Evaluating Nubian Population Structure from Cranial Nonmetric Traits: Gene Flow, Genetic Drift, and Population History of the Nubian Nile Valle

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Evaluating Nubian Population Structure from Cranial Nonmetric Traits: Gene Flow, Genetic Drift, and Population History of the Nubian Nile Valley

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Short Title: Evaluating Nubian Population Structure

KEYWORDS: MAHALANOBIS D2, BIOLOGICAL DISTANCE, DISCRETE TRAITS
Abstract  Paleolithic archaeological and skeletal remains from the Nile Valley have yielded a complex picture of life along the river. Sociocultural and sociopolitical events during this timeframe shaped population structure, while gene flow and genetic drift further developed it. In this paper, we take a population genetics approach to modeling Nubian biological relationships in an effort to describe how an accumulation of events formed Nubian population structure. A variety of Nubian samples were utilized, spanning the Mesolithic-Christian time periods, and geographically, from just above the first through the third cataracts. Population genetics statistics were employed to estimate and depict biological affinities (Mahalanobis $D^2$ with a tetrachoric matrix, principal coordinates analysis, $F_{st}$, and Relethford Blangero residuals) and supplemented by spatial-temporal modeling (Mantel tests and PROTESTs). Variation is high amongst these groups, indicating an intricate pattern of relationships in their population history where similar levels of gene flow probably stemmed from extensive cultural contact with Egypt and other populations in a variety of contexts. Genetic drift is also apparent in some of these sites, which is consistent with social and political histories of these groups. Traditional modeling of spatial-temporal patterning was not successful, which may be attributed to the non-linear, loose clustering of Nubian groups by site. Collectively, the archaeological, biological, and environmental evidence support the ideas of multiple populations living in Lower Nubia during the Paleolithic, and/or a new population entering the area and shaping Nubian population structure.
The Paleolithic inhabitants of the Nile Valley are of interest to scholars as population events during this timeframe have implications for later population structure in Nilotic populations. Migrations and population replacements in Nubia have been hypothesized based on archaeological (e.g., Wendorf 1968) and biological (Irish 2005; Irish and Turner 1990; Turner and Markowitz 1990) findings. Alternatively, others (e.g., Carlson and Van Gerven 1979) have proffered there was great homogeneity in Nubian skeletal remains and the level of variation is mainly a product of in situ evolution without much influence from other populations. Later Nubian biological material shows evidence of great heterogeneity (Godde 2009; Godde 2013a; Irish 2005), which supports the idea of increased gene flow over time through contact with other populations, among other hypotheses, but is a simplistic explanation for describing the source of heterogeneity.

In this paper, we will explore a more synthesized view, looking at environmental influences that may have promoted gene flow, as well as the moderation of gene flow with demonstrable genetic drift as a result of sociopolitical factors. This notion is in line with ideas postulated by Keita (2005) for Egyptians. Keita (2005) proposes an aggregation of events (e.g., language family dispersals, environmental pressures) is responsible for the high heterogeneity found in Egypt. He stresses the necessity in constructing narratives using historical evidence to interpret statistical findings, an inferential strategy imperative to strong interpretations of population genetics studies (559). Here, Nubia can be examined in a similar vein, focusing on multiple lines of evidence from the environmental, archaeological, historical, mortuary, and skeletal records in an effort to demonstrate a lack of population replacements after the Paleolithic, sustained extraregional gene flow over time, and signatures of genetic drift. To do
this, a history of the population, combined with findings from biological material, will provide
the framework for the population genetics analysis and interpretations.

**Early Nubia: The Paleolithic, Mesolithic, and Neolithic**

The earliest time period of modern human occupation along the Nile begins with the
Paleolithic. The material culture of the human remains in some cemeteries at Jebel Sahaba (Late
Paleolithic) suggest they were part of the long-lasting Qadan culture (Edwards 2004; Phillipson
2005), whose microlithic industry is known in Lower Nubia (Trigger 1976). Those interred there
died brutally, likely from surviving and subsisting in harsh environmental conditions (Close and
Wendorf 1990). A climate shift occurred between the Paleolithic and Mesolithic (Edwards
2004), which appears to correspond to a less tumultuous time in Nubia. The Khartoum
Mesolithic toolkit was pervasive (Edwards 2004; Manning and Timpson 2014; Trigger 1976),
expanding across the Sahara around 8,000 B.C (Manning and Timpson 2014) and lasting 2-3,000
years (Edwards 2004).

Pottery and artifacts primarily define two Neolithic Nubian groups (Khartoum Neolithic
Variant and Abkan). The pottery of the Khartoum Variant is interpreted as demonstrating a
logical evolution from the Khartoum Mesolithic (Trigger 1976). Further support of this transition
comes from the continuous occupation at el-Barga (east of Kerma), spanning 6000-5500 BC,
which extends from the late Mesolithic into the Neolithic and demonstrates continuity across
these times (Honegger 2004). Later in the Neolithic, the A-Group appeared in the Lower Nubian
archaeological record and probably arose from the indigenous people already in the area (Adams
The A-Group, a complex stratified chiefdom (Smith 1998), was wealthy (e.g., grave goods at el-Barga (Honegger 2004)), possibly due to exportation of raw materials and control of trade into Egypt from further south (Edwards 2004; Trigger 1976). With time, the A-Group wealth waned, probably as a result of Egyptian economic changes (Smith 1998) that led to establishing of alternate trade routes to southern areas (Trigger 1976). It has been said that Egyptian interference may have led to the abandonment of Lower Nubia by the A-Group (Adams 1977; Edwards 2004; Trigger 1976; Trigger et al. 1983), with the conflict possibly revolving around these trade routes (Trigger 1976). The Nile underwent changes during this time, marked by a lowering of flood levels (Adams 1977; Trigger 1976) due to increased aridity and variability in rainfall (Kröpelin et al. 2008) that Manning and Timpson (2014) link to a corresponding decrease in biodiversity (Hély and Lézine 2014). These climatic changes are ideal for spurring population movement, which is consistent with recent evidence of a movement of A-Group occupations to a more widespread placement, rather than an abandonment of the area (Edwards 2004).

While nothing from the archaeological record suggests a new population definitively migrated to Nubia after the Mesolithic and became the A-Group, studies have found a reduction in the craniofacial complex over time (Small 1981; Van Gerven 1982; Van Gerven et al. 1977), sometimes attributing the changes to shifting masticatory stress as a result of the transition from hunting and gathering to agriculture after the Mesolithic (Carlson 1976; Carlson and Van Gerven 1977), or as a product of natural selection (Van Gerven 1982). An alternative view posits a diminution of body size could account for these changes (Macchiarelli and Bondioli 1986). Both mechanisms for gracilization are consistent with the environmental, archaeological, and mortuary evidence. However, most recently, Galland et al. (2016) noted a discontinuity between
Mesolithic and Neolithic Nubian groups derived from geometric morphometric analyses, which requires an evaluation in the context of the results of the current analysis (see Discussion for more details).

**Middle Nubian Horizon**

The Middle Nubian Horizon is represented by three culturally distinct, but roughly contemporary, groups spread across Nubia: the C-Group, Kerma, and Pan-Grave people. The C-Group is a more northern population, occupying the area of the Nile above the second cataract (Edwards 2004). The C-Group pottery appears to represent a natural evolution from the A-Group (Adams 1977) and overlaps with ceramics from Kerma and the Pan-Grave cultures (Trigger 1976). Most recently, several lines of archaeological evidence (including pottery and burial superstructures) at Kerma demonstrated an overlap or close affinity between the C-Group and Kerma peoples (Honegger 2012). Further indications of a connection between the C-Group, Kerma, and Pan-Grave peoples comes from the placement of bucrania adjacent to tumuli (see Chaix, L. et al. 2012).

Biologically, a straightforward relationship of the A- and C-groups has been difficult to model. When cranial nonmetrics are considered, the A-Group shows biological continuity with the post-Neolithic C-Group (Godde 2013b; Prowse and Lovell 1995), which is also supported by dental nonmetrics (Johnson and Lovell 1995). Complimentarily, Godde (2013b) showed the same C-Group sample utilized in Prowse and Lovell (1995, 1996) and Johnson and Lovell (1995) was more biologically related to a Mesolithic Nubian sample than to the A-Group also examined in these earlier studies, a finding that on the surface appears contradictory to the results of Galland et al. (2016) (see Discussion).
The Pan-Grave culture, which appears very different than either the C-Group or Kerma culture, has been found in Egypt, C-Group occupations, and in the desert near the C-Group (Trigger 1976). The Pan-Grave people dug unique, oval shaped, shallow burial pits at the perimeter of C-Group cemeteries, as well as in the desert (Trigger 1976). It is suggested they were Medjay, stationed by Egypt to keep track of the C-Group (Trigger 1976), but this may not have been the sole purpose of their presence (Edwards 2004). Adams (1977) cites literature that identifies the Medjay/Pan-Grave people as another cultural group of nomadic Nubians that lived in the Eastern Desert.

The Kerma culture was found further south, below the third cataract (Edwards 2004). Kerma was a state civilization (Smith 1998) and massive trade center along the Nile, overseeing the importation of goods from areas south to more northern areas (Welsby 1996) and providing the conditions needed for interaction with other peoples. The downfall of Kerma likely came during the New Kingdom with a conquest by Thutmosis III (Edwards 2004). The necropolis was abandoned and the people moved south (Edwards 2004). The C-Group likely disappeared as a result of Egyptianization, which is an over-simplified description of colonialism that is often used to describe the adoption of Egyptian customs, materials, and practices (Edwards 2004). The Pan-Grave people appear to have been more resistant to this force as their graves held little Egyptian material (Edwards 2004).

**Later Time Periods: The Meroitic, X-Group, and Christian**

Kush can be divided into two chronological time periods: the Napatan and Meroitic (Welsby 1996). Kush represents a state-level society that arose after the downfall of Kerma and subsequent Egyptian occupation of the area (Edwards 2004). The royal cemeteries at Napata...
ceased royal entombment at around the time the royal cemeteries arose at Meroë (Edwards 2004). The people of the Meroitic time period utilized a new language that has only recently been deciphered (Rilly and de Voogt 2012). The Meroitic civilization dealt in trade and their influence reached far north to the strategic areas in the trade route between Meroë and Egypt (Edwards 2004), allowing for contact and relationships with other populations. The settlements in the north were small, spread out, and likely served as “outposts” on the trade route (Edwards 2004).

Archaeologically, it appears the Meroitic culture smoothly transitioned to the X-Group (Adams 1977; Edwards 2004) and later to the Christian Nubians (Adams 1977), although foreign influences have also been noted (Nielsen 1970). The name X-Group derives from original interpretations of the X-Group as a foreign population spreading across Nubia after the Meriotic period (c.f., Adams 1977). Because later interpretations debunked this hypothesis, Adams (1977) refers to the X-Group as Ballana, following the suggestion by Trigger (1965). We use X-Group and Ballana interchangeably here as many of the studies cited utilize X-Group or Ballana to refer to the Ballana culture. The conversion to Christianity marks the Christian time period. During this time period, there was an immigration of northern peoples to areas in Lower Nubia (Van Gerven 1995). Skeletally, later Nubian groups (Meroitic, X-Group, Christian) and a Middle Nubian Horizon group (Kerma) cluster together, showing biological affinity (Godde 2010).

Aims

As is demonstrated above, Nubian history shows regular contact with other peoples, providing opportunity for extraregional gene flow, but the environmental, archaeological, mortuary, and skeletal evidence do not suggest population replacement after the Paleolithic. This
paper was developed from the work in Godde (2009), providing an updated method to modeling population structure in Nubia with parameter estimates of gene flow and genetic drift and combined with multiple lines of evidence, which is novel in research studying Nubians. A model-bound approach (Relethford and Lees 1982) is applied whereby population genetics parameters are estimated and interpreted from Nubian skeletal material. Samples from across the first three cataracts of Nubia and across time are analyzed to assess biological evolution, representing one of the most comprehensive data sets used to examine Nubian population structure, and its relatively complete coverage of time periods from the Mesolithic will provide new information about biological relationships in Nubia.

We first hypothesize that the Nubian samples included here will show strong biological affinity to one another (via biological distances) with similar levels of external gene flow among most groups (with the exception of the Sayala C-Group, which has been established to be biologically distinct in Godde (2013b)), and with no indications these groups moved into the area as a population replacement. Further, a smaller research question can also be addressed by looking at the biological distances among the different contemporary Middle Nubian Horizon cultures (what was the biological relationship among these overlapping, distinctive groups?).

Second, we apply the spatial-temporal model of Konigsberg (1990) (an extension of the isolation by distance model) to these samples, where it is hypothesized samples that are spatially proximate to one another are more closely related and time will have an inverse relationship to biological affinity. The null hypothesis we test, then, is that these samples will follow the expectations under this model and the alternative hypotheses are that a more complex spatial and/or temporal data structure is present (first alternative hypothesis) or there is no spatial and temporal patterning (second alternative hypothesis). Finally, our third hypothesis is that we
anticipate finding levels of gene flow through the population genetics analyses that are consistent with maintaining a level of heterogeneity established sometime near the Paleolithic, mediated by genetic drift, and supported over time by a variety of contact, but with no evidence of population replacement/disruption after the Paleolithic.

Materials and Methods

The data set analyzed in this investigation is comprised of original observations and those contributed by several scholars (Dr. Tsunehiko Hanihara, Dr. Nancy Lovell, and Dr. Eugen Strouhal), which enabled the building of a highly representative sample of Nubians. Time periods extending from the Mesolithic through Christian Nubia and geographic locations from Upper Egypt through Upper Nubia are present (Table 1, Fig. 1). The map (Fig. 1) was created in R (R Core Team 2013) using geographic coordinates from Google Earth (Google Inc. 2013), data from Natural Earth, and implementing the maptools (Bivand and Lewin-Koh 2013), maps (Becker et al. 2013), and mapproj (McIlroy et al. 2013) packages. Confidence in assignment of time period from burials where multiple Nubian groups utilize cemeteries is facilitated by the mortuary distinctiveness of each group. A brief description of the sites will further illuminate the sample composition.

Hesa and Biga. The islands of Hesa and Biga are found just south of Philae in the Nile. Reisner supervised the Archaeological Survey of Nubia’s excavation of the cemeteries. According to Elliot Smith and Wood-Jones (1910), the el-Hesa cemeteries were established first, with Biga reserved for Temples built during the Ptolemaic-Roman period. Later, cemeteries were also built on Biga. The remains analyzed here are from el-Hesa, Cemetery 2 (Francigny et al. 2014). Although multiple time periods are represented at this site, the Christian burials were
distinctive from prior burials, which allows confidence in assigning a time period to the skeletons analyzed here. While the Christian time period is quite broad (500-1100 AD), this site has been dated as early within the time period (Francigny et al. 2014), overlapping with the X-Group (Table 1).

**Kerma.** Reisner was also involved in the excavations at Kerma for Harvard University and the Boston Museum of Fine Arts. The burials date to the Kerma time period. Originally, the skeletons found at this site were interpreted as Egyptian (Collett 1933), but later conclusions regarding the skeletal material categorized the individuals as a culturally separate C-group of Nubians (Adams 1984).

**Kulubnarti.** The Kulubnarti site consisted of mainland (West bank) and island cemeteries (Turner et al. 2007) located in the Batn el Hajar. The island was created by the installation of the Aswan High Dam, where prior to its construction the island was part of the mainland at Kulubnarti (Adams et al. 1999; Kilgore et al. 1997). The island cemetery contained Christian, Islamic and X-Group burials, although the Christian remains are the focus of this investigation. The cemeteries’ dates are inconclusive; inconsistencies in artifacts and surrounding structures are responsible for the issues with dating (Adams et al. 1999). The site has been continuously occupied since the medieval time period (Edwards 2004). While the island cemetery was established first, these cemeteries possibly overlap in time to some extent (Van Gerven 1995).

**Sayala.** Both C-Group and Pan-Grave burials were excavated on the eastern bank of the Nile at Sayala (Strouhal and Jungwirth 1984). The Pan-Grave burials were located further inland than the C-Group (Bietak and Bauer 1966). Strouhal and Jungwirth (1984) contend the pan graves did not exhibit influences from other cultures, which is consistent with Smith’s (1998)
description of a resistance to Egyptian acculturation during Egyptian occupation of Lower Nubia in the Middle Kingdom and Edward’s (2004) description of Pan-Grave burials after the downfall of Kerma.

**Semna South.** At Semna South, remains from the Meroitic, X-Group, and Christian time periods were excavated as part of the salvage efforts from the construction of the Aswan High Dam. The cemeteries were located on the West bank of the Nile. North of the fort at Semna South, a cemetery contained the remains from individuals of all three time periods (žabkar and žabkar 1982). Because the graves were distinct in structure, orientation, and grave goods culture to which each burial belongs.

**Sesebi.** Sesebi (not depicted on the map as it will not be included in spatial-temporal analyses) is located on the west bank of the Nile, south of Kulubnarti and north of Kerma. The town was protected by fortress-like walls (Blackman 1937) and was originally purported to have contained evidence of great influence from Egyptian Pharaohs (Blackman 1937; Fairman 1938). The most recent research identifies Sesebi as an Egyptian colonial town built during Akhenaten’s reign (as Amenhotep IV) with evidence of Egyptian occupation dating to the early New Kingdom (Spence and Rose 2009; Spence et al. 2011). Some skulls were found on the surface having lost their provenience (Lisowski 1952b). The composition of this sample appears to contain New Kingdom Egyptian remains mixed with Nubians from Meroitic, 5th-7th Century, and unknown time periods (Lisowski 1952a), although this cannot be fully documented. Thus, this sample is problematic due to its composite nature and was only included initially to look at its relationship with other Nubian samples.

**Wadi Halfa.** Three samples from this relative area are represented in this study. A sample from the Mesolithic was excavated from 2.5 km inland at Wadi Halfa (Saxe 1971). This cemetery has

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highly variable mortuary practices and is interpreted as representing a sedentary group of
Mesolithic hunter-gatherers (Saxe 1971). North of Wadi Halfa was site 179 and whose C-Group
skeletal remains were sampled by Dr. Lovell for discrete traits (Prowse and Lovell 1995) and
provided to the junior author for inclusion in this paper. To the south of Wadi Halfa was an A-
Group cemetery, site 277, also observed by Dr. Lovell (Prowse and Lovell 1995) for cranial
nonmetric traits (also provided to the junior author).

Methodology

The first author (KG) scored six Nubian samples housed at University of Colorado,
Boulder and Arizona State University (see Table 1) for a series of 8 cranial nonmetric traits using
the scores and definitions of Hanihara et al., (1998), and Hanihara and Ishida (2001 a,b,c,d,e)
(Table 2). These observations were combined with data provided by Nancy Lovell, Tsunehiko
Hanihara, and published by Eugen Strouhal. All 4 observers (Godde, Hanihara, Lovell, Strouhal)
collected these same 8 traits across the samples. While this number of nonmetric traits is low,
others have used 8 traits successfully to assess ancient population relationships (e.g., Konigsberg
1990) and it is a necessity considering the age and fragmentary nature of the Mesolithic sample
preventing many traits from being scored across the entire sample. Cranial nonmetric
observations made by Dr. Lovell (Prowse and Lovell 1995; Prowse and Lovell 1996) and Dr.
Strouhal (Strouhal and Jungwirth 1984) were meticulously scrutinized to ensure the same
technique was employed by all four observers. Any trait that was scored as polychotomous
(multiple levels of expression) was converted to dichotomous (present/absent) for consistency
among observers (Hallgrimsson et al. 2005) and for running through biodistance statistics. An

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interobserver analysis (see Inter- and Intraobserver Error section below) indicates any bias from multiple observers is probably low.

Cranial discrete traits provide an avenue for assessing phenotypic biological distances that approximately reflect genetic distances. Heritability is a measure of the strength of genetic vs. environmental influence in the expression of the phenotypic traits. In the past, cranial nonmetric trait heritability has been calculated as approximately in line with craniometrics, depending on the characteristic (Cheverud 1981; Cheverud and Buikstra 1981a; Cheverud and Buikstra 1981b; Cheverud and Buikstra 1982; De Papa and Perez 2007; Grüneberg 1952; McGrath et al. 1984; Richtsmeier and McGrath 1986; Sjøvold 1984), although the estimates of nonmetric trait heritabilities are lower in a more recent appraisal of these data (Carson 2006). Despite the lower estimates of heritability, two newer papers have shown excellent concordance of molecular and discrete cranial traits (Herrera et al. 2014; Ricaut et al. 2010), indicating the phenotype can be an excellent proxy for the genotype.

Several biases must be controlled, tested, and acknowledged when working with cranial nonmetric traits. Age (subadult vs. adult), sex, and intertrait correlations can all influence the expression of cranial discrete traits. With age, some traits develop during adolescence, but are stable with adulthood (Saunders and Popovich 1978). Age was not of concern in this study, as it was controlled through the elimination of subadults. As in Hanihara et al. (2003), third molar eruption and fusion of the sphenooroccipital synchondrosis were used as indicators of adulthood.

Postmarital residence patterns can be reflected in the phenotype, causing differential expression of traits along sex lines. Further, knowledge about genetic inheritance is limited, with preliminary research suggesting craniometrics and nonmetrics may be sex-linked (mtDNA and Y-chromosome, respectively) in some populations (Herrera et al. 2014). Thus, testing for sex
dependency in a trait was necessitated and performed with chi-square tests in SAS 9.1.2 (SAS 2002-2004), using a Bonferroni corrected 0.05 significance level (0.007).

Intertrait correlations can be problematic as the presence/absence of one trait may influence the expression of another. Typically, localized traits are more greatly affected than traits spaced further apart (Hertzog 1968). The statistic assessing biological distance here, Mahalanobis $D^2$ with a tetrachoric matrix, corrects for intercorrelations among the cranial variables (Konigsberg 1990), and thus eliminates biases resulting from intertrait correlations.

**Population Structure**

To quantify population relationships and heterozygosity, a set of population genetics statistics were completed. As the observations from the crania were categorical, Mahalanobis $D^2$ with a tetrachoric matrix was applied, which allows for computation of biological distances from data measured as present or absent (Bedrick et al. 2000; Blangero and Williams-Blangero 1991; Konigsberg 1990):

$$d_{ij}^2 = (z_{ik} - z_{jk})' T^{-1}(z_{ik} - z_{jk})$$  \hfill (1)

where $z$ is a threshold value for $i$ or $j$ populations with trait frequency $k$. The tetrachoric matrix is denoted by $T$. Biological distances provide estimates of how related two populations are to one another and can be input into population genetics statistics to further model population structure.

Principal coordinates analysis (PCO) (Gower 1966) was selected as the ordination method to depict population relationships from the Mahalanobis $D^2$ matrix. PCO allows the matrix to be depicted in graphical space, facilitating the visual interpretation of population relationships. PCO was calculated and plotted using the Ecodist package (Goslee and Urban 2015) in R.
An R matrix (kinship matrix) was generated from a codivergence matrix and minimum $F_{st}$. The codivergence matrix, an estimation of variance around the centroid, is calculated as follows (Konigsberg 2006):

$$C = -0.5(I - 1w')D^2(I - 1w')$$  \hspace{1cm} (2)

where $I$ is an identity matrix with $g$x$g$ dimensions ($g$= number of groups) and $w$ is a column vector ($g$x$1$) of the relative weights of the populations. Relative weights can be calculated from census information, if known (Relethford and Harpending 1994). As these are archaeological populations and a census is lacking (a concept developed more in the Discussion, below), equal weights were given to the samples.

Minimum $F_{st}$ is derived from the codivergence matrix:

$$\text{minimum } F_{st} = \frac{w'\text{diag}(C)}{2t+w'\text{diag}(C)}$$  \hspace{1cm} (3)

where $\text{diag} (C)$ are values on the diagonal of the $C$ matrix, converted to a column vector and $t$ is the number of traits in the analysis. Minimum $F_{st}$ informs the biostatistician how much variation is found within and among subpopulations. A heritability of 1 was invoked as exact heritability estimates of cranial nonmetric traits are under question and this is the conservative approach (Relethford 1994; Relethford and Blangero 1990). The R matrix equation, therefore, is (Konigsberg 2006):

$$R = C(1 - \text{minimum } F_{st})/2t$$  \hspace{1cm} (4)

The computed R matrix allows for a modified Relethford-Blangero residual analysis, which looks at rate and magnitude of gene flow by subtracting the expected within-group phenotypic variation ($E(\bar{V}_{Gi})$) from the observed within-group phenotypic variation ($\bar{V}_{Gi}$). These values approximate the Relethford-Blangero residuals generated by RMET (Relethford and Blangero 2005) and are a multivariate extension of the Harpending and Ward (1982) model:

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\[ E(\bar{V}_{Gi}) = \bar{V}_{Gw}(1 - r_{ii})/(1 - r_0) \]  

where \( \bar{V}_{Gw} \) is the pooled average within-group phenotypic variation among populations, \( r_{ii} \) represents the diagonal elements of the R matrix (which is the distance of population \( i \) to the centroid), and \( r_0 \) is the \( F_{st} \) in equation 3. \( \bar{V}_{Gi} \) is calculated as the trace of the within group additive genetic variance-covariance matrix, divided by the number of traits \( t \). The additive genetic variance-covariance matrix \( (G) \) has been shown to be proportional to the phenotypic variance-covariance matrix \( (P) \) in craniometrics (Konigsberg and Ousley 1995), and in light of its strong concordance with molecular data (Herrera et al. 2014; Ricaut et al. 2010), cranial nonmetric trait \( P \) should also be proportional to \( G \). Thus, \( \bar{V}_{Gi} \) was calculated from the within group phenotypic variance-covariance matrix. Steadman (1998, 2001) applied a Relethford-Blangero analysis to diachronic samples and established the model’s utility in assessing populations over time. Harpending and Ward (1982) plots were created to provide a graphic for depiction of the modified Relethford-Blangero analysis. Interpretations of the plots revolve around outliers; outliers below the regression line indicate isolation from extraregional gene flow in relation to the rest of the group (indicating lower rates of gene flow and possibly genetic drift), while those outliers above the regression line are affected by extraregional gene flow. To our knowledge, this is one of the first applications of the Relethford-Blangero methodology to binary phenotypic data derived from skeletal material, joining Godde (2009), Harle (2010), and Godde (2013b). This approach provides additional information that can be gleaned from the biological material, allowing for a more complete understanding of population structure in Nubians. All statistical analyses were calculated in R (R Core Team 2013), using coding created by Dr. Lyle Konigsberg unless otherwise specified.
Spatial-Temporal Isolation Model

The spatial-temporal model, as put forth by Konigsberg (1990), provides the theoretical and mathematical framework with which to analyze population relationships across space and time. This model analyzes space and time concurrently for non-contemporary samples that incorporates the expectations under the island model (Wright 1951), unidimensional stepping stone model (Kimura and Weiss 1964), and a migration matrix (e.g., Harpending and Ward 1982). Under the expectations of this model, populations separated by geographic space will display a positive association, whereby they will become less related as the distance between them increases. Conversely, an inverse relationship exists in time; as temporal space increases, genetic dissimilarity decreases. Konigsberg (1990) proffers this is as a result of gene flow acting as a homogenizing force on the genetic structure of the population (65).

Approximate linear geographic distances (in kilometers) were calculated using the geographic coordinates from Google Earth (Google Inc. 2013). River distances were also derived from Google Earth (Google Inc. 2013) as Buikstra (1977) found river distances were positively associated with biodistances. In the past, studies have tested both linear and river distances, finding similar results with each (Godde 2009; Godde 2013a; Godde 2013b; Konigsberg 1990). Temporal distances were generated from subtracting the median dates assigned to each sample (c.f., Konigsberg 1990). Geographic distances are presented in Table 3 and temporal distances are found in Table 4.

To test whether these samples are consistent with the expectations of the spatial-temporal isolation model, a three-way Mantel test (Smouse et al. 1986) was applied to the biological distance matrix simultaneously with a geographic distance matrix (testing for correlations between geographic space and biodistance), while controlling for time with the temporal matrix.
Likewise, to test for correlations between biodistance and time, the biodistance matrix was tested against the temporal matrix, while controlling for a geographic space with the corresponding spatial matrix. These tests were also completed in R (R Core Team 2013) using the Ecodist package (Goslee and Urban 2015).

The Mantel test has endured recent criticism, allowing for another test to receive attention, the PROTEST. PROTEST is more sensitive to detecting relationships among matrices (Peres-Neto and Jackson 2000), and thus can detect associations that Mantel tests cannot. With most previous work unable to model spatial and temporal associations in Nilotic populations (Godde 2009; Godde 2013a; Godde 2013b; Zakrzewski 2007), the more sensitive PROTEST was deemed an appropriate statistic with which to run the spatial-temporal isolation model.

The PROTEST has been applied in anthropology by Relethford (2009) and more recently, Herrera et al. (2014). The PROTEST is a Procrustean superimposition (Gower 1971) where the ordinations can be scaled and rotated to find the best fit (Peres-Neto and Jackson 2000). The statistical association is computed using a permutation approach, testing the sum of squares residuals ($m^2$). The first two principal coordinates from PCO were calculated for the temporal and geographic matrices to be used in combination with the first two dimensions derived from the R matrix. The ordination results were input into PROTESTs to calculate the significance for modeling space and time.

**Interobserver Error**

Interobserver error is of concern in cranial nonmetric trait analysis (Finnegan and Rubison 1980; Gualdi-Russo et al. 1999; Ishida and Dodo 1990). While it has been proposed that authors not share work to eliminate interobserver bias (Ishida and Dodo 1990), others believe
that experience (Finnegan and Rubison 1980; Gualdi-Russo et al. 1999), and strict adherence to standardized definitions (Gualdi-Russo et al. 1999) will help alleviate the effects of multiple practitioners. While in the past concerns over standardization of scoring were warranted, since the advent of well-defined diagnostic criteria and detailed definitions accompanied by photographs, this concern is less as data collectors have the necessary tools to make decisions normalized to the technique and thusly to other practitioners employing the same methodology of scoring. Other scientific methodology employs a similar approach relying on practitioners learning to accurately and consistently apply diagnostic criteria, standardized process, and the scientific method to develop a conclusion based on methodological rigor, e.g., DNA. This paper relies on the same assumptions as together they are a fundamental, commonly used scientific practice and promote the development of large, robust datasets, derived from multiple observers, that allow for the forward progression towards a stronger scientific foundation of the discipline, facilitating a holistic approach to examining anthropological questions no longer limited by funding constraints introduced by collecting one’s own data. This helps to eliminate or limit the bias imposed by a lack of monetary support, which leads to an artificial selection of samples based on non-scientific principles lending to fragmentarily constructed research designed around access to skeletal material. This study uses a combination of qualitative and quantitative approaches to examine and estimate the impact of interobserver error as evaluated in three ways: 1) examination of definitions used among observers to ensure the same standards were used to collect data, 2) the pictures in Strouhal and Jungwirth (1984) were independently scored by KG to verify traits were recorded similarly, 3) consideration of affinity patterns in biodistance plots to investigate whether samples cluster by observer.
Results

Measures to avoid interobserver error appear successful; the Nubian groups do not cluster by observer in the PCO plots (Figs. 2 & 3) on either the X or Y axis, which would be expected if the observers scored the cranial discrete traits differently (i.e., the sites would cluster by observer, and thusly by the methodology they applied). Only one observer appeared to cluster along the X axis (Lovell: A- and C-Group). However, population history indicates these samples should cluster together, and thus the pattern is not likely due to interobserver bias. The conclusion is further supported by the placement of the Hanihara-observed sample (Hesa & Biga) in between the two Lovell samples on the Y axis. All other samples did not cluster by observer.

Qualitatively, accessory mental foramen appears to have been recorded differently when comparing trait definitions, and thus it was dropped from further analysis. Moreover, KG’s scores of Strouhal and Jungwirth’s (1984) pictures yielded complete concordance (which further supports the idea that standardization of the method has significantly reduced interobserver bias). Turning to quantitative methods, two groups of traits were identified by Ishida and Dodo (1990) as providing a low level of interobserver error, as evidenced in a Phi coefficient (□) threshold of 0.7. Table 2 displays the Phi coefficients associated with each trait in this study. Two traits in this study fell below this threshold (asterionic bone, tympanic dehiscence) and required further scrutiny. To provide further support interobserver bias is low in the remainder of the traits, they underwent the same quantitative scrutiny as the two traits below the threshold. The 7 remaining traits were subject to analyses where each trait was dropped and the remaining six variables were used to generate a distance matrix. Mantel tests on the distance matrix from which all population interpretations are derived in this paper showed a high concordance against a distance matrix.
without each trait in turn: without accessory infraorbital foramen ($r = 0.9754$, $p = 0.01$), asterionic bone ($r = 0.9900$, $p = 0.01$), tympanic dehiscence ($r = 0.9726$, $p = 0.01$), ossicle at lambda ($r = 0.9947$, $p = 0.01$), parietal notch bone ($r = 0.9947$, $p = 0.01$), precondylar tubercle ($r = 0.9856$, $p = 0.01$), and supraorbital foramen ($r = 0.691$, $p = 0.01$).

An interpretation of the PCO graphs from the resulting biodistance matrices (Supplementary Information; Figs. S1-S7) confirm the sample relationships stayed relatively the same, with the exception of supraorbital foramen. The removal of supraorbital foramen altered a small portion of the sample relationships, but did not cause the samples to cluster by observer. The samples that changed relationships were all collected by the same observer (KG), and were only a portion of the samples that the observer scored. Intraobserver error is not likely as a standardized definition of supraorbital foramen was used by KG during the data gathering process (c.f., Molto, 1979), and data scored later was comparable to data gathered earlier, which most likely indicates intraobserver error was not at fault. Collectively, these results indicate interobserver error is minimal and not detectable, otherwise changes in population relationships and clustering by observer would have been identified.

Sex differences were negligible among these samples (Table 6). Thus, there was no need to separate by sex for this population genetics investigation. Mahalanobis $D^2$ revealed that one sample separated from the remaining groups: the C-Group at Sayala (Tables 4 & 5). This is evident in both iterations: one distance matrix without Sesebi and one with. This relationship was visually confirmed when plotted with PCO on the first 2 principal coordinates (representing 94% of the variation across both analyses in Figs. 2 & 3). Moreover, the samples appear to mostly cluster by site and in some cases by temporal distances. The Relethford-Blangero residuals (Table 7) demonstrate the gene flow among these groups was close to zero, indicating no sample
experienced higher or lower extraregional gene flow in relation to other groups in this study. The Harpending and Ward (1982) plot (Fig. 4) depicts the placement of these populations around the regression line with the axes zoomed in to look at the pattern among the tightly clustered samples. The $F_{st}$ hovers between 0.10 and 0.09 among the groups in this analysis excluding Sesebi and including Sesebi, respectively. An examination into the Middle Nubian Horizon relationships is complex; the C-Group at Wadi-Halfa is biologically similar to Kerma. However, the C-Group at Sayala is depicted as an outlier to all of the Nubian groups. As the $F_{st}$ values are high in relation to other cranial nonmetric studies of Native American groups (Harle 2010; Herrmann 2002; McCarthy 2011) and the Sayala C-Group was an outlier on the Harpending and Ward (1982) plot, the sample was removed to test how its elimination affected $F_{st}$ estimate in the analysis without Sesebi. $F_{st}$ was reduced (0.07), but still high in relation to other populations.

Quantitative spatial-temporal modeling failed to detect geographic and time patterns as expected under the model. Both Mantel tests and PROTEST $p$-values (Table 8) indicate we should fail to reject the null hypothesis that the space/time matrices are not correlated to the biological distance matrix. The trend noted earlier in the PCO plots may be the reason spatial and temporal patterning could not be detected; the biodistances roughly cluster by site, irrespective of geographic distance. Thus, the results here violate the assumptions of the spatial-temporal isolation model. Despite a lack of statistical spatial-temporal patterning when looking at the data set as a whole (likely due to the nonlinear distribution of geographic and corresponding biological distances), most groups show evidence of spatial clustering in the PCO plot. For example, the sites of Hesa-Biga and Wadi Halfa cluster together on PC1, creating a northern group (Sayala C-Group and Pan-Grave people are not included in this cluster, a finding which is

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consistent with the archaeological record and explained in the Discussion). Moreover, Kulubnarti roughly clustered with their neighbors at Semna South on PC1.

**Discussion**

The in-depth population genetics analysis revealed a closely related group of samples that show no evidence of population replacement within this dataset (support for hypotheses 1 and 3) and do not support the null hypothesis of the spatial-temporal model explaining the patterning in these data. Instead, one of our alternative hypotheses appears to be supported (see Spatial Structure and Social Isolation in Nubia: Support of Genetic Drift, below). Therefore, our hypotheses are only somewhat supported by these results. In sum, with the exception of the C-Group from Sayala, the Nubian samples all clustered together, showing a strong biological relationship (thus, supporting hypothesis 1). The Middle Nubian Horizon relationship is complex, with only the Kerma and Wadi Halfa C-Group demonstrating a close affinity (which addresses the research question), while most other contemporary samples did not cluster together (hypothesis 2). Instead, the Nubian groups mostly clustered by site with some deviations (e.g., Sayala C-Group). To understand the results in the context of our hypotheses/research question, questions of extraregional gene flow, genetic drift, and population replacements should be examined.

While the modified Relethford-Blangero residual analysis suggests there was little to no extraregional gene flow, as is evidenced by the residuals that hovered at zero and the placement of the populations around the regression line, the $F_{st}$ is quite high, exceeding estimates from populations around the world (Jorde 1980), a documented admixed population (Relethford and Blangero 1990), estimates of North and Southern Africa regions (Hubbe et al. 2009), and on par
with other regional groupings of populations, e.g., South and Northwest Asia (Hubbe et al. 2009). However, population sizes can affect $F_{st}$ estimates making raw comparisons across populations biased.

The estimate of $F_{st}$ reveals that 9-10% of variation in these samples lies between the Nubian groups, and around 90% within. As genetic drift was not statistically removed from the population structure analyses, the 9-10% thusly reflects influences from both extraregional gene flow and genetic drift. These results appear to be conflicting on first glance, but upon further inspection they more likely indicate almost all the groups engaged in longstanding extraregional gene flow from other population(s) that exerted a homogenizing effect (c.f., Konigsberg 1990) on the residuals and biological distances. In other words, the residuals are approximately the same across most of the groups because extraregional gene flow was similar among the samples at some point in their population history. These results are in agreement with the molecular data; mtDNA studies have found evidence for gene flow into (Fox 1997) and among (Krings et al. 1999) Nilotic populations. To investigate the roles of extraregional gene flow in combination with genetic drift, the generated population genetics parameters must be interpreted against the historical record (c.f., Keita 2005).

**Contact with Extraregional Populations Post-Paleolithic: Support of Gene Flow**

While evidence from the archaeology and mortuary archaeology discussed in the Introduction does not demonstrate any population replacements from the Mesolithic and on, Nubian history is punctuated by extensive contact with other peoples, including the Romans and Egyptians (c.f., Krings et al. 1999; Smith 1998), which might explain the maintenance of similar levels of extraregional gene flow over time from our first hypothesis. In addition to known trade
with the Egyptians, the A-Group also displayed similarities in artifacts from the Upper Egyptian predynastic Naqada culture (Bard 1994; Keita 2005; Nordström 1972; Smith 1991), which is suggestive of cultural diffusion. The Egyptians occupied Lower Nubia during the Middle Nubian Horizon, and evidence suggests amicable interactions between them and the C-Group (Smith 1998; žabkar and žabkar 1982). Moreover, Kerma’s establishment as a strategic trade center, with Egyptian fortresses established in the vicinity at the Second Cataract (2050-1750 BC) (Welsby 1996) would provide opportunity for gene flow between the populations. Similarly, the documented presence of the Pan-Grave people in Egypt at Hierakonpolis (Friedman 2001) and employment by the Egyptians would also provide the means for gene flow if it occurred.

Tombos is located south of Semna South and appears to have experienced, while unusual (c.f., Kerma), long-term, peaceful interactions with the Egyptians during the Napatan period (Smith and Buzon 2014). Analyses of the skeletal material demonstrated a movement towards biological homogeneity from distinctly different groups of Nubians and Egyptians at Tombos (Smith and Buzon 2014). The Meroitic time period brought with it another major trade center at Meroë, where there was opportunity for gene flow. However, the Meroitic group here was from north of Meroë, at Semna South (an outpost). At Semna South the most compelling evidence of contact with other peoples or groups of Meroitic Nubians is exemplified by a few bronze mirrors attributed to the Roman empire and found at Meroitic sites (e.g., Meroë, Faras) (žabkar and žabkar 1982). While Meroitic presence north of Semna South at Dodekaschoinos seems inconsistent over time, Meroitic kings were building in this region, including at Philae where Egyptian kings were also building (Edwards 2004). Evidence of Roman military garrisons during the Meroitic period have been found at Qasr Ibrim (executed by Petronius) and in the
Dodekaschoinos (Edwards 2004), both north of Semna South. At nearby Philae, inscriptions describe contact between the Romans and Meroites (Edwards 2004).

At the more northern sites of Qustul and Ballana, trade is suggested during the Ballana culture (X-Group) from the imported grave goods found in their two cemeteries (Edwards 2004). However, not much information exists regarding the trading of goods below this site (Edwards 2004). The conversion to Christianity during the Christian period took place in the Northern areas prior to the more southerly sites, potentially starting North in the later fifth century (Edwards 2004). At Philae (near el-Hesa), the temple of Isis was utilized as a Pagan cult center (Welsby 2002) until 551 AD (Francigny et al. 2014), providing plenty of opportunities for interaction between Nubians and different peoples. Lastly, texts from the Byzantine empire document the movement of Christianity into Lower Nubia (Edwards 2004). Such examples of sustained contact with the Romans and Egyptians are demonstrated through the archaeological and mortuary (i.e., grave goods) evidence, which supports the biological conclusions of sustained extraregional gene flow in this paper. But, was gene flow the predominant evolutionary mechanism driving the statistical results here, or was genetic drift also operating in this population?

**Spatial Structure and Social Isolation in Nubia: Support of Genetic Drift**

Statistical analysis did not detect the patterning of the spatial-temporal model, and therefore our second hypothesis (the null) was rejected. Evidence from other research, an examination of the PCO plots, and analysis of the archaeological record leads us to conclude there is support for the first alternative hypothesis of a more complex, untested spatial and temporal structure in these groups. We take the approach offered by Keita (2005) where spatial
patterning and interpretations of the biodistances are inferentially derived by concurrently using the environmental, archaeological, and mortuary records.

Spatial patterning has been difficult to model along the Nile, with some studies modeling it well (Schillaci et al. 2009; Van Gerven 1982), and others not as successful (Godde 2004; Godde 2005; Godde 2013a; Zakrzewski 2007). Zakrzewski (2007) partially attributes this to the model not being applicable in this region. We agree with this assertion; it may be due to identified and unidentified factors that violate the assumptions of the model. For example, differing routes and trajectories among the sites may not be accounted for in the reconstructed geographic structure. The archaeological record yields a clue to this issue where, “… goods were largely funneled along the Nile valley before the dramatic rise of maritime goods in the later first millennium BC and of cross-desert trade in the Islamic period” (Welsby 1996: 12). The most likely reason, though, for the lack of significance in Mantel tests and PROTESTs is the assumption of linearity; more complex spatial and temporal patterns are not detected by tests with linear assumptions (Goslee and Urban 2007). Likewise, the isolation by distance and spatial temporal models assume linearity. In the PCO plots, we see a more complex, non-linear structure where samples cluster mainly by site. For the samples that do not group by site (i.e., island Kulubnarti, Pan-Grave people, and Sayala C-Group), social isolation, which occurs in several forms (Jorde 1980), might explain this pattern. Jorde (1980) provides examples of social isolation, including class and clan differences, which are factors that could potentially be found in these groups and should be detectable by interpreting the archaeological and historic records.

Here, it is of use to also interpret the diagonal of the R matrix ($r_{ii}$) (Supplementary Information; Table S1), which represents the distance of the samples to the centroid (Harpending and Ward 1982) and is a kinship coefficient (Harpending and Jenkins 1973). The same groups
that deviate from site clustering in the PCO plot (Kulubnarti (island), Pan-Grave, and Sayala C-Group) appear to be affected more greatly by either differing levels of gene flow, or potentially by genetic drift, (Table 7). Increased genetic drift and/or reduced gene flow might explain the values on the diagonal of the R matrix and clustering of samples by site when taking into account archaeological, historic, and mortuary evidence (the environment was relatively constant during these times and therefore will not be further explored); these three Nubian groups were isolated by social boundaries.

The Kulubnarti (Batn el Hajar region) samples are representative of the Christian time period where the island cemetery was established at the beginning of the Christian period and the mainland cemetery was established later, probably around 1100 AD (Van Gerven 1995). During the late Christian period, immigration to the Batn el Hajar area from the North is evident (Adams 1977), although the impetus for the population movement has not been established (Van Gerven 1995). The increased level of genetic drift (or reduced extraregional gene flow) in the island Kulubnarti sample vs. the mainland sample may be due to its earlier date, prior to the influx of immigrating peoples (making them isolated to the genetic material contributed at the later time), and which also would allow for differences in genetic composition between the two Kulubnarti samples. Increased extraregional gene flow was not detected by the residuals in either sample, suggesting the immigrating population was one with which Nubians had engaged in gene flow in the past, which is supported by the historic record detailed in the section above.

Sociocultural factors, suggestive of social isolation, may have caused the drift in the Pan-Grave people; while most likely Nubian, they were culturally and socially distinct from other Nubian groups (including their positions as Medjay and burial practices), which may have led to their genetic isolation and the patterning of Middle Nubian Horizon biological relationships (c.f.,
our research question). Strouhal and Jungwirth (1984) attribute these practices to cultural seclusion in the desert where they resided, lending support to the genetic differentiation of this sample being as a result of genetic drift, rather than gene flow.

Strouhal and Jungwirth (1984) also provide information that explains the genetic profile of the C-Group at Sayala, and their separation from other Middle Nubian Horizon groups, supporting the kinship coefficient value, and the idea they were significantly affected by genetic drift/and or low levels of extraregional gene flow in relation to the other samples examined here. The authors describe the nature of the C-Group settlements as comparatively small, spatially separated, endogamous groups, providing ideal conditions for genetic drift (187-8). The archaeological and historic records further indicate the Egyptians occupied Lower Nubia during the partially contemporary C-Group (Nubian)/Middle Kingdom (Egyptian) periods (Smith 1998). While Kerma crumbled from Egyptian intervention, the C-Group were stable from, “emphasizing their own culture and excluding Egyptian influences” (Smith, 1998: 277 citing Säve-Söderbergh, 1989: 6-14 and Williams 1991). As a result of this solidarity, and social isolation, it is probable that genetic drift could take hold in some C-Group occupations. In combination, the historical evidence and the outlying nature of the Sayala C-Group biodistance here and in Godde (2013b) point to local genetic drift, and/or greatly reduced extraregional gene flow, stemming from the spatial distribution of their settlements and cultural practices.

Galland et al. (2016) provide alternative evidence, showing biological continuity between the A- and C-Groups, but with neither group showing a close affinity to a Mesolithic sample from Wadi Halfa. While on the surface this may appear to contradict the results here and in Godde (2009, 2013b), the differing perspectives may be as a result of study design and the different information the data types contain. Godde (2009, 2013b) and the current paper both
examine the relationships among the Mesolithic, A-, and C-Group of a single region (Wadi Halfa), while Galland et al. (2016) investigates the relationship between the same Mesolithic sample from Wadi Halfa and the A- and C-Groups from a slightly more southern area, Gamai. If genetic drift was operating due to the sociopolitical factors and spatial patterning described in the C-Group, the differentiation of the Mesolithic and C-Group samples from different regions would be expected, the findings of Galland et al. (2016) would still be congruent with the results here, and supported by the archaeological interpretations of Strouhal and Jungwirth (1984) and Smith (1998). Moreover, it is expected that disparities exist between the studies due to the genetic information provided by each type of data; they follow different inheritance patterns (craniometrics/geometric morphometric data follow a polygenic inheritance and cranial nonmetric are polygenic threshold traits) and the results should be considered complimentarily, rather than competitively. Therefore, collectively, the evidence suggests extraregional gene flow and genetic drift appear to both be the major contributors to the biodistance patterns in these samples.

**Population Origins and the Paleolithic**

Is the variation found in this study contributed to by population events during the Paleolithic? There is evidence of great competition for resources in the Nile Valley during the Late Paleolithic (Close and Wendorf 1990) and the aridity of the climate probably forced more peripheral populations to move toward the river to survive (Edwards 2004), placing a number of populations in the region. The climate changed in the Nile Valley between the Late Paleolithic and the Mesolithic, moving from the hyperaridity of the Last Glacial Maximum into the African Human Period. Prior to the change in climate, the flow of the Nile was approximately 10-20% of
its current output (Edwards 2004). With the climate shift, the environment became wetter
(Edwards 2004; Manning and Timpson 2014) and environmental pressures would have changed
at this time, potentially lowering competition for resources and allowing populations to spread
out from the floodplain.

After the African Humid Period took hold in the region there was a population increase
(Manning and Timpson 2014), which would likely have been the result of one or more
populations migrating to the northern and eastern Sahara. Moreover, several different lithic
assemblages in the Nile Valley have been interpreted as representing distinct populations moving
into the area (Wendorf 1968). Adams (1977) also notes the variety of lithic technologies
identified in Wendorf (1968), and the lack of ancestor-descendant relationships among all types,
but he is skeptical that this is definitely evidence of multiple populations in the area. However, if
taken together, the climate, demographic (increase in population), and lithic evidence points to
multiple peoples inhabiting the Paleolithic at around the same time.

In combination, the dental nonmetrics (Irish 2005; Irish and Turner 1990; Johnson and
Lovell 1995; Turner and Markowitz 1990) and the cranial nonmetrics (Godde 2013b) have
narrowed down any population replacements or other major population events that affected the
genetic structure of the Nubians to before the Mesolithic and near the Late Paleolithic, although
the work from Galland et al. (2016) disagrees (see below). This is supported by limb proportions
data (Holliday 2013) from individuals interred at Jebel Sahaba (Late Paleolithic), which
represents a morphologically dissimilar population to later Nubian groups. Thus, taken in
combination, the preponderance of evidence from archaeology (possibly more than one
population in Nubia during the Late Paleolithic), climatic changes, the among group variation,
the findings from Jebel Sahaba, and the continuity after the Late Paleolithic in population
relationships, support our third hypothesis and suggest multiple populations from the Late Paleolithic may be ancestral to modern Nubians or a new population moved into the area and became ancestral.

**Biases and Conclusions**

The exclusion of the Sesebi sample from the second analysis demonstrates the effect it had on $F_{st}$. $F_{st}$ lowered with the inclusion of Sesebi. This makes sense in relation to sample structure; Sesebi is a composite of several Nubian groups (represented by other samples in the analysis) at a single site (Kerma, Meroitic, and Christian). Thus, the composite sample exerted a homogenizing effect on this population structure investigation and biodistance interpretations of Sesebi should be framed on a limited scale, such that the conclusions are only that Sesebi groups most closely align with its geographic neighbor to the south: Kerma.

The discovery of Nubian samples mostly clustering by site may explain the inability of some studies to meet the expectations under the spatial-temporal model in Nilotic populations (e.g., Godde 2013 a,b; Zakrzewski 2007). Other factors may have potentially muddied the analyses in this paper, including estimates of effective population size, the effects of long-term effective population size differences (c.f., Relethford and Harpending 1995), small sample size (e.g., the Mesolithic sample numbers 11), and the effects of genetic drift itself (c.f., Relethford 1996). While scaling the R matrix by sample size seems like an effective way to solve many of these issues, these numbers are unknown in many of these groups and remnants suggestive of population size have been potentially destroyed by rising water levels after the installation of the Aswan Dam. The Harpending and Ward (1982) model is theoretically constructed around the assumption that an equal proportion of gene flow affects each sample. This study may have
violated the concept, thus affecting the outcome of the R matrix and Harpending and Ward (1982) analyses. However, the excellent concordance of the environmental, archaeological, mortuary, and biological evidence suggest these biases had a negligible effect.

In this paper, the population structure of Nubians, as constructed from the skeletal record, was examined in relation to the environmental, archaeological, and mortuary evidence in order to interpret population genetics parameters in conjunction with the historic record. It was discovered that the samples mostly clustered by site, which in combination with the archaeological evidence of social isolation operating on some samples, balanced with their biological similarity to other samples that display evidence of extensive contact with different peoples, suggest that extraregional gene flow was probably punctuated with genetic drift, at least in three of the samples we examined. Our results also discount a population replacement happening during the range of time examined in this study.
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**Table 1. Sample information for 13 Nubian groups**

<table>
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<tr>
<th>Time Period</th>
<th>Site</th>
<th>Referred to as</th>
<th>Dates</th>
<th>Median Date</th>
<th>Sample size</th>
<th>Researcher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesolithic</td>
<td>Wadi Halfa South of</td>
<td>Mesolithic</td>
<td>9050-6050 BC</td>
<td>7550 BC</td>
<td>11</td>
<td>Godde</td>
</tr>
<tr>
<td></td>
<td>Wadi Halfa North of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-Group</td>
<td>Wadi Halfa</td>
<td>A-Group/AGroup</td>
<td>3300-2800 BC</td>
<td>3050 BC</td>
<td>34</td>
<td>Lovell</td>
</tr>
<tr>
<td>C-Group</td>
<td>Wadi Halfa</td>
<td>C-Group/CGroup</td>
<td>2300-1800 BC</td>
<td>2050 BC</td>
<td>41</td>
<td>Lovell</td>
</tr>
<tr>
<td>Kerma</td>
<td>Sayala</td>
<td>Sayala C-Group/Sayala</td>
<td>1786-1550 BC</td>
<td>1668 BC</td>
<td>20</td>
<td>Strouhal</td>
</tr>
<tr>
<td>Pan-Grave</td>
<td>Kerma</td>
<td>Kerma</td>
<td>2000-1550 BC</td>
<td>1775 BC</td>
<td>224</td>
<td>Hanihara</td>
</tr>
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<td></td>
<td>Sayala</td>
<td>Pan-Grave</td>
<td>1786-1550 BC</td>
<td>1668 BC</td>
<td>9</td>
<td>Strouhal</td>
</tr>
<tr>
<td>Meroitic</td>
<td>Semna South</td>
<td>Meroitic</td>
<td>0-350 AD</td>
<td>175 AD</td>
<td>268</td>
<td>Godde</td>
</tr>
<tr>
<td>X-Group</td>
<td>Semna South</td>
<td>X-Group/Ballana/XGroup</td>
<td>350-550 AD</td>
<td>450 AD</td>
<td>28</td>
<td>Godde</td>
</tr>
<tr>
<td>Christian</td>
<td>Semna South</td>
<td>Christians/SS_Christians</td>
<td>550-1500 AD</td>
<td>1025 AD</td>
<td>11</td>
<td>Godde</td>
</tr>
<tr>
<td>Christian</td>
<td>Islands of el-Hesa/Biga</td>
<td>Hesa/Biga/Hesa_Biga_Kulubnarti</td>
<td>395-640 AD</td>
<td>527.5 AD</td>
<td>139</td>
<td>Hanihara</td>
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<tr>
<td>(mainland)</td>
<td>Kulubnarti (mainland)</td>
<td>Mainland/Kulubnarti_M_Kulubnarti</td>
<td>1100-1500 AD</td>
<td>1300 AD</td>
<td>81</td>
<td>Godde</td>
</tr>
<tr>
<td>(island)</td>
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<td>Island/Kulubnarti_I</td>
<td>550-800 AD</td>
<td>675 AD</td>
<td>42</td>
<td>Godde</td>
</tr>
</tbody>
</table>

Kerma, Meroitic, Christian, and unknown  Sesebi Sesebi 1800 BC-1500 AD 1150 AD 89 Hanihara

**Total:** 997

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**Table 2.** List of cranial nonmetric traits collected and interobserver error rates.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Definition of Traits</th>
<th>$\phi^2$</th>
<th>Error Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accessory Infraorbital Foramen</td>
<td>Berry and Berry (1967); Hanihara and Ishida (2001e)</td>
<td>0.7307</td>
<td>4.75%</td>
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<tr>
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¹ Trait dropped due to interobserver bias

² Ishida and Dodo (1990)
Table 3. Geographic distances (in kilometers). Linear distances are in the lower triangle and river distances are in the upper triangle

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<th>Kulubnarti_M</th>
<th>Meroitic</th>
<th>Mesolithic</th>
<th>PanGrave</th>
<th>Sayala</th>
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<th>XGroup</th>
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Table 4. Temporal distances in years (upper triangle) and biological distances (lower triangle)

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Pre-print version. Visit http://digitalcommons.wayne.edu/humbiol/ after publication to acquire the final version.
Table 5. Biological distances of Nubians including Sesebi

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**Table 6.** Chi-square tests evaluating sex differences by trait in each Nubian group. *p*-values are reported and 0.00 indicates a zero frequency of the trait in the sample.

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Table 7. Modified Relethford Blangero analysis

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<th>( V_{gi} )</th>
<th>( E(V_{gi}) )</th>
<th>Residual</th>
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<td>0.01</td>
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<td>0.14</td>
<td>0.14</td>
<td>0.00</td>
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<td>Kerma</td>
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<td>0.02</td>
<td>0.14</td>
<td>0.14</td>
<td>0.00</td>
</tr>
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<td>Kulubnarti_I</td>
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<td>0.13</td>
<td>0.00</td>
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<td>-0.03</td>
</tr>
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<td>Mesolithic</td>
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<td>0.02</td>
<td>0.11</td>
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<td>-0.03</td>
</tr>
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<td>0.09</td>
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<td>0.11</td>
<td>0.14</td>
<td>-0.03</td>
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</table>

\( F_{ST} = 0.0957 \)

Standard error \( F_{ST} = 0.0102 \)
Table 8. Mantel and PROTEST results utilizing 999 permutations

**Mantel Equation**

<table>
<thead>
<tr>
<th>Equation</th>
<th>Mantel r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>biodistance = temporal + river</td>
<td>-0.12</td>
<td>0.43</td>
</tr>
<tr>
<td>biodistance = temporal + linear</td>
<td>-0.13</td>
<td>0.41</td>
</tr>
<tr>
<td>biodistance = river + temporal</td>
<td>0.04</td>
<td>0.82</td>
</tr>
<tr>
<td>biodistance = linear + temporal</td>
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</table>

**PROTEST**

<table>
<thead>
<tr>
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<th>$m^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>biodistance = temporal</td>
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<td>0.14</td>
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<tr>
<td>biodistance = river</td>
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</tr>
<tr>
<td>biodistance = linear</td>
<td>0.98</td>
<td>0.88</td>
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</tbody>
</table>
Table S1. R matrix of all Nubian groups except Sesebi.

<table>
<thead>
<tr>
<th></th>
<th>AGroup</th>
<th>CGroup</th>
<th>Hesa_Biga</th>
<th>Kerma</th>
<th>Kulubnarti_I</th>
<th>Kulubnarti_M</th>
<th>Meroitic</th>
<th>Mesolithic</th>
<th>PanGrave</th>
<th>Sayala_C</th>
<th>SS_Christians</th>
<th>XGroup</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.0355</td>
<td>0.0071</td>
<td>-0.0655</td>
<td>-0.0665</td>
<td>-0.0274</td>
<td>0.0091</td>
<td>-0.0444</td>
<td>0.1238</td>
<td>-0.0484</td>
<td>-0.0508</td>
</tr>
<tr>
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<td>0.0123</td>
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<td>-0.0321</td>
<td>-0.0058</td>
<td>0.0191</td>
<td>-0.0573</td>
<td>0.0686</td>
<td>-0.0378</td>
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</tr>
<tr>
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<td>0.0123</td>
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<td>0.0041</td>
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<td>-0.0278</td>
<td>0.0013</td>
<td>0.0053</td>
<td>-0.0338</td>
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<td>-0.0194</td>
</tr>
<tr>
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<td>-0.0148</td>
<td>0.0028</td>
<td>-0.0183</td>
<td>0.0088</td>
<td>-0.0158</td>
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<td>-0.0370</td>
<td>-0.0552</td>
<td>0.0058</td>
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<td>0.0159</td>
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<td>0.0389</td>
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<tr>
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<td>-0.0183</td>
<td>-0.0244</td>
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<tr>
<td>SS_Christians</td>
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<td>0.0365</td>
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<tr>
<td>XGroup</td>
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<td>0.0297</td>
<td>-0.1004</td>
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<td>0.0584</td>
</tr>
</tbody>
</table>

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Figure 1. Map of Nubian sites (excluding Sesebi).

Figure 2. PCO plot of Nubian groups (excluding Sesebi).

Figure 3. PCO plot of all Nubian groups.

Figure 4. Harpending and Ward (1982) plot of heterozygosity. Note the plot zooms in on the axes in order to demonstrate the spread of points in such a tight clustering.

Figure S1. PCO plot of distance matrix without AIOF.

Figure S2. PCO plot of distance matrix without ASB.

Figure S3. PCO plot of distance matrix without TD.

Figure S4. PCO plot of distance matrix without OL.

Figure S5. PCO plot of distance matrix without PNB.

Figure S6. PCO plot of distance matrix without PCT.

Figure S7. PCO plot of distance matrix without SOF.
Pre-print version. Visit http://digitalcommons.wayne.edu/humbiol/ after publication to acquire the final version.
Figure 2.
Figure 3.
Figure 4.
Figure S1.
Figure S2.
Figure S3.
Figure S4.
Figure S5.
Figure S6.
Figure S7.