tend towards unilaterality though they are still significantly correlated. This confounding result is probably due to the influence of the common absence cell on the Phi values. To account for this, the odds ratio was calculated with the elimination of the common absence cell. Only two traits had odds ratio point estimate greater than one, divided superior facet and divided foramen transversarium of C6 which are the same two traits that had a bilateral index greater than 50.

5.3 Phi coefficient and Intertrait Correlations

The use of the phi coefficient is again recommended for testing correlation (Sjøvold 1977, Molto 1980). Significant comparisons at the 0.05 level will be reported in this section. A total of 408 trait pair comparisons for the female, male and whole population,

in addition to the 546 trait comparisons for left and right side expression in the female, male and whole population (a total of 954 trait pairs) were analyzed. Right and left side expressions of the same trait are excluded from this list as these types of correlations have already been looked at and are known to be highly correlated. It is expected at the 0.05 level, seven correlations will be significant based on chance and 18 correlations when right and left side are taken into account will be significant based on chance. The expected and the observed will be reported.

5.3.1 Female Phi- coefficient

Using grave data gathered from Ain Tirghi, Kellis Town site, Kellis 1 and Kellis 2, 136 pairwise comparisons and an additional 182 comparisons based on right and left side expression were tested using phi coefficient. The results of the phi coefficient for the female subset are found below.

Table 5.5. Significant intertrait correlations of nonmetric traits (P<0.05) among females

Trait	Phi coefficient	P-value
Sup Fac/Pos Bri	0.283	0.00
Lat Bri/Vert	0.369	0.00
Pos Bri/SBO	0.257	0.00
Pos Arf/IncFT C1	0.176	0.03
Pos Arf/DivFT C7	0.177	0.04
IncFT C1/C1 cleft	0.228	0.01
IncFT C1/SpoL4	0.192	0.02
C1 cleft/IncFT C6	0.176	0.04
C1 cleft/SpoL4	0.345	0.00
C1 cleft/SBO	0.164	0.05
DivFT C7/SpoL4	0.181	0.03
SpoL5/SBO	0.288	0.00

For the female subset, which involved a study of 136 pairwise comparisons, it is expected that seven intertrait correlations would be significant due to chance; however 12 pairwise correlations were found to be statistically significant. It can be hypothesized that there is some genetic factor(s) that could account for the large number of significant correlations. Further review of significant correlated traits will be found in Chapter 6.

Table 5.6 Significant right side intertrait correlations of nonmetric traits (P<0.05) among females

Trait	Phi coefficient	P-value
R. Sup Fac/R. Lat Bri	0.187	0.02
R. Sup Fac/R. Pos Bri	0.260	0.00
R. Lat Bri/R. Pos Bri	0.237	0.00
R. Lat Bri/R. IncFT C2	0.208	0.01
R. Pos Arf/L. IncFT C1	0.189	0.02
R. IncFT C1/L. DivFT C7	0.192	0.03
R. IncFT C2/L. IncFT C6	0.214	0.01
R. IncFT C2/R. IncFTC7	0.232	0.01

Table 5.6 shows the phi results from examining intertrait correlations on the right side for the 10 bilaterality scored traits. Eight significant correlations were found, which is one less trait correlation than expected to occur based on chance. Of these eight correlations, five appeared only on the right side.

Table 5.7. Significant left side intertrait correlations of nonmetric traits (P<0.05) among females

Trait	Phi coefficient	P-value
L. Sup Fac/R. Lat Bri	0.183	0.03
L. Sup Fac/R. Pos Bri	0.191	0.02
L. Pos Bri/R. Pos Arf	0.199	0.02
L. Pos Arf/L. IncFT C1	0.162	0.05
L. Pos Arf/R. DivFT C7	0.210	0.02
L. IncFt C1/R. DivFT C6	0.197	0.02
L. IncFT C2/L. IncFT C7	0.213	0.01
L. DivFT C7/SacL5	0.204	0.02
L. IncFT C6/C1 cleft	0.175	0.04
L. IncFT C6/R. IncFT C7	0.389	0.00

Table 5.7 shows the results of the left side testing for the females. Unlike the right side, the female subset based on the left side expression yielded ten significant correlations, one more significant correlation found than the expected. Four of the significant correlations were duplicates with the right side results, while six were different.

Totaling the right and left side expression data, 18 pairwise comparisons were expected to occur and 18 were found. Therefore, it cannot be suggested that there is a strong heritable genetic influence; these correlations can be described as stochastic although some of the correlations may have genetic meaning.

5.3.2 Male Phi coefficient

Like the female subset, 136 and 320 pairwise comparisons using the same regional sample were conducted on the male subset of the population using phi coefficient. All significant comparisons are reported below.

Table 5.8. Significant intertrait correlations of nonmetric traits (P<0.05) among males

Trait	Phi coefficient	P-value
Sup Fac/Pos Bri	0.191	0.05
Lat Bri/Pos Bri	0.227	0.02
Lat Bri/Pos Arf	0.191	0.05
Lat Bri/OsAp Lig	0.217	0.03
Lat Bri/Vert	0.302	0.00
Pos Bri/IncFT C7	0.351	0.00
Pos Bri/SpoL4	0.255	0.01
Pos Arf/OsAp Lig	0.277	0.01
Pos Arf/SacL5	0.201	0.04
OsAp Lig/Vert	0.248	0.01
DivFT C6/DivFT C7	0.319	0.00
SpoL4/SpoL5	0.316	0.00
SacL5/Vert	0.208	0.03

For the male subset, 13 pairwise comparisons were found to be significant which is almost double the amount of comparisons expected by chance.

In Tables 5.9 and 5.10 the right and left male subset of intertrait correlations are respectively shown. There were 11 significant correlations on the right side which is two more than expected to occur by chance, whereas on the left there were only six significant correlations or three less than expected by chance. Two common correlations appear between the left and right side, the posterior bridge and incomplete foramen transversarium of C7 and the posterior arch foramen and the ossified apical ligament (see

asterisks). The remaining correlations are not symmetrical. The total amount of right and left significant trait pairs expected to occur is 18 but actually 17 occurred. This is close to chance expectation. When the side expression criterion is removed, the number of significant correlations exceeds what is expected.

Table 5.9. Significant right side intertrait correlations of nonmetric traits (P<0.05) among males

Trait	Phi coefficient	P-value
R. Sup Fac/Vert	0.215	0.03
R. Lat Bri/SpoL4	0.298	0.00
R. Pos Bri/R. IncFT C7*	0.294	0.00
R. Pos Bri/L. IncFT C7	0.366	0.00
R. Pos Bri/Vert	0.195	0.05
R. Pos Arf/OsAp Lig*	0.249	0.01
R. Pos Arf/Vert	0.208	0.04
R. IncFT C1/R. IncFt C2	0.204	0.04
R. DivFT C6/R. DivFT C7	0.257	0.01
R. DivFT C6/L. DivFT C7	0.308	0.00
R. IncFT C7/SacL5	0.242	0.01

Table 5.10. Significant left side intertrait correlations of nonmetric traits (P<0.05) among males

Trait	Phi coefficient	P-value
L. Sup Fac/L. Pos Bri	0.228	0.02
L. Lat Bri/L. Pos Arf	0.205	0.04
L. Lat Bri/R. DivFT C7	0.210	0.04
L. Pos Bri/L. IncFT C7*	0.384	0.00
L. Pos Arf/SpoL4	0.197	0.05
L. Pos Arf/OsAp Lig*	0.224	0.02

5.3.3 Composite Population

Intertrait correlation data with the sexes pooled is shown in Table 5.11. Only the significant comparisons are shown. Eighteen pairwise comparisons were found to be significant. A negative significant correlation found with the c1 cleft and ossified apical

ligament (*) means that the traits are independent and thus this combination can be removed. The 17 significant correlations still is nearly 2 and half times more than expected by chance. A genetic commonality could explain the large number of correlations, although each pair of traits has to be analyzed for their potential biological relevance.

Table 5.11. Significant intertrait correlations of nonmetric traits (P<0.05) among the composite population.

Trait	Phi coefficient	P-value
Sup Fac/Pos Bri	0.234	0.00
Lat Bri/Pos Bri	0.217	0.00
Lat Bri/OsAp Lig	0.141	0.03
Lat Bri/SpoL4	0.209	0.00
Pos Bri/IncFT C7	0.148	0.02
Pos Bri/SpoL4	0.189	0.00
Pos Bri/SBO	0.180	0.01
Pos Arf/IncFT	0.127	0.04
Pos Arf/OsAp Lig	0.153	0.02
Pos Arf/DivFT C7	0.132	0.04
IncFT C1/C1 cleft	0.176	0.01
C1 cleft/OsAp Lig	-0.134	0.04
OsAp Lig/Vert	0.161	0.01
DivFT C6/DivFT C7	0.203	0.00
DivFT C7/IncFT C6	0.143	0.03
SpoL4/SpoL5	0.216	0.00
SpoL5/SBO	0.194	0.00
SacL5/Vert	0.161	0.01

Tables 5.12 and 5.13 respectively show the significant correlations for the right and left side in the composite sample. For the right side, nine significant correlations were found; exactly what is expected. There can be some biological meaning for the traits that are correlated even though those trait pairs have occurred based on chance. Four of the traits pairs showed expression on only the right side. On the left side, there were 20 significant correlated trait pairs, which is more than double the expected number of

correlations. These data could obviously reflect the impact of the male-female differences along with some potential genetic influences.

Table 5.12. Significant right side intertrait correlations of nonmetric traits (P<0.05) among the population

Trait	Phi coefficient	P-value
R. Sup Fac/R. Pos Bri	0.171	0.01
R. Lat Bri/R. Pos Bri	0.181	0.00
R. Pos Bri/L. IncFT C7	0.185	0.01
R. Lat Bri/SpoL4	0.268	0.00
R. Pos Arf/L. IncFT	0.134	0.03
R. IncFT C2/C1 cleft	0.143	0.03
R. IncFT C2/R. IncFT C7	0.150	0.02
R. DivFT C6/R. DivFT C7	0.160	0.01
R. DivFT C6/L. DivFT C7	0.186	0.00

.

Table 5.13. Significant left side intertrait correlations of nonmetric traits (P<0.05) among the population

Trait	Phi coefficient	P-value
L. Sup Fac/R. Pos Bri	0.136	0.03
L. Sup Fac/L. Pos Bri	0.201	0.00
L. Lat Bri/R. Pos Bri	0.135	0.03
L. Lat Bri/L. Pos Bri	0.138	0.03
L. Lat Bri/R. Pos Arf	0.125	0.05
L. Lat Bri/OsAp Lig	0.135	0.03
L. Lat Bri/R. DivFT C7	0.132	0.05
L. Lat Bri/SpoL4	0.132	0.04
L. Lat Bri/SpoL5	0.128	0.04
L. Pos Bri/R. Pos Arf	0.134	0.03
L. Pos Bri/L. IncFT C7	0.228	0.00
L. Pos Bri/SpoL4	0.166	0.01
L. Pos Bri/SBO	0.178	0.01
L. Pos Arf/OsAp Lig	0.144	0.02
L. Pos Arf/R. DivFT C7	0.136	0.04
L. DivFT C6/R. DivFT C7	0.201	0.00
L. DivFT C6/L. DivFT C7	0.151	0.02
L. DivFT C7/L. IncFT C6	0.155	0.02
L. DivFT C7/SacL5	0.174	0.01
L. IncFT C6/R. IncFT C7	0.262	0.00

It appears that intertrait correlations among this population far exceed what is expected, but when side expression criteria are removed the number of significant correlations drops to a value more closely similar to what is expected. Overall the large numbers of significant correlations suggest that there could be a strong heritable genetic influence accounting for the high number of correlations.

5.4 Correlations by Trait Type

The nonmetric traits examined for this thesis can be divided by four trait types, hypostotic, hyperstotic, foramina and other. The prevalence of these types of traits can differ within a population and traits with similar developmental origins may have greater probability of sharing genetic factors. To further examine the possible varying occurrence of the type traits, the results of the correlations between traits among the male and female population using phi coefficient will be reported. As these traits often have male-female differences, the influence of sex must be considered. Significant correlations will be further analyzed in Chapter 6.

5.4.1 Hypostotic

From the 17 traits, seven hypostotic traits (divided superior facet, incomplete foramen transversarium of C1, C1 cleft, incomplete foramen transversarium of C2, incomplete foramen transversarium of C6, incomplete foramen transversarium of C7 and spina bifida occulta) are analyzed. Correlations between the different hypostotic traits within the male and female populations using phi coefficient are found below. Phi coefficients are found above the dashed line and p-values are found below the dashed line.

Table 5.14. Male hypostotic trait correlations using phi coefficient

	Sup Fac	IncFT C1	C1 Cleft	IncFT C2	IncFT C6	IncFT C7	SBO
Sup Fac	-	-0.105	0.005	0.057	0.032	-0.073	-0.126
IncFt C1	0.28	-	0.080	0.148	-0.052	-0.083	-0.087
C1 cleft	0.96	0.41	-	0.072	-0.036	-0.057	-0.002
IncFT C2	0.57	0.14	0.47	-	0.042	-0.041	0.021
IncFT C6	0.75	0.60	0.72	0.67	-	-0.050	-0.089
IncFT C7	0.47	0.41	0.57	0.69	0.61	-	0.078
SBO	0.20	0.37	0.98	0.83	0.36	0.42	-

Of the 21 pairwise comparisons shown in Table 5.14, there were no significant comparisons found at 0.05 or even at the 0.10 level although it was expected that there would be one or two significant comparisons found at each level respectively.

Table 5.15. Female hypostotic trait correlations using phi coefficient

	Sup Fac	IncFT C1	C1 Cleft	IncFT C2	IncFT C6	IncFT C7	SBO
Sup Fac	-	0.018	0.042	0.038	-0.066	-0.150	-0.004
IncFT C1	0.82	-	0.228	-0.048	0.071	-0.121	-0.117
C1 cleft	0.61	0.00	-	0.130	0.176	-0.069	0.164
IncFT C2	0.65	0.56	0.12	-	0.059	0.154	0.049
IncFT C6	0.41	0.41	0.04	0.48	-	0.151	-0.061
IncFT C7	0.08	0.16	0.42	0.07	0.07	-	-0.025
SBO	0.66	0.16	0.05	0.56	0.48	0.75	-

Unlike the males, there were three significant correlations found at 0.05 (Table 5.15); three times as many traits than expected to be significant. At 0.10, six correlations were found to be significant, however, one correlation is negative (Sup Fac/ IncFT C7), which is an indication of independence and must then be removed. The removal of the trait leaves five significant positive correlations. The hypothesis that there is independence among correlated traits is rejected. There could possibly be some genetic underlying cause leading to the large correlations found among the traits. However, the fact that hypostotic traits generally have a higher prevalence in females (Ossenberg 1969; Saunders 1978; Molto 1985), suggests the finding may have a genetic meaning.

5.4.2 Hyperstotic traits

From the 17 traits, three are hyperstotic, lateral bridge, posterior bridge and ossified apical ligament. Correlations between the different hyperstotic traits among the male and female populations using phi coefficient are respectively shown in Tables 5.16 and 5.17. Again the Phi coefficients are found above the dashed line and the p-values are found below the dashed line.

Table 5.16. Male hyperstotic trait correlations using phi coefficient

	Lat Bri	Pos Bri	OsAp Lig
Lat Bri	-	0.227	0.217
Pos Bri	0.02	-	0.155
OsAp Lig	0.03	0.12	-

At the 0.05 level, it is expected that there will be no significant correlations among the three traits, but there were two significant correlations Lat Bri/Pos Bri and Lat Bri/OsApLig (0.227, 0.019 and 0.217, 0.029 respectively). The two significant correlation pairs among the only three pairwise comparisons lead to possible genetic causations. The Lat Bri/Pos Bri correlations will be further discussed in the following chapter.

Table 5.17. Female hyperstotic trait correlations using phi coefficient

	Lat Bri	Pos Bri	OsAp Lig
Lat Bri	-	0.125	0.071
Pos Bri	0.127	-	0.102
OsAp Lig	0.383	0.221	-

Unlike the males, there are no significant correlations at both the 0.05 and 0.10 level which was expected based on only three hyperstotic comparisons. The fact that hyperstotic traits are generally common in males (Ossenberg 1969, Saunders 1978, Molto 1980) likely accounts for these findings.

5.4.3 Foramina

Three foramina traits are in the database, posterior arch foramen and divided foramina transversarium of C6 and C7. Tables 5.18 and 5.19 report the data for males and females respectively. Phi coefficients are found above the dashed line and the p-values are found below the dashed line.

Table 5.18. Male foramina trait correlations using phi coefficient

	Pos Arf	DivFT C6	DivFT C7
Pos Aft	-	0.059	0.081
DivFT C6	0.56	-	0.319
DivFT C7	0.42	0.00	-

Out of the three comparisons there is one significant correlation between the divided foramina of C6 and C7, (0.422, 0.00) which is the same trait found on adjacent vertebra. It was expected 0 to 1 significant correlations to be found at 0.05, making this correlation somewhat expected.

Table 5.19. Female foramina trait correlations using phi coefficient

	Pos Arf	DivFT C6	DivFT C7
Pos Aft	-	0.026	0.177
DivFT C6	0.76	-	0.106
DivFT C7	0.04	0.22	-

Among the females, there is one significant correlation found between Pos Arf and DivFT C7 (0.117, 0.04) at the 0.05 level. This one correlation was expected to occur at 0.05 level. Independence of foramina traits cannot be rejected.

5.4.4 "Other" Type

There are four traits in the "other" category, one with mixed etiologies: spondylolysis of L4, and L5, sacralization of L5 and vertebral number. Like the other types of traits, male and females will each be examined for significant correlations using phi coefficient. Phi

coefficients are found above the dashed line and the p-values are found below the dashed line for the male and female data respectively shown in Tables 5.20 and 5.21. In these comparisons, the expected number of significant correlations is between zero and one.

Table 5.20. Male other trait correlations using phi coefficient

	SpoL4	SpoL5	Sac L5	Vert
SpoL4	-	0.316	-0.057	-0.029
SpoL5	0.00	-	-0.103	-0.050
Sac L5	0.52	0.25	-	0.208
Vert	0.77	0.59	0.03	-

For the males there are two significant correlations between SpoL4/SpoL5 (0.316, 0.00) and SacL5/Vert (0.208, 0.03). Correlation between Spondylolysis of L4 and L5 is expected as they are essentially the same trait occurring adjacent to each other. It would be difficult to suggest a genetic link between these types of traits among the male population as there are not enough significant correlations found in this population.

Table 5.21. Female other trait correlations using phi coefficient

	SpoL4	SpoL5	Sac L5	Vert
SpoL4	-	-0.023	-0.037	-0.029
SpoL5	0.77	-	-0.073	0.095
Sac L5	0.63	0.35	-	0.140
Vert	0.74	0.27	0.11	-

Among the females there are no significant correlations as expected when looking at significance at 0.05. It appears that among this type of traits, the hypothesis that traits would be independent cannot be rejected.

5.5 Regional Correlations

As described in previous chapters, Barnes (1994) asserts that a number of the variations that appear on the vertebral column are the result of cranial and caudal shifting. If this is true, it is expected that traits that exhibit cranial or caudal shifting would be correlated

with other traits that have also experienced cranial or caudal shifting. Ossified apical ligament and sacralization of the L5 are two traits which Barnes believes are caused by cranial shifting. Using phi coefficient, correlations between the two traits were analyzed. None of the correlations of these two traits among males (-0.043, 0.66), females (0.004, 0.96) and the entire population (-0.011, 0.86) were found to be statistically significant. This will be further discussed in Chapter 6.

5.6 Odds Ratio

The odds ratio has been recently cited as a good tool, in conjunction with phi coefficient, for examining intertrait correlations (Brown 2013) and will be used in this thesis to examine intertrait correlations. The odds ratio, like the phi coefficient, was calculated for each sex and the entire population in addition to right and left side correlations. All trait pairs with an odds ratio of two or higher are considered significant. Confidence intervals that do not encompass one are also deemed significant. All significant pairwise comparisons are found below.

5.6.1 Female Odds Ratio

Table 5.22 shows the results of significant intertrait correlation. Among the female population, 27 significant intertrait correlations out of 136 pairwise comparisons were found when using the odds ratio compared to the 12 significant correlations found with phi coefficient; more than double the number of pairwise comparisons. However only five comparisons when using odds ratio have a confidence interval that do not include one, although the confidence intervals for many traits are very wide.

Table 5.22. Significant odds ratio intertrait correlations of nonmetric traits (P<0.05) among the females

Trait	Odds Ratio	Confidence Interval
Sup Fac/Lat Bri	3.900	0.237, 64.178
Sup Fac/Pos Bri	5.143	1.905, 13.887
Sup Fac/SacL5	2.520	0.837, 7.586
Lat Bri/Pos Bri	6.737	0.404, 112.277
Lat Bri/IncFt C2	5.174	0.312, 85.726
Pos Bri/Pos Arf	2.276	0.849, 6.098
Pos Bri/IncFt C2	2.524	0.858, 7.425
Pos Bri/SBO	4.936	1.651, 14.756
Pos Arf/IncFT C1	2.692	1.067, 6.791
Pos Arf/DivFT C7	2.585	1.012, 6.600
IncFt C1/C1 cleft	6.421	1.480, 27.865
IncFt C1/DivFT C7	2.395	0.808, 7.100
IncFt C1/IncFT C6	2.714	0.235, 31.302
C1 cleft/IncFt C2	3.136	0.698, 14.088
C1 cleft/DivFT C6	2.451	0.477, 12.602
C1 cleft/IncFT C6	9.143	0.737, 113.421
C1 cleft/SBO	4.129	0.904, 18.863
IncFt C2/IncFT C6	2.333	0.203, 26.787
IncFt C2/IncFT C7	3.303	0.860, 12.684
OsAp Lig/Vert	3.065	0.588, 15.971
DivFT C6/SpoL5	4.932	0.578, 42.009
DivFT C7/IncFT C6	4.542	0.274, 75.197
IncFT C6/IncFT C7	7.222	0.597, 87.433
IncFT C7/Vert	2.600	0.274, 24.706
SpoL5/SBO	11.167	2.290, 54.440
SpoL5/Vert	3.333	0.344, 32.272
SacL5/Vert	3.867	0.676, 22.123

In Tables 5.23 and 5.24, the right and left side significant correlations among the female population are respectively shown. Twenty-two significant correlations were found on the right side with only five of these correlations with a confidence interval not containing one. In total only four trait pairs were found solely on the right side. The left side yielded more significant correlations; only eight of the 26 significant pairs did not contain unity and seven trait pairs were expressed only on the left side.

Table 5.23. Significant odds ratio right side intertrait correlations of nonmetric traits (P<0.05) among the females

Trait	Odds Ratio	Confidence Interval
R. Sup Fac/R. Pos Bri	5.261	1.732, 15.986
R. Sup Fac/L. Pos Bri	3.314	0.888, 12.369
R. Sup Fac/C1 cleft	2.145	0.391, 11.765
R. Sup Fac/SacL5	2.632	0.822, 8.427
R. Pos Bri/OsAp Lig	2.048	0.686, 6.113
R. Pos Bri/SBO	2.500	0.710, 8.803
R. Pos Arf/R. IncFT C1	2.036	0.596, 6.952
R. Pos Arf/L. IncFT C1	3.734	1.132, 12.321
R. Pos Arf/C1 cleft	4.341	0.951, 19.820
R. Pos Arf/R. DivFT C7	2.474	0.772, 7.931
R. Pos Arf/L. IncFT C6	3.222	0.278, 37.388
R. IncFt C1/C1 cleft	2.583	0.480, 13.901
R. IncFt C1/L. DivFT C7	4.077	1.077, 15.435
R. IncFt C1/L. IncFT C6	3.688	0.316, 43.015
R. IncFt C2/C1 cleft	3.967	0.872, 18.042
R. IncFt C2/L. IncFT C6	12.316	1.064, 142.565
R. IncFt C2/R. IncFT C7	6.333	1.453, 27.610
R. IncFt C2/L. IncFT C7	3.600	0.794, 16.321
R. DivFT C6/C1 cleft	2.212	0.507, 9.655
R. DivFT C7/C1 cleft	2.059	0.384, 11.050
R. DivFT C7/L. IncFT C6	5.895	0.353, 98.318
R. DivFT C7/SacL5	2.386	0.677, 8.408

Table 5.24. Significant odds ratio left side intertrait correlations of nonmetric traits (P<0.05) among the females.

Trait	Odds Ratio	Confidence Interval
L. Sup Fac/R. Pos Bri	3.537	1.151, 10.871
L. Sup Fac/L. Pos Bri	3.129	0.841, 11.639
L. Sup Fac/L. IncFT C1	2.477	0.777, 7.900
L. Sup Fac/C1 cleft	2.035	0.372, 11.135
L. Pos Bri/R. Pos Arf	4.610	1.206, 17.613
L. Pos Bri/L. IncFt C2	2.034	0.396, 10.411
L. Pos Bri/SpoL5	2.352	0.255, 21.705
L. Pos Bri/SBO	6.330	1.721, 23.313
L. Pos Arf/L. IncFT C1	2.918	0.961, 8.866
L. Pos Arf/R. DivFT C7	3.519	1.196, 10.354
L. IncFt C1/C1 cleft	3.543	0.628, 19.993
L. IncFt C1/R. DivFT C6	3.491	1.140, 10.697
L. IncFt C1/L. DivFT C7	3.682	0.851, 15.929
L. IncFT C2/C1 cleft	3.154	0.577, 17.352
L. IncFT C2/R. IncFT C7	3.306	0.600, 18.219
L. IncFT C2/L. IncFT C7	6.000	1.274, 28.254
L. IncFT C2/SpoL5	3.785	0.667, 21.489
L. DivFT C6/C1 cleft	2.436	0.558, 10.639
L. DivFT C6/SpoL5	3.846	0.719, 20.566
L. DivFT C6/SBO	2.139	0.799, 5.726
L. DivFT C7/C1 cleft	3.767	0.670, 21.162
L. DivFT C7/L. IncFT C6	9.154	0.540, 155.165
L. DivFT C7/SacL5	4.520	1.198, 17.050
L. IncFT C6/C1 cleft	9.071	0.731, 112.540
L. IncFT C6/R. IncFT C7	43.667	3.457, 551.519
L. IncFT C7/Vert	3.400	0.348, 33.185

The number of significant correlations far exceeds what is expected for the female population and the with the phi coefficient results which could be an indicator that there may be genetic processes acting on these traits which is causing such a large number of correlations. More in-depth analysis of the individual trait pairs will need to be done in order to confirm or refute this claim and to better understand the discrepancies between the two statistical tests.

5.6.2 Male Odds Ratio

Table 5.25. Significant odds ratio intertrait odds ratio correlations of nonmetric traits (P<0.05) among males

Trait	Odds Ratio	Confidence Interval
Sup Fac/Pos Bri	2.523	0.983, 6.475
Lat Bri/Pos Bri	4.500	1.170, 17.304
Lat Bri/Pos Arf	3.571	0.994, 13.313
Lat Bri/OsAp Lig	4.111	1.074, 15.737
Lat Bri/DivFT C7	2.630	0.670, 10.315
Pos Bri/IncFT C7	18.818	2.147, 164.932
Pos Bri/SpoL4	11.385	1.216, 106.555
Pos Arf/OsAp Lig	3.911	1.449, 10.554
Pos Arf/SacL5	4.606	0.958, 22.148
IncFT C1/C1 cleft	2.528	0.254, 25.108
IncFT C1/IncFt C2	2.741	0.699, 10.743
C1 cleft/DivFT C7	3.524	0.468, 26.537
IncFtC2/SpoL5	2.535	0.740, 8.678
IncFtC2/Vert	3.500	0.210, 58.252
DivFT C6/DivFT C7	7.000	1.937, 25.299
DivFT C7/IncFT C6	7.273	0.630, 83.975
DivFT C7/SacL5	3.810	0.716, 20.255
IncFT C6/SpoL5	4.227	0.354, 50.503
IncFT C7/SpoL4	3.917	0.364, 42.129
SpoL4/SpoL5	14.864	2.237, 98.742
SacL5/Vert	12.875	0.735, 225.635

Twenty-one significant trait pairs were found compared to the 13 found using phi coefficient. The discrepancies between the number of correlations between the two statistical tests are quite large as eight correlations differ between the two methods (see Chapter 6). Confidence intervals of traits with an odds ratio point estimate greater than 10 are very wide.

Table 5.26. Significant odds ratio right side intertrait correlations of nonmetric traits (P<0.05) among males

Trait	Odds Ratio	Confidence Interval
R. Sup Fac/R. DivFT C6	2.221	0.769, 6.416
R. Lat Bri/R. Pos Bri	3.375	0.694, 16.413
R. Lat Bri/L. Pos Bri	2.591	0.539, 12.458
R. Lat Bri/L. Pos Arf	2.050	0.365, 11.507
R. Lat Bri/OsAp Lig	2.813	0.582, 13.602
R. Lat Bri/L. IncFT C7	2.361	0.243, 22.926
R. Lat Bri/SpoL4	12.533	1.692, 92.850
R. Lat Bri/SBO	3.118	0.643, 15.117
R. Pos Bri/R. IncFT C6	4.444	0.265, 74.457
R. Pos Bri/L. IncFT C6	4.444	0.265, 74.457
R. Pos Bri/L. IncFT C7	13.929	2.455, 79.015
R. Pos Bri/SpoL4	2.842	0.443, 18.214
R. Pos Arf/L. IncFT C1	2.132	0.363, 12.506
R. Pos Arf/OsAp Lig	3.643	1.290, 10.288
R. Pos Arf/L. IncFT C6	4.158	0.249, 69.526
R. Pos Arf/SacL5	3.375	0.694, 16.413
R. IncFT C1/C1 cleft	3.958	0.381, 41.153
R. IncFT C1/R. IncFt C2	4.788	0.960, 23.885
R. IncFT C1/OsAp Lig	4.909	0.017, 0.861
R. IncFt C1/SpoL5	3.200	0.553, 18.517
R. IncFT C2/C1 cleft	2.964	0.495, 17.743
R. IncFT C2/R. DivFT C6	2.344	0.760, 7.232
R. IncFT C2/R. IncFT C6	5.125	0.305, 86.248
R. IncFT C2/SpoL4	2.594	0.438, 15.377
R. IncFT C2/Vert	5.188	0.308, 87. 291
R. DivFT C6/R. DivFT C7	4.488	1.388, 14.514
R. DivFT C6/L. DivFT C7	14.477	1.819, 115.223
R. DivFT C7/C1 cleft	4.647	0.611, 35.341
R. DivFT C7/R. IncFT C6	4.474	0.268, 74.761
R. DivFT C7/L. IncFT C6	4.474	0.268, 74.761
R. IncFT C7/SacL5	16.333	0.906, 294.413

Table 5.26 shows that right side expression among the males yielded 31 significant correlations; 20 more correlations than the 11 found using phi coefficient. Seven of the trait pairs were found be expressed on only the right side and only five trait pairs did not encompass one in the confidence interval although there are a few trait pairs that have a

high odds point estimate with the inclusion of one in the confidence interval.

Table 5.27. Significant odds ratio left side intertrait correlations of nonmetric traits (P<0.05) among males

Trait	Odds Ratio	Confidence Interval
L. Sup Fac/L. Pos Bri	3.143	1.172, 8.427
L. Sup Fac/R. IncFT C6	3.217	0.194, 53.495
L. Sup Fac/L. IncFT C6	3.217	0.194, 53.495
L. Sup Fac/R. IncFT C7	3.130	0.188, 52.063
L. Lat Bri/R. Pos Bri	4.556	0.849, 24.435
L. Lat Bri/L. Pos Bri	3.545	0.668, 18.811
L. Lat Bri/R. Pos Arf	4.556	0.849, 24.435
L. Lat Bri/L. Pos Arf	5.250	0.971, 28.374
L. Lat Bri/R. IncFT C1	3.800	0.371, 38.969
L. Lat Bri/L. IncFT C1	3.800	0.371, 38.969
L. Lat Bri/OsAp Lig	3.800	0.712, 20.275
L. Lat Bri/R. DivFT C7	5.267	0.969, 28.624
L. Lat Bri/L. DivFT C7	3.417	0.564, 20.716
L. Lat Bri/L. IncFT C7	2.900	0.290, 28.951
L. Lat Bri/SpoL4	4.700	0.440, 50.215
L. Lat Bri/SpoL5	4.000	0.655, 24.427
L. Pos Bri/OsAp Lig	2.276	0.816, 6.348
L. Pos Bri/R. IncFT C6	3.083	0.186, 51.203
L. Pos Bri/L. IncFT C6	3.083	0.186, 51.203
L. Pos Bri/L. IncFT C7	23.053	2.615, 203.188
L. Pos Bri/SpoL4	5.571	0.873, 35.538
L. Pos Arf/OsAp Lig	3.297	1.132, 9.599
L. Pos Arf/L. DivFT C7	2.057	0.564, 7.497
L. Pos Arf/L. IncFT C6	4.764	0.284, 79.992
L. DivFT C6/R. DivFT C7	4.917	1.709, 14.147
L. DivFT C6/L. DivFT C7	7.333	1.906, 28.215
L. DivFT C7/R. IncFT C6	7.000	0.412, 118.864
L. DivFT C7/L. IncFT C6	7.000	0.412, 118.864
L. DivFT C7/SacL5	3.750	0.619, 22.711
L. IncFT C6/OsAp Lig	3.261	0.196, 54.212
L. IncFT C7/SpoL4	3.917	0.364, 42.129

Table 5.27 has similar results to the right side where 31 significant correlations were found, largely exceeding the six traits found with phi coefficient. The large

discrepancies between the two methods will be later discussed in Chapter 6. Thirteen trait pairs were expressed solely on the left side.

5.6.3 Composite Population

As seen in both the male and female population, a large number of significant correlations were uncovered. Table 5.28 shows 32 significant comparisons compared to the 17 correlations found with phi coefficient; nearly double the significant phi pairwise traits. Eighteen trait pairs had confidence interval which excludes 1, which closely resembles the same traits found to be significant with phi.

Table 5.28. Significant odds ratio intertrait correlations of nonmetric traits (P<0.05) among the population**.**

Traits	Odds Ratio	Confidence Interval
Sup Fac/Pos Bri	3.497	1.791, 6.828
Lat Bri/Pos Bri	6.512	1.973, 21.490
Lat Bri/Pos Arf	2.266	0.693, 7.411
Lat Bri/OsAp Lig	3.519	1.089, 11.370
Lat Bri/DivFT C7	2.225	0.639, 7.753
Lat Bri/SpoL4	11.650	1.904, 71.297
Lat Bri/SpoL5	2.444	0.497, 12.016
Lat Bri/Vert	7.926	1.400, 44.874
Pos Bri/IncFT C7	3.133	1.124, 8.730
Pos Bri/SpoL4	9.045	1.606, 50.946
Pos Bri/SBO	2.794	1.346, 5.802
Pos Arf/IncFT	2.179	1.014, 4.684
Pos Arf/OsAp Lig	2.153	1.145, 4.049
Pos Arf/DivFT C7	2.019	1.010, 4.035
IncFT C1/C1 cleft	4.754	1.454, 15.547
C1 cleft/IncFt C2	2.526	0.806, 7.924
C1 cleft/DivFT C7	3.101	0.937, 10.263
C1 cleft/SpoL4	3.933	0.425, 36.360
C1 cleft/SBO	2.327	0.682, 7.946
IncFT C2/IncFT C6	2.022	0.359, 11.379
IncFT C2/SpoL5	2.456	0.923, 6.537
OsAp Lig/Vert	5.278	1.221, 22.810
DivFT C6/DivFT C7	3.113	1.502, 6.448
DivFT C7/IncFT C6	6.163	1.001, 37.961
DivFT C7/SpoL4	2.021	0.359, 11.369
DivFT C7/SacL5	2.167	0.824, 5.700

IncFT C6/IncFT C7	2.624	0.290, 23.747
IncFT C6/SpoL5	2.478	0.275, 22.364
IncFT C7/SpoL4	3.139	0.333, 29.581
SpoL4/SpoL5	11.083	2.303, 53.338
SpoL5/SBO	4.173	1.666, 10.453
SacL5/Vert	5.475	1.272, 23.565

Table 5.29. Significant odds ratio right side intertrait correlations of nonmetric traits (P<0.05) among the entire population

Trait	Odds Ratio	Confidence Interval
R. Sup Fac/R. Lat Bri	3.090	0.710, 13.451
R. Sup Fac/R. Pos Bri	2.880	1.312, 6.323
R. Sup Fac/L. Pos Bri	2.133	0.941, 4.834
R. Lat Bri/R. Pos Bri	6.455	1.539, 27.069
R. Lat Bri/L. Pos Bri	3.836	0.875, 16.813
R. Lat Bri/L. IncFT C7	2.173	0.250, 18.921
R. Lat Bri/SpoL4	19.667	2.998, 128.996
R. Lat Bri/SBO	3.062	0.703, 13.341
R. Lat Bri/Vert	5.143	0.544, 48.640
R. Pos Bri/R. IncFT C6	5.853	0.357, 95.824
R. Pos Bri/R. IncFT C7	2.668	0.655, 10.871
R. Pos Bri/L. IncFT C7	4.414	1.463, 13.317
R. Pos Bri/SpoL4	3.074	0.542, 17.435
R. Pos Arf/L. IncFT C1	2.722	1.051, 7.312
R. Pos Arf/C1 cleft	2.531	0.740, 8.658
R. Pos Arf/R. DivFT C7	2.095	0.917, 4.784
R. Pos Arf/L. IncFT C6	3.514	0.567, 21.758
R. Pos Arf/SpoL4	2.875	0.508, 16.280
R. IncFt C1/C1 cleft	2.986	0.764, 11.666
R. IncFt/L. IncFT C6	2.386	0.255, 22.302
R. IncFtC2/C1 cleft	3.507	1.105, 11.132
R. IncFtC2/R. IncFT C6	5.378	0.329, 87.910
R. IncFtC2/L. IncFT C6	3.667	0.592, 22.723
R. IncFtC2/R. IncFT C7	4.434	1.135, 17.332
R. IncFtC2/L. IncFT C7	2.183	0.648, 7.351
R. IncFtC2/SpoL4	2.216	0.414, 11.853
R. DivFT C6/R. DivFT C7	2.438	1.189, 4.997
R. DivFT C6/L. DivFT C7	3.520	1.436, 8.631
R. DivFT C7/C1 cleft	2.706	0.772, 9.489
R. DivFT C7/R. IncFT C6	5.077	0.311, 82.905
R. DivFT C7/L. IncFT C6	5.184	0.708, 37.944

R. DivFT C7/SpoL4	2.605	0.461, 14.732

Table 5.30. Significant odds ratio left side intertrait correlations of nonmetric traits (P<0.05) among the entire population

Trait	Odds Ratio	Confidence Interval
L. Sup Fac/R. Pos Bri	2.309	1.065, 5.008
L. Sup Fac/L. Pos Bri	3.291	1.537, 7.048
L. Sup Fac/R. IncFT C6	3.833	0.235, 62.408
L. Lat Bri/R. Pos Bri	4.676	1.002, 21.816
L. Lat Bri/L. Pos Bri	4.841	1.037, 22.608
L. Lat Bri/R. Pos Arf	4.236	0.911, 19.707
L. Lat Bri/L. Pos Arf	3.443	0.744, 15.934
L. Lat Bri/OsAp Lig	4.568	0.992, 21.039
L. Lat Bri/R. DivFT C7	4.295	0.919, 20.075
L. Lat Bri/L. DivFT C7	3.478	0.638, 18.957
L. Lat Bri/L. IncFT C7	2.560	0.288, 22.754
L. Lat Bri/SpoL4	7.833	0.789, 77.730
L. Lat Bri/SpoL5	4.956	0.897, 27.364
L. Lat Bri/Vert	5.143	0.544, 48.640
L. Pos Bri/R. Pos Arf	2.397	1.051, 5.470
L. Pos Bri/R. IncFT C6	5.657	0.346, 92.567
L. Pos Bri/R. IncFT C7	2.481	0.610, 10.080
L. Pos Bri/L. IncFT C7	5.793	1.954, 17.178
L. Pos Bri/SpoL4	6.774	1.309, 35.067
L. Pos Bri/SpoL5	2.200	0.745, 6.495
L. Pos Bri/SBO	2.983	1.349, 6.597
L. Pos Arf/L. IncFT C1	2.205	0.844, 5.758
L. Pos Arf/OsAp Lig	2.184	1.099, 4.341
L. Pos Arf/R. DivFT C7	2.299	1.026, 5.151
L. Pos Arf/SpoL4	2.302	0.409, 12.975
L. IncFT C1/C1 cleft	2.433	0.492, 12.042
L. IncFT C1/L. DivFT C6	2.079	0.840, 5.149
L. IncFT C1/SpoL4	2.076	0.232, 18.611
L. IncFT C2/C1 cleft	2.375	0.619, 9.113
L. IncFT C2/SpoL5	2.228	0.686, 7.233
L. DivFT C6/R. DivFT C7	2.963	1.468, 5.980
L. DivFT C6/L. DivFT C7	2.569	1.146, 5.761
L. DivFT C7/R. IncFT C6	7.815	0.475, 128.590
L. DivFT C7/L. IncFT C6	8.077	1.091, 59.793
L. DivFT C7/SacL5	3.955	1.380, 11.331
L. IncFT C6/C1 cleft	5.023	0.517, 48.783
L. IncFT C6/R. IncFT C7	19.250	2.814, 131.701

L. IncFT C7/SpoL4	3.563	0.376, 33.772

Table 5.29 and Table 5.30 show the results of significant right and left side correlations respectively. The right side yielded 32 significant correlations where there is a difference of nine trait pairs between phi and odds. Approximately one-third of the traits pairs were found to be expressed on the right side only. The left side yielded the largest number of significant correlations found in this study, 38. The phi coefficient also yielded a large number of significant correlations (20) but this is nearly half of what was found to be significant using odds ratio. The number of significant odds ratio has far exceeded the expected and this could be an indicator that genetic influences are acting on these traits.

5.7 Spatial Analysis

To better understand the distribution of traits and to determine if any clustering found among the traits could be used as an indicator of family relationships or society organization, a number of spatial analyses were conducted on individuals only interred in the Kellis 2 cemetery. Kellis 2 was the only cemetery where available coordinates could be accessed and this was done for all 17 traits. One grave (453), found in the southwest corner of the Kellis 2 site was removed from analysis as this was a spatial outlier. Three burials (141, 166, and 189) were also removed as their burial coordinates could not be verified. The total number of burials analyzed at the Kellis 2 cemetery was 213, where 127 of those burials belong to females and 86 belong to males.

The traits were examined in two steps; traits were first analyzed ignoring sex using Keron's Proximity Probability and Hodder and Okell's A statistic. The traits were then analyzed accounting for sex differences using cross nearest neighbour and Keron's cross proximity. Maps of all 17 traits highlighting the presence and absence of traits by sex can be found in Appendix C.

The first test, Keron's Proximity Probability (Keron 2014) was utilized to determine if any apparent cluster groups found among the traits were statistically

significant. The second test, Hodder and Okell's A statistic (1978) was used to determine if individuals with a trait are separated from individuals without the trait in the cemetery.

The cross nearest neighbour, which determines the average distance from each grave to its nearest neighbour based on the criteria of sex, works to determine if a set of graves based on sex criteria are clustered, evenly spaced or found in a random distribution. The last test Keron's cross proximity probability looks at clustering of traits comparing individuals of the same and opposite sex. Grave pairs are counted again. The last two statistics make sense in the context of spatial analysis of a cemetery as these two statistics were designed specifically for Kellis (Keron 2014).

Divided Superior Facet

Table 5.31. Proximity probability of divided superior facet

Distance (m)	Count	Probability
3	19	0.01
5	43	0.04
7	76	0.01
10	141	0.00

Table 5.31 shows that clustering of the divided superior facet was found at all distances. With only 38 individuals (24 females and 14 males) who have the trait in this cemetery, the counts of graves are quite high at all the distance radii. The high pair grave counts are attributed to the significant clustering, causing the counts at all the distances to be greater than the number of individuals with the trait.

The Hodder and Okell's A statistic for divided superior facet yielded a value of 0.85 with a p-value of 0.00. This value shows that there is a trend towards segregation between the location of burials with the trait and without the trait; this segregation is statistically significant.

Table 5.32 Cross nearest neighbour of divided superior facet

	Actual AvgNN	Rand AvgNN	NNRatio	p=
Male to Male	5.22	5.88	0.89	0.26
Male to Female	3.41	4.30	0.79	0.14
Female to Female	4.04	4.55	0.89	0.17
Female to Male	4.58	5.82	0.79	0.05

Females with divided superior facets tend to be closer to males (0.79, 0.05) but in the opposite direction (male to female), they are not statistically found close together (0.79, 0.14). Females are not found close to each other significantly (0.89, 0.17) nor are males (0.89, 0.26).

Table 5.33. Cross proximity probability of divided superior facet

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	2	0.38	5	0.44	12	0.04	18	0.07
Male to Female	12	0.00	22	0.00	35	0.02	66	0.01
Female to Female	5	0.41	16	0.06	29	0.08	57	0.01
Female to Male	12	0.01	22	0.05	35	0.08	66	0.03

The cross proximity probability in Table 5.33 shows that clustering of the divided superior facet variant is significant at various distances across the sexes. Clustering is first found significant at 3m and 5m for both males with females and females with males. At 7m, clustering is significant for both males with other males and males with other females while the significance is slightly lower for females with other females and with other males (29, 0.08; 35, 0.08 respectively).

Incomplete Foramen Transversarium C2

Table 5.34. Proximity probability of incomplete foramen transversarium of C2

Distance (m)	Count	Probability
3	15	0.02
5	31	0.06
7	49	0.12
10	75	0.60

Among the 35 individuals with the trait (20 females and 15 males), significant clustering is found at the smallest radius distance of 3m (15, 0.02), with fairly significant clustering also found at 5m (31, 0.06). As the radius distance increases, significant clustering decreases. Individuals with the trait appear to be buried very close to each other.

Table 5.35. Cross nearest neighbour of incomplete foramen transversarium of C2

	Actual AvgNN	Rand AvgNN	NNRatio	p=
Male to Male	5.06	6.36	0.80	0.11
Male to Female	4.96	4.74	1.05	0.38
Female to Female	3.47	4.93	0.70	0.00
Female to Male	6.26	5.98	1.05	0.32

Females with the incomplete foramen transversarium of the C2 trait are closer than expected (0.70, 0.00) and males with this trait are also fairly close to each other (0.80, 0.11). Males to females and females to males with this trait appear to be evenly distributed but this is not significant.

Table 5.36. Cross proximity probability of incomplete foramen transversarium of C2

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	2	0.28	4	0.52	7	0.53	12	0.40
Male to Female	4	0.80	12	0.59	21	0.39	32	0.95
Female to Female	9	0.00	15	0.00	21	0.06	31	0.27
Female to Male	4	0.60	12	0.33	21	0.40	32	0.85

Females tend to cluster to other females at various smaller ranges (3 to 7 meters). Among males, males and females as well as females and males there is no significant clustering at any of the four distances. Clustering is only limited to females.

Ossified Apical Ligament

Table 5.37. Proximity probability of ossified apical ligament

Distance (m)	Count	Probability
3	5	0.97
5	27	0.15
7	54	0.02
10	95	0.03

Clustering of the ossified apical ligament among 47 individuals, where 27 of those are females and 20 are males, is found to be significant at 7 meters based on the significant probabilities at 0.05. In addition, the larger distances have large pair grave counts that exceed the 47 individuals that were found to have the ossified apical ligament trait, another indication of clustering. Individuals with the trait, while they may cluster together do not do so at smaller distances.

Hodder and Okell's A statistic for ossified apical ligament yielded a value of 0.98 with a p-value of 0.12. This value of 0.98 with such close proximity to one reveals that there is a randomized mixing of individuals with and without the trait however, this value of 0.98 is only significant at the 0.15 level.

Table 5.38. Cross nearest neighbour of ossified apical ligament

	Actual AvgNN	Rand AvgNN	NNRatio	p=
Male to Male	4.42	6.16	0.72	0.04
Male to Female	5.16	4.87	1.06	0.36
Female to Female	4.35	4.88	0.89	0.21
Female to Male	5.60	6.24	0.90	0.28

Males with ossified apical ligament are closer than expected to each other (0.72, 0.04) but females are not found to be closer at a statistically significant level. While males with

females appear to be randomly spaced than would be expected and females with males are more clustered, these results are not statistically significant.

Table 5.39. Cross proximity probability of ossified apical ligament

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	2	0.44	4	0.45	7	0.51	13	0.32
Male to Female	1	0.99	14	0.17	28	0.06	47	0.19
Female to Female	2	0.81	9	0.45	19	0.13	35	0.11
Female to Male	1	1.00	14	0.52	28	0.12	47	0.25

The results found in Table 5.39 show males tend to fairly cluster to females at 7m (28, 0.06), while there is a fair amount of clustering among females at 10m (35, 0.11) and female and males at 7m (28, 0.12). There is no clustering at any distance solely among the males.

Divided Foramen Transversarium C7

Table 5.40. Cross nearest neighbour of divided foramen transversarium of C7

	Actual AvgNN	R and AvgNN	NNRatio	p=
Male to Male	5.58	5.35	1.04	0.39
Male to Female	4.04	4.94	0.82	0.08
Female to Female	5.77	4.95	1.17	0.09
Female to Male	4.48	5.14	0.87	0.14

With 17 males and 20 females with this trait, males with the divided foramen transversarium of C7 trait tend to be closer to females than would be expected (0.82, 0.08) while males appear to be randomly distributed from other males but not at a significant level. Females are found to be statistically randomly distributed in the cemetery (1.17, 0.09).

Table 5.41. Cross Proximity Probability of Divided Foramen Transversarium of C7

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	1	0.81	5	0.79	9	0.89	23	0.25
Male to Female	6	0.29	15	0.12	27	0.28	61	0.10
Female to Female	1	0.97	7	0.68	11	0.85	33	0.18
Female to Male	6	0.35	15	0.39	27	0.62	61	0.20

Table 5.41 shows that there is no significant clustering of the divided foramen transversarium of C7 among the various distances. There is clustering at 10m among males with females but this is only significant at 0.10. At 3m and 7m among the males and among the females, the trait tends towards a more even distribution versus clustering. Interestingly, significant clustering of divided foramen transversarium of C7 was found only when the data were further analyzed using sex, no significant results were found when the population was analyzed as a whole.

Incomplete Foramen Transversarium C7

Table 5.42. Proximity Probability of Incomplete Foramen Transversarium of C7

Distance (m)	Count	Probability
3	2	0.22
5	4	0.35
7	9	0.07
10	12	0.17

None of the five distances examined contained significant clustering at the 0.05 level however at 0.10, significant clustering can be found at only 7m (12, 0.07). With only 12 individuals found to exhibit the trait (6 males and 6 females), pair grave counts are not large.

Hodder and Okell's A statistic for incomplete foramen transversarium yielded a value of 0.84 with a p-value of 0.01. A trend towards segregation between the location of burials with the trait and without the trait is occurring within the cemetery significantly.

Table 5.43. Cross nearest neighbour of incomplete foramen transversarium of C7

	Actual AvgNN	R and AvgNN	NNRatio	p=
Male to Male	11.65	10.71	1.09	0.33
Male to Female	9.77	9.44	1.03	0.37
Female to Female	8.94	10.31	0.87	0.28
Female to Male	6.53	9.57	0.68	0.07

Table 5.43 shows females with incomplete foramen transversarium of C7 were found to appear closer to other males (0.68, 0.07). Males with the trait (1.09) and males with females with the trait (1.03) indicates a tendency towards even distribution but this is not statistically significant, while females appear to cluster but this is also not found to be statistically significant.

Table 5.44. Cross proximity probability of incomplete foramen transversarium of C7

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	0	1.00	0	1.00	1	0.76	2	0.62
Male to Female	1	0.12	3	0.04	7	0.00	8	0.02
Female to Female	1	0.25	1	0.43	1	0.71	2	0.63
Female to Male	1	0.75	3	0.35	7	0.09	8	0.26

There are various pockets of clustering among the sexes as clustering is quite significant at distances ranging from 5m to 10m. Significant clustering is found among males with other females at 5m (3, 0.04), 7m (7, 0.00) and 10m (8, 0.02). Clustering is also found among females with other males at 7m.

Spondylolysis L4

Table 5.45 Proximity probability of spondylolysis L4

Distance (m)	Count	Probability
3	0	1.00
5	2	0.07
7	2	0.24
10	3	0.36

While no distance was found to display significant clustering at the 0.05 level, at 0.10, clustering can be found at 5m (2, 0.07). The low counts are indicative of the two females and five males with the trait.

The Hodder and Okell's A statistic for spondylolysis of L4 yielded a value of 0.84. This value reveals that those with the trait and those without the trait tend towards segregation which is not significant at 0.05 or 0.1.

The cross nearest neighbour and the cross proximity statistics could not be run due to the small number of individuals with the trait. The low numbers are not appropriate to run these two tests as the results will not be a true representation of Kellis 2.

Spondylolysis of L5

Table 5.46. Proximity probability of spondylolysis L5

Distance (m)	Count	Probability
3	2	0.26
5	4	0.26
7	7	0.19
10	17	0.00

With 12 individuals found with the trait, 4 of 114 females and 8 of 76 males, significant clustering was only found at only 10m, (17, 0.00). This distance is large in relation to the size of the cemetery; however, because only 12 individuals were found with the trait, this result is telling of the clustering taking place in the cemetery.

Hodder and Okell's A statistic for spondylolysis of L5 yielded a value of 0.83 with a p-value of 0.01. This value shows that there is segregation between the location of burials with the trait and without the trait; as well, it shows that this segregation is statistically significant.

Table 5.47. Cross nearest neighbour of spondylolysis of L5

	Actual AvgNN	R and AvgNN	NNRatio	p=
Male to Male	5.47	9.00	0.61	0.01
Male to Female	7.00	12.47	0.56	0.00
Female to Female	14.97	13.45	1.11	0.41
Female to Male	5.72	8.39	0.68	0.13

Males with spondylolysis of L5 are closer to both males (0.61, 0.01) and females (0.56, 0.00) with spondylolysis of the L5. Females are not statistically close to other males nor other females with the trait.

Table 5.48. Cross proximity probability of spondylolysis of L5

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	1	0.29	2	0.27	4	0.09	7	0.05
Male to Female	1	0.40	2	0.41	3	0.516	10	0.01
Female to Female	0	1.00	0	1.00	0	1.00	0	1.00
Female to Male	1	0.41	2	0.51	3	0.57	10	0.00

Clustering of spondylolysis L5 is significant at 10m among males, females and males and between males and females with the trait (7, 0.05; 10, 0.00; 10; 0.01) respectively. A majority of the clustering occurs at distances that are large; based on the size of the cemetery, this distance may not be ideal for examining clustering.

Spina Bifida Occulta

Table 5.49. Proximity probability of spina bifida occulta

Distance (m)	Count	Probability
3	7	0.64
5	29	0.04
7	50	0.09
10	86	0.11

Significant clustering at the 0.05 level was only found at 5m (29, 0.04). The third

distance interval tested, 7m, also yielded clustering that approaches significance (50, 0.09). In addition to the significant probability, there are high pair grave counts found among the 33 individuals with the trait, an indicator of clustering. Individuals with the trait are buried fairly close to others with the trait in the cemetery.

Spina Bifida Occulta yielded a Hodder and Okell's A statistic value of 0.96 with a p-value of 0.02. The value is significant indicating that those with the trait and those without the trait slightly tend to segregate from each other throughout the cemetery.

Table 5.50. Cross proximity probability of spina bifida occulta

Radius within =	3		5		7		10	
	n-	p=	n-	p=	n-	p=	n-	p=
Male to Male	2	0.56	3	0.92	7	0.81	18	0.29
Male to Female	4	0.56	18	0.01	31	0.05	46	0.15
Female to Female	1	0.89	8	0.21	12	0.36	22	0.36
Female to Male	4	0.74	18	0.19	31	0.05	46	0.10

Clustering of spina bifida appears at various distances and between the different sexes. At 5m clustering is only significant between males and other females with the trait but at 7m clustering extends from male to female to female to males as well. Significant clustering is also found at the largest distance among males and females with other males.

Approximately half of the traits (divided superior facet, incomplete foramen transversarium of C7, ossified apical ligament, and divided foramen transversarium of C7, spondylolysis of L4 and L5 and spina bifida occulta) showed significant clustering. The rest of the traits analyzed for this thesis, lateral bridge, posterior bridge, posterior arch foramen, incomplete foramen transversarium of C1 and C6, divided foramen transversarium of C6, sacralization of L5 and extra vertebral element did not show any significant clustering among the various tests. However the first two statistics, proximity probability and Hodder and Okell's A statistics can be found for each trait on the maps in Appendix C.

The results of the spatial analysis have revealed some interesting patterns among the traits. Because previous studies (Molto 2002, Haddow 2012) have shown that Kellis 2

may be organized by kinship, this idea needs to be further tested. While some traits appear to show random distributions, half of the traits analyzed appear to show signs of clustering which strongly favours a kin-based deposition. The traits that have been looked at in this section of this chapter seem to support that Kellis 2 may be organized by kinship. In order to better understand the results in the context of what they mean for kinship and social organization, three traits, superior divided facet, incomplete foramen transversarium of C7, and spondylolysis of L5, which had significant results across all four spatial analysis, and two other traits ossified apical ligament and spina bifida occulta, will be of particular focus for further in-depth discussion in chapter 6.

Chapter 6

Discussion and Conclusion

6.1 Introduction

This thesis examined the efficacy of epigenetic vertebral nonmetric traits in the study of paleogenetics in a population sample (N = 303 fairly complete vertebral columns) from the Dakhleh Oasis, Egypt. The samples used spanned the Third Intermediate (circa 1000 to 800 B.C.) to the Roman Periods (circa 50-450 A.D.) that previous research has shown to represent an evolving deme, thus facilitating the pooling of the vertebral data. The prevalence data of a select battery of 17 traits were analyzed for their epigenetic characteristics (i.e., age, sex, symmetry, and intertrait correlations) using a number of accepted statistical measures (i.e., Phi coefficient, G-test, and Odds Ratio). By convention, the .05 level of statistical significance was used to determine if the prevalence data was due to stochastic variation or if, in the case of rejecting the H_0 correlations were significant in terms of having biological meaning. The genetic meaning of these traits was examined by studying the spatial distribution of these variants in the Kellis 2 cemetery, which was constructed during the early to middle part of the Roman occupation of Kellis (circa 50-450 A.D.). The interpretation of the epigenetic data and the spatial analysis is reviewed herein in order to assess vertebral traits paleogenetic value, including potential avenues for future research.

6.2 Significance of the Epigenetic Data

Age and sex are the two variables that have received the most attention in epigenetic research, although there has been limited data on infracranial variants. The Dakhleh material is well-preserved, and most elements of the skeleton are present, errors in sexing adults are minimal, and most skeletons could be aged with reasonable accuracy as multiple methods were used (Molto 2001). Also, as segments of the vertebral column have extremely variant developmental patterns, very broad age categories were used - adolescent (12-19), young adult (20 to 35) and older adult (36+). Due to the small sample size of individuals in the adolescent age category, the data were not analyzed herein.

In testing for age associations the key question is: when does the prevalence of a trait become age-stable? This facilitates their use for both intra- and inter-sample comparison. Of the 17 traits, 12 did not show statistical differences between the adult age categories. Overall, the prevalence data, as expected, show that hypostotic traits are age regressive and hyperstotic traits are age-progressive in terms of prevalence.

Statistical testing showed that two traits, the ossified apical ligament and spondylolysis (L4), became age-stable once adulthood was reached. The only other vertebral variant that showed an age effect was vertebral number though the overall result was not significant (e.g., odds ratio was > than 2 but the 95% confidence included unity). From a purely theoretical perspective the vertebral number should not show an age pattern, since the number of vertebrae has an embryonic origin. These data are deemed stochastic. Theoretically, for paleogenetic research only adult data should be used for hypostotic and hyperstotic traits. Traits in the other categories can be pooled for comparative research.

The prevalence data by sex only showed a statistical dichotomy between hyperstotic and not hypostotic traits, although the hyperstotic traits were higher in males overall and lower in females. Three of the hyperstotic traits, the posterior and lateral bridge of the atlas and spondylolysis of L5 yielded significant differences, where they had higher prevalence in males. Of interest, is the fact that the odds ratio shows that the lateral bridge is 7 times more common in males than females. It may be also noteworthy that these atlas variants are two of the three vertebral traits described in Buikstra and Ubelaker (1994). For intergroup comparative purposes, when samples are pooled by sex, the posterior and lateral bridge can be scored together as was recommended by Saunders (1978).

The data for the 10 bilaterally scored traits were analyzed to determine if there were side prevalence tendencies. Studies have shown that hypostotic traits appear more on the right and hyperstotic traits more on the left (Ossenberg 1969, Saunders 1978, Winder 1981). This pattern was not found in the Dakhleh data. However, all the traits had a significant correlation using the Phi coefficient, which could be a statistical artifact of the high common absence cell for each trait. To better understand trait symmetry the

'index of bilaterality' was calculated (Molto 1980). This test showed that two of the ten traits, the divided superior facet and divided foramen transversarium of C6, tended towards bilaterality, while the other eight traits tended towards unilaterality. The odds ratio was also used to test the likelihood that traits would appear bilaterally. In the calculation of the odds ratio, the common absence cell (individuals who do not have the trait) is culled. Interestingly, no odds ratio estimate was significant (using 95% confidence interval) including the two traits that had ratios >1. Thus, symmetry is not a factor for the use of these ten traits in population studies. Given the predominance of unilateral expression, it is recommended that the side method should be used in population research. However, patterns of unilateral expression may have some enhanced meaning for future intragroup genetic interpretations. For example, in a small sample a trait could occur only on one side in the skeletons whereas in the large composite sample, side prevalence does not occur.

Fundamental to using epigenetic traits to explore biological relationships between and within past population samples is that the traits have to be independent of each other. Statistically correlated traits provide redundant genetic data that can distort the meaning of intergroup distance statistics. Conversely, traits that are correlated in a sample population may suggest a higher genetic meaning. For example endogamy can increase the probability of traits being associated that are normally independent of each other in a larger population sample. Traits that have statistical associations with sex are very important in this area of research, as residence practices can profoundly influence the pattern of intrasample variation. The phi coefficient examining intertrait interactions in the male and female subsamples found 12 and 13 significant correlations respectively, which exceeded chance expectations (>.05 x 136 = 7). Due to the fact that sex differences in the number of correlations per sex are almost equal, the sexes were pooled creating a much larger sample (N = 303). The number of significant correlations was still more than expected (17 pairwise associations were positive and significant). The H_0 for trait independence is thus rejected. These results demonstrate that the reductionist model, where traits are culled from the research, should be applied to avoid overstating the genetic influence of the traits. It is very important to consider intertrait correlations for research designs for subsequent studies as their effects on biodistance can be extreme

(Molto 1980). It is important to note that the results of this study might be population specific (for the Dakhleh sample).

As chance can be involved in creating significant associations, it is important to examine which correlations make biological sense in light of the reductionist model. In both male and female subsamples, two traits were found to be significant (Sup Fac/ Pos Bri and Lat Bri/#Vert). Despite the fact that the divided superior facet and the posterior bridge are different developmentally, the fact that they are atlas variants, suggests a meaningful regional genetic effect may account for this association. However, a previous study by Edwards (2005) using a smaller sample size from the same Dakhleh population did not find these traits to be statistically associated. Sjøvold (1977) noted that when sample sizes are in the hundreds, significant correlations would then be revealed. Edwards (2005) examined only 156 atli with only 72 with known sex and age compared to over 300 individuals of known sex and age used in this study. Also, significant correlation between these two traits has not been found in other population samples.

The lateral bridge and extra vertebral element was also found to be significantly correlated in both sexes using the Phi test. Closer examination shows that there is only one individual with both traits in both the females and males. The large absent/absent cell (individuals who do not express either trait) could be skewing these results leading to the significant phi correlation. The odds ratio however was not significant. This exemplifies the importance of looking at the raw data as well as using the odds ratio to further analyze and confirm other statistical results. Also, as noted above, the extra vertebra is a rare embryonic event and does not include the cervical region. This and the above suggest that this is a stochastic correlation.

There were three trait pairs that were not common or significant in the sex subsamples, but were significant in the composite sample (Lat Bri/SpoL4), (OsAp Lig/Vert), (DivFT C7/IncFT C6). This again shows the impact of sample size. Among the three trait pairs, none of the correlated traits are categorically the same and based on our current knowledge, none appear to have similar etiologies. One trait pair (DivFT C7/IncFT6) however, occurs on adjacent cervical vertebra. This could be a regional ‘developmental genetic’ effect.

Intertrait correlations were also examined by trait category. Among hypostotic traits, seven were tested in males and females. Among the males, there were no significant trait comparisons, however, in females there were three trait pairs significantly correlated when only one was expected to occur by chance. These were: C1 cleft and SBO of S1, C1 cleft/IncFT and C1/IncFT C6. As hypostotic traits are generally more common in females this finding was not unexpected. Moreover, two of the three involved variations in the cervical vertebrae (e.g., a regional effect), while C1 clefting and SBO of the S1 involve the agenesis of the neural arch. Though Barnes (1994) does not consider C1 cleft to be SBO, this association would suggest otherwise; they have the same neural tube developmental pathway despite different closure times for the neural arch (Saunders 1978).

Among hyperstotic traits, females had no significant correlations while two significant correlations were present in the males. This again is not unexpected as hyperstotic traits are more common in males. The lateral bridge-posterior bridge association represents traits on the atlas and this association have been reported previously (Buikstra 1972, Saunders 1978). As noted, Saunders (1978) previously suggested that both atlas bridging traits should be combined together for distance studies, a view that is supported herein.

Examining pairwise correlations based on trait type is a useful strategy to better illustrate the meaning of intertrait correlation. Saunders (1978) however, cautions that many traits (e.g., spondylolysis, posterior arch) have multiple classifications. For example, spondylolysis is correlated with males because it is hypothesized to be influenced by biomechanical stress on the pars interarticularis. It also has a higher prevalence in older age cohorts. Males in ancient Dakhleh worked the fields and were involved in heavy labour which put chronic stress on the lower back, a cumulative stress that would increase with age. These data support a functional, as well as, a potential genetic etiology for spondylolysis.

As noted, Barnes (1994) has hypothesized that many axial skeletal traits are influenced by cranial shifting during embryogenesis. Thus, it can be hypothesized that variations influenced by the same shift should be found in association, particularly those

that are in closest spatial proximity (e.g., found on the same bone). Two traits that Barnes included as being influenced by cranial shifting, sacralization of L5 and ossification of the apical ligament were used herein to address her hypothesis. All statistical tests showed these two traits were not significantly correlated in both the male and female subsamples. These traits should have been significantly correlated if cranial shifting was the key etiological mechanism involved. Thus it is more likely that a common genetic influence is operant in their etiologies. A similar result and conclusion was reached by Brown (2013) using cranial nonmetric traits. Also, Schwartz (1995) has suggested that sacralization of the L5 should be classified as accelerated closure or union, as the variant involves a reduction of the lumbar vertebrae as it is incorporated into the sacrum. Schwartz's hypothesis which contrasts Barnes' model has yet to be tested. The above data suggest that cranial shifting can be rejected as a hypothesis to explain the occurrence of the vertebral traits examined in this thesis.

6.3 Kinship Analysis

The final portion of the analysis involved mapping burials of individuals with, and without, the traits using four different point analysis statistics. This was done to determine if there were clusters that could indicate kinship patterns or other social organization practices (e.g., residence practices). As noted, this analysis only involved the K2 cemetery for two reasons. Firstly, there are adequate and confirmed burial locations for most graves; and secondly, Kellis 2 is believed to have been a Christian cemetery. A Christian cemetery is supported by the presence of three churches in the village as well as the burials showing Christian burial traditions (e.g., lack of grave goods, and single interments with heads to the west. Christian burial practices found at Kellis 2 are evidence that Kellis may have been organized by kinship lines dictated by patrilineal and patrilocal practices (Molto 2002). Among the 17 traits, the clearest patterns were found for the following five traits; spondylolysis of L5, incomplete foramen transversarium of C7, ossified apical ligament, divided superior facet and spina bifida occulta. Visual inspection of the map shows clustering of spondylolysis of L5 on the south section of the cemetery. This clustering was found to be significant based on the results of the proximity count and Hodder and Okell's A, a statistic where those with and without the

trait are segregated in the cemetery. In addition to traits clustering among the population, individuals with this trait tended to be found adjacent among males and females and males were adjacent to other males, further evidence that burials followed a patrilocal organization. The males and females could be husbands and wives buried together and the males could be related (sons, brothers, cousins). Clustering of the traits are found at a distance radius of 10 m which is large for the size of the cemetery, but the results are still telling of the importance of burial locations and the individuals interred in these locations. Another trait where significant clustering was found is the incomplete foramen transversarium of C7 where 12 individuals express the trait. Visually, clustering seems to be limited to the northern portion of the cemetery. This clustering was found to be significant and the Hodder and Okell's A statistic also confirmed this finding, as those with and without the trait, tended towards segregation from each other. In addition, significant clustering was only found among the females and males, which could be evidence for a patrilocal burial pattern (male clustering). The ossified apical ligament presented interesting spatial results. A large number of individuals with this trait are found clustered in the north-western section of the cemetery. Additionally, a small clustering appears in the south section of Kellis 2. Significant clustering was found among individuals at larger distances with this trait, but Hodder and Okell's A statistic shows randomization rather than segregation; individuals with and without the trait appear randomly in the cemetery, though this is significant at the 0.15 level. When organized by sex, males were found to be statistically close together. However, when cross proximity was conducted on the data, males were not found to statistically cluster together at any distance, though conversely the males and females clusters were significant. The cross proximity analysis provides a finer review of the sex breakdown showing strong clustering among males and females. The males found in close association could be related, and this is strong additional support of patrilocal mode of organization (Usher 2005).

Of all the traits analyzed, the divided superior facet has the most consistent significance across the spatial statistics used. Visual inspection of the map for this trait (Appendix C) reveals a major clustering at the north-east section and a minor clustering at the north-west region of the cemetery. Not only is the clustering in the cemetery found

to be significant, there is clustering especially among males and females, with the clustering of the sexes appearing only at larger distances. Additionally, males and females with this trait cluster closer together when looking at adjacent burials. This type of burial pattern may reflect a patrilocal type lineage at Kellis where husbands and wives are buried together. Individuals with, and without, spina bifida occulta of S1 showed strong visual clustering in the eastern section of the cemetery with little clustering in the north-west region of the cemetery. This could also reflect consanguinity as SBO has a known genetic etiology. Cross Proximity demonstrated that clustering is statistically significant among individuals with the trait, and Hodder and Okell's A statistic reveals that the individuals with and without SBO tended to be segregated in the cemetery. This again supports the familial model and contrasts a cemetery that was built by accretion (Molto 2002). The distribution of SBO by sex yielded interesting results; males and females with the trait tended to cluster together significantly at relatively short distances. However, when "cross nearest neighbour" was analyzed, there was no significant clustering or particular sex(es) close to each other. Despite the unequivocal clustering present, the sex pattern found confounds the interpretation. Based on the results alone, it would be difficult to say that the distribution of spina bifida represent closely related individuals, as clustering of the traits along adjacent graves is not found.

The large number of individuals with SBO, usually a rare trait but with a high prevalence in the Kellis population (p/N = %), has been argued by Molto (2002) as provisional evidence of high endogamy. This could be a function of their relative isolation from the other Oases. However, there are additional data that counter this interpretation. Parr (2002), for example, found a higher than expected number of mtDNA lineages in a small number of burials from K2 suggesting influxes of females from elsewhere. Moreover, there is evidence that males of Kellis travelled to the Nile for work and trade (Gardner et al. 1999) and it is possible that they brought back females for marriage which would account for this high maternal genetic diversity. It is also possible that inbreeding in the population is indicative that there are some husbands and wives that are buried adjacent to each other, sharing a female ancestor (Usher 2005), though there is limited evidence supporting this hypothesis. The abundant literary evidence for the influx of Christians from other parts of Egypt during the Roman period (Molto 2001), finds

additional biological data support whereby several of the lepers found at Kellis 2 had lived outside the Oasis (probably the Nile region) prior to coming back to die at Kellis among family members (Molto 2002). The nonmetric and metric cranial variants clearly place the first 4 lepers found at Kellis in the local population, while stable isotope data showed that the lepers had oxygen (0^{16}) and nitrogen (N^{15}) signatures that were outliers to the local population. The Christians at Kellis presumably were more tolerant of this debilitating disease than people elsewhere, reminiscent of the pattern of the early AIDs epidemic in North America, whereby AIDs patients moved back with their relatives (Brown and Powell-Cope 1991). Better resolution of what is occurring at Kellis and the type of relationships taking place among individuals in the cemetery will be dependent on additional (nuclear, Y-chromosome and mtDNA) aDNA analyses (Just recently {2014}; a complete mtDNA genome was verified for a male at Kellis using next generation sequencing).

At this point the spatial analysis of these vertebral traits has provided a valuable first step in revealing some burial patterns in the Kellis population. Though Haddow (2012) has suggested that mapping individual traits to attempt to discern intracemetery kin-groups may not be ideal, I disagree, although this research has shown that there are many confounding factors that need to be addressed. One of the more promising strategies is mapping multiple rare traits together such as sacralization of L5 with SBO, as this could potentially provide increased resolution of the spatial trends and could potentially assist in understanding trait etiology. Mapping multiple traits at the same time can only be done with accuracy if more programs are created to compute the statistical tests employed in this study. In addition, in order to map more than one trait, individuals need to be observable equally for both traits. All these factors need to be addressed when creating research designs for spatial analysis using traits. This thesis has shown that looking at individual traits to infer related individuals though not optimal is the best current method. The recent success with mtDNA noted above provides a means of integrating rare nonmetric traits with DNA to determine relationships within the Kellis population.

6.4 Conclusion

The null hypothesis that infracranial traits cannot be useful for understanding past populations is rejected. Proper description of the traits and investigating their epigenetic influences (sex, age, symmetry, and intertrait correlations) as well as, the culling of traits that appear to be strongly influenced by these factors, must be part of all research designs. As noted in the introduction, a study by Edwards (2005) using a battery of 6 atlas traits from Ain Tirghi and Kellis, supported the hypothesis, based on craniometrics and nonmetric traits, that these temporally disjunct samples were from the same deme. This result facilitated the combining of vertebral data from both samples used herein which has improved the investigation of the epigenetic factors influencing the development of vertebral variants. One key statistical finding from this study is the application of the odds ratio to address statistical results based on cross tabbing absent/present data. With epigenetic traits, the common absence cells are large and confound interpretations (e.g., Type 1 errors are commonplace in most nonmetric trait studies which use the chi-square test). In removing the common absence cell the odds ratio deals with trait presence only, making clearer understanding of the influences of the epigenetic factors being investigated.

This thesis has also further advanced the genetic meaning of vertebral infracranial traits by illustrating their value for intracemetery analyses. The integration of epigenetic traits with molecular data (both nuclear and mtDNA) is the future. As well, the recently developed spatial statistics used herein represent another first step in an area that will be integral to understanding the social and genetic dynamics of past populations. In my opinion, it is fundamental that researchers understand the factors operant at the intrasample level before the genetic meaning of broad intersample comparisons, the norm for the field of epigenetic research, can be understood. I would also like to reiterate that, in modern medical and disease research, epigenesis is the emerging cornerstone of understanding the etiological basis of normal and aberrant variations. Skeletal research in epigenetic traits in biological anthropology should be a part of this new paradigm shift. This thesis has demonstrated that infracranial nonmetric traits of the vertebral column should be a major part of future paleogenetic research.

References

Akabori, E. 1933. “Crania Nipponica Recentia. 1: Analytical Enquires into the Nonmetric Variations in the Japanese Skill According to Age and Sex”. *Japanese Journal of Medical Science. Anatomy* 4:61-318.

Allbrook, DB. 1955. “The East African Vertebral Column”. *American Journal of Physical Anthropology* 13: 489-514.

Alt KW and W Vach. 1991. “The Reconstruction of ‘Genetic Kinship’ in Prehistoric Burial Complexes-Problems and Statistics.” In: Bock HH and P Ihm (eds.), Classification, Data Analysis, and Knowledge Organization. Berlin: Springer-Verlag. p 299–310.

--- 1992. “Non-spatial Analysis of ‘‘Genetic Kinship’’ in Skeletal Remains.” In: Schader M, (ed.), Analysis and Modeling Data and Knowledge. Berlin: Springer-Verlag. p 247–256.

--- 1995. “Detection of Kinship Structures in Skeletal Remains.” In: Jacob B and W Bonte (eds.), Advances in Forensic Sciences: Vol. 7. Forensic Odontology and Anthropology. Berlin: Köster. p 27–34.

--- 1998. “Kinship Studies in Skeletal Remains—Concepts and Examples.” In: Alt KW, FW Rösing and M Teschler-Nicola (eds.), Dental anthropology: Fundamentals, limits, and prospects. Wien: Springer. p 537–554.

Anderson JE. 1968. “Skeletal Anomalies as Genetic Indicators.” In: Brothwell DR (ed.), The Skeletal Biology of Earlier Human Populations. Oxford: Pergamon Press. p 135-147.

Armelagos GJ and DP Gerven. 2003. “A Century of Skeletal Biology and Paleopathology: Contrasts, Contradictions, and Conflicts” *American Anthropologist* 105(1): 53–64.

Bagnall RS. 1993. “Egypt in Late Antiquity”. Princeton University Press: Princeton.

Barge, JA. 1918. “Die Entwickklung der Craniovertebral grenze beim Schaf”. *Anat. Hefte* 55:415.

Barnes, E. 1994. “Developmental Defects of the Axial Skeleton in Paleopathology”. University Press of Colorado.

Berry, AC and RJ Berry. 1967. "Epigenetic Variation in the Human Cranium." *Journal of Anatomy* 101(2): 361–79.

Bick, EM and JW Copel. 1950. "Longitudinal Growth of the Human Vertebra: A Contribution to Human Ostegeny." *The Journal of Bone & Joint Surgery* 32(4): 803-814.

Birrell, M. 1999. "Excavations in the Cemeteries of Ismant el-Kharab." In: Hope CA and AJ Mills (ed.), *Dakhleh Oasis Project: Preliminary Reports of the 1992-1993 and 1993-1994 Field Seasons.* Oakville, CT: Oxbow Books. p 29–41.

Birx, JH. 2010. "Biological Anthropology" In: Birx H (ed.), 21st Century Anthropology: A Reference Handbook. CA: SAGE Publications. p 2-13.

Bland, JM and DG Altman. 2000. "Statistics Notes: The Odds Ratio." *British Medical Journal* 320(7247): 1468.

Bornstein, PE and RR Peterson. 1966. "Numerical Variation of the Presacral Vertebral Column in Three Population Groups in North America." *American Journal of Physical Anthropology* 25:139-146.

Boulle EL. 2001. "Evolution of Two Human Skeletal Markers of the Squatting Position: A Diachronic Study from Antiquity to Modern Age." *American Journal of Physical Anthropology* 115:50–56.

Bowen, GE. 2003. "Some Observations of Christian Burial Practices at Kellis." In: Bowen GE and CA Hope (ed.), *The Oasis Papers 3: Proceedings of the Third International Conference of the Dakhleh Oasis Project.* Oxford: UK Oxbow Books. p 167–182.

Boyd, JD and JC Trevor. 1953. "Problems in Reconstruction." In: Simpson K. (ed.), Modern Trends in Forensic Medicine. London. p. 133-152.

Brooks, S and JM Suchey. 1990. "Skeletal Age Determination Based on the Os Pubis: A Comparison of the Acsadi-Nemeskeri and Suchey-Brooks Methods." *Human Evolution* 5(3): 227–238.

Brothwell, DR. 1981. "Digging Up Bones: The Excavation, Treatment, and Study of Human Skeletal Remains". Cornell University Press.

Brown, LC. 2013. "Statistical Analysis of Nonmetric Cranial Trait Interactions in a Skeletal Population Sample from the Dakhleh Oasis, Egypt." Master's Thesis,

Department of Anthropology. University of Western Ontario.

Brown, MA and GM Powell-Cope. 1991. "AIDS Family Caregiving: Transitions through uncertainty." *Nursing Research* 40(6): 338-345.

Buikstra, JE. 1976. Hopewell in the Lower Illinois Valley: A Regional Approach to the Study of Human Biological Variability and Prehistoric Behavior. Evanston, Il: Northwestern Archeological Program Scientific Papers.

Buikstra, JE, SR Frankenberg, and LW Konigsberg. 1990. "Skeletal Biological Distance Studies in American Physical Anthropology: Recent Trends." *American Journal of Physical Anthropology* 82(1): 1–7.

Buikstra, JE, and DH Ubelaker. 1994. "Standards for Data Collections from Human Skeletal Remains: Proceedings of a Seminar at the Field Museum of Natural History". Fayetteville, Arkansas: Arkansas Archaeological Survey.

Carpenter, JC. 1976. "A Comparative Study of Metric and Nonmetric Traits in a Series of Modem Crania." *American Journal of Physical Anthropology* 45:337-344.

Case DT, NS Ossenberg and SE Burnett. 1998. "Os Intermetatarseum: A Heritable Accessory Bone of the Human Foot." *American Journal of Physical Anthropology* 107:199–209.

Chandravadiya, LS Patel, J Goda, V Chavda and S Ruparelia. 2013. "Double Foramen Transversarium in Cervical Vertebra□: Morphology and Clinical Importance" *International Journal of Medical Sciences* 2(1): 103–105.

Cheverud, JM and JE Buikstra. 1981. "Quantitative genetics of skeletal nonmetric traits in the Rhesus macaques on Cayo Santiago. I. Single trait heritabilities." *American Journal of Physical Anthropology* 54: 1:43-49.

Clark, PJ. 1952. "An Extension of the Coefficient of Divergence for Use with Multiple Characters". *Yearbook Physical Anthropology* 8:209-264 (reprinted from Copeia 1952, No. 2).

Clemente, IC. 1984. 'Tesis de licenciatura". Universitat Autònoma de Barcelona.

Corruccini, RS. 1974. "An Examination of the Meaning of Cranial Discrete Traits for Human Skeletal Biological Studies." *American Journal of Physical Anthropology*, 40: 425-446.

---. 1976. "The Interaction between Nonmetric and Metric Cranial Variation." *American Journal of Physical Anthropology* 44:285-294.

Cybulski, JS. 1972. "Skeletal Variability in British Columbia Coastal Populations: A Descriptive and Comparative Assessment of Cranial Morphology". PhD Dissertation, Department of Anthropology. University of Toronto.

Das, S., R Suri and V Kapur. 2005. "Double Foramen Transversaria: An Osteological Study with Clinical Implications." *International Medical Journal-Tokyo* 12(4): 311-313.

Daya, S. 2000. "Understanding Statistics: Odds Ratio." *Evidence-based Obstetrics and Gynecology* 2:84-85.

Deol, MS., H Grüneberg, AG Searle and GM Truslove. 1957. "Genetical Differentiation Involving Morphological Characters in Inbred Strains of Mice. I. A British Branch of the C57BL Mice." *Journal of Morphology* 100:345-375.

Deol, MS, and GM Truslove. 1957. "Genetical Studies on the Skeleton of the Mouse. XX. Maternal Physiology and Variations in the Skeleton of C57BL Mice". *Journal of Genetics* 55:288-312.

Donlon, DA. 2000. "The Value of Infracranial Nonmetric Variation in Studies of Modern *Homo sapiens*: An Australian Focus." *American Journal of Physical Anthropology 113*(3) 349-368.

Dugdale, LM. 1981. "The Ponticulus Posterior of the Atlas." *Australasian Radiology 25*(3): 237-238.

Edwards, J. 2005. "An Investigation of Nonmetric Discontinuous Trait Variation of the Atlas in Ancient Egyptian Population Samples from the Dakhleh Oasis, Egypt". Honour's thesis, Department of Anthropology. Lakehead University.

Enticott, JC, RK Kandane-Rathnayake and LE Phillips. 2012. "Odds Ratios Simplified". *Transfusion 52*(3): 467-469.

Evangelopoulos, DS. 2013. "Embryology" In: Korres DS (ed.) *The Axis Vertebra.* Milano: Springer Milan. p 3-5,

Fairgrieve, SI and JE Molto. 2000. "Cribra Orbitalia in Two Temporally Disjunct Population Samples from the Dakhleh Oasis, Egypt." *American Journal of Physical Anthropology* 111(3): 319–31.

Fazekas, IG and F Kósa. 1978. "Forensic Fetal Osteology" Budapest: Akadémiai Kiadó.

Finnegan, M. 1972. "Population Definition on the Northwest Coast by Analysis of Discrete Character Variation." PhD Dissertation, Department of Anthropology. University of Colorado.

---1973. "Discrete Nonmetric Variation of the Post-Cranial Skeleton in Man." *American Journal of Physical Anthropology* 40:135-136.

---1978. "Nonmetric Variation of the Infracranial Skeleton". *Journal of Anatomy 125*(1): 23-37.

Flander, LB. 1978. "Univariate and Multivariate Methods for Sexing the Sacrum". *American Journal of Physical Anthropology* 49(1): 103–10.

Fredrickson BE, D Baker, WJ McHolick, HA Yuan and JP Lubicky. 1984. "The Natural History of Spondylolysis and Spondylolisthesis". *Journal of Bone and Joint Surgery* 66:699–707.

Frey, R. 1986. "Dakhleh Oasis Project Interim Report on Excavations at the Ein Tighi Cemetery." *Journal for the Society for the Study of Egyptian Antiquities* 16:92-102.

Gaherty G.G. 1970. "Skeletal Variation in Seven Africa Populations". PhD Dissertation, Department of Anthropology. University of Toronto

--- 1973. "Discrete Traits, Cranial Measurements and Non-Biological Data in Africa." *American Journal of Physical Anthropology.* 4:136

Gardner, IM and SN Lieu. 1996. "From Narmouthis (Medinet Madi) to Kellis (Ismant El-Kharab): Manichaean Documents from Roman Egypt." *Journal of Roman Studies* 86: 146-169.

Gardner IM, A Alcock and WP Funk. 1999. Introduction. In: Gardner I, Alcock A, and Funk WP (eds.), Coptic Documentary Texts from Kellis Volume 1. Oxford: Oxbow Publishing. p 4-95.

Grogan JP, S Hemminghytt, AL Williams, GF Carrera and VM Haughton. 1982. "Spondylolysis Studied With Computed Tomography" *Radiology* 145:737–742.

Grüneberg, H. 1952. "Genetical Studies on the Skeleton of the Mouse IV. Quasicontinuous Variations." *The Journal of Genetics* 51: 95–114.

Haddow, SD. 2012. "Dental Morphological Analysis of Roman Era Burials from the Dakhleh Oasis, Egypt". PhD Dissertation, Department of Anthropology. University College London.

Hauser, G and GF De Stefano. 1989. "Epigenetic Variants of the Human Skull." Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung.

Hefner, JT. 2009. "Cranial Nonmetric Variation and Estimating Ancestry." *Journal of Forensic Sciences*. *54*(5):985-995.

Hodder, I and E. Okell. 1978. "A New Method for Assessing the Association between Distributions of Points in Archaeology". In: Hodder I (ed.), Simulation Studies in Archaeology. Cambridge: Cambridge University Press.

Hooton, EA, HY Rihan and E Reynolds E. 1930. The Indians of Pecos Pueblo: a study of their skeletal remain*s*. Department of Archaeology, Phillips Academy, Andover, Massachusetts.

Hrdlička, A. 1932. "The Humerus: Septal Apertures*"*. *Anthropologie Prague* X: 31-96.

---1934. "The Hypotrochanteric fossa of the Femur." *Smithsonian Miscellaneous* Collections. 92(1): 49.

İşcan, MY, SR Loth and RK Wright. 1984. "Age Estimation from the Rib by Phase Analysis: White Males." *Journal of Forensic Science* 29: 1094-1104.

James, CCM and Lassman, LP. 1972. "Spinal dysraphism: spina bifida occulta. London: Butterworths.

--- 1981. "Spina Bifida Occulta: Orthopaedic, Radiological and Neurosurgical Aspects". London: Academic Press

Kavaklı, A, A Aydınlıoğlu, H Yeşilyurt, I Kus, S Diyarbakırlı, S., Erdem, and O Anlar. 2004. "Variants and Deformities of Atlas Vertebrae in Eastern Anatolian people". *Saudi Medical Journal* *25*(3):322-325.

Kaya, S, Yilmaz ND, Pusat S, Kural C, Kirik, A, Izci Y. 2011. "Double Foramen Transversarium Variation in Ancient Byzantine Cervical Vertebrae: Preliminary Report of an Anthropological Study." *Turkish neurosurgery 21*(4): 534-538.

Kellock, WL and PA Parsons. 1970. "A Comparison of the Incidence of Minor Non-metrical Cranial Variants in Australian Aborigines with Those of Melanesia and Polynesia." American Journal of Physical Anthropology 33: 235-240.

Keron, J. 2014. Spatial Statistics Archaeology Unpublished Draft of PhD Dissertation, Chapters 2 and 3 in possession of Tiffany Sarfo. Western University, London Ontario.

Kintigh, KW. 1990. "Intrasite Spatial Analysis: A commentary on major methods." *Mathematics and Information Science in Archaeology: A Flexible Framework, Studies in Modern Archaeology* 3: 165-200.

Kiyono, K. and H. Miyamoto. 1926. "Anthropological Studies on Human Bones from the Neolithic Shells Mounds of Tukumo (of Ko)". *Journal of Anthropological Society Tokyo* 41:3, 1, 8, 12.

Knip, AS. 1970. "Metrical and Non-metrical Measurements on the Skeletal Remains of Christian Populations from Two Sites in Sudanese Nubia. Part I and II. Proceedings. Koninklijke nederlandse akademie van wetenschappen, Series C" *Biological and Medical* Sciences 73:433-468.

Konin, GP and DM Walz. 2010. "Lumbosacral Transitional Vertebrae: Classification, Imaging Findings, and Clinical Relevance." *American Journal of Neuroradiology 31*(10): 1778-1786.

Korey, KA. 1970. "Characteristics of the Distributions of Nonmetric Variants". M.A. Thesis, Department of Anthropology. University of Chicago.

--- 1980. "The Incidence of Bilateral Nonmetric Skeletal Traits: a Reanalysis of Sampling Procedures." *American Journal of Physical Anthropology* 53(1): 19–23.

Khüle, K.1934. "Symmetrieverhältnisse und die Absbrietungszentren in der Variabilität der regionalen Grenzen der Wirbelsäule des Menschen." *Zeitschrift für Morphologie und Anthropologie* 34:191-206.

Khüle, K. 1936. “Die Zwillingswirbelsäule.” *Zeitschrift fur Morphologie und Anthropologie* 35:1-375.

Lane, RA and AJ Sublett. 1972. “Osteology of Social Organization: Residence Pattern”. *American Antiquity* 37(2): 186-201

Laughlin, WS and JB Jorgensen. 1956. “Isolate Variation in Greenladic Eskimo Crania”. *Acta Genetica* 6: 3–12.

LeDouble, AF. 1912. “Traité des Variations de la Colonne Vertebrale de l'Homme et de leur Signification au point de vue de l 'Anthropologie Zoologique”. Paris.

Leone, A, A Cianfoni, A Cerase, N Magarelli and L. Bonomo. 2011. “Lumbar Spondylolysis: A Review.” *Skeletal Radiology 40*(6): 683-700.

LeVeau, BF, and DB Bernhardt. 1984. “Developmental Biomechanics. Effect of Forces on the Growth, Development, and Maintenance of the Human Body”. *Physical Therapy* 64(12): 1874–1882.

Little, MA and RW Sussman. 2010. “History of biological anthropology” In: Larsen CS(ed.) A Companion to Biological Anthropology MA: Wiley-Blackwell. p 13-38.

Long, JC and RA Kittles. 2009. Human Genetic Diversity and the Nonexistence of Biological Races. *Human Biology* 81(5/6): 777-798.

Ludwig, KS. 1953. Die Frühentwicklung des Dens epistropheiund seiner Bänder beim Menschen. Morph. Jb. 93:98

MacAlister, A. 1868. “Notes on the Homologies and Comparative Anatomy of the Atlas and Axis.” *Journal of Anatomy and Physiology* 3:54-64

Macalister, A. 1893. “Notes on the Development and Variations of the Atlas”. *Journal of Anatomy and Physiology 27*(4): 519-542.

Mahato, NK. 2010. "Complete Sacralization of L5Vertebrae: Traits, Dimensions, and Load Bearing in the Involved Sacra." *The Spine Journal* 10(7): 610-615.

McDonald, JH. 2009. “Handbook of Biological Statistics.” Baltimore, MD: Sparky House Publishing.

McGrath, JW, JM Cheverud and JE Buikstra. 1984. “Genetic Correlations between Sides and Heritability of Asymmetry for Nonmetric Traits in Rhesus Macaques on Cayo Santiago.” *American Journal of Physical Anthropology 64*(4):401-411

McNally, E, B Sandin, and RA Wilkins. 1990. “The Ossification of the Costal Element of the Seventh Cervical Vertebra with Particular Reference to Cervical Ribs”. J*ournal of Anatomy*, *170*:125.

Merbs, CF. 1974. “The Effects of Cranial and Caudal Shift in the Vertebral Columns of Northern Populations.” *Arctic Anthropology* 11: 12-19.

--- 1989. “Spondylolysis: Its Nature and Anthropological Significance.” *International Journal of Anthropology 4*(3): 163-169.

Mills, A J. 2010. “Dakhleh Oasis Project.” http://www.arts.monash.edu.au/archaeology/excavations/dakhleh/.

Molto, JE. 1980 “Biological Relationships of Southern Ontario Woodland Peoples: The Evidence of Discontinuous Cranial Morphology”. PhD Dissertation, Department of Anthropology. University of Toronto.

--- 1985. Simultaneous Occurrence of Discontinuous Cranial Traits: Some Theoretical and Practical Consideration for Population Studies. *Canadian Journal of Anthropology 4*: 57-65.

--- 2001. “The Comparative Skeletal Biology and Paleoepidemiology of the People from Ein Trighi and Kellis, Dakhleh, Egypt.” In: Marlow CA and AJ Mills (ed.), The Oasis Papers 1: Proceedings of the First International Symposium of the Dakhleh Oasis Project. Oxbow Monograph. p 81–100.

--- 2002. "Bio-archaeological research of Kellis 2: An Overview." In: Hope CA and GE Bowen (ed.), Dakhleh Oasis Project: Preliminary Reports on the 1994-1995 and 1998-1999 Field Seasons Oxbow Monograph. p 239-255.

Monash University. 2013. Ancient Kellis http://artsonline.monash.edu.au/ancient-kellis/north-tomb-group/

Murlimanju, BV, LV Prabhu, K Shilpa, R Rai, KV Dhananjaya and PJ Jiji. 2011. Accessory Transverse Foramina in the Cervical Spine: Incidence, Embryological Basis, Morphology and Surgical Importance. *Turkish Neurosurgery* 21(3): 384–7.

Netter, F. H. (1987). Musculoskeletal System: A Compilation of Paintings. Anatomy, Physiology, and Metabolic Disorders; Russell T. Woodburne, Edmund S. Crelin and Frederick S. Kaplan, Consulting Editors; Regina V. Dingle, Managing Editor. Crelin, ES FS Kaplan and RT Woodburne (eds.), Ciba-Geigy Corporation.

Ogden, JA.1983. "Radiology of Postnatal Skeletal Development. XI. The First Cervical Vertebra." *Skeletal radiology*, *12*(1), 12-20

Ossenberg, NS. 1969. "Discontinuous Morphological Variation in the Human Cranium". University of Toronto

--- 1976. "Within and Between Race Distances in Population Studies Based on Discrete Traits of the Human Skull." *American Journal of Physical Anthropology* 45 (3): 701–15.

--- 1981. "An Argument for the Use of Total Side Frequencies of Bilateral Nonmetric Skeletal Traits in Population Distance Analysis: The Regression of Symmetry on Incidence." *American Journal of Physical Anthropology* 54(4): 471–9.

O'Sullivan, D and DJ Unwin. 2003. "Geographic Information Analysis". John Wiley & Sons

Parr RL. 2002. Mitochondrial DNA Sequence Analysis of Skeletal Remains from the Kellis 2 cemetery. In: Hope CA, and Bowen GE (eds.), Dakhleh Oasis Project: Preliminary Reports on the 1994-1995 to 1998-1999 Field Seasons. Oxford: Oxbow. p 257-261.

Petronis, A. 2010. "Epigenetics as a Unifying Principle in the Aetiology of Complex Traits and Diseases." *Nature 465*(7299), 721-727.

Phenice, TW. 1969. "A Newly Developed Visual Method of Sexing the Os Pubis." *American Journal of Physical Anthropology* 30 (2): 297–302.

Prowse, TL. 1994. "Biological Affinities of Ancient Egyptians and Nubians: An Analysis of Cranial Nonmetric Traits." M.A. Thesis, Department of Anthropology. University of Alberta.

Regan MH, DT Case and JC Brundige. 1999. "Articular Surface Defects in the Third Metatarsal and Third Cuneiform: Nonosseous Tarsal Coalition." *American Journal of Physical Anthropology* 109:53–65.

Richtsmeier, JT and JW McGrath. 1986. Quantitative genetics of cranial nonmetric traits in randombred mice: Heritability and etiology. American Journal of Physical Anthropology, 69(1): 51-58.

Riggs, RE and AJ Perzigian. 1977. "Nonmetric Skeletal Trait Analysis of Prehistoric Burial Groups." *American Journal of Physical Anthropology* 47:156.

Rightmire, GP. 1972. Cranial Measurements and Discrete Traits Compared in Distance Studies of African Negro Skulls. *Human Biology* 44: 263-276.

Roberts, C and K Manchester. 2005. "The Archaeology of Disease". Ithaca, New York: Cornell University.

Sani, V, R Singh, M Bandopadhyay, S Tripathi and S Shamal. 2009. "Occipitalization of the Atlas: Its Occurrence and Embryological Basis." *International Journal of Anatomical Variations 2*: 65-68.

Saunders, SR. 1978. "The Development and Distribution of Discontinuous Morphological Variation of the Human Infracranial Skeleton". PhD Dissertation, Department of Anthropology. University of Toronto.

Saunders, SR and F Popovich. 1978. "A Family Study of Two Skeletal Variants: Atlas Bridging and Clinoid Bridging." *American Journal of Physical Anthropology* 49(2): 193-203.

Saunders SR. 1989. Nonmetric skeletal variation. In: Isc□an MY and KAR Kennedy (eds.), Reconstruction of life from the skeleton. New York: Alan R. Liss. p 95–21.

Saunders, SR, and DL Rainey. 2008. "Nonmetric Trait Variation in the Skeleton: Abnormalities, Anomalies, and Atavisms." In: Katzenberg AM and SR Saunders(eds.), *Biological Anthropology of the Human Skeleton*, Second Ed. Hoboken, NJ: Wiley-Liss p 533-560.

Scheuer, L and S Black. 2000. "Development and Ageing of the Juvenile Skeleton". In: Cox M and M Simon (eds.), Human Osteology in Archaeology and Forensic Science. p 9-22.

--- (2004). *The Juvenile Skeleton*. Academic Press.

Schoenwolf, G, SB Bleyl, PR Brauer and PH Francis-West. 2009. "Larsen's Human Embryology". 4th Ed Elsevier Inc, Churchill Livingstone

Schulze PJ, R Buurman. 1980. “Absence of the Posterior Arch of the Atlas”. *American Journal of Roentgenology 134*(1):178-180.

Schwartz, JH. 1995.” Skeleton Keys: An Introduction to Human Skeletal Morphology, Development, and Analysis”. New York, NY: Oxford University Press.

Scott, JH. 1893. “Osteology of the Maori and Moriori.” *Transactions New Zealand Institute* 26: 1-64.

Searle, A.G. 1954a. “Genetical Studies on the Skeleton of the Mouse. IX. Causes of Skeletal Variation Within Pure Lines”. *Journal of Genetics* 52:68-102.

---1954b. “Genetical Studies on the Skeleton of the Mouse. XI. The Influence of Diet on Variation Within Pure Lines. *Journal of Genetics* 52:413-424.

Selby, S, SM Garn, and V Kanareff. 1955. “The Incidence and Familial Nature of a Bony Bridge on the First Cervical Vertebra.” *American Journal of Physical Anthropology 13*(1): 129-141.

Senoglu, M, D Ozbag and Y Gumusalan. 2008. “Two cases of Klippel-Feil Syndrome.” *International Journal of Anatomical Variations* 1(1): 6-7.

Shennan, S. 1997. *Quantifying archaeology*. University of Iowa Press.

Shore, LR. 1930. “Abnormalities of the Vertebral Column in a Series of Skeletons of Bantu Natives of South Africa.” *Journal of Anatomy* 64: 206-238.

Sim E, AR Vaccaro, A Berzlanovich, H Thaler, CG Ullrich. 2001 “Fenestration of the Extracranial Vertebral Artery: Review of the Literature.” *Spine* 26:139–142.

Singh, S. 1965. “Variations of the Superior Articular Facets of Atlas Vertebrae.” *Journal of Anatomy 99*(3):565.

Sjøvold, T. 1977. “Nonmetrical Divergence between Skeletal Populations: The Theoretical foundation and Biological Importance of C.A.B. Smith's Mean Measure of Divergence”. *Ossa* 4:1-133.

-----1984. “A Report on the Heritability of Some Cranial Measurements and Nonmetric Traits.” In: Van Vark GN and WW Howells (eds.), Multivariate Statistical Methods in Physical Anthropology. Boston: D. Reide. p 223–246.

Snow, CE, WS Webb and WG Haag. 1948. "Indian Knoll Skeletons of Site Oh 2, Ohio County, Kentucky". University of Kentucky.

Sokal, RR. And FJ Rohlf. 1981. Biometry (2nd edn). *New York: WH Feeman and Company*, *668*.

Stewart, JD, JE Molto and PJ Reimer. 2003. "The Chronology of Kellis 2: The Interpretative Significance of Radiocarbon Dating of Human Remains." In *The Oasis Papers 3: Proceedings of the Third International Conference of the Dakhleh Oasis Project*, ed. Gillian E Bowen and Colin A Hope. Oxford, UK: Oxbow Books.

Stojanowski, CM, and MA Schillaci. 2006. "Phenotypic Approaches for Understanding Patterns of Intracemetery Biological Variation." *American Journal of Physical Anthropology* 49: 49–88.

Sublette, A. 1966. "Seneca Physical Type and Changes Through Time." PhD Dissertation, Department of Anthropology. State University of the New York at Buffalo

Suchey, J.M. 1975. "Biological Distance of Prehistoric Central California Populations Derived from Nonmetric Traits of the Cranium." PhD Dissertation, Department of Anthropology. University of California Riverside.

Susanne, C. 1975 "Genetic and Environmental Influences on Morphological Characteristics." *Annals of Human Biology* 2:279-287.

Szumilas, M. 2010. "Explaining Odds Ratios". *Journal of the Canadian Academy of Child and Adolescent Psychiatry* 19(3): 227-229.

Teplick JG, PA Lafley, A Berman and ME Haskin. 1986. "Diagnosis and Evaluation of Spondylolisthesis and/or Spondylolysis on Axial CT." *American Journal of Neuroradiology* 7: 479–91.

Taitz, C, H Nathan and B Arensburg. 1978. "Anatomical Observations of the Foramina Transversaria." *Journal of Neurology, Neurosurgery & Psychiatry* 41(2): 170-176.

Töndury, G. 1958. "Entwicklungsgeschichte und Fehlbildungen der Wirbelsäule". Hippokrates, Stuttgart.

Trinkaus, E. 1978. “Bilateral Asymmetry of Human Skeletal Non-metric Traits”. *American Journal of Physical Anthropology* 49(3): 315-318.

Troup JDG. 1976. “Mechanical Factors in Spondylolisthesis and Spondylolysis.” *Clinical Orthopaedics* 147:59–67.

Tubbs, RS, P Grabb, A Spooner, W Wilson, WJ Oakes. 2000. “The Apical Ligament: Anatomy and Functional Significance.” *Journal of Neurosurgery Spine* 92(2): 197-200.

Tyrrell A. 2000. “Skeletal Nonmetric Traits and the Assessment of Inter- and Intra-Population Diversity: Past Problems and Future Potential”. In: Cox M, Mays S, editors. Human Osteology in Archaeology and Forensic Science. Cambridge: Cambridge University Press. pp. 289–306.

Ubelaker, DS. 1999. “Human Skeletal Remains, Excavation, Analysis, Interpretation”. 3rd edition Taraxacum, Washington.

Usher, BM. 2005. “Anabaptist Cemetery Spatial Organization reflects patrilocal household structure and endogamous corporate groups: A model for prehistory”. Paper Presented at the Society for American Archaeology Annual Meeting. Salt Lake City.

von Hayek, H. 1927. “Untersuchungen über Epistropheus, Atlas und Hinterhauptsbein”. Morph. Jb. 58:269.

von Roy, P, E Barbaix, M de Maesseneer, C Ponders and JP Clarys. 2006. “The Anatomy of the Neural Arch of the Lumbar Spine with References to Spondylolysis, Spondylolisthesis, and Degenerative Spondylolisthesis”. In: Gunzberg, R and M Szpalski (eds.), Spondylolysis, Spondylolisthesis, and Degenerative Spondylolisthesis. Philadelphia: Lippincott Williams and Wilkins. p 1-11.

von Torklus, D and W Gehle. 1972. “The Upper Cervical Spine” Grune and Stratton, New York.

Waldron, T. 2009. “Palaeopathology”. New York: Cambridge University Press.

Washburn, SL. 1951. "The New Physical Anthropology." *Transactions of the New York Academy of Sciences* 13 (7): 298-304.

Wheeler, SM. 2012. “Nutritional and Disease Stress of Juveniles from the Dakhleh Oasis, Egypt.” *International Journal of Osteoarchaeology* 22 (2):219–234.

Wheeler, SM, L Williams, P Beauchesne and JE Molto. 2007. “Fractured Childhood: A Case of Probable Child Abuse from Ancient Egypt.” *SAS Bulletin* 30 (4): 6–10.

Wheeler, SM, L Williams, TL Dupras, MW Tocheri and JE Molto. 2011. “Childhood in Roman Egypt: Bioarchaeology of the Kellis 2 Cemetery, Dakhleh Oasis, Egypt.” In: ed. Mike Lally M and A Moore (eds.), *(*Re)Thinking the Little Ancestor: New Perspectives on the Archaeology of Infancy and Childhood. Oxford: Archaeopress. p 110–121.

White, TD, MT Black and PA Folkens. 2012. “Human Osteology”. California: Academic press.

Wight, S, N Osborne, and AC Breen. 1999. “Incidence of Ponticulus Posterior of the Atlas in Migraine and Cervicogenic Headache.” *Journal of Manipulative and Physiological Therapeutics 22*(1): 15-20.

Winder, CS. 1981. “Infracranial Nonmetric Variation: An Assessment of its Value for Biological Distance Analysis. PhD Dissertation, Department of Anthropology. Indiana University.

Yates JR, MA Ferguson-Smith, A Shenkin, R Guszman-Rodriguez, M White and BJ Clark. 1987. “Is Disorded Folate Metabolism the Basis for Genetic Predisposition to Neural Tube Defects?” *Clinical Genetics* 31(5): 279-287.

Appendix A

Photographs of Nonmetric Vertebral Traits

Photos Courtesy of JE. Molto

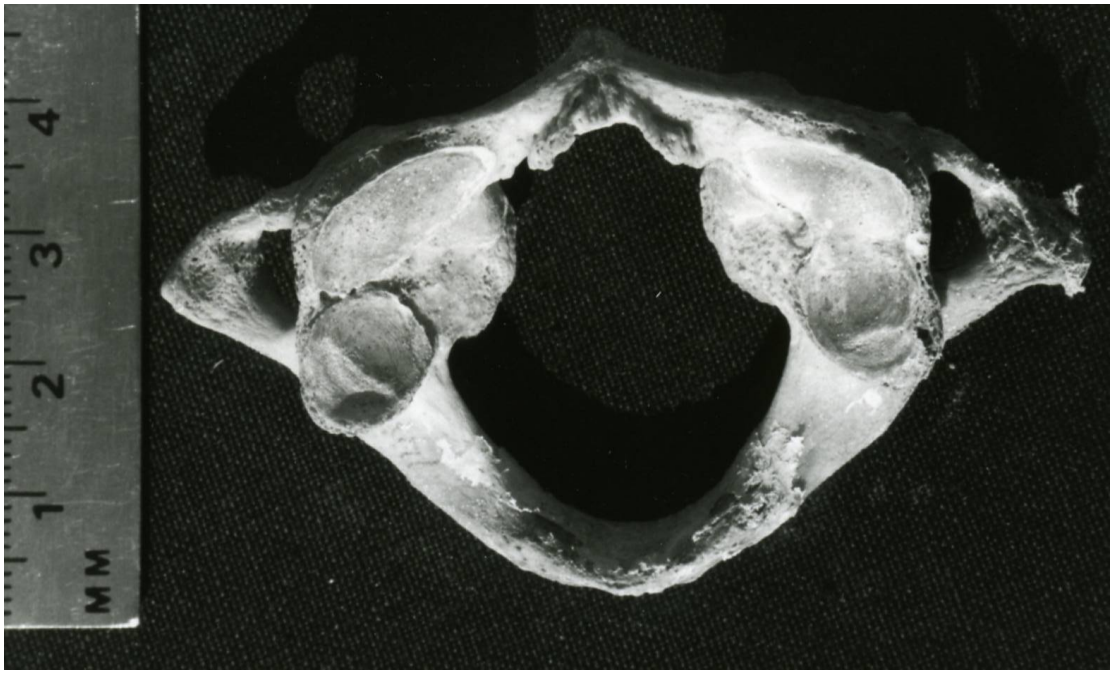

Figure A.1 Divided Superior Facet (Sup Fac)

Figure A.2 Lateral Bridge (Lat Bri)

Figure A.3: Posterior Bridge (Pos Bri)

Figure A.4 Posterior Arch Foramen (Pos Arf)

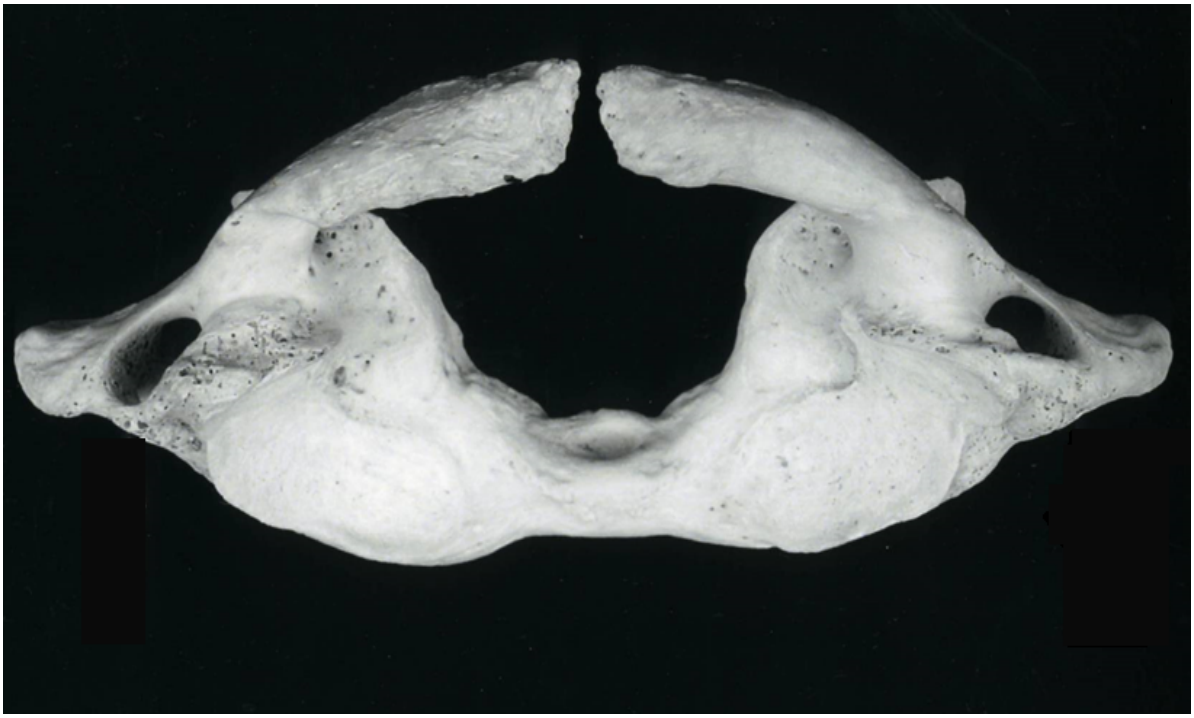

Figure A.5 Posterior Cleft of C1 (C1 Cleft)

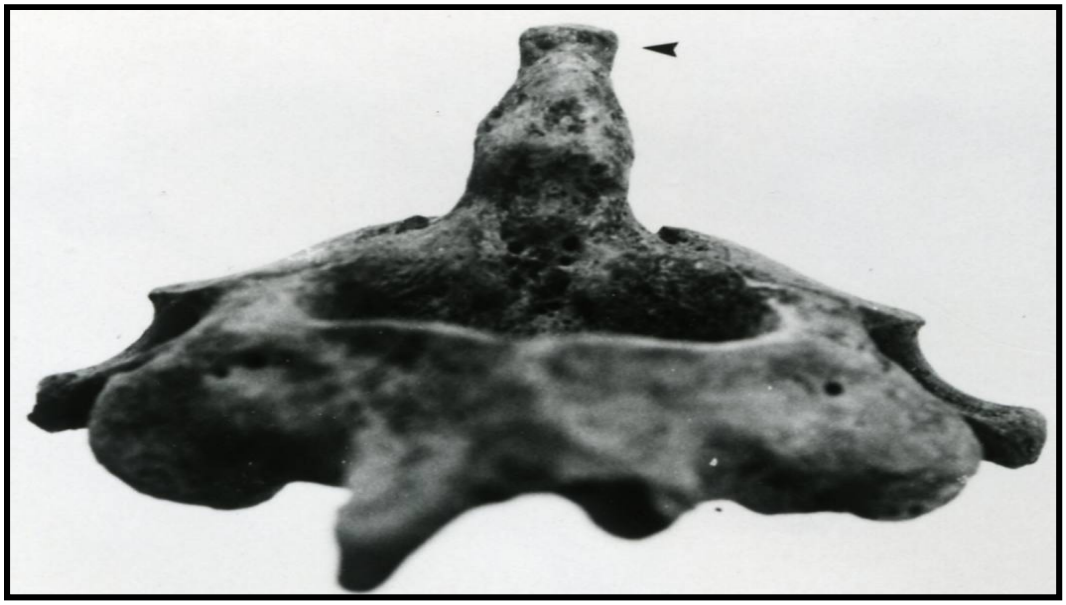

Figure A.6 Ossified Apical Ligament (OsAp Lig)

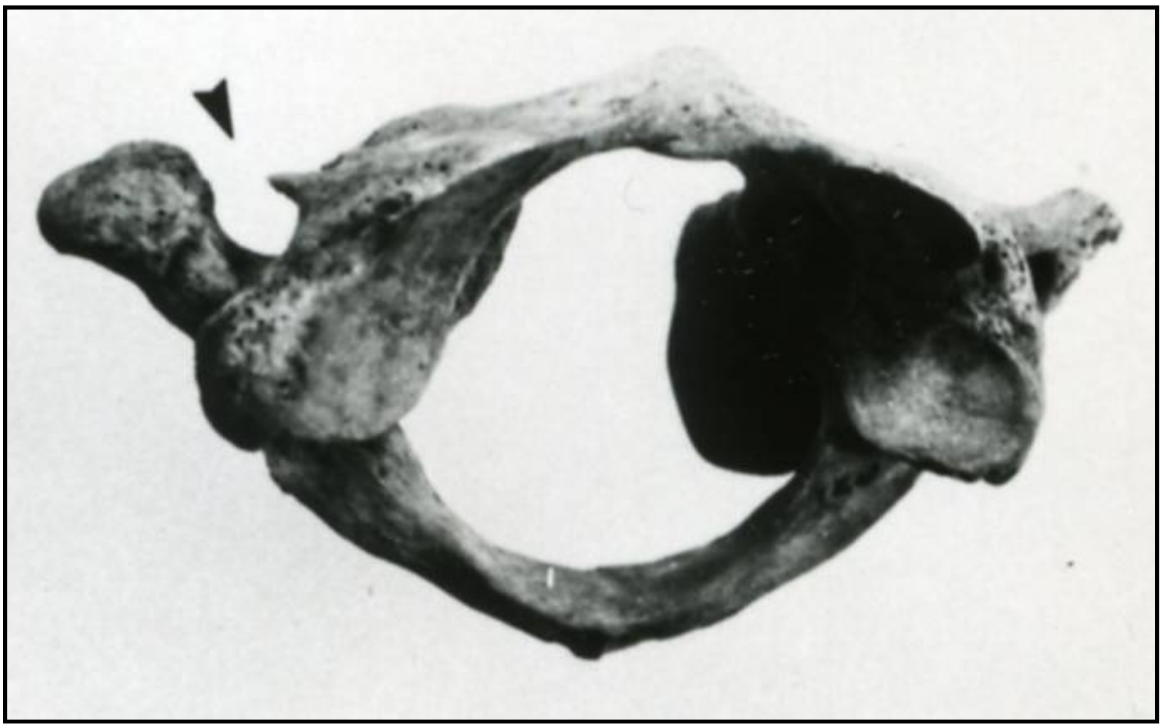

Figure A.7 Incomplete Foramen Transversarium (IncFT C1)

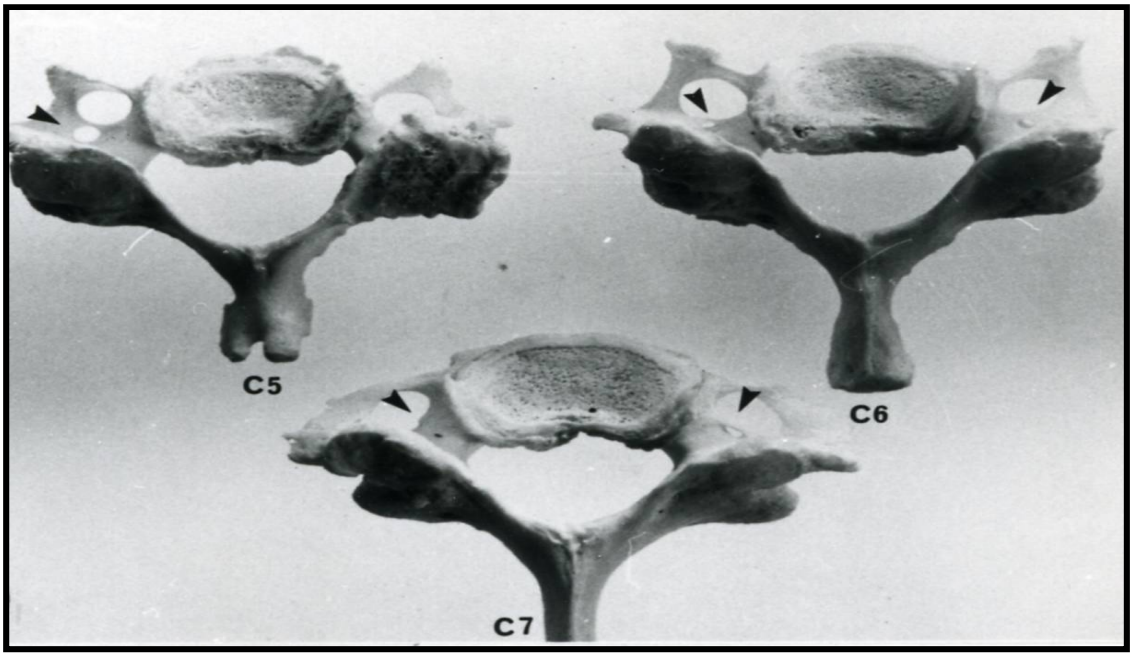

Figure A.8 Divided Foramen Transversarium of C6 and C7 (DivFT C6 and DivFt C7)

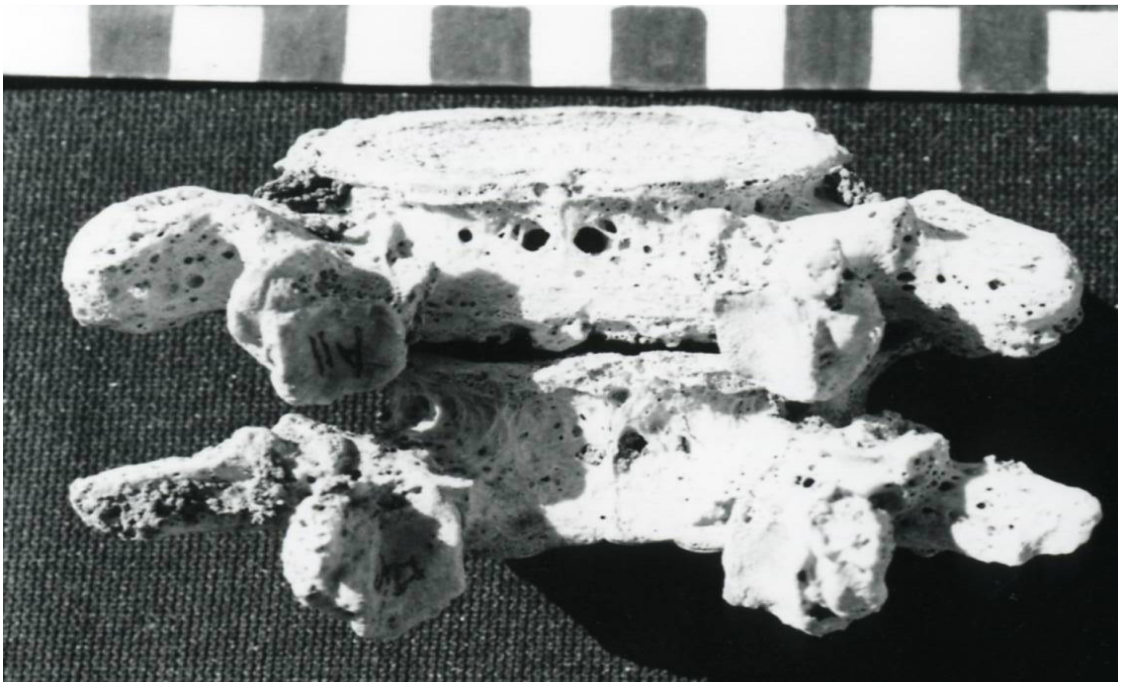

Figure A.9 Spondylolysis of L4 and L5 (SpoL4 and SpoL5)

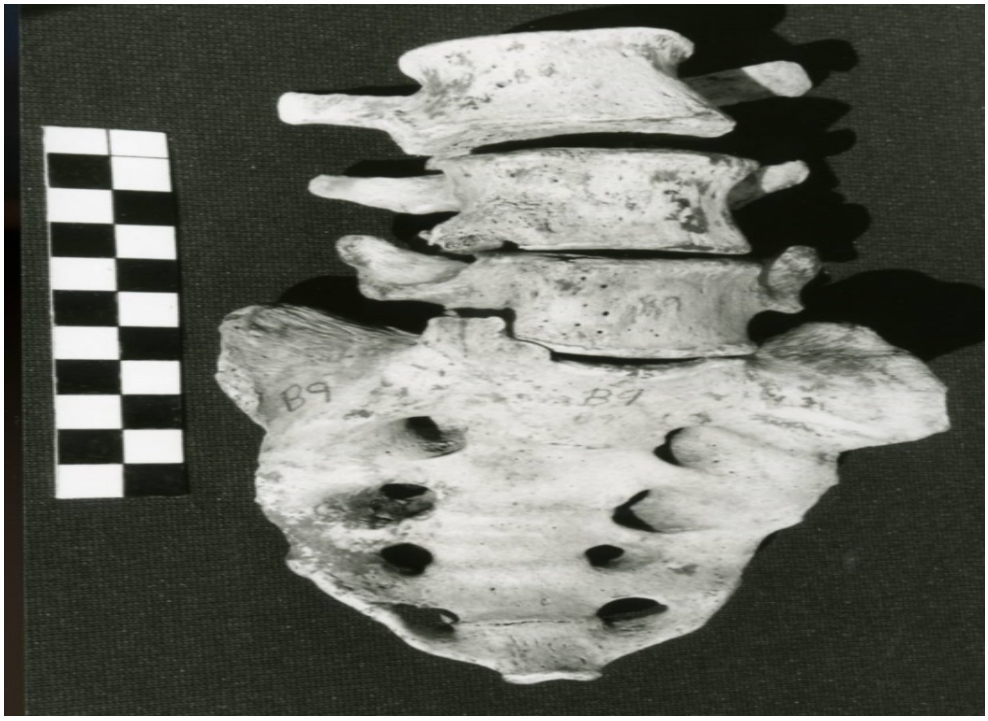

Figure A.10 Sacralization of L5 (Sac L5)

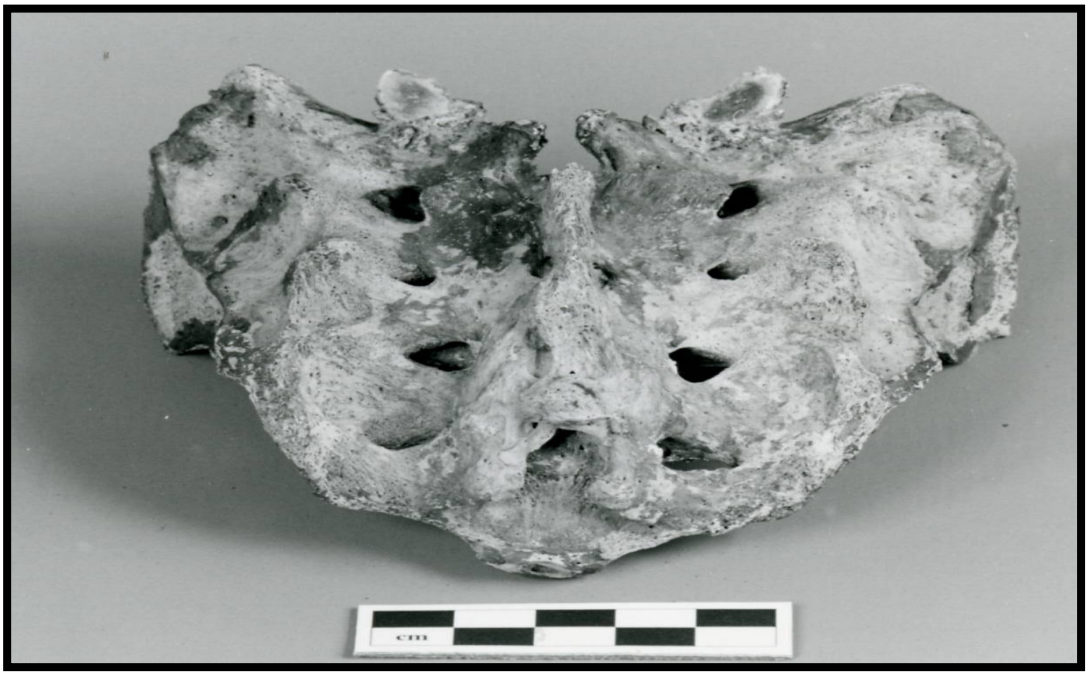

Figure A.11 Spina Bifida of S1 (SBO)

Appendix B

Statistical Calculations

G-test

Example

Sex * Sup Fac Cross tabulation

Count

		Sup Fac		Total
		0	1	
Sex	F	118	30	148
	M	80	24	104
Total		198	54	252

118*Ln(118)	30*ln(30)	148*ln(148)
80*ln(80)	24*ln(24)	104*ln(104)
198*ln(198)	54*ln(54)	252*ln(252)
= 562.9408	= 102.0359	= 739.5874
= 350.5621	= 76.27329	= 483.0167
= 1047.077	= 215.4051	= 1393.416

S1 = 562.9408 +102.0359 + 739.5874 +
350.5621 + 76.27329 + 483.0167

S2 = 739.5874 + 483.0167

S3 = 1047.077 + 215.4051

S4 = 1393.416

G = 2 * S1 - S2 - S3 + S4

= 0.284359

Phi coefficient- Intertrait Correlation

Example

Crosstab

Count

		Lat Bri		
		0	1	Total
Sup.Fac	0	190	8	198
	1	52	4	56
Total		242	12	254

$$\varphi = \frac{ad - bc}{\sqrt{(a+b)(a+c)(b+d)(c+d)}}$$

$$= \frac{(190)(4) - (8)(52)}{\sqrt{(190+8)(190+52)(8+4)(52+4)}}$$

$$= 0.061$$

Odds Ratio - Intertrait correlations

Example

Crosstab

Count

		Lat Bri		
		0	1	Total
Sup.Fac	0	190	8	198
	1	52	4	56
Total		242	12	254

$$= \frac{ad}{bc}$$

$$= \frac{(190)(4)}{(8)(52)}$$

$$= 1.827$$

Confidence Interval (95%) for Odds Ratio

$$Ln\,(OR) \pm 1.96\,x\,\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$$

$$= Ln\,(1.827) \pm 1.96\,x\,\sqrt{\frac{1}{190}+\frac{1}{8}+\frac{1}{52}+\frac{1}{4}}$$

= (-0.63602, 1.841420)

$= e^{(-0.63602)}$, $e^{(1.841420)}$

= (0.529 , 6.306)

Odds Ratio – Elimination of common absence cell

Example

Crosstab

Count

		L. Sp Fac		
		0	1	Total
R. Sp Fac	0	196	13	209
	1	7	36	43
Total		203	49	252

$$Ln\ (OR) \pm 1.96\ x\ \sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$$

$$= Ln\ (1.8) \pm 1.96\ x\ \sqrt{\frac{1}{36}+\frac{1}{56}+\frac{1}{56}+\frac{1}{20}}$$

= (-0.07250956, 1.280829)

$e^{(-0.072510)}$, $e^{(1.280829)}$

= (0.930, 3.600)

Appendix C

Maps for all 17 traits at Kellis 2 cemetery

Courtesy of James Keron

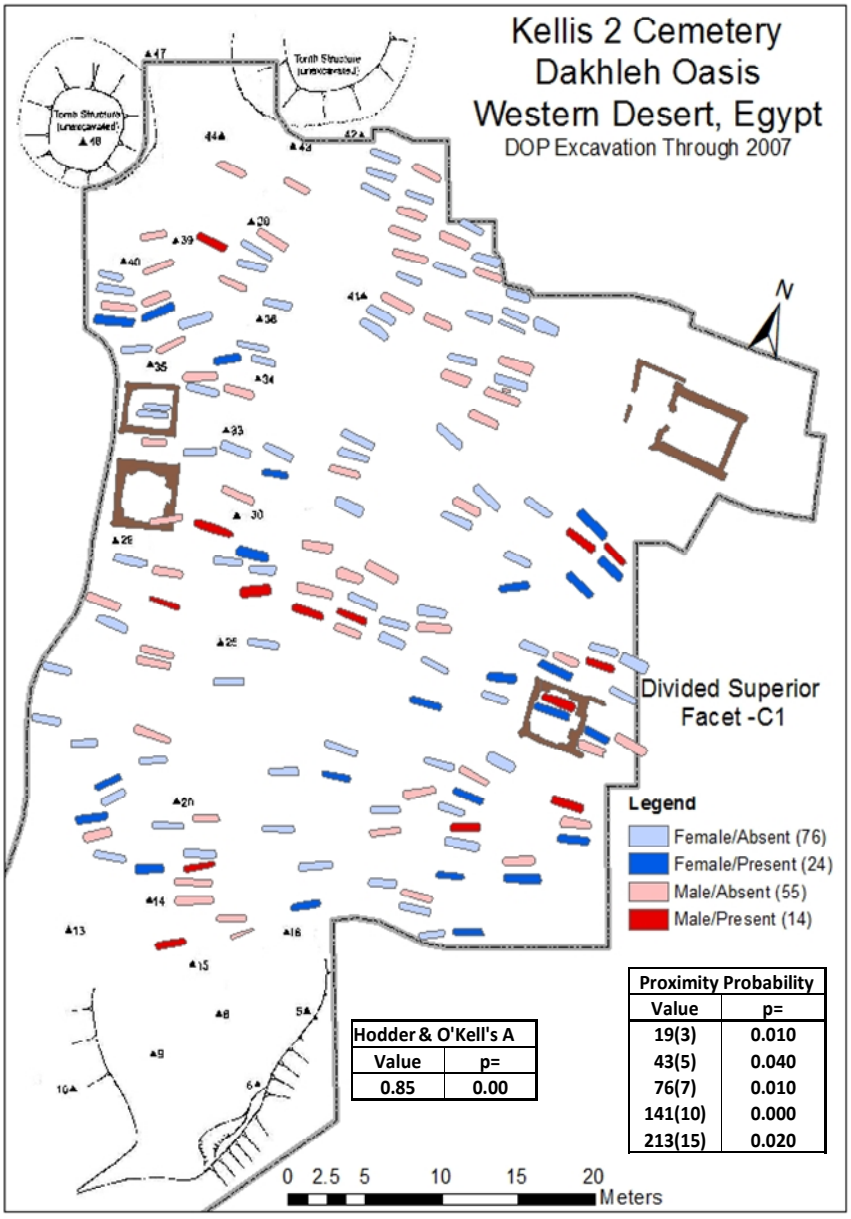

Figure C.1 Divided Superior Facet

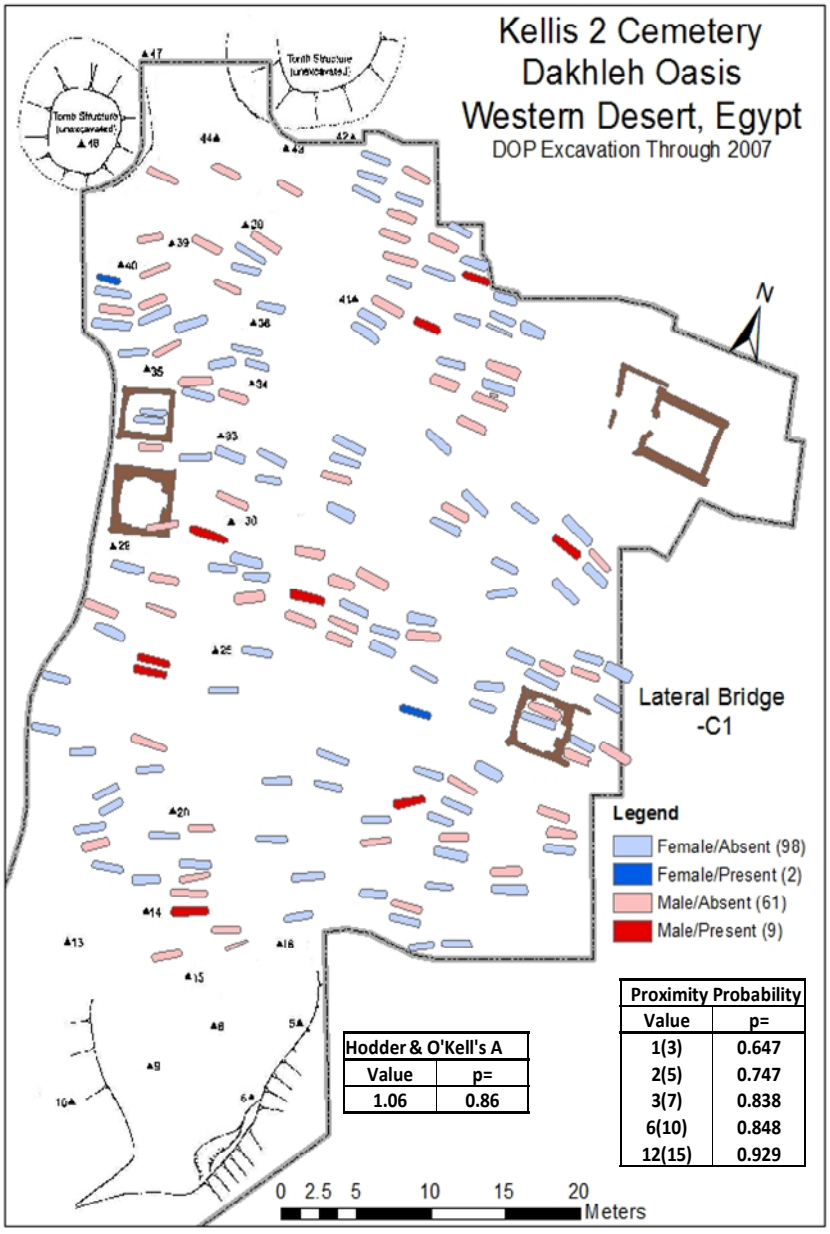

Figure C.2 Lateral Bridge

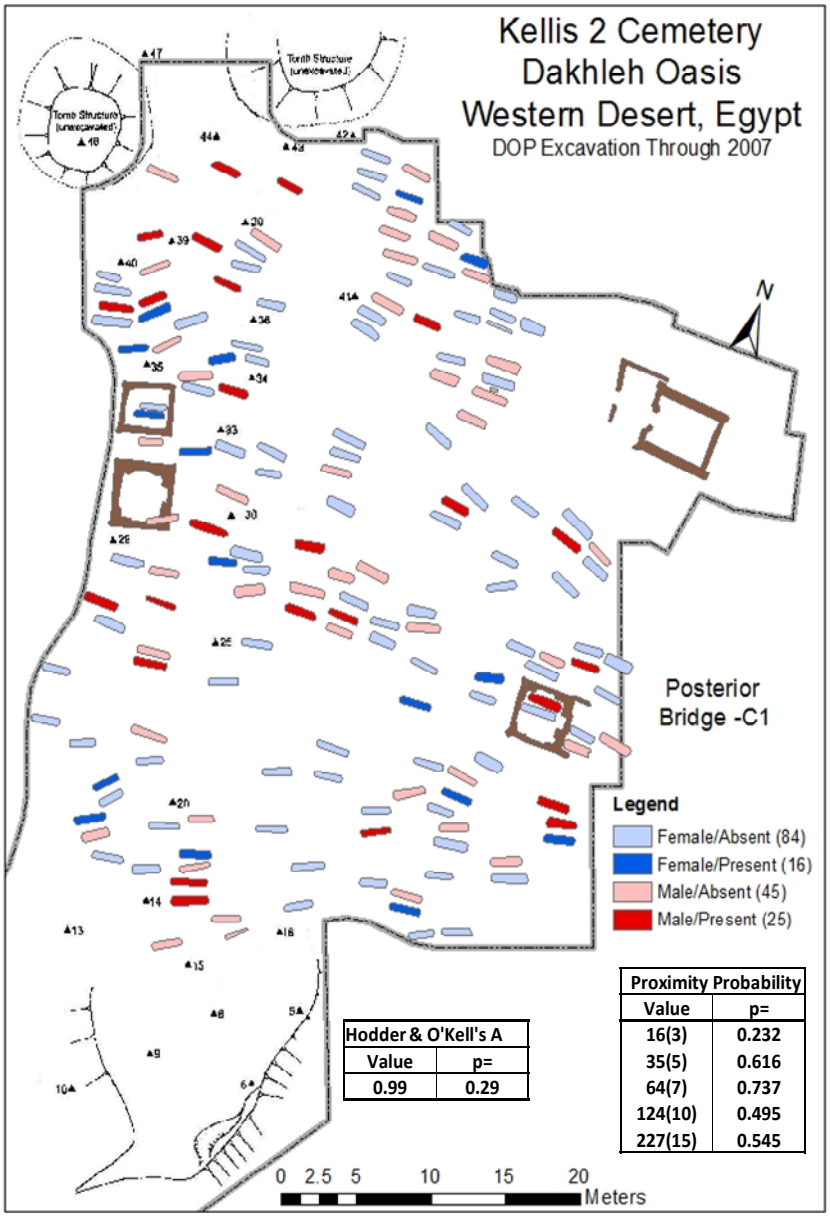

Figure C.3 Posterior Bridge

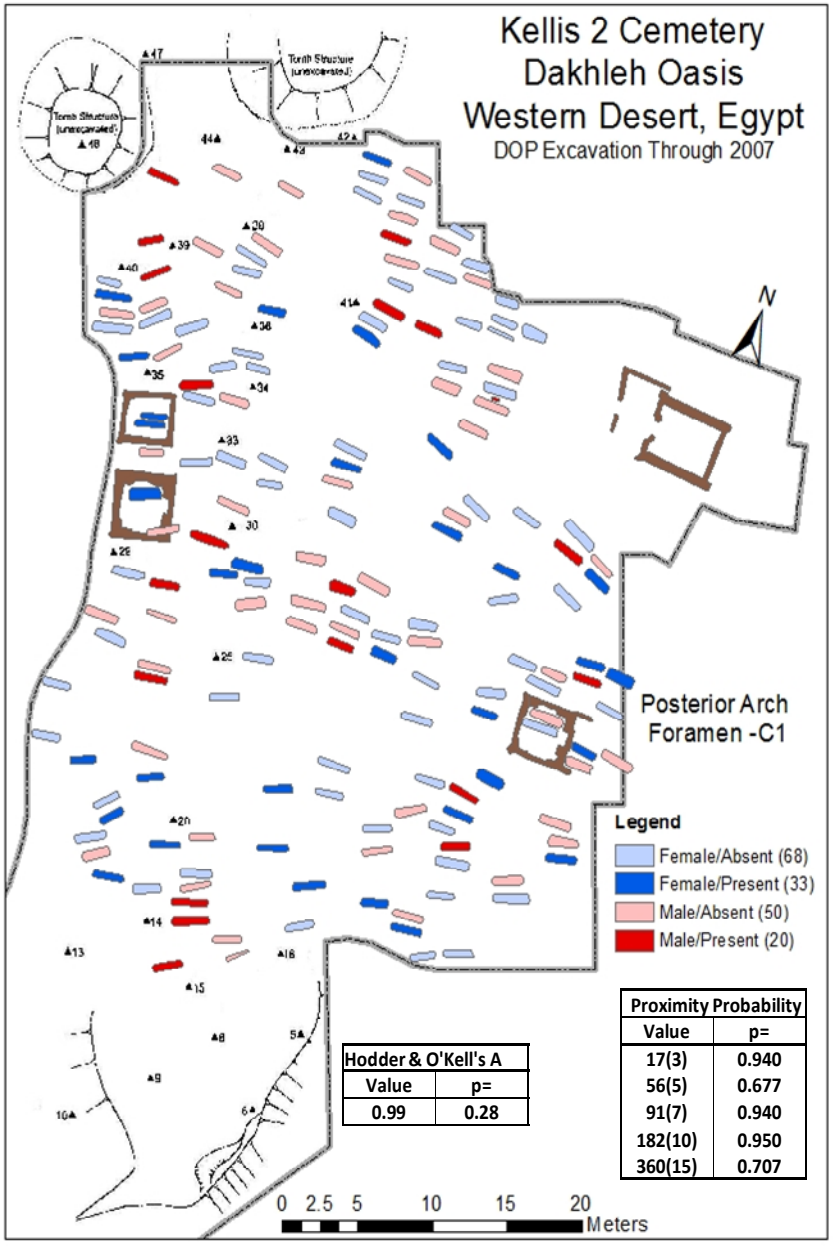

Figure C.4 Posterior Arch Foramen

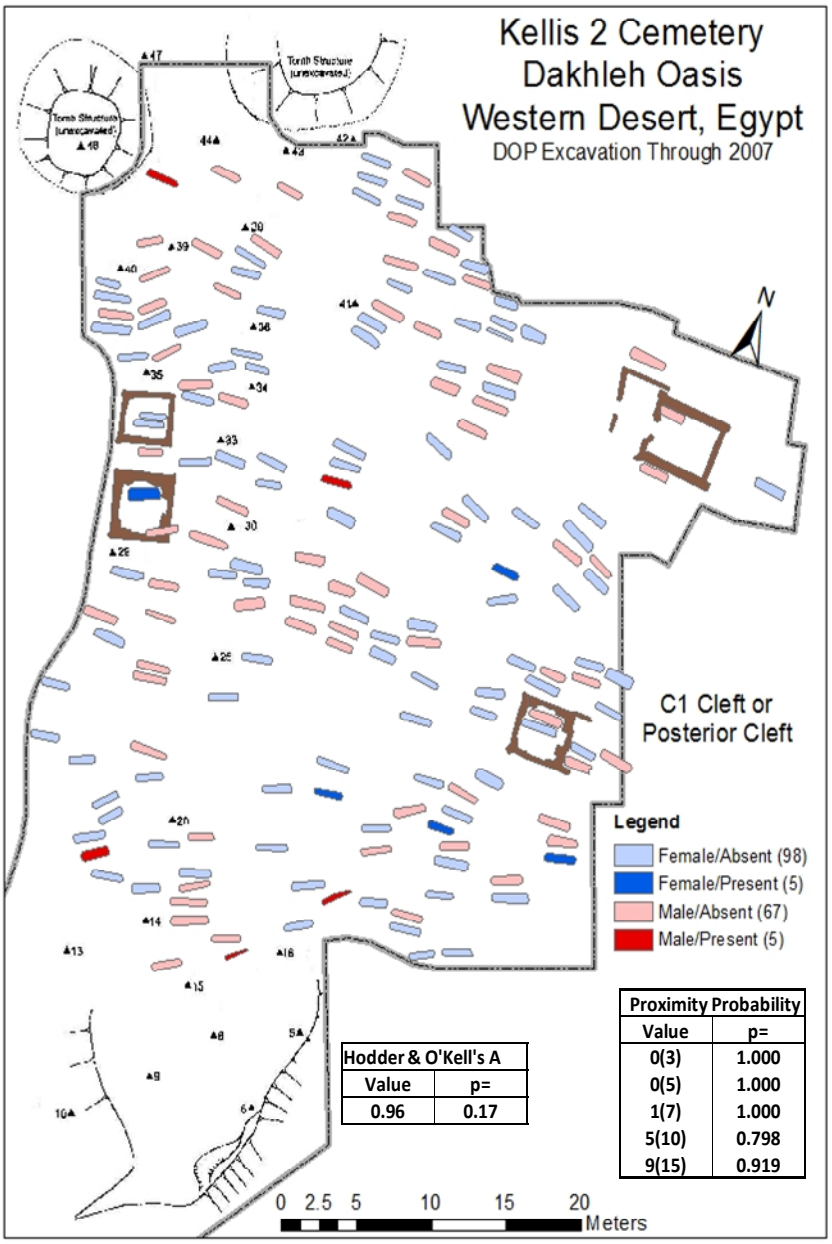

Figure C.5 Posterior Cleft

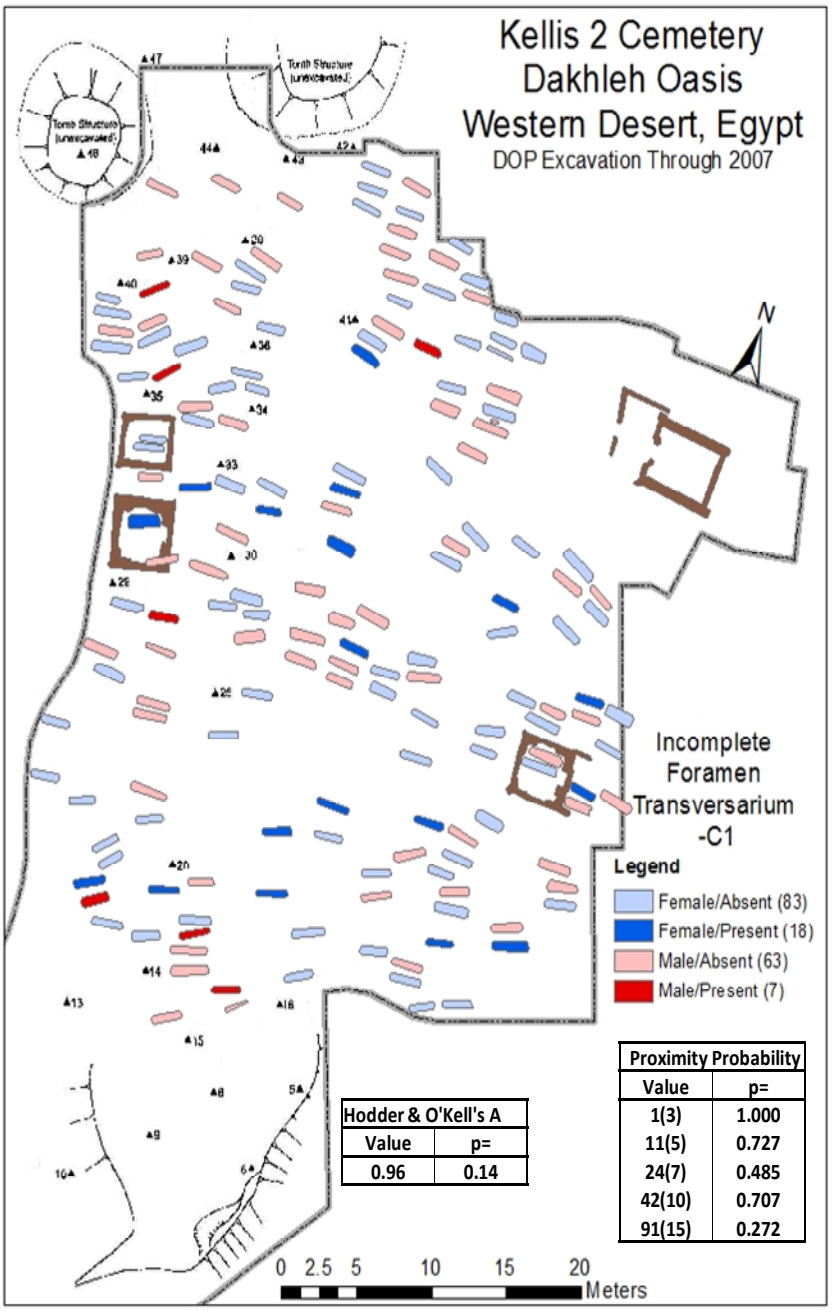

Figure C.6 Incomplete Foramen Transversarium C1

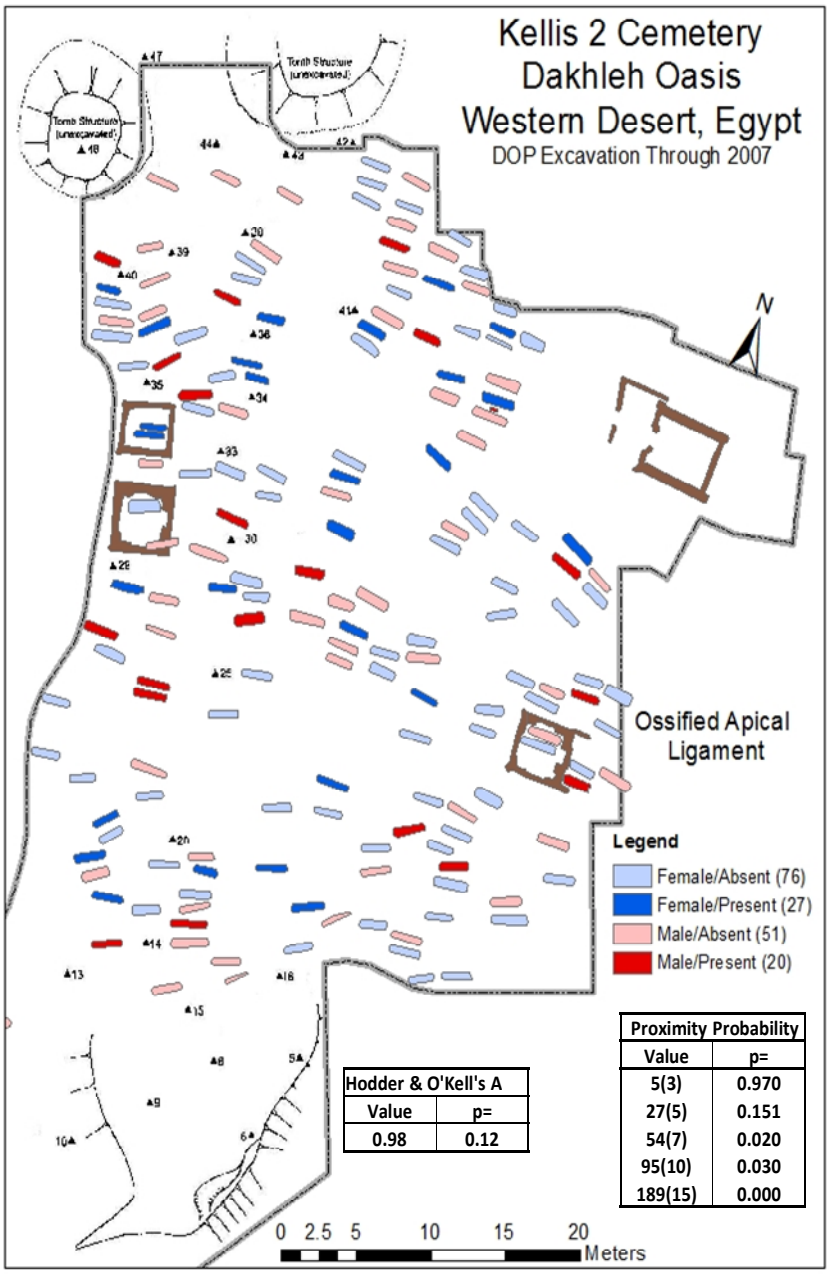

Figure C.7 Ossified Apical Ligament

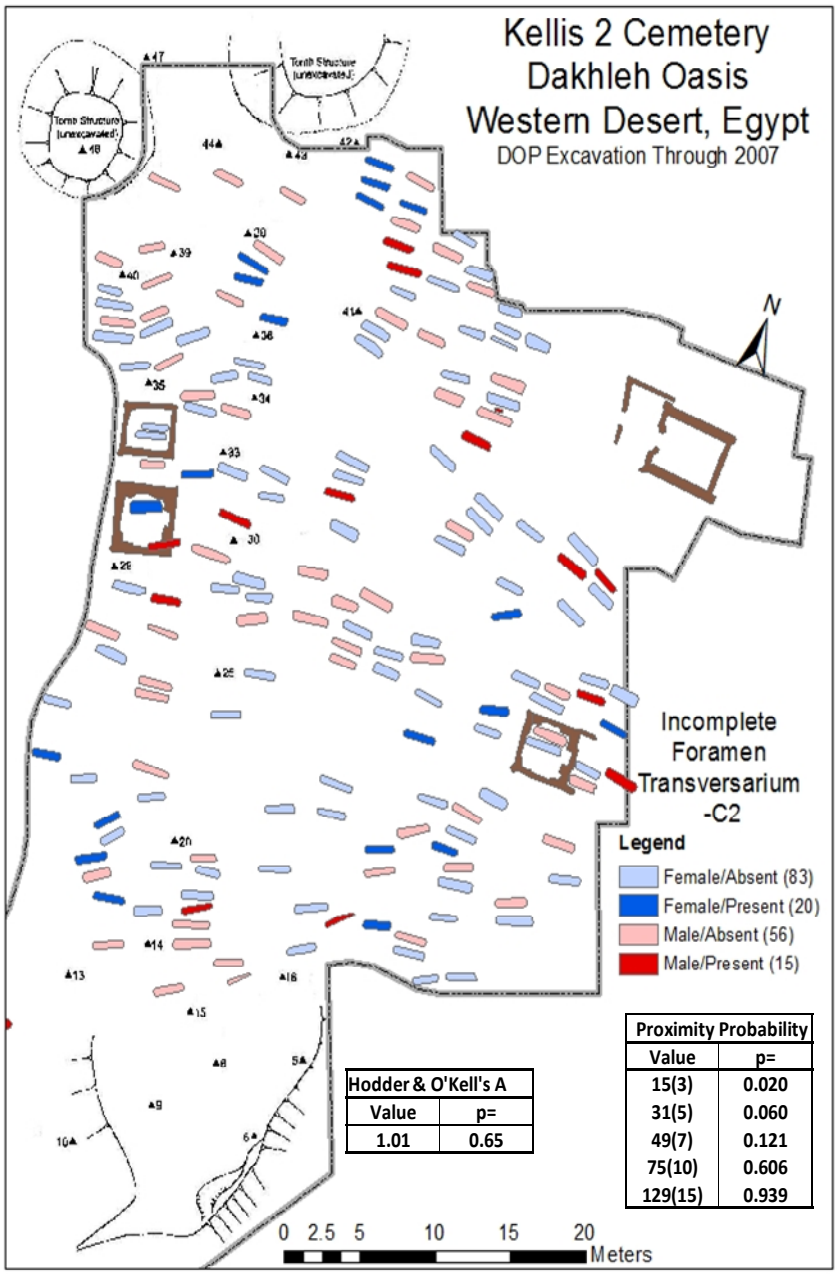

Figure C.8 Incomplete Foramen Transversarium C2

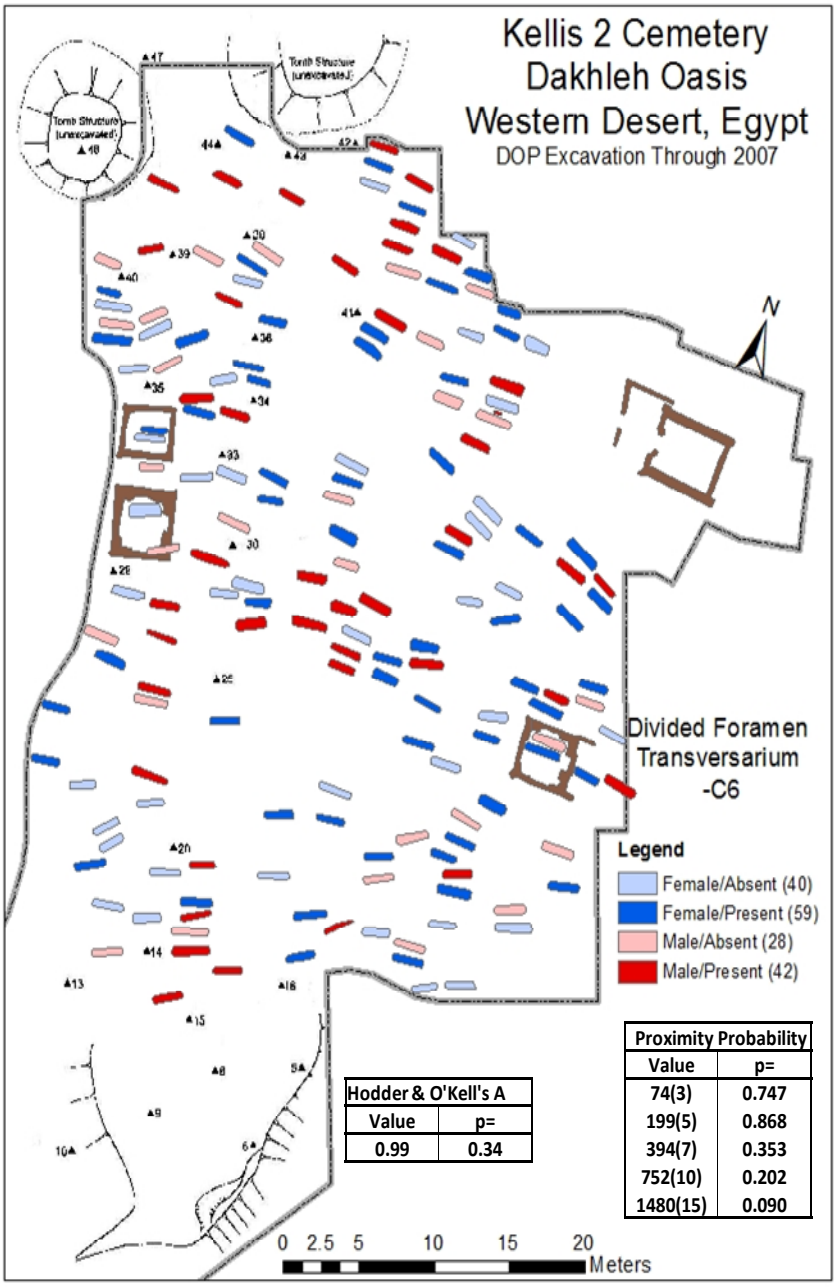

Figure C.9 Divided Foramen Transversarium C6

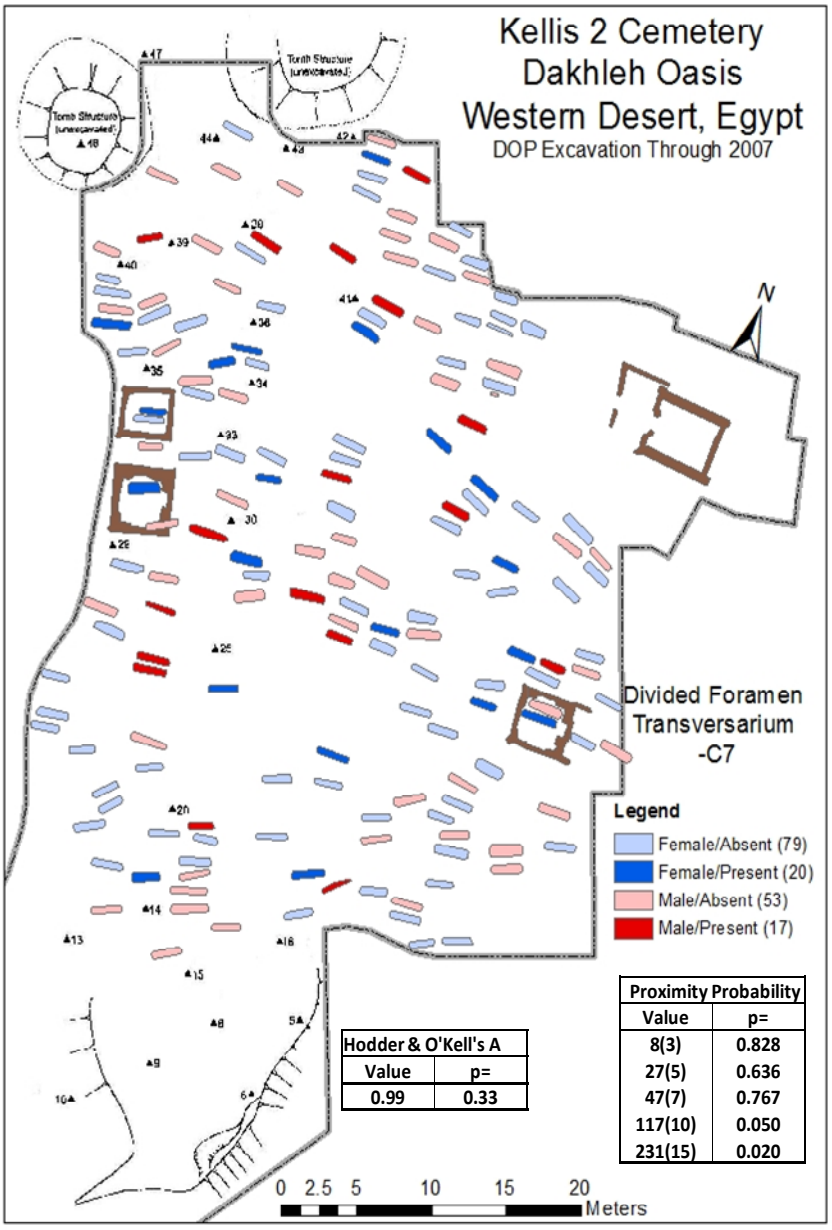

Figure C.10 Divided Foramen Transversarium C7

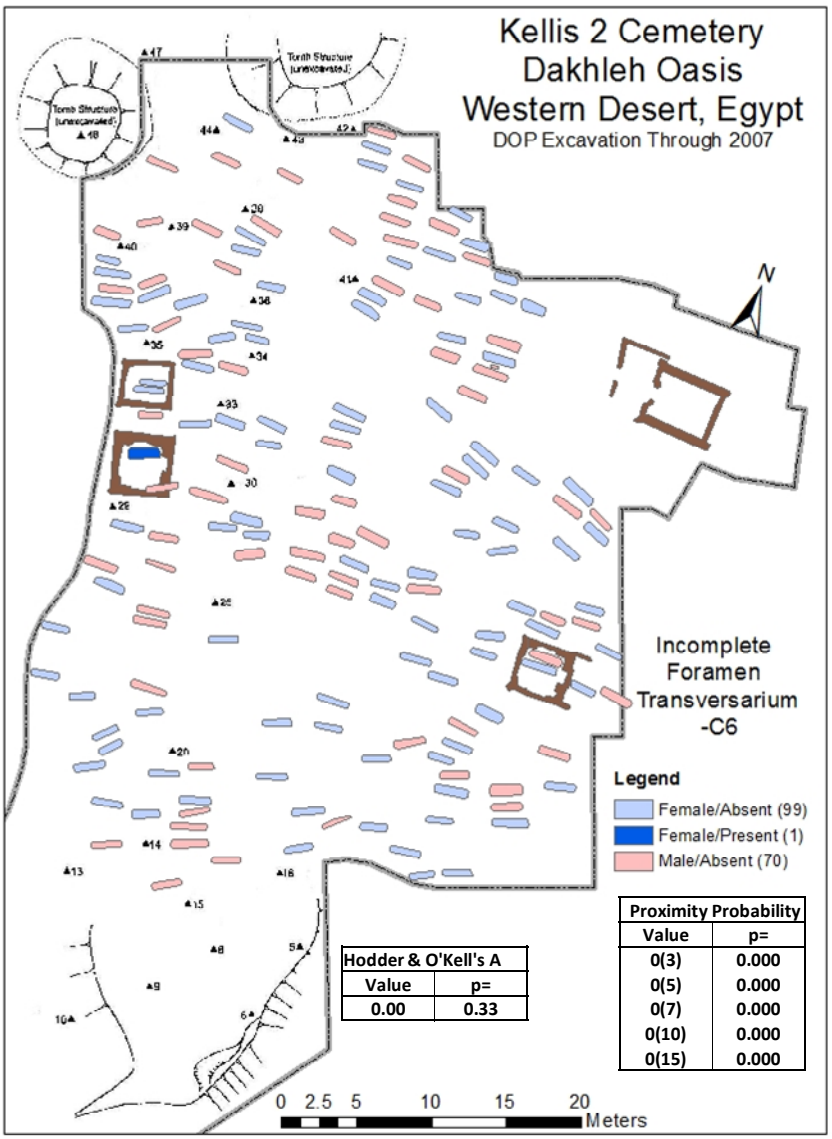

Figure C.11 Incomplete Foramen Transversarium C6

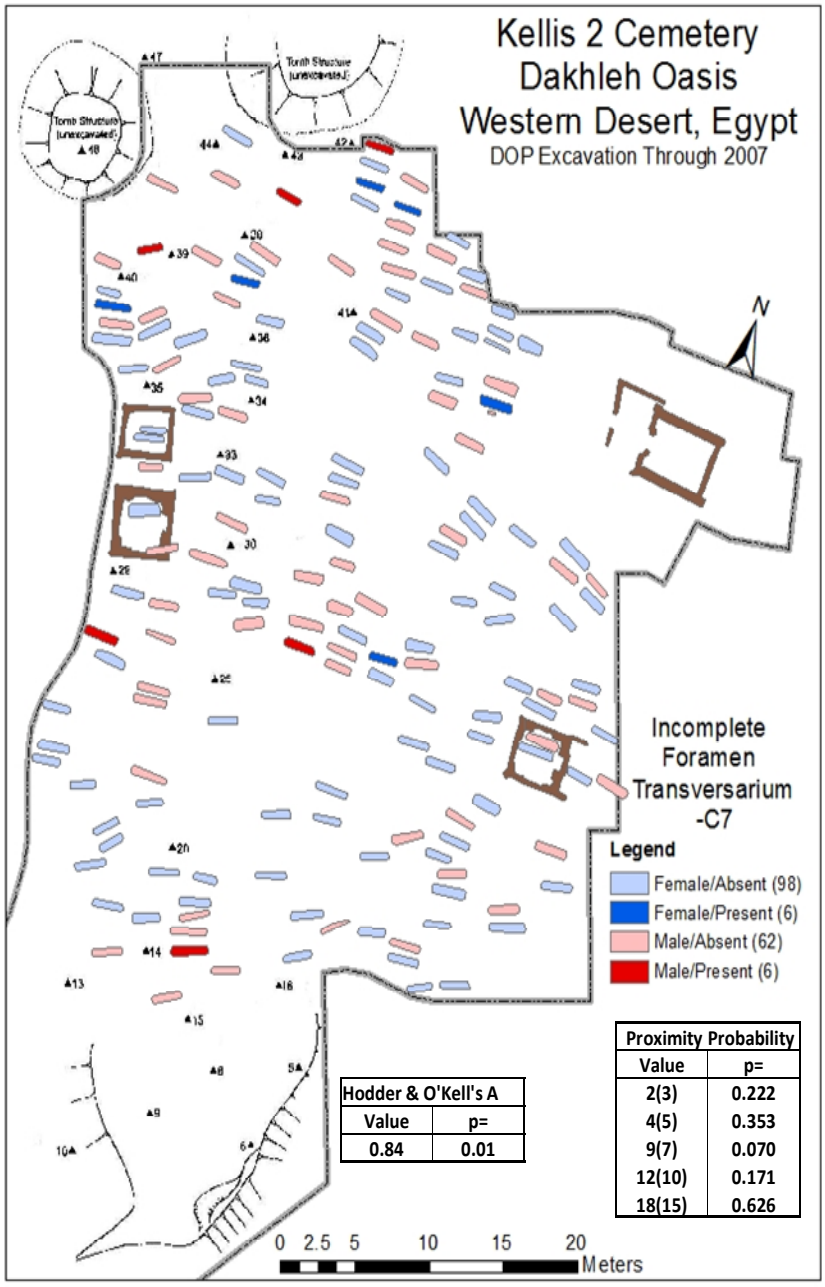

Figure C.12 Incomplete Foramen Transversarium C7

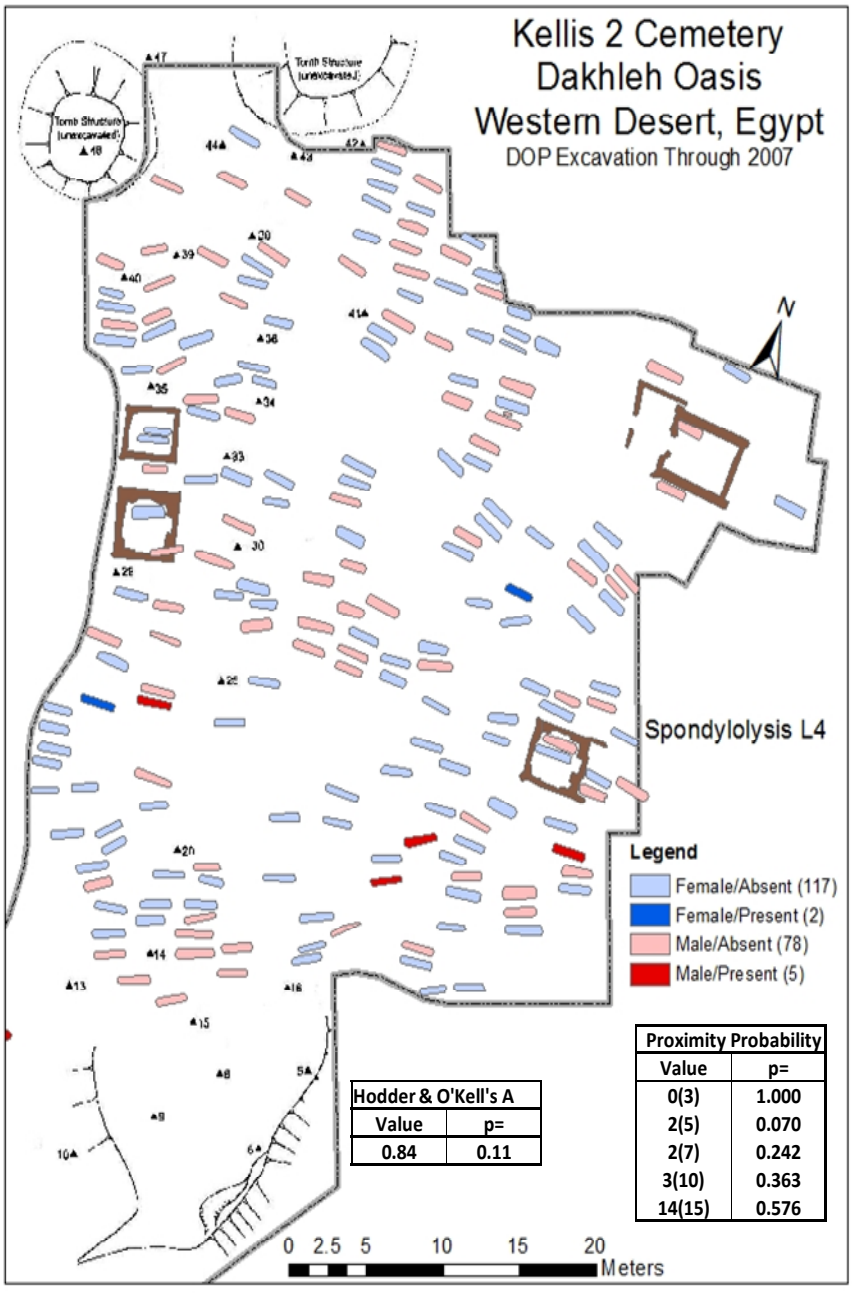

Figure C.13 Spondylolysis L4

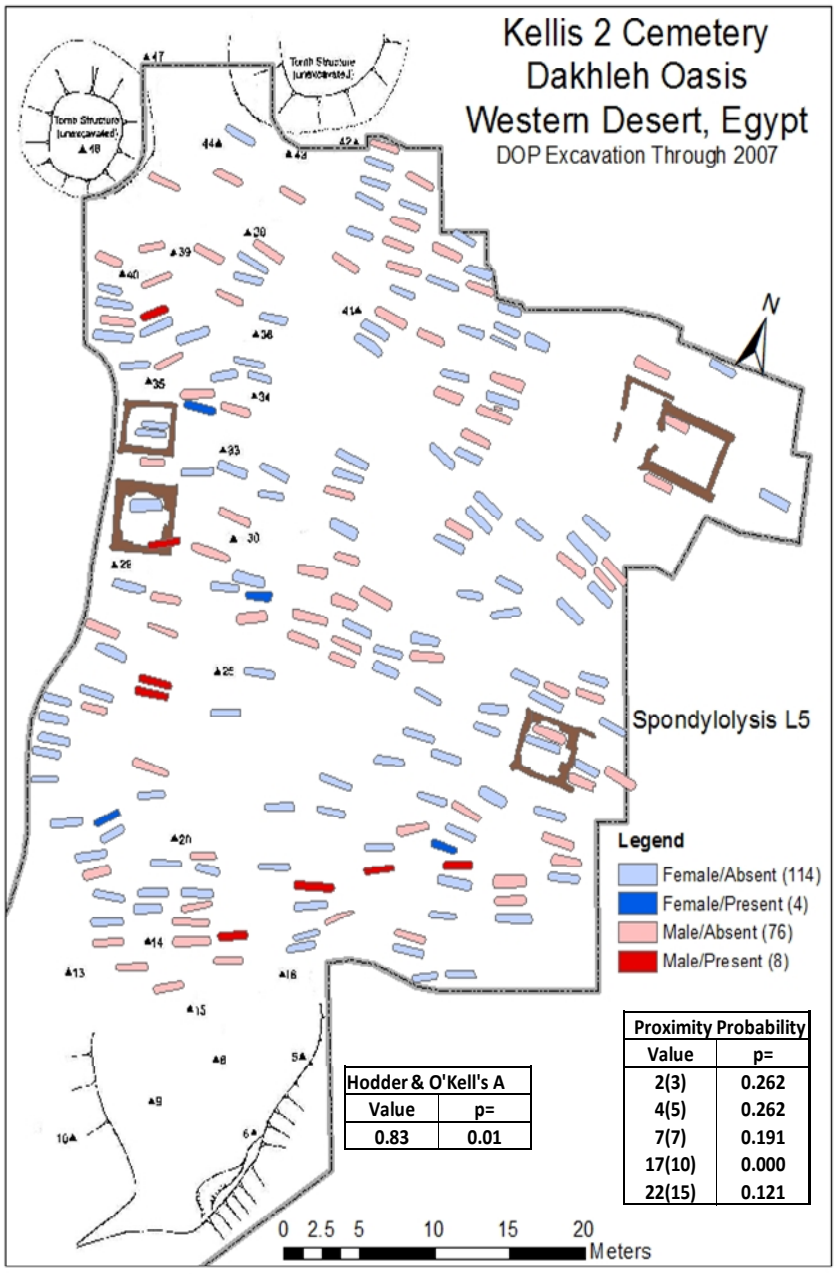

Figure C.14 Spondylolysis L5

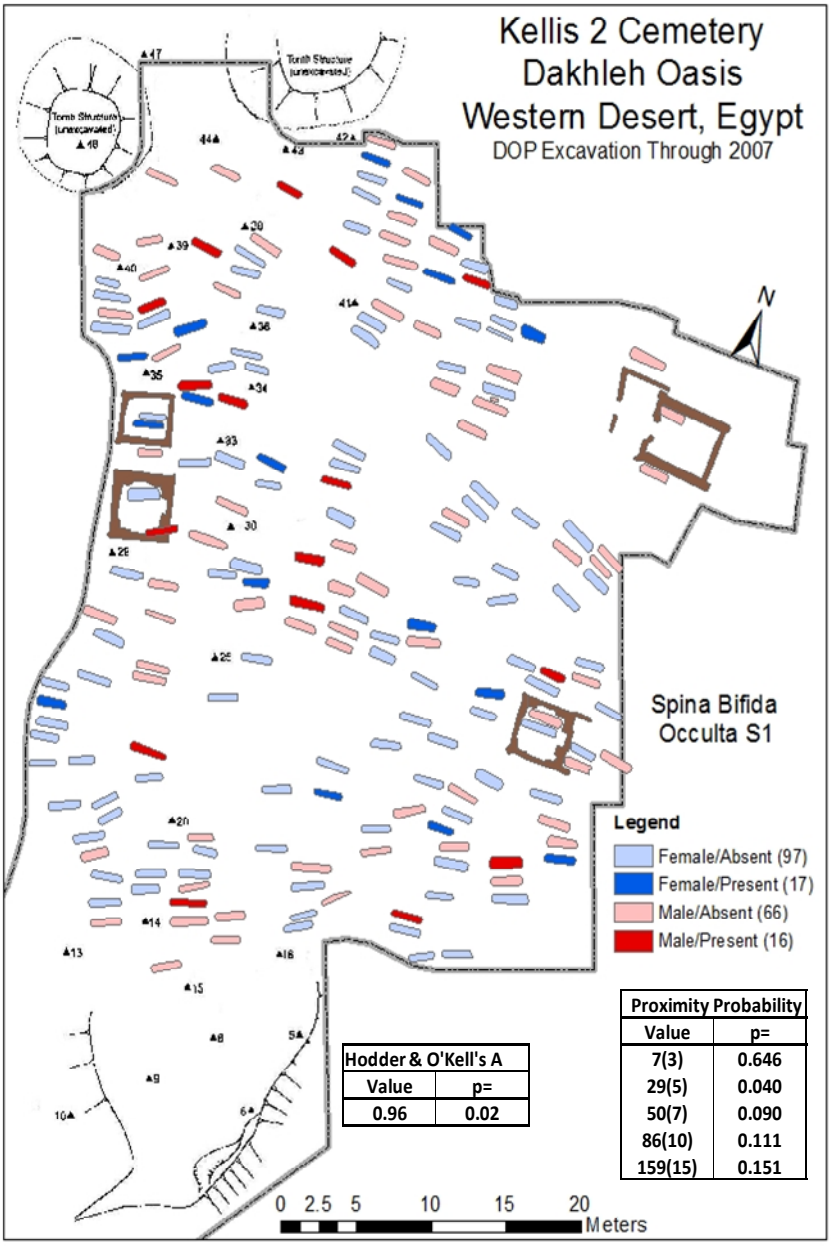

Figure C.15 Spina Bifida Occulta

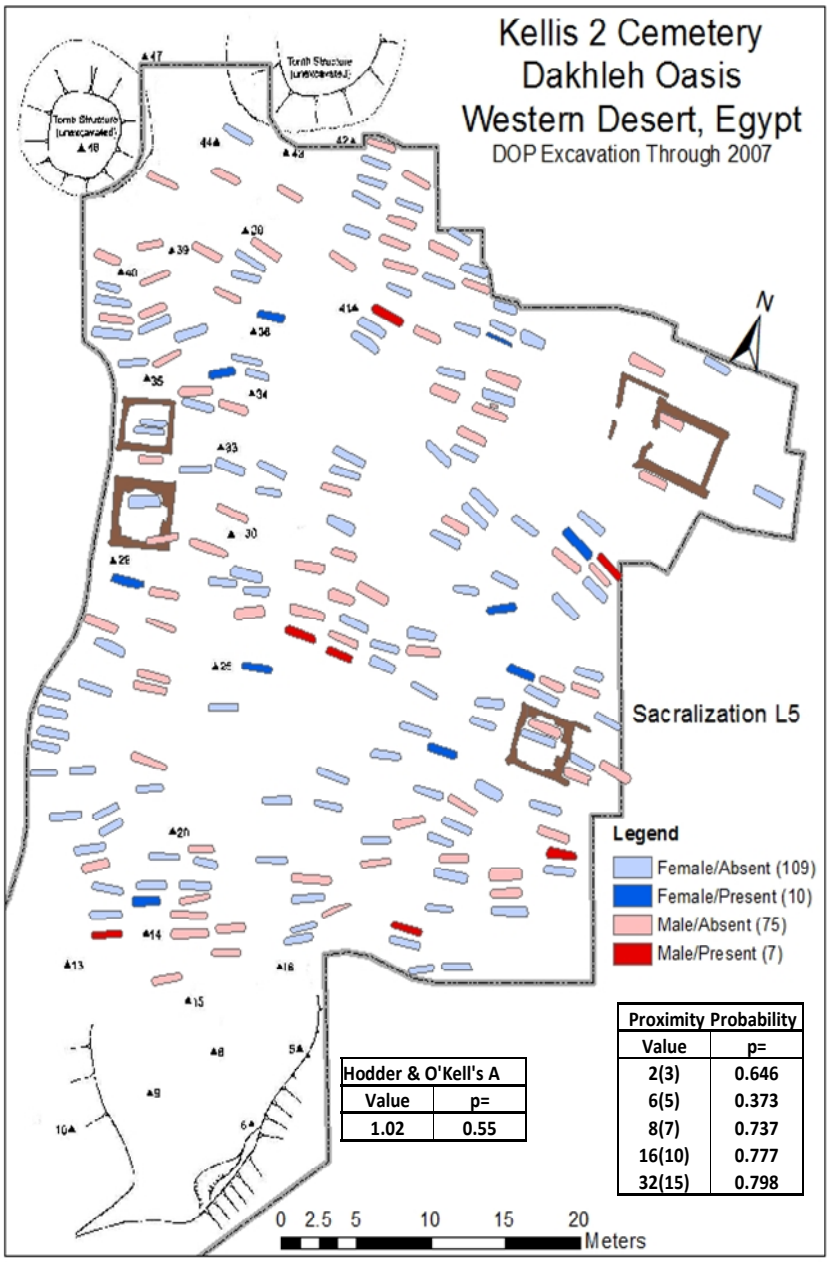

Figure C.16 Sacralization L5

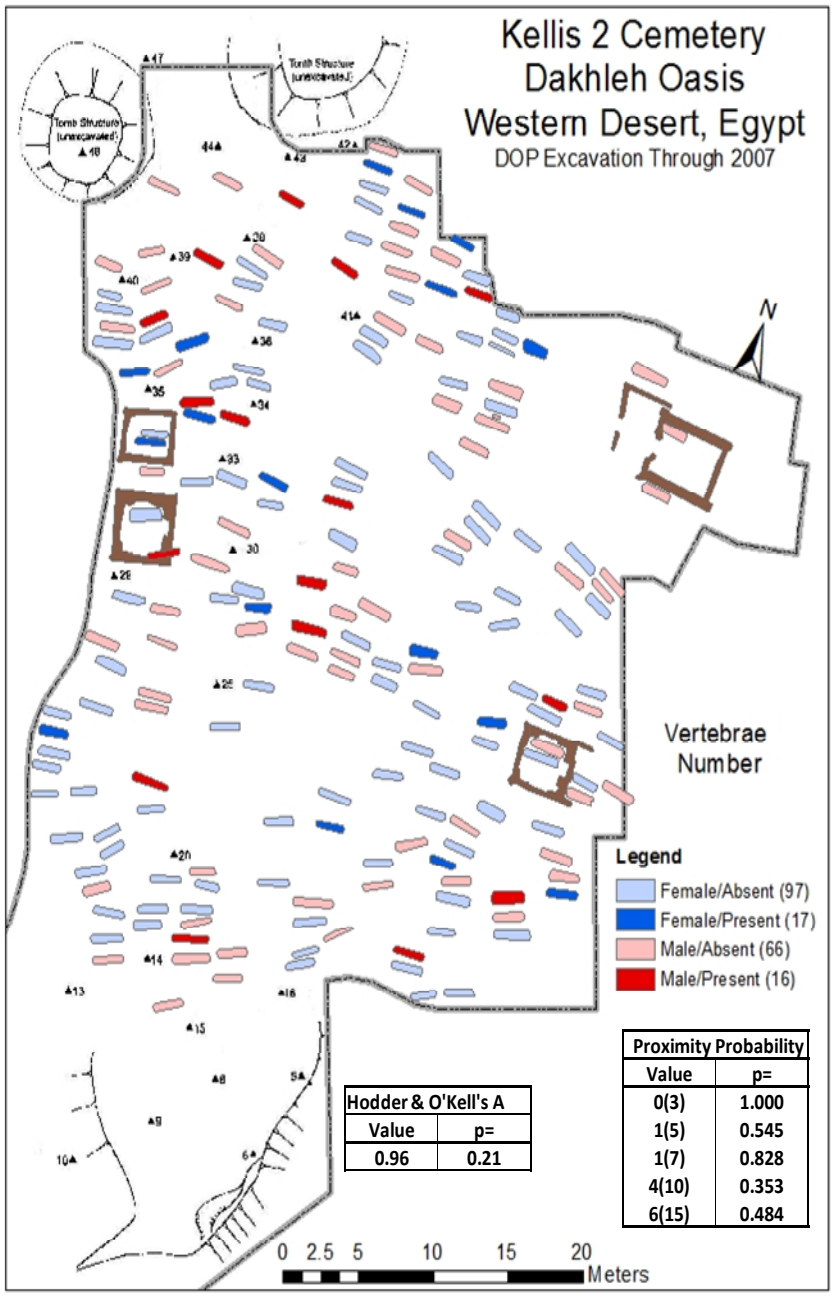

Figure C.17 Vertebrae Number

Curriculum Vitae

Tiffany Sarfo

EDUCATION:

M**asters in Archaeology and Bioarchaeology**
Faculty of Social Science - Anthropology
Western University, London, ON
2012- 2014

B.Sc Biology and Bioarchaeological Anthropology
Graduated with Honours
Faculty of Science and Social Science
Western University, London, ON
2008-2012

HONOURS AND AWARDS:

Dean List
Western University
2012

RELAVENT WORK EXPERIENCE:

Graduate Teacher Assistant
Western University
2012- 2014

Archaeology Field Technician
Stantec Consulting Ltd
2013

Archaeology Field Technician
Golder Associates
2012