A Treatment Plan for Archaeological Findings at the Buckeye Knoll Site, 41VT98, Victoria County Texas

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Abstract

This document is the Corps of Engineers, Galveston District's treatment plan for archaeological materials excavated at the Buckeye Knoll site (41VT98), Victoria County, Texas. The Buckeye Knoll site is unique in the archaeological record of North America, and holds the potential for addressing major research topics of broad interest to the scientific community, Native American tribes and the general public. All aspects of the analyses are discussed, but emphasis is on the analyses of the human remains and associated artifacts, most of which come from a unique Early Archaic cemetery component. The discussions are formulated within the context of a series of Research Issues presented in the Data Recovery Plan, prepared prior to initiation of field work by Coastal Environments, Inc. for the U.S. Army Corps of Engineers, Galveston District. Additional important research issues that arise from the unexpected discovery of the Early Archaic cemetery are formulated in this Treatment Plan, with recommended analytical approaches. Attention is given to the comparative data bases currently available for standard, non-destructive bioarchaeological studies and minimally destructive analyses such as AMS dating, stable isotope assays and DNA sequencing.

Organization of this Document

This document combines information previously presented in the original Data Recovery Plan (Ricklis 2000) and in a Draft Proposal for analyses distributed to consulting parties by the U.S. Army Corps of Engineers, Galveston District (U.S. Army COE, Galveston District, 2002). The Data Recovery Plan was part of a technical proposal for site investigation submitted to Galveston District, while the Draft Proposal was written for an audience consisting of professional and avocational archaeologists and Native American tribal representatives. Thus, there were significant differences in the wordings with which issues were formulated and in the degrees of technical details provided.

This Treatment Plan, which is technically oriented, discusses the research issues and potentials that were presented in the two earlier documents, though the greatest emphasis is placed on recommendations for the treatment and analyses of mortuary and bioarchaeological materials. Additionally, space is devoted to discussions of the rationales, methodologies, and available comparative data bases for both non-destructive and destructive analyses.

Basically, the present document is built around a series of 13 research issues, along with annotations explaining the significance of each issue and the analytical approaches appropriate to each. The first eight research issues were formulated in the original Data Recovery Plan (Ricklis 2000), while the last five are newly formulated in response to the unexpected discovery and partial excavation of an Early Archaic cemetery. Research Issues 1-5 are to be addressed through analyses of non-mortuary artifacts and ecofacts, while Research Issues 6-13 require study of mortuary remains. While non-destructive analyses of human remains comprise the greatest recommended efforts, in terms of time required, the minimally destructive techniques discussed herein are of crucial importance as they provide basic information otherwise unattainable.

The order of presentation here is largely based on the dichotomy between those Research Issues formulated in the original Data Recovery Plan and those that are newly defined here based on the findings in the Early Archaic cemetery. A concise summary of this order has been presented in the preceding Table of Contents.

The Project and Research Issues Formulated in the Original Data Recovery Plan

In the spring of 2000, the U.S. Army Corps of Engineers, Galveston District (COE) requested that Coastal Environments, Inc. (CEI) prepare a Data Recovery Plan for archaeological investigations at 41VT98 (the Buckeye Knoll Site). A Scope of Work provided to CEI by the COE served as a guideline for the development of this plan. The catalyst for this work was the anticipated widening of the Victoria Barge canal, an artificial waterway that is used by various petrochemical plants for transport of materials, and that transects 41VT98. It was anticipated at the time that this activity would promote increased bankline erosion and thus endanger possible significant cultural resources within the bounds of the site. The Data Recovery Plan antipated a phased approach to mitigation consisting of an initial phase of additional assessment excavations, and a follow-on data recovery phase. It was intended that site information obtained from this assessment would be used to revise the list of research issues initially proposed.

The original Data Recovery Plan (Attachment 1), entitled *Proposal for Archaeological Data Recovery, 41VT98, Victoria County, Texas*, provided a list of eight specific research issues designed to address current, significant questions in regional prehistory (Ricklis 2000). Initial testing at the site, directed for CEI in 1989 by Richard A. Weinstein, had found evidence of stratified midden deposits representing cultural components spanning most of the Archaic and into the Late Prehistoric (Weinstein 1992). In combination with good faunal preservation, these findings suggested that 41VT98 contained unusual potential to address basic issues of chronology, paleoenvironment, human ecology, and cultural/adaptive change in the region. In brief, the research issues formulated in the Data Recovery Plan are:

<u>Research Issue 1</u>. *Prehistoric cultural chronology of the lower Guadalupe Basin*. While it has long been recognized that the area around the lower Guadalupe River contains

numerous archaeological resources, a dearth of professional excavations and analyses has hampered development of a detailed culture chronology.

Research Issue 2. *Diachronic patterns of human adaptation/ecology on the central coastal prairie of Texas*. The potential here derives from the stratified nature of the site deposits, good faunal preservation, the potential of obtaining macrobotanical samples via flotation from midden deposits and features, plus the possibility of acquiring long-term palynological data from cores in the nearby Guadalupe River floodplain sediments.

Research Issue 3. *Possible variable intensity of site occupation through time*. Again, this issue is keyed to the presence of stratified midden deposits.

Research Issue 4. *Horizontal intrasite patterning*. This research issue hinges on the use of standard distribution studies of various artifact classes, in association with topographic variables and locations of cultural features such as hearths, pits, evidence of structure floors, etc.

Research Issue 5. *Prehistoric patterns of mobility, exchange and interaction*. This question was to be approached through seasonality analyses as reflective of mobility (or relative sedentism), and presence/absence of materials of artifact styles indicative of extra-regional origins.

Research Issue 6. *The demographic dimension of prehistoric adaptation at 41VT98*. It was recognized that if prehistoric human burials were encountered within excavations, information could be obtained on age/sex structure of populations, health of the burial population and physical characteristics.

Research Issue 7. *Coastal vs. inland adaptations as revealed by stable isotope analysis*. The long-term diet of individuals can be ascertained using this technique, if human bone is available for study.

Research Issue 8. *Social and ideological patterns*. It was recognized that additional aspects of culture and adaptation can be investigated if burials were encountered in quantity; such questions can be explored where data can be obtained on age-sex correlations with various kinds and quantities of mortuary artifacts.

Summary of the Results of Fieldwork

Many aspects of the Buckeye Knoll site revealed by the 2000-01 fieldwork proved to meet the expectations summarized in the Data Recovery Plan. As expected, the site produced intact, stratified deposits that spanned most of the known prehistoric cultural sequence. Quite unexpected, however, was the discovery of an Early Archaic cemetery, unique in the region, and one of only three sizeable cemeteries of comparable age in all of North America.

The Buckeye Knoll site, 41VT98, is, then, unique in several important ways. First, it is the largest Early Archaic cemetery (ca. 5,000 B.C.) ever found west of the Mississippi River and, as just noted, one of the three largest early cemeteries with preserved human remains in North America. Second, the assemblage of mortuary artifacts found associated with these burials represents a previously undiscovered early culture. Third, the site has yielded the most complete sequence of stratified or layered cultural deposits yet found in southern Texas, a sequence estimated to represent more than 10,000 years, almost the entire span of known regional prehistory.

Each of the unique aspects of the site offers possibilities for understanding early lifeways and demographics. Study of the Buckeye Knoll remains would set the baseline data on fundamental questions of human biology, demography and economy, for the Early Archaic in the south-central U. S. The age of the early burials and their number (approximately 73 individuals recovered) offers a rare opportunity to learn about the biological affinity, health, diet, disease, activity-related stress and/or trauma, life expectancy and demography of a very early North American population. Additionally, the intact stratified midden deposits provide an unusually complete record of the culture history of the region from Paleo-Indian times, around 9,000 B.C. through the long Archaic period, from about 7,000 B.C. to 700 A.D., and into the Late Prehistoric period, which dates to about 700 to 1600 A.D. The associated mortuary artifacts, which are varied and reflect considerable craftsmanship, offer unique possibilities for learning about early technology, styles of workmanship in stone, shell and bone material, early trade networks, and the kinds and quantities of different materials placed with individuals of both sexes and varying ages.

It is important to note that no Early Archaic midden component was identified during the extensive excavations at the site; the early cemetery component is the only representation of this period at the site. Thus, crucial questions concerning the age of the cemetery and the dietary patterns of the burial population can only be answered through those direct analyses of human remains that are discussed further on.

Very briefly summarized, the broad cultural periods represented by time-diagnostic artifacts from more or less discrete stratigraphic contexts are:

1. Paleo-Indian, ca. 10,000 B.P. The latter part of the Paleo-Indian sequence, as currently defined in Texas and adjacent regions, is represented by unstemmed lanceolate point types such as Golondrina and Dalton (cf. Turner and Hester 1999), as well as specimens of the stemmed Wilson type (Kerr and Dial 1998). Other associated materials include a Dalton adze and various lithic scraping/cutting tools, plus a large sample of chert debitage. Paleo-Indian occupation (camp) components were found in Zone 3-A in the Knoll Top Block Excavation, and in the basal levels of the East Midden Area.

2. Early Archaic, ca. 9,000-6,000 B.P. This period is clearly represented only by the early cemetery component in the Knoll Top Block Excavation, which produced the remains of a minimum of 73 individuals and a unique assemblage of mortuary artifacts. No non-mortuary component of this period was identified in any of the excavations at the site. This is highly significant as it precludes using materials from non-mortuary contexts to define the early cemetery chronologically or in terms of cultural-ecological patterns. This early cemetery was contained within Zone 3, being intrusive into the earlier Paleo-Indian component in that stratum. The cemetery was clearly separated from later components by a geologic unconformity, above which were found Middle Archaic materials.

3. Middle Archaic, ca. 6,000-4,000 B.P. This period is well represented by occupation (camp) debris and features in Zone 3 in the West Slope Block Excavation by an apparently "pure" assemblage of unstemmed dart points (Early Triangular and Refugio types) in association with other lithic tools, debitage, hearth features, and what appears to be pole/grass-impressed daub. In the Knoll Top Block, Middle Archaic diagnostics were found along with other camp debris resting on the aforementioned geologic unconformity at the interface of Zones 2 and 3-A.

4. Late Archaic, ca. 4,000-1,200 B.P. This period is well represented by occupation debris at the site, particularly in Zone 2 and the lower part of Zone 1 in the West Slope Block, and in the lower part of Zone 2 in the Knoll Top Block. Several dart point types (Pedernales, Morhiss, Lange, Fairland, Ensor) span the full time range of the period. Field observations and initial inventorying of the materials suggest that vertical segregation of components within the period is possible through identification of distribution patterns of time-diagnostic artifacts. A number of hearth features and pits appear to pertain to this period.

The Late Archaic is additionally represented by five burials, four within (or in pits originating in) Zone 2 in the Knoll Top Block and one isolated interment in Zone 2 in the West Slope Block. The Late Archaic provenance of these burials is indicated by their stratigraphic positions and, in two cases, by their direct associations with Late Archaic time-diagnostic mortuary items.

5. Late Prehistoric, ca. 1,200-300 B.P. This period is well represented at the site. The earlier part of the period (ca. 1,200-700 B.P.) is particularly in evidence on the Knoll Top, where abundant Scallorn arrow points were found in the upper part of Zone 2. Ceramic sherds are also a trait of this component. A component of the later Rockport Phase (ca 700-300 B.P.), represented by lithics and pottery, was identified in the East Midden Area.

New Research Issues Emerging from Findings

Any major archaeological field effort is likely to result in findings that require (re)formulation of research questions not fully anticipated in the original research design. This is especially true at Buckeye Knoll, where the large Early Archaic cemetery represents an unexpected set of potential data bases suited to addressing important questions of regional and extra-regional significance. These questions, formulated as additional Research Issues, are:

<u>Research Issue 9</u>. *Are the human remains and associated mortuary artifacts from the Buckeye Knoll site culturally or archaeologically related to present-day Indian tribes or groups?*

<u>Research Issue 10</u>*:* *What are the chronological parameters of the Early Archaic cemetery?*

<u>Research Issue 11:</u>. *How close is the biological relationship between the Early Archaic population represented at Buckeye Knoll and other populations of similar antiquity in North America?*

<u>Research Issue 12</u>. *Were the Early Archaic people represented by the Buckeye Knoll cemetery ancestral to later inhabitants of the Texas coastal plain region?*

<u>Research Issue 13</u>. *Were social roles and/or statuses achieved or were they linked by heredity to lineage memberships?*

Research Issues Amenable to Investigation through Analyses of Non-Mortuary Materials

As the site is unique, so is the collection obtained during the Corps of Engineers-sponsored work. While other prehistoric cemeteries have been found in Texas, none are of comparable age to Buckeye Knoll. The earliest large cemetery known in the state prior to the work at this site was at the Ernest Witte site in Austin County, where the oldest burials are some 3,000 years later (and most still more recent, dating to the last 2,500 years or so and thus post-dating the early Buckeye Knoll cemetery by 4,500-5,000 years). Thus, existing samples of human remains from the state cannot provide answers to the key issues of early population, demography and culture listed above.

The sample size at Buckeye Knoll provides the best opportunity scientists have ever had to address biological questions of early populations west of the Mississippi River. Older specimens do exist, both in Texas and other parts of North America, but they are much smaller samples, many are less well preserved, and many contain no associated artifacts or other pertinent information for socio-cultural interpretation. Only in Florida and Illinois do samples approximating this antiquity and size exist out of approximately 500,000 known North American archaeological sites (a rough estimate of the number of sites).

The artifact assemblage from the early cemetery is unique and therefore cannot be found in existing collections. Some of the individual traits (or artifact types) found at Buckeye Knoll have been sporadically reported before, but never have they been found in association with the quantity and variety of materials at Buckeye Knoll as an integral material-culture assemblage. The following list of the kinds of mortuary objects recovered shows the range and uniqueness of this assemblage:

- Lanceolate stone dart points similar to Angostura and other early forms
- Stemmed dart points of types found elsewhere in non-burial contexts
- Large flint bifacial blades, probably of non-mundane significance
- Various forms of flaked flint tools
- Ground stone artifacts including "Waco sinkers", bannerstones, and plummets
- Marine and freshwater shell ornaments, including perforated pendants and marginella and netrite shell beads
- Canid canine-tooth beads
- Tool kits containing bone and antler flint-working tools, sandstone abraders and flint preforms
- Caches of flaked flint preforms (unfinished points or tools)
- Nodules of asphaltum with basketry impressions
- Red and yellow ochre paint in nodule and powder forms

Outside of Texas, only two other large cemeteries are of comparable age to Buckeye Knoll. The Carrier Mills site in Illinois is about the same age, while the Windover site in Florida is about 1,000-1,500 years older. The information from these two sites does not substitute for that from Buckeye Knoll, due to the great geographic separation of the sites. On the contrary, the three sites are complementary in that they can provide important comparative information on culture and human biology on a continental scale during the Early Archaic period.

The above-listed cultural periods, as mentioned, are defined on the basis of stratigraphy and time-diagnostic lithics. While an abundance of faunal bone and shell offers ample opportunity to radiocarbon date the non-mortuary components at the site, no assays on these materials have, as yet, been obtained. It is important to note, however, that a series of optically stimulated luminescence (OSL) dates on sedimentary matrices from the Knoll Top and West Slope areas (Frederick and Bateman 2002), obtained as part of the Corps' geoarchaeological investigation of the site, generally support the chronological summary presented above.

Given the fact that the site produced an approximately 10,000-11,000-year record of occupation within good stratigraphic contexts, a number of the Research Issues formulated in the original Data Recovery Plan, and listed herein above, clearly can be addressed by using standard analytical techniques. These are:

Research Issue 1: Prehistoric cultural chronology of the lower Guadalupe Basin

As listed above, the excavations at Buckeye Knoll have occupations during the Late Paleo-Indian, Early, Middle and Late Archaic, and the Late Prehistoric periods. Detailed analysis of stratigraphic and arbitrary unit-level proveniences, as well as an array of standard typological and functional analyses, are expected to produce the most complete chronological record of material culture and associated ecofacts yet obtained at a single site in southern Texas. Components can be dated with a series of standard radiocarbon assays on associated faunal bone and shell samples.

Research Issue 2: Diachronic patterns of human adaptation/ecology on the central Texas coastal prairie

Diachronic trends and/or fluctuations in the use of key resources over the 10,000-year use of the site should be identifiable, once the various components are temporally bracketed by radiocarbon dates.

With the expected identification of chronologically defined components, various kinds of analyses will result in the definition of patterns of resource use for the various periods represented. Zooarchaeological identification and quantification of faunal taxa will permit assessment of the relative importance of terrestrial and aquatic fauna in terms of minimum numbers of individuals and proportional dietary biomass. Further, fluctuations in the significance and availability of estuarine fish and mollusks can be linked to the paleogeography of the coastline, as fluctuations in Holocene sea level influenced the extent and biotic productivity of coastal estuaries downstream from the site Ricklis 1993, 1995; Ricklis and Blum 1997).

The palynological analyses of dated sediments from cores in the nearby Guadalupe floodplain have already provided a 9,000-year record of vegetation and climatic change in the environs of the site (Albert 2002). Importantly, these data form an extremely useful baseline for placing the economic patterns evidenced at the site within a long-term environmental context.

Research Issue 3: Possible variable intensity of site occupation through time

This question can be addressed through comparisons of the biomass represented by faunal materials between temporally bracketed site components. Additionally, inferences concerning occupational intensity may be possible using data on the relative quantities and functional range of various artifacts classes. The ecological basis for possible fluctuations in intensity of occupation may be inferred from the zooarchaeological and palynological data.

Research Issue 4: Horizontal intrasite patterning

This can be explored by spatial analysis of the variable density of different artifact and debris classes in relation to features such as hearths and pits.

Research Issue 5: Prehistoric patterns of mobility, exchange and interaction

As originally defined, this question is to be addressed through seasonality studies and by identification of artifact materials and styles that indicate extra-local origins. The fieldwork at Buckeye Knoll produced a large sample of well preserved faunal material that can be used for seasonality analyses inluding molluskan bivalve shells, fish otoliths and deer bone. Identification of seasonal patterns of resource use at the site will provide information on season(s) of occupation during various time periods, thus contributing to an understanding of the degree of mobility vs. sedentism that characterized site occupations. A large sample of stone tools and debitage are available with which to identify possible importation of raw materials through identification of source areas (e.g., ultra-violet lighting on cherts to identify materials originating in the Edwards Plateau area of central Texas).

Research Issues Amenable to Investigation Using Mortuary and Bioarchaeological Data

A number of key research questions arise from the fact of the age, size, and unique nature of the Early Archaic mortuary sample from Buckeye Knoll. Therefore, the scientific goal in studying the archaeological materials from the site should be to maximize the recovery of highly significant information, while treating human remains and associated artifacts with respect. This be followed by reinterment of human remains and associated grave goods. We estimate that the necessary studies can be accomplished within a two-year time frame, and that a draft technical report can be generated within an additional six- to twelve-month period.

In order to achieve these goals with minimal disturbance to the remains, we recommend the following:

1. All human remains will be handled with care and cleaned only to the extent necessary for making critical visual and metric observations. It is not necessary to wash these materials; cleaning need only involve dry brushing. All soil removed by such cleaning will be kept with the original remains. All of the human remains recovered from the Buckeye Knoll site will be studied by non-destructive methods involving standard measurements and observations, microscopic examination, and photodocumentation. X-ray

and CAT scans may also be performed on selected specimens. Specific measurements and observations are described in more detail in Table 2.

2. Native American tribal representatives are invited to observe and, if they so wish, to participate in, the study of the remains.

3. While several lines of analysis are necessary to obtain important information that can be acquired in no other way (e.g., radiocarbon, DNA, stable isotope studies), these will be done in such a way as to minimize disturbance of the remains. In order to ensure that the smallest, statistically meaningful sample is subjected to destructive analyses, a series of empirical tests for statistical optimization was performed to determine recommended sample sizes. This analysis is presented as an appendix to this treatment plan. Based upon this analysis, the District proposes a phased sampling approach for all destructive bioarchaeological analyses. All sample sizes referenced in the following sections are based on this analysis. For radiocarbon and stable isotopes, Galveston District proposes an initial analysis of 20 samples from the Early Archaic interments. If a definite pattern is not discernable due to wide variability or data inconsistencies, then a second phase of testing will be performed on 10 additional samples, for a total of 30 Early Archaic samples. For the Late Archaic interments, 3 samples (50%) will be analyzed with no follow-up phase. For DNA analysis, the first phase would consist of a preliminary test for preserved DNA in the same 20 interments sampled for radiocarbon/stable isotope analyses. If preserved DNA is found in some or all of these samples, a second phase consisting of full DNA analysis will be performed on a minimum of 5 and a maximum of 20 samples, with the final number determined by cost. For the Late Archaic interments, the preliminary test for preserved DNA will be run on the same 3 samples obtained for radiocarbon/stable isotope analyses, followed by a maximum of 3 samples submitted for full DNA analysis.

The amount of bone tissue removed from each set of remains for destructive analyses will be actively managed to ensure the least disturbance possible. It is the District's intent to obtain one sample from a range of 20 to 30 burials and use that sample for all three analyses. Critical information can be obtained using only minute amounts of material (i.e., <1 gram per sample) present in pulp tissue from one tooth. It is possible that additional bone will be required from some of these burials in order to adequately perform one or more of the analyses, but those decisions will be made on a case-by-case basis and the amount obtained will minimized to the greatest extent possible. Close coordination between the various analytical laboratories will be necessary in order to ensure that a sample is not rendered unsuitable for succeeding analyses. If reconstruction of bone elements is essential to obtain specific data, it will be accomplished using only natural organic materials.

According to the preliminary field inventory, the Buckeye Knoll sample consists of 75 numbered burials with an estimated minimum of 79 individuals. Of these, at least 73 are believed to pertain to the Early Archaic period, ca. 7,000 B.P. As such, the site provides the largest mortuary sample from any known location west of the Mississippi River. Only two other sites, Windover in Florida (Doran 2002), and Carrier Mills in southern Illinois, provide samples of this magnitude and age in North America.

Human skeletal material and the information it provides has long been recognized as one of the most important components of the archaeological record (Steele and Bramblett 1988; Buikstra and Ubelaker 1994). Such materials provide a series of essentially unique perspectives on the past through considerations of population biology, population comparisons, population change through time, population health and demography. A brief summary of these perspectives is provided as a way of framing the potential importance of the Buckeye Knoll materials.

Skeletal material provides the only medium through which direct information on a population's physical and biological characteristics (e.g., cranial, postcranial and dental metrics and nonmetrics) can be obtained. This information is used to assess a population's morphological features vis-a-vis other comparative information (Buikstra 1980; Steele and Powell 1993), an approach that has driven a majority of the osteological data collection in the last century. Through statistical comparison of available attributes, we are able to address issues of population evolution that have both chronological and spatial components. These analyses allow for assessment of population affinity, migration patterns and patterns of interaction relating to the broader issue of diachronic population and technological change. In the continental United States, such strategies have been particularly useful in the Midwest where researcher interest and preservation (and consequently larger sample sizes) has proven to be an integral component for understanding not only biological aspects of human populations but also cultural aspects (see studies by J. Buikstra and her colleagues and students centered on sites in the Illinois Valley spanning the Archaic and Mississippian periods; Buikstra 1984; Charles et al. 1988, and many others). Until recently, this approach was the only method for assessing a population's 'genetic' relationships. However, recent developments have initiated a redirected emphasis on the assessment of population affinity through direct access to human genetic material in the form of modern and ancient DNA comparisons (Doran et al. 1986; Smith et al. 1999).

In addition to population genetic issues, a major focus of modern osteological investigation has been the assessment of population health profiles through identification of pathological and degenerative changes visible in the skeleton. Identification of specific pathogens, sometimes through differential diagnosis as well as, more recently, identification of surviving bacteria, has also been useful in this approach. More directly observable features such as linear enamel hypoplasia (evidence of growth disruption in teeth), cribra orbitalia, porotic hyperostosis, and growth arrest lines, as well as actual disease (viral or bacterial based pathogens) states, continue to provide important information on levels of health and disease loads in prehistoric contexts (Buikstra and Cook 1980). Collectively, these approaches have proven useful for developing biological profiles of "success" or "stress" in populations with an emphasis on how "health" relates to cultural and environmental adaptation strategies. This has been one of the critical elements in understanding major shifts in subsistence as well as the interaction of such strategies with reconstructed environmental features (see Cohen and Armelagos 1984).

A further focus of skeletal investigation is the assessment of population demographic (or paleodemographic) features. This approach, optimally applied in concert with both biodistance- and health-related studies, as discussed above, minimally requires reliable assessments of age and sex in relatively large skeletal samples (Verano and Ubelaker 1992). For analytical purposes, most researchers feel a minimum of 50 individuals can effectively be used in this approach allowing observations of life expectancy, mortality rates, population growth rates, fertility, generation length, family size and other demographic parameters (Buikstra et al. 1986). The strategies discussed above are all nondestructive and require little more than the collection of appropriate observable features involving an array of measurement tools and possibly radiographic analyses (x-ray).

Some techniques of investigation require the destruction of small bone samples (approx. 1 gram or less). Some of these approaches require bone samples so small that their extraction can be accomplished with unidentifiable bone fragments (Tuross et al. 1994) or alternatively can be extracted from teeth as the pulp underlying the tooth enamel. These minimally destructive techniques provide highly important information that can be obtained in no other way. Stable isotope analyses provide information on prehistoric dietary patterns that cannot be obtained from study of the faunal and floral samples from midden deposits. This is because the faunal/floral remains on a site represent completely, or overwhelmingly, those resources extracted from the environment within the site catchment area, but not from entire range of food resources available to the population that used the site. This is especially true in the case of more or less mobile hunter-gatherer populations, who procured and processed important resources at locations some distance from a given site that is the focus of archaeological investigation. That is, the site being investigated may represent only one of a number of environmental resource zones within the total territory occupied and exploited by a prehistoric group or population. In the case of the Early Archaic population represented in the Buckeye Knoll mortuary sample, coastal resources may have been of some importance in the diet. The site, however, is located some distance from the coast, so that the residues of shellfish and/or fish extraction will have been deposited at shoreline locations, not at the Buckeye Knoll site. Even if some fraction of such residues, (e.g, mollusk shells or fish bones) were brought to Buckeye Knoll, it is unlikely that they would accurately reflect the actual proportional significance of those resources within the overall diet. By contrast, stable isotope studies will show if marine/estuarine foods were important dietary components, since they in effect offer biochemical signatures of the total diet of the population over the lifetimes of each sampled individual.

The subsistence economy of the Early Archaic folk who were interred at Buckeye Knoll cannot, in any case, be even partly assessed using faunal or floral remains present at the site. This is simply because, as mentioned earlier, there are no midden deposits at the site that represent this time period; the Early Archaic is represented only by the cemetery component (which suggests that the site was a separate mortuary precinct, as opposed to a domestic campsite, during the Early Archaic).

Another analytical technique that provides information that cannot be obtained in any other way is DNA sequencing. In the last 15 years, major advances in extraction, characterization, and direct observation of genetic markers have created a potentially much more precise method of assessing population genetic relationships, familial connections, and even sex in either poorly preserved or subadult remains where traditional sex assessment criteria are difficult if not impossible (Smith et al. 2002; Stone et al. 1996; Tuross 1997). Increasingly, this approach is targeting biological residues in tooth pulp cavities where the risk of contamination is minimized (Kohlman and Tuross 2000). Most of the genetic studies are targeting genetic haplotype morphology which are identifiable in the mitochondrial DNA (as opposed to the nucleic DNA).

The primary benefit of DNA analyses is that it permits assessment of degrees of biological relatedness that cannot be ascertained using standard metric data due to preservation factors, and also provides a level of resolution in assessing genetic relatedness between and within populations unobtainable in other ways. While genetic relatedness can be assessed through metric and non-metric observations of teeth, at least half of the relevant measurements and attributes are on occlusal surfaces that have been removed by the tooth wear common among hunter-gatherer populations (and such is definitely the case in the Buckeye Knoll sample); thus, DNA analysis offers a more reliable avenue of addressing questions of biological relatedness within and between populations. The importance of accuracy and reliability of data on genetic relatedness is crucial to understanding ancient movements of, and/or relationships between, populations on the one hand, and intra-societal differentiation based on lineages on the other.

Another important aspect of DNA analysis is identification of sex, which is often ambiguous in poorly preserved or incomplete skeletons and indeterminate in the case of juvenile individuals. DNA data, however, can provide ready identification of sex using minute quantities of bone or tooth pulp. Thus DNA analysis can provide crucial information on the possible social roles of male vs. female children that has previously been completely inaccessible.

Research Issues Amenable to Investigation Using Non-Destructive Analyses

Research Issue 6. The demographic dimension of prehistoric adaptation at 41VT98

The sizeable number of individuals represented by recovered human remains (MNI=79) offers a unique opportunity to study population age/sex structure, health, and physical characteristics during the Early Archaic. As explained above, these issues can be addressed using standard non-destructive methods of metric and non-metric analysis where bone/tooth preservation permits (e.g., Bass 1995; Doran et al. 2001; Steele and Bramblett 1988).

As regards the non-destructive analyses, the number of specific observations (metric and non-metric) for different parts of the skeleton are as follows:

Cranial		
	Metric:	116
	Non-Metric:	50
Long Bones		
	Metric:	91
Teeth		
	Metric:	34

These provide basic information on physical appearance as determined genetically and as influenced environmentally through diet and individual activity patterns. Accordingly, they provide important information on population biology, subsistence economy and various aspects of culture. Not all of the total of 291 observations can be made on each individual represented at Buckeye Knoll-- the number will depend on the preservation of any given skeleton. Because of the importance of statistically valid results, and because these observations are entirely non-destructive, all individual remains can and should be studied to the extent allowed by preservation.

In addition, various other observations are important for assessing the health and lifeways of the Buckeye Knoll people. These include indicators of health (or diseases) that leave distinctive imprints in the skeleton, plus evidence for stress and trauma (for example, healed breaks in bone elements). To these observations can be added evidence for disease registered in preserved DNA, as discussed further on.

Comparative Data Bases

Several databases are available with which to develop the scientific potential of the Buckeye knoll skeletal materials in light of research issues at the local, regional and continental scales. These databases have been under development over the last 15 years and provide a variety of chronological and geographic perspectives for comparing skeletal samples. All of the databases were initiated as part of a comparative effort specifically for Florida State University archaeological investigations of the Early Archaic Windover site near the east coast of Florida (Windover dates to ca. 8,100 cal. years BP and is the largest sample of human skeletal material of this antiquity in any of the databases considered).

The four most important databases will be briefly described and then compared in more detail to the Buckeye Knoll materials. These databases include an inventory of North American skeletal samples (NORTH), a craniometric database (CRAN), a postcranial database (PCRAN), and a dental metric database (DENT). A separate database constructed specifically for providing a comparative regional framework for Buckeye Knoll is based on materials from Texas (TEXAS). All of these databases are described in more detail in Attachment 2: The Place of Buckeye Knoll (41VT98) Material with Respect to Bioarcheology. Excluding the TEXAS database these datasets minimally contain some kind of information on over 60,000 individuals. While the greatest emphasis has been on the North American database and on the craniometric database, we continue to expand these databases as more information is obtained. Even if an international database was considered, the conclusions would be essentially the same and is predicated upon the fact that human skeletal material as old as that from Buckeye Knoll is exceedingly rare regardless of geographic context.

<u>Research Issue 7</u>. Social and ideological patterns

It is an axiom in archaeological study of mortuary patterns that the variable treatment of individuals at death reflects in some ways the roles and social positions of those persons during life. A common approach to defining social roles and statuses in burial populations has been to assess the differential degrees of material "wealth" that accompanied different individuals (e.g., Binford 1964, 1971; Brown 1981; Chapman and Randsborg 1981; O'Shea 1984; Peebles and Kus 1977). Where there are discernible correlations between the quantities and kinds of materials and identifiable differences between individuals, such as age or sex, or when there are spatial patterns of differential disposal of materials within or between cemeteries, then social and/or socioeconomic distinctions can be inferred (Brown 1981; Freid 1967; Mainfort 1985; Service 1971; O'Shea 1984).

The preliminary inventory of the Buckeye Knoll burials shows that a majority (59%) of the interred individuals in the recovered sample was accompanied by grave artifacts. A wide range of artifacts is represented including the items listed above.

The materials associated with individual graves ranged from a single item to a numerous and varied array of materials, thus suggesting a significant variability in the kinds and quantities of items that were interred. The combination of a sizeable number of graves and a large and varied assemblage of mortuary artifacts offers a unique opportunity to explore questions of social roles and statuses in the south-central United States at this early time period. Many of the artifacts represent considerable investments of time and energy (e.g., the finely formed and polished ground stone implements and the oversize bifaces), and their placement within graves likely expresses some degree of status or societal significance for the associated individual. Moreover, the range of materials and functions represented in the mortuary artifact assemblage probably represents, in some fashion, the technoeconomic roles associated with certain individuals (e.g., tight clusters of flint-knapping tools with certain individuals may express part-time craft specialization). These artifacts will be subjected to standard archeological analysis and recordation before being reintered with associated human remains. Quality resin casts will be prepared for selected specimens of appropriate size and significance. The casts will be curated with the remainder of the collection to facilitate future research and interpretion.

In order to understand these factors, it is necessary to idetify, through macroscopic observations of bone elements, the age and sex of individuals. Additionally, DNA studies can aid in accurate identification of sex where preservation of diagnostic bone elements is poor.

Research Issue 8: Potential for association with present-day Indian tribes.

Examination of the question of whether the archeological remains from the Buckeye Knoll site can be associated with any extant Indian tribe or groups hinges upon comparative studies of artifact styles and modes of burial (i.e., body position, primary vs. secondary interments) with known patterns among present-day groups or their historic-era ancestors. This issue, therefore, requires the investigation of historical and ethnohistorical information, as stated below.

Historical and Ethnographic Studies

Historical and ethnographic studies can gather evidence of the possible cultural and historical association of Native American tribes or groups to the Buckeye Knoll site and the lower Guadalupe River region. The purpose of these studies would be to identify extant Native American tribes, groups and organizations that may have ties to the prehistoric archeological sites and cultures of the lower Guadalupe River region, specifically, and the central Texas coastal plain, generally.

As part of this historical study, Native American tribes and organizations could assist in the identification of specific questions the Tribes believe could be answered by analysis of the Buckeye Knoll site. Appropriate tribal representatives could be consulted to determine their willingness to participate in this effort. Those tribes willing to participate would be asked to identify specific questions they would like to see pursued during the site analysis.

These studies involve a search of the published ethnographic and historical data, a limited search of archival and other primary data, consultation with appropriate tribal representatives and other experts, and an evaluation and assessment of the ethnographic and historic database.

Research Questions Amenable to Investigation Using Minimally Destructive Analytical Techniques

Stable Isotope Studies

Research issue 9. Coastal vs. inland adaptations as revealed by stable isotope analyses

The Buckeye Knoll site lies close to a major boundary between inland and coastal peoples and adaptations that archeological and ethnohistorical research has shown for Late Prehistoric and Early Historic times (Ricklis 1992, 1996). While it is unclear, at present, how far back in time such a cultural and human-ecological boundary may have existed, recent research has shown shoreline shell middens on the central Texas coast to have basal components dating to between ca. 8,200 and 6,800 years B.P. (Ricklis 1993, 1995; Weinstein 1994, 2003). Thus, emergent early-to-middle Holocene estuarine resources may have played a role in supporting early populations along the coastal prairies. This is an important research issue related to the nature of early Holocene adaptations in North America (e.g., see Russo 1996), which can be addressed though stable isotope studies of human bones from the Early Archaic mortuary component at Buckeye Knoll. An optimal sample size for these studies was determined to be 20-30 samples by an optimization analysis provided as an appendix to this plan.

As noted above, Buckeye Knoll is too far from the coast for faunal remains in its various midden strata to fully and accurately represent the detritus of a possible coastal aspect in regional adaptive strategies. However, stable isotopic data represent lifetime diets and thus can signify a significant marine dietary component, if one were in fact present due to group mobility between the coast and the interior. It must be emphasized that, due to the mobility of hunter-gatherer societies, the only means of determining if group foraging patterns included significant use of coastal food resources is stable isotope analysis, given that the bulk of fish/shellfish residues would have been left at shoreline extraction loci. The mobile nature of hunter-gatherer land-use patterns makes this true for the Late Archaic and the Early Archaic mortuary samples regardless of the fact that there are Late Archaic domestic debris deposits with well-preserved faunal remains at the site. In distinction from standard zooarchaeological and macrobotanical studies, stable isotope analysis measures the nutritional sources actually used by an individual over the course of his or her lifetime (DeNiro and Epstein 1978, 1981). Both stable carbon (13C) and stable nitrogen (15N) values can be employed to estimate the relative significance of terrestrial vs. marine foods during the lifetime of prehistoric individuals (Schoeninger et al. 1984). Carbon isotope values in marine foodwebs are enriched in comparison with most terrestrial ecosystems, due to the use of bicarbonate as the carbon source in marine photosynthesis (Boutton 1991). However, 13C values can also be enriched due to consumption of certain kinds of terrestrial plants (e.g., those with C4 photosynthetic pathways) that that do not follow the more common C3 photosynthetic pathway (e.g., plants such as maize, certain grasses, and opuntia cacti) (e.g., O'Leary 1988; Smith and Epstein 1971; Tieszen and Boutton 1989).

While a significantly marine diet may be confused with one that includes C4 plants, consideration of nitrogen (15N) can serve to distinguish marine-based from terrestrial diets. This is because nitrogen isotope values, like carbon isotopes, are enriched in marine environments (Wada 1980). Dissolved nitrate in seawater is used by phytoplankton and macrophytes and is more enriched with 15N than the atmospheric N2 and soil nitrate. Also, marine food chains usually have more intermediate tropic levels than do terrestrial ones (Macko 1981; Schoeninger and DeNiro 1984), and nitrogen isotopes are enriched by some 2.3-3o/oo at each trophic level (Schwarcz 1991).

The Regional Comparative Data Base for Stable Isotope Studies

Current stable isotopic research by Robert J. Hard (The University of Texas at San Antonio) suggests that a clear coastal-inland dichotomy in adaptive patterns revealed archaeologically for the final centuries of the Late Prehistoric (e.g., Ricklis 1992, 1995, 1996) has considerable time depth, reaching at least as far back as ca. 4,000 B.P. (Hard pers. comm. 2002; Hard and Francis 2000; Hard et al. 2002). The Buckeye Knoll site, which lies inland of the Late Prehistoric boundary, provides the first significant sample of human remains from the region for the Early Archaic period and thus offers a unique opportunity to test whether a clear demarcation between coastal and inland adaptive systems existed as early as 7,500 B.P. The nearly completed research by Hard, conducted under a grant from the National Science Foundation, examines 13C and 15N values in human remains from various Archaic and Late Prehistoric sites along the Texas coast and the adjacent inland prairie-riverine environment. This work suggests that the inland-coastal dichotomy has considerable time depth, going back at least to the Late Archaic. As such, the study provides an excellent comparative data base for isotope data from the Buckeye Knoll site.

Additional comparative data are found in a series of published stable isotope analyses on the Texas coast and adjacent coastal plain conducted by Jeffrey Huebner (1994; Huebner and Comuzzie 1993). Samples came from prehistoric mortuary sites dated to the Middle Archaic through Late Prehistoric periods.

Purified-Collagen AMS Dating

Research Issue 10. What are the Chronological Parameters of the Early Archaic Cemetery*?*

It is important to accurately place the early cemetery component at Buckeye Knoll within a reliable chronological framework. As discussed above, the complete absence of domestic debris pertaining to the Early Archaic makes direct dating of the human remains in the Early Archaic cemetery necessary for this component to be accurately dated. The most obvious need for accurate chronological placement of the cemetery is that diachronic cultural processes can only be understood when the temporal dimension of change is reasonably well established There presently are only four AMS dates on human remains from this early component, which in combination span a calibrated age range of 7420-6290 years B.P. With this small sample size, it is difficult to know whether the period of cemetery use spans over a millennium, or if the age range reflects only the margins of error inherent in the dating technique in combination with possible errors due to organic contamination of the bone samples. A larger number of dates will be required to address these questions; a sample size of 20-30 was established by an optimization analysis presented as an appendix to this plan. If sample ages tend to be dispersed fairly evenly along the temporal spectrum, then it is probable that the cemetery was used for a relatively long period. If, on the other hand, the ages tend to cluster, a shorter duration of use, or at least a period of most intensive use, is more probable.

Reliable definition of the age range of the cemetery has significant implications for demographic interpretations. Based upon the percentage of the cemetery area excavated and the minimum number of individuals identified with the excavation, it is possible to make a reasonable minimum estimate of the total number of individuals buried there. At this preliminary stage (with only field observations to go on), in light of the estimate that only 25-30% of the cemetery area was excavated, a minimum of 150-200 individuals may have been interred in the Early Archaic cemetery. With our presently limited chronological control, this figure could represent a sizeable living population for a relatively short time span or, alternatively, a smaller group that used the cemetery for centuries or perhaps a millennium or more. Additional chronological data are needed to reliably address this basic issue.

There is some reason to believe that the four presently available AMS dates may be somewhat too young, as they were not run on purified collagen. Recent research suggests that younger organic matter can be introduced into bone samples via groundwater, such that samples of considerable antiquity may produce ages that are several hundred to over 3,000 years too young. Careful sample preparation that removes contaminants, if present, is necessary (e.g., Stafford 2002; Stafford et al. 1991). In the case of the Buckeye Knoll cemetery, certain of the lanceolate dart points that are clearly associated with burials are morphologically similar to types such as Angostura and Thrall (e.g., Kerr and Dial 1998) estimated to fall within an 8,000-9,000-year-old time range. This contributes to our suspicion that the four existing dates may indeed be somewhat too young, and further supports the need for additional AMS assays on purified bone collagen.

DNA Analysis:
Synchronic and Diachronic Genetic Comparisons

The Early Archaic mortuary sample from Buckeye Knoll offers a unique opportunity to assess the biological relationship between this population and other relatively early samples from North America. In large part, this question can be addressed through comparisons of metric data on skulls and teeth. If viable DNA can be extracted from the Buckeye Knoll remains, an additional important comparative data base can be developed.

The primary research questions that can be addressed through DNA studies are:

<u>Research Issue 11</u>: How close is the biological relationship between the Early Archaic population represented at Buckeye Knoll to other populations of similar antiquity in North America?

Comparison of metric and DNA data from the Buckeye Knoll population with other samples from North America, particularly from the Carrier Mills site (Illinois) and the Windover site (Florida) will address this significant issue. Some of the most qualified scientists in the United States have already expressed great interest in contributing to the acquisition and interpretation of the necessary comparative data.

This bears directly on the important question of early population migrations. If the Buckeye Knoll people were biologically closely related to other early groups (such as at Carrier Mills or Windover), then broad migration patterns during the Early Archaic might be indicated. On a the other hand, if there is no close biological relationship, then the populations must have been regionally isolated and might represent distinctly different migrations into each region at a still earlier time, perhaps during the Paleo-Indian period.

The question of degrees of biological relatedness among Early Archaic populations in North America is directly linked to fundamental issues of the timing and routes of early population entries into major regions of the continent. Understanding the spatial/geographic dimension of early population movements and migrations plays a key role in modeling the demographic aspect of early cultural patterns, and is of self-evident importance as a first principle in paleo-anthropological and prehistoric cultural studies. Given the long tradition of these basic questions (going back to the beginning of archaeological inquiry in North American and elsewhere; e.g., Trigger 1990), and the ever-present challenge of obtaining robust data on the subject, any opportunity to elucidate these issues is important. The Buckeye Knoll site is a rare, outstanding example of just this sort of opportunity.

While non-destructive metric and non-metric data play a crucial role in defining between-population biological relationships, DNA studies are offering the possibility to both broaden and refine the relevant data bases to a degree previously unattainable. DNA sequencing can provide a direct window on genetic relationships, whereas the observations/measurements of physical characteristics observable in skeletal materials often reflect environmental and technoeconomic influences to varying degrees.

Research Issue 12: Were the Early Archaic people at Buckeye Knoll related to later inhabitants of the region?

A number of large samples of Late Archaic skeletal populations from the western Gulf coastal plain have been studied and reported, but the unusually early Buckeye Knoll findings give us the first opportunity to examine the possible ancestry of these later peoples and thus to provide a case study in population continuity (or discontinuity) over several thousand years. How closely related the Buckeye Knoll population was to later groups in the region can be evaluated by comparing metric data on teeth and crania from the site with those from later sites such as Ernest Witte (Hall 1981) as well as the Late Archaic individuals from Buckeye Knoll itself. Particularly informative would be comparative DNA from the Early Archaic population at Buckeye Knoll, the later Buckeye Knoll population represented by several burials, and other sites such as Ernest Witte and Loma Sandia (Taylor and Highley 1995).

Thus, DNA analysis provides a unique opportunity to address the question, stated above, of whether the Early Archaic culture and population of the Gulf Coastal Plain can be linked to historic tribal populations of the region. From the perspective of any scientific testing of possible long-term cultural affiliations, this technique potentially offers unprecedented opportunities for clarification. Standard archaeological comparisons between artifact assemblages are more limited, given the obvious shifts in styles and functional forms over the millennia; in all known instances, artifact forms/styles either drop out of the archaeological record, or are too generalized to provide specific information relevant to questions of cultural continuity. As regards bioarchaeological analyses, observable phenotypic characteristics of skeletons may, as noted above, reflect environmental and/or technoeconomic changes as much or more than they do genetic continuity or discontinuity. Given these variables, the potentials inherent in DNA studies should be actualized in the

Buckeye Knoll sample, which is the only one in North America that has produced human skeletal remains that span at least 5,000 years.

Research Issue 13: Was social status achieved during the individual's lifetime, or was it based on membership in a particular lineage?

Given the range and quantity of materials associated with the burials, the Buckeye Knoll site holds the best opportunity from North America to explore this question in any Early Archaic context. This question may be addressed through DNA and other studies. If individuals who had special status (as indicated by accompanying mortuary artifacts), are found to be closely related biologically, this would suggest status according to lineage. If, on the other hand, such individuals are no more closely related to each other than to other members of the group, status was more likely to have been based on the person's social standing achieved during his or her lifetime, regardless of lineage. In addition to DNA studies, observations on specific bone elements, especially teeth and crania, may help to identify biological relatedness between individuals with the group.

Overview of Objectives for DNA Studies of the Buckeye Knoll Samples

A series of published DNA analytic results from human bone are available in existing publications (see Table 1). Additionally, ongoing DNA studies at the University of California and the University of Arizona, funded by the National Science Foundation, will provide an important comparative data base. A comparative data base of several hundred samples from North and Middle America has been generated from this work, and more samples will become available in the near future (David G. Smith, UC-Davis, pers. comm. 2002). Samples in excess of 8,000 years old are few, presently numbering around two dozen. Larger samples for the Early Archaic are available from the Snake River of Idaho, the western Great Basin, and Florida, and more data are expected shortly from Tennessee and Kentucky. A sizeable sample dating to around 4,000 years old is available from the Central Valley of California. More recent samples (dating to later prehistory and early Historic times) are available from across North America, and these may be useful in assessing genetic relatedness between the early population at Buckeye Knoll and later ones in other regions.

Twenty samples will be tested for the presence of preserved DNA. If DNA cannot be extracted from these tests, then no further efforts along these lines are to be recommended. However, if the tests prove positive, a minimum of 5 and a maximum of 20 samples will be submitted for full DNA analysis, with the final number constrained by cost. This sample size was selected on the basis of the optimization analysis presented as an appendix to this plan. The best material for this purpose is tooth pulp (David G. Smith, pers. comm. 2002), which is less likely to be contaminated than bone. A single tooth should provide enough material from a given individual for analysis, and this often can be extracted without otherwise damaging the tooth.

The typical strategy in examinations of ancient DNA (aDNA) is to focus on mitochondrial DNA (mtDNA) rather than nucleic DNA (nDNA) and to focus on haplogroup identification sometimes with secondary sequencing confirming and bolstering haplogroup assignments. However, it should be remembered that other biomolecules also can be identified in archaeological material and other DNA markers are increasingly of scientific interest (Smith et al. 2000; Tuross 1993). Techniques of DNA extraction and isolation are rapidly advancing, but as noted earlier, it is still virtually impossible to predict, *a priori*, if a sample or samples will contain useful DNA.

The majority of both living Native Americans and archaeological materials so far examined typically fall into one of four haplogroup lineages, referred to simply as lineage A, B, C or D (Lorenz and Smith 1994, 1997). A small minority of individuals have also been found to fall either into a fifth group, sometimes referred to as haplogroup X , or simply referred to as 'other'. Some of these 'X' or 'other' cases may be 'real' DNA polymorphism which fail to properly sequence due to molecular damage, some may result from contamination, and some may simply reflect molecular damage associated with specific micro climatic environments and are in fact one of the other more frequently encountered haplogroup types (A,B,C or D) (Hughes et al. 1986;Schmidt et al. 1995).

Objective 1: Sample/Population Characterization

Studies of within-sample diversity provide basic information on the morphological variants within the populations. This is useful information as a purely descriptive element, but also has been shown to be of analytical use in and of itself. Hauswirth et al. (1994) were able to use DNA samples from Windover brain tissue to conclusively demonstrate the genetic relatedness of numerous individuals, showing that Windover represented a single closely related population and was not the accumulation of individuals from different populations over a half millennium. Such interpretations support more traditional archaeological models of mobility and sedentism and population diversity. Additionally, when a large enough series is run, and when detailed sequencing is carried out, distinct genetic lineages within a single population can be identified and this provides unsurpassed information on within population structure and diversity (Stone and Stoneking 1993). This kind of information can be extremely important in understanding internal population structure and can be valuable in partitioning the sample into units for more traditional osteological analysis.

Objective 2: Within-Population Differences

Questions of local population adaptive success and population movements can also be addressed by comparing DNA profiles of the early and late samples at Buckeye Knoll. If they are sufficiently different it could indicate population replacement or changes important to archaeological interpretations. Alternatively, and more likely, and certainly more in concert with the existing archaeological information, quite similar DNA profiles for the time intervals represented at Buckeye Knoll would support a proposition both of population adaptive success and in-place continuity with no population replacement. We know of no other existing context where this specific question could be asked within a single-site sample. Most studies looking at the issue of continuity have examined samples from different sites. In these cases the questions of direct continuity are not as obvious. Hypothetically, careful sequencing of a variety of DNA markers would be able to provide the most precise assessment of 'in-place' evolutionary change.

Objectives 3: Between-Population Comparisons

Population-based analysis of DNA variation/polymorphism has for years been a standard comparative approach in anthropology and human biology (Cavalli-Sforza and Edwards 1967). A variety of statistical tools that allow for the evaluation of population affinity and relatedness have until the last decade been applied to the study of modern populations. Some of these phylogenetic algorithms provide methods of assessing ancestor-descendant population relatedness (Evison 2001). These studies have been important in efforts to understand population history, migration and colonization events and have clear archaeological implications (Greenberg et al. 1986; Malhi et al. 2002; Mauricio et al. 2000; Omoto et al. 1997; Suarez et al. 1985). With the relatively recent identification of ancient DNA (Doran et al. 1986; Paabo 1985) entirely new opportunities to validate and expand such population comparative studies exist. These studies can provide a much greater level of detail with new insights into prehistoric population movements and colonization processes than was heretofore possible (Kaestle and Smith 2001; Malhi and Smith 2002; Schurr et al. 1989).

Some of the broader continental studies of modern DNA polymorphism (in living people), particularly those focusing on European/Middle Eastern issues, contain data on literally thousands of individuals distributed across continents (Goicoechea et al. 2002; Tommasseo-Ponzetti et al. 2002; Torroni et al. 2001). In other studies, more localized and specific questions of population change, movement, etc. are addressed with much smaller samples often numbering in the hundreds of individuals from much smaller and geographically circumscribed regions (Lell et al. 2002; Quintana-Murci 2001).

It is increasingly obvious that sample size, both within populations, and from greater numbers of locations across the landscape, are a critical concern for analytical and interpretive rigor (validity) of the spatial and chronological patterns inferred from DNA analysis. Richards et al. (1993; and many others) call for larger sample sizes to increase interpretive clarity. Proportionally, the issue of sample sizes is a greater problem in the New World where samples even of modern populations rarely exceed five hundred individuals (Batista et al. 1999; Lane et al. 2002; Malhi et al. 2002; Mauricio et al. 2000). Some European studies use DNA data from over 10,000 individuals (Torroni et al. 2001). Increases in sample size and distribution, as well as the diversity of DNA markers studied, will increase analytical rigor in both broad scale and localized interpretations of population change.

Tuross and Kohlman (2002, Table 2), basing inclusions criterion on sample integrity, size and sequencing precision, identified only 19 North American groups, five Central American groups, four South American groups, six African groups, fourteen European samples, nine Asian/Pacific Island samples, and 13 Siberian samples as providing optimal comparative potentials. In reality, using less restrictive inclusion criteria would substantially expand this relatively small list. Regardless of existing sample limitations, perhaps more problematic in the New World than elsewhere, ‘new’ populations are being sequenced all the time and this rapid increase in sample size, diversity and geographic coverage will improve analytical precision and interpretive potentials (Bert et al. 2001; Gonzalez-Oliver 2001; Merriweather et al. 1997). Tuross and Kohlman (2002:Table 2) included only six archaeological samples from North America (regardless of sample size) which could be used in these kinds of studies. In addition to the Tuross and Kohlman's (2002, Table 2) inventory of archaeological samples, other New and Old World samples have been sequenced and are presented in Table 1 (see below). David G. Smith (University of California, Davis), in particular, has been focusing on North American Paleoindian and Early Archaic samples and has significantly expanded the number of samples of potential comparative interest. Furthermore, there are additional samples which are going to be sequenced shortly which are not included in Table 1.

One thing that is clear from Table 1 is that fewer North American samples, in contrast to Central and particularly South American specimens, are being sequenced (Brandt et al. 2002; Lalueza et al. 1997; Varela et al. 2002). Even so, the analytical opportunities for Buckeye Knoll comparative work is expanding rapidly. Eleven sites as old as or older than Buckeye Knoll (representing roughly 40 individuals) have been identified. This also includes at least one other Texas sample and several more are in the process of being sequenced. Additional early materials from South America, Japan, Asia and Siberia are also being sequenced. Sampling is always critical in these studies but Buckeye Knoll's addition to the list of New World early materials will be a valuable contribution to understanding New World genetic evolution and diversity.

Objective 4: Sex Assessment

One of the more promising consequences of expanded DNA analysis is the enhanced ability to address issues of sex assessment in skeletal samples. This is particularly valuable in cases of either poorly preserved skeletal material where normal features and assessment of sexual dimorphism are compromised by deterioration. This can also be valuable in cases were gender assignment is simply ambiguous due to an absence of strong features of sexual dimorphism. Enhancements in sex identification based on DNA are also valuable in assessing gender in subadults where normal skeletal features limit the accuracy of sex assignments because sexually diagnostic features are poorly expressed in immature skeletal systems (Stone et al. 1996). Several strategies have been effective in this approach. Microsatellite differences, Y-chromosome morphology, and sequencing of the amelogenin gene also present approaches to sex assessment in skeletal material (Quintana-Murci et al. 2001). Such strategies may be particularly appropriate for some of the specimens from Buckeye Knoll.

Objective 5: Identification of Diseases

As Tuross and Kohlman (2002) note, there is, when examining archaeological materials, a unique opportunity to provide information on prehistoric pathogenes. This is particularly advantageous because many diseases leave similar gross anatomical records in the skeletal system. In some cases careful differential diagnosis can narrow the pathogens to a smaller list but sometimes such efforts are far from conclusive. Additionally, some diseases do not leave obvious markers in the skeletal system even though they may be endemic. In a few cases, the process of preservation has even been illuminated by the identification of specific bacteria (Rollo et al. 1999). A number of studies are beginning to appreciate the unique potential provided by archaeological specimens. Salo et al. (1994) sequenced the tuberculosis bacteria from prehistoric archaeological samples in South America and the TB baceterium has also been identified in specimens from Europe. Guhl et al. (1999) identified treponemal agents (related generically to syphilis and yaws) in Chilean materials.

Conclusions

There are a variety of very clear analytical potentials with respect to the Buckeye Knoll samples. Preservation of biomolecules is unpredictable and it is not clear any such materials survived the passage of time. If they have, however, a variety of analytical possibilities would be archaeological and scientifically important. Some of these focus entirely on within-sample analysis. Others however, expand the utility of the Buckeye Knoll information to both a continental, New World and even global basis. The opportunity to examine these materials within a multidisciplinary framework is scientifically significant on a regional, national and even global scale (Thomas 1993).

Table 1. Partial Inventory of Archaeological DNA samples (bibliography available upon request).

SITE	DATE	STATE	HAPLOGROUP IDENTIFIED	SAMPLE SIZE	REFERENCE
Kennewick	8410 - 5750 BP	Washington	no recovery	1	Tuross and Kolman 2002
Hourglass Cave	8000 BP	Colorado	Haplogroup B	1	Stone and Stoneking 1996
Pyramid Lake	9515 ± 60 BP	Nevada	A,B,C,D other	21	Kaestle 1997
Norris Farm	700 BP	Illinois	A,B,C,D, other + amelogenin gene	108	Stone and Stoneking 1998; Stone et al. 1996
Unspecified Plains	A.D. 760 to 1200 - 1600	Unspecified	B,C other	5	Kolman and Tuross 2000
Windover	7410 BP	Florida	albumin, A, B, & other	10	Hauswirth et al. 1994; Smith et al. 2002
Ventana Cave	?	?	B & C	3	Handt et al. 1996
Fremont	4000 - 1000 BP	Utah	B,C,D, & other	32	Parr et al. 1996
Arlington Springs	9,300 BP	California	B	1	Smith personal communication

Browns Valley	9049 BP	Minnesota	D	1	Smith personal communication
Cutler Sink	9620 BP	Florida	B	3	Smith personal communication
Horn Shelter	10300-9500	Texas	B	2	Smith personal communication
Pelican Rapid	7840 BP	Minnesota	C	1	Smith personal communication
On Your Knees Cave	9730 BP	Alaska	other	1	Smith personal communication
Wizard's Beach, Pyramid Lakes	9200 BP	Nevada	C	1	Smith personal communication
Little Salt Springs	6500 BP	Florida	xx	1	Paabo et al. 1989
Kaweskar	Mainly contact period	Tierra del Fuego/Patagonia	C,D	19	Fox 1996; Lalueza et al. 1997
Aonikenk (Laguna del Juncal and others)	Mainly contact period	Tierra del Fuego/Patagonia	C,D	15	Fox 1996; Lalueza et al. 1997
Yamaha	Mainly contact period	Tierra del Fuego/Patagonia	C,D	11	Fox 1996; Lalueza et al. 1997
Selk'nam (Cueva Lago Sofia	4030 - 5000 BP	Tierra del Fuego/ Patagonia	C,D other	13	Fox 1996; Lalueza et al. 1997
Pampa Grande	1000-1500 BP	Argentina	A,B,C,D & other	18	Demarchi et al. 2001
Puna region	500 - 900 BP (ambiguous)	Argentina	A,B,C,D & other	8	Demarchi et al. 2001

Tres Arroyos	7000 BP	Argentina	A,B,C,D & other	3	Demarchi et al. 2001
Cordoba	500 - 900 BP	Argentina	A,B,C,D & other	1	Demarchi et al. 2001
Chubut	500 - 900 BP	Argentina	A,B,C,D & other	1	Demarchi et al. 2001
Peru (5 sites)	A.D.115 - 1450	Peru	plasma proteins (albumin, AHSG and PI)	100	Brandt et al. 2002
Maya	?	Mexico	C,D	30	Merriweather et al. 1997
Brazilian Amazon	?	Brazil	A,B,C,D, OTHER	18	Ribeirao-dos-Santos et al. 1996
Japanese			other	10	Horai et al. 1991
Chinese, Yixi Site	2000 BP	China, Shandong Province	B,C, other	23	Oota et al. 1999
Japan, Jomon	6000 - 3000 BP	Saitama and Hokkaido	sequence	5	Horai et al. 1989, Oota et al. 1999
Japan, Yayoi	2000 BP	?	sequence	14	Oota et al. 1995
			TOTAL	481	

General Summary

The Buckeye Knoll site has provided a large sample of archaeological materials from the Texas coastal plain that represent some 10,000 years of culture history. The non-mortuary materials from the stratified midden deposits, when radiocarbon dated and fully analyzed, are expected to offer many fundamental insights into regional chronology, ecology and culture history. The human remains and mortuary artifacts from the Early Archaic component provide a unique record of that period for Texas, and hold considerable potential to elucidate issues of demography, migration and biological affinity in North America through metric analyses of cranial and dental attributes. These broader issues will be more susceptible to investigation if DNA can be recovered from human bone. Questions of the health, stress/trauma and diet in this early population are amenable to investigation through a range of non-destructive and minimally destructive analyses. The basic research issues to be addressed at Buckeye Knoll are listed in Table 2, along with a summary of the materials and analytical techniques more appropriate in each case.

Table 2. Summary of Major Research Issues and Analytical Approaches, the Buckeye Knoll Site, 41VT98.

Research Issue	Materials	Analysis	Recommend-ed No. of Samples
Prehistoric cultural chronology Age/duration of E. Archaic cemetery component	Faunal bone, unmodified shells Human tooth pulp*	Standard radiocarbon dating Purified collage AMS dating	20-30
Diachronic patterns of human adaptation/ecology	Faunal bone	Zooarchaeological analysis	TBD
	Macrobotanical samples (flotation)	Species identifications	TBD
	Floodplain sediment cores	Palynology	Completed
	Unmodified shells, fish otoliths	Species identifications; seasonality studies	TBD
	Lithic, bone, shell and ceramic artifacts	Typological, functional identifications;	All diagnostics
		Residue analyses	30 tools, 15 clay
		Use wear, lithic tools	50
		Debitage analysis (lithic technological org.)	TBD
Possible variable intensity of site occupation (diachronic)	Based on variable densities of ecofacts & artifacts by identifiable components	Quantifications based on collection inventory in conjunction with component identification	TBD
Horizontal intrasite patterning	Based on horizontal variability artifact classes by components	Identification of spatial distributions of materials in relation to features	TBD
Demographic dimension of prehistoric adaptation	Human bone (non-destructive)	Age/sex identifications (metric)	All burials
Population health	Population health	Physical attributes of bone; DNA*	All burials
Stress, trauma	Human bone (non-destructive)	Physical attributes of bone	All burials
Physical characteristics	Human bone, teeth (non-destructive)	Metric attributes of teeth	All burials
Coastal vs. inland adaptations	Human bone	Stable isotopes	20-30
Diet of mortuary population	Sediment from abdominal cavities	Pollen, phytolith identifications	20

Social and ideological patterns	Field data on associations, plus bio-archaeological data on age and sex of individuals	Age/sex data on burials, inventory of associated mortuary artifacts (non-destructive)	All burials

Research Issue	Materials	Analysis	Recommend-ed No. of Samples
Relatedness between E. Archaic populations	Human bone (non-destructive)	Comparative metric data	All burials
	Human teeth*	DNA analysis	5-20
Relatedness of E. Archaic and L. Archaic populations	Human bone (non-destructive)	Comparative metric data	TBD
	Human teeth*	DNA analysis	3

* Requires destruction of small (1 gram) sample; all destructive analyses will be performed on human tooth pulp, if possible. The same sample may be used for AMS, DNA and stable isotope analyses.

TBD = Number of samples to be determined based on preliminary analyses in the case of non-mortuary materials and on preservation in the case of DNA.

References Cited

Albert, B. M.
2002 *Holocene Environmental Change and Changing Conditions for Human Subsistence as Inferred from Geo-Botanical Data from Two Cores in the Lower Guadalupe River Valley, by the Buckeye Knoll Site (41VT98), near Victoria, Texas*. Draft report submitted to the U.S. Army Corps of Engineers, Galveston District.

Bass, W. M.
1995 *Human Osteology: A Laboratory and Field Manual* (Fourth Edition). Special Publication No. 2, Missouri Archaeological Society, Columbia, Missouri.

Batista Dos Santos, Sidney E., J.D. Rodrigues, A.K.C. Ribeiro-Dos-Santos, and M.A. Zago
1999 Differential contribution of indigenous men and women to the formation of an urban population in the amazon region as revealed by mtDNA and Y-DNA. *American Journal of Physical Anthropology* 109:175-180.

Binford, Lewis R.
1964 *Archaeological Investigations of Wassam Ridge*. Southern Illinois Museum Archaeological Salvage Report 71. Carbondale.

1971 Mortuary practices: their study and potential. In*: Approaches to the Social Dimensions of Mortuary Practices*, J. A. Brown, editor. Pp. 6-38. Society for American Archaeology Memoir 25. Washington, D. C.

Bert, Francesco, Alfons Corella, Manel. Gene, Alejandro Perez-Perez, and Daniel Turbon Borrega
2001 Major mitochondrial DNA haplotype heterogeneity in Highland and Lowland Amerindian populations from Bolivia. *Human Biology* 73:1- 16.

Boutton, T. W., M.J. Lynott and M. P. Bumsted
1991 Stable carbon isotope and the study of prehistoric human diet. *Critical Reviews in Food Science and Nutrition* 30:373-385.

Brandt, Elisabeth, Ingrid Wiechmann and Gisela Grupe
2002 How reliable are immunological tools for the detection of ancient proteins in fossil bones? *International Journal of Osteoarchaeology* 12:307-316.

Brown, J. A.
1981 The search for rank in prehistoric burials. In*: The Archaeology of Death, R. Chapman, I. Kinnes and K. Randsborg*, eds., pp. 25-68. Cambridge University Press.

Brown, T. A., R. G. Allaby, K. A. Brown, and M. K. Jones
1993 Biomolecular archaeology of wheat: past, present and future. *World Archaeology* 25(1):67-71.

Buikstra, J. E.
1980 Epigenetic distance: a study of biological variability in the Lower Illinois River Region. In *Early Native Americans*, edited by D. Browman, pp. 271-299. Mouton, The Hague.

1984 The Lower Illinois River Region: a prehistoric context for the study of ancient diet and health. In: *Paleopathology at the Origins of Agriculture*, edited by Cohen, M.H. and Armelagos, G.J. New York: Academic Press, Inc.

Buikstra, J. E. and Cook, D.C.
1980 Paleopathlogy: an American account. *Annual Review of Anthropology* 9:433-470.

Buikstra, J. E., L. W. Konigsberg, and J. Bullington
1986 Fertility and the development of agriculture in the prehistoric Midwest. *American Antiquity* 51:528-546.

Buikstra, J. E. and D. H. Ubelaker

1994 *Standards for Data Collection From Human Skeletal Remains*. Arkansas Archeological Survey Research Series No. 44. Fayetteville, Arkansas.

Cavalli-Sforfza, LL and Edwards, AWF
1967 Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics* 19:233-257.

Chapman, R. and K. Randsborg
1981 Approaches to the archaeology of death. In: *The Archaeology of Death,* R. Chapman, L. Kinnes and K. Randsborg, eds., pp. 1-24. Cambridge University Press.

Charles, D. K., S. R. Leigh and J. E. Buikstra (editors)
1988 *The Archaic and Woodland Cemeteries at the Elizabeth site in the Lower Illinois Valley*. Research Series, Vol. 7. Illinois Dept. of Transportation and Center for American Archaeology, Kampsville, Illinois.

Cohen, M.H. and G.J. Armelagos (editors)
1984 *Paleopathology at the Origins of Agriculture*, edited by Cohen, M.H. and Armelagos, G.J. New York: Academic Press, Inc.

De Niro, M. J. and S. Epstein
1978 Carbon isotopic evidence for different feeding patterns in two Hydrax species occupying the same habitat. *Science* 201:906-907.

1981 Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:3411-351.

Doran, G. H. (editor)
2002 *Windover: Multidisciplinary Investigations of and Early Archaic Florida Cemetery*. University Press of Florida.

Doran, G. H., D. N. Dickel, W. E. Ballinger, Jr., O. F. Agee, P. J. Laipis and W. W. Hauswirth
1986 Anatomical, cellular and molecular analysis of 8,000-yr-old human brain tissue from the Windover archaeological site. *Nature* 323(6091):803-806.

Doran, G. H., C. Stojanowski, and R. Smith
2001 *The Place of Buckeye Knoll (41VT98) Material with Respect to Bioarchaeology*. Draft report prepared for Coastal Environments, Inc. and submitted to the U.S. Army Corps of Engineers, Galveston District.

Evison, Martin P.
2001 Population studies using HLA. *Ancient Biomolecules* 3:167-180.

Fox, C.L.
1996 Mitochondrial DNA haplogroups in four tribes from Tierra del Fuego-Patagonia:

inferences about the peopling of the Americas. *Human Biology* 68:855-871.

Frederick, C. D. and M. D. Bateman
2002 *Geoarchaeological Investigations at 41VT98*. Draft report prepared for Coastal Environments, Inc. and submitted to the U.S. Army Corps of Engineers, Galveston District. On file, Coastal Environments, Inc. (Corpus Christi) and the Galveston District, U.S. Army Corps of Engineers.

Goicoechea, A.S., F. R. Carnese, C. Dejean, S.A. Avena, T.A. Weimer, M.H.L.P. Franco, S.M. Callegari-Jacques, A.C. Estalote, M.L.M.S. Simoes, M. Palatnik, and Francisco M. Salzano
2002 Genetic relationships between Amerindian population of Argentina. *American Journal of Physical Anthropology* 115:133-143.

Gonzalez-Oliver, Angelica, Lourdes Marquez-Mortin, Jose C. Jimenez, and Alfonso Torre-Bianco
2001 Founding amerindian mitochondrial DNA lineages in ancient Maya from Xcaret, Quintana Roo. *American Journal of Physical Anthropology* 116:230-235.

Greenberg, Joseph, Christy Turner, and Stephen Zegura
1986 The settlement of the Americas: a comparison of linguistic, dental, and genetic evidence. *Current Anthropology* 27(5):477-497

Guhl, F.C. Jaramillo, G.A. Valleho, R. Yockteng, F. Cardenal-arroyo, G. Fornaciari, B. Arriaza and Arthur C. Aufderheide
1999 Isolation of trypanosoma cruzi DNA in 4,000-year-old mummified human tissue from northern Chile. *American Journal of Physical Anthropology* 108:401-407.

Hall, G. D.
1981 *Allens Creek: A Study in the Cultural Prehistory of the Lower Brazos River Valley, Texas*. Research Report 61, Texas Archeological Survey, The University of Texas at Austin.

Hard, Robert J. and J. R. Francis
2000 *An Isotopic Dietary Reconstruction of the Texas Hunter-Gatherers*. Proposal to the National Science Foundation. Funded, NSF grant BCS-4459.

Hard, Robert J., N. Katzenberg, T. Stafford and M. Schurr
2002 *Multiple analyses (stable isotopes, radiocarbon, fourine, trace elements) of samples form the Texas coastal plains, Middle Archaic to Early Historic*. Poster presentation, Annual Meeting of the Texas Archeological Society, Laredo, Texas, October 25-27, 2002.

Hauswirth, William W., C.D. Dickel, D.J. Rowold and M.A. Hauswirth
1994 Inter- and intrapopulation studies of ancient humans. *Experientia* 50:585-591.

Heubner, J. A.
1994 Stable isotope analysis of human diets at Mitchell Ridge. In*: Aboriginal Life and Culture on the Upper Texas Coast: Archaeology at the Mitchell Ridge Site, 41GV66,* Galveston Island, by R. A. Ricklis, ed. Pp. 406-416. Coastal Archaeological Research, Inc.

Huebner, J. A. and A. G. Comuzzie
1993 *The Archeology and Bioarcheology of Blue Bayou, A Late Archaic and Late Prehistoric Mortuary Locality in Victoria County, Texas*. Studies in Archeology 9, Texas Archeological Research Laboratory, The University of Texas at Austin.

Hughes, Margaret H., David S. Jones and Robert C. Connolly
1986 Body in the bog but no DNA. *Nature* 323:208.

Kaestle, F.A. and David G. Smith
2001 Ancient mitochondrial DNA evidence for prehistoric population movement: the Numic expansion. *American Journal of Physical Anthropology* 115:1-21.

Kerr, A. C. and S. W. Dial
1998 Statistical analysis of unfluted lanceolate and early bifurcate stem projectile points. In: *Wilson Leonard, An 11,000-year Archeological Record of Hunter-Gatherers in Central Texas, Vol.II: Chipped Stone Artifacts,* M.B. Collins editor and assembler. Pp. 447-506. Studies in Archeology 31, Texas Archeological Research Laboratory, The University of Texas at Austin, and Archeology Studies Program, Report 10, Texas Department of Transportation, Environmental Affairs Division. Austin.

Kolman, C. J. and N. Tuross
2000 Ancient DNA analysis of human populations. *American Journal of Physical Anthropology* 111:5-23.

Lalueza, Carles, Alejandro Peres-Perez, Eva Prats, Luis Cornudella and Daniel Turbon
1997 Lack of founding Amerindian mitochondrial DNA lineages in extinct aborigines from Tierra del Fuego-Patagonia. *Human Molecular Genetics* 6:41-46.

Lambert, JB, Vlasak, SM, Thometz, AC and Buikstra, JE
1982 A comparative study of the chemical analysis of ribs and femurs in Woodland populations. *American Journal of Physical Anthropology* 59:289-294.

Lane, A.B., S. Soodyal, M.E. Ratshikhpha, E. Jonker, C. Freeman, L. Young, B. Morar and L. Toffie
2002 Genetic substructure in South African Bantu-speakers: evidence from autosomal DNA and Y-Chromosome studies. *American Journal of Physical Anthropology* 119:175-185.

Lell, Jeffrey T., Rem I. Sukernik, Yelena B. Starikovskaya, Bing Su, Li Jin, Theodore G. Schurr, Peter A. Underhill, and Douglas C. Wallace
2002 The dual origin and Siberian affinities of Native American Y Chromosomes. *American Journal of Human Genetics* 70:192-206.

Lorenz, J. and D.G. Smith
1994 Distribution of the 9 bp mitochondrial DNA region V deletion among North American Indians. *Human Biology* 66:777-788.

Lorenz, J.G. and D.G. Smith
1997 Distribution of sequence variation in the mtDNA control region of Native North Americans. *Human Biology* 69: 749-776.

Mainfort, R. C., Jr.
1985 Wealth, space and status in a historic Indian cemetery. *American Antiquity* 50(3):555-579.

Mako, S. A.
1981 *Stable Nitrogen Isotope Ratios as Tracers of Organic Geochemical Processes*. Ph.D. dissertation, Department of Marine Sciences, The University of Texas at Austin.

Malhi, Ripan S. and David Glenn Smith
2002 Brief communication: haplogroup X confirmed in prehistoric North America. *American Journal of Physical Anthropology* 119:84-86.

Malhi, Ripan S., Beth A. Schultz, and David Glenn Smith
2001 Distribution of mitochondrial DNA lineages among Native American tribes of northeastern North America. *Human Biology* 73:17 - 55.

Malhi, Ripan S., Jason A. Eshleman, Jonathan A. Greenberg, Deborah A. Weiss, Beth A. Schultz Shook, Frederika A. Kaestle, Joseph G. Lorenze, Brian M. Kemp, John R. Johnson, and David Glenn Smith
2002 The structure of diversity within New World mitochondrial DNA haplogroups: implications for the prehistory of North America. *American Journal of Human Genetics* 70:905-919.

Mauricio, L Moraga, Paola Rocco, Juan F. Miquel, Flavio Nervi, Elena Llop, Ranajit Chakraborty, Franciso Rothhammer and Pilar Carvallo
2000 Mitochondrial DNA polymorphisms in Chilean aboriginal populations: implications for the peopling of the Southern Cone of the Continent. *American Journal of Physical Anthropology* 113:19-30.

Merriweather, D.A. D.M. Reed and R.E. Ferrell
1997 Ancient and contemporary mitochondrial DNA variation in the Maya. In: *Bones of the Maya: Studies of Ancient Skeletons,* S.L. Whittington and D.M. Reed editors, pp. 208-217. Smithsonian Institution Press, Washington, D.C.

O'Leary, M. H.
1988 Carbon isotopes in photosynthesis. *Bioscience* 38:328-336.

O'Shea, J. M.
1984 *Mortuary Variability: An Archaeological Investigation*. Academic Press, Orlando.

Omoto, Keiichi and Naruya Saitou
1995 Genetic origins of the Japanese: a partial support of the dual structure hypothesis. 102:437-446.

Oota, Horoki, Naruya Saitou, Takayuki Matsushita, and Shintaroh Ueda
1999 Molecular genetic analysis of remains of a 2,000-Year-Old human population in China - and its relevance for the origin of the modern Japanese populations. *American Journal of Human Genetics* 64:250-258.

Oota, Kiroki, Naruya, Takayuki Matsushita, and Shintaroh Ueda
1996 A genetic study of 2,000--Year-Old human remains from Japan using mitochondrial DNA sequences. *American Journal of Physical Anthropology* 98:133-145.

Paabo, Svante
1985 Preservation of DNA in ancient Egyptian mummies. *Journal of Archaeological Science* 12:411-417.

Paabo, Svante, John A. Gifford and Allan C. Wilson
1989 Mitochondrial DNA sequences from a 7000-year-old brain. *Nucleic Acids Research*.

Parr, Ryan L., Swawn W. Carlyle, and Dennis H. O'Rourke
1996 Ancient DNA analysis of Fremont Amerindians of the Great Salt Lake wetlands. *American Journal of Physical Anthropology* 99:507-518.

Peebles, C. S. and S. M. Kus
1977 Some archaeological correlates of ranked societies. *American Antiqu*ity 42:421-448.

Quintana-Murci, Luis, Csilla Krausz, Tatiana Zerjal, S. Hamid Sayar, Michael F. Hammer, S. Qasim Mehdi, Qasim Ayub, Raheel Qamar, Aisha Mohyuddin, Uppala Radhakrishna, Mark A. Jobling, Christ Tyler-Smith and Ken McElreavey
2001 Y-Chromosome lineages trace diffusion of people and languages in southwestern Asia. *American Journal of Human Genetics* 68:537-542.

Richards, M., K. Smalley, B. Sykes, and R. Hedges
1993 Archaeology and genetics: analyzing DNA from skeletal remains *World Archaeology* 25(1):18-28.

Richards, Martin and Vincent Macaulay
2001 The mitochondrial gene tree comes of age. *American Journal of Human Genetics* 68:1315-1320.

Ricklis, Robert A.
1992 Aboriginal Karankawan adaptation and Colonial Period acculturation: archeological and ethnohistorical Evidence. *Bulletin of the Texas Archeological Society* 63:211-243.

1993 *A Model of Holocene Environmental and Human Adaptive Change on the Central Texas Coast: Geoarchaeological Investigations at White's Point, Nueces Bay, and Surrounding Area*. Coastal Archaeological Studies, Inc. Corpus Christi, Texas.

1995 Prehistoric occupation of the Central and Lower Texas Coast: a regional overview. *Bulletin of the Texas Archeological Society* 66: 265-300.

1996 *The Karankawa Indians of Texas: An Ecological Study of Cultural Tradition and Change*. University of Texas Press. Austin.

2000 *Proposal for Archaeological Data Recovery, 41VT98, Victoria County, Texas.* Prepared for the U.S. Army Corps of Engineers, Galveston District. Coastal Environments, Inc.

Ricklis, Robert A. and M. D. Blum
1997 The geoarchaeological record of Holocene sea level change and human occupation of the Texas Gulf Coast. *Geoarchaeology* 12(4):287-314.

Rollo, Franco, Stefania Suciani, Adriana Canapa and Isolina Marota
1999 Analysis of bacterial DNA in skin and muscle of the Tyrolean iceman offers new insight into the mummification process. *American Journal of Physical Anthropology* 111:211-219.

Russo, Michael
1996 Southeastern Mid-Holocene coastal Settlements. In*: Archaeology of the Mid-Holocene Southeast*, K. D. Sassaman and D. G. Anderson, eds. University Press of Florida.

Salo, W.L, A.D. Aufderheide, J. Buikstra, and T.A. Holcomb
1994 Identification of Mycobacetrium tuberculosis DNA in a pre-Columbian Peruvian mummy. *Proceedings of the National Academy of Science USA* 91:2091-2094.

Schmidt,T., S. Hummel and B. Herrmann
1995 Evidence of contamination in PCR laboratory disposables. *Naturwissenschaften* 82423-82431.

Schoeninger, M. J. and M. J. De Niro
1984 Nitrogen and carbon isotope composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* 48:625-639.

Schurr, T.G. S.W. Ballinger, Y. Gan, J.A. Hodge, D.A. Merriwether, D.N. Lawrence, W.C. Knowler, K.M. Weiss, D.C. Wallace
1989 Paleoindian Mitochondrial DNAs have rare Asian mutations at high frequencies suggesting a limited number of founders. *American Journal of Human Genetics* 46:613-623.

Schwarcz, H. P.
1991 Some theoretical aspects of isotope paleodiet studies. *Journal of Archaeological Science* 18:261-275.

Service, E.
1971 *Primitive Social Organization: An Evolutionary Perspective* (second edition). Random House. New York.

Silva, Jr., Wilson A., Sandro L. Bonatto, Adriano J. Holanda, Andrea K. Ribeiro-dos-Santos, Beatriz M. Paixao, Gustavo H. Goldman, Kyoko Abe-Sandes, Luis Rodgriquez-Delfin, Marcela Barbosa, Mari Luiza Paco-Larson, Maria Luiza Petzl-Erler, Valeria Valente, Sidney E.B. Santos, and Marco A. Zago
2002 Mitochondrial genome diversity of Native Americans supports a single early entry of found populations in America. *American Journal of Human Genetics* 71:187-192.

Smith, B. N. and S. Epstein
1971 Two categories of 13C/12C ratios for higher plants. *Plant Physiology* 47:380-384.

Smith, David Glen, Joseph Lorenz, Becky k. Rolfs, Robert L. Bettinger, Brian Green, Jason Eshlemen, Beth Schultz, and Ripan Malhi
2000 Implications of the distribution of Albumin Naskapi and Albumin Mexico for New World prehistory. *American Journal of Physical Anthropology* 111:557-572.

Smith, David Glenn, Ripan S. Malhi, Jason Eshleman, Joseph G. Lorenz and Frederika A. Kaestle
1999 Distribution of mtDNA haplogroup X among Native Americans. *American Journal of Physical Anthropology* 110:271-284.

Smith, David Glen, B.K. Rolfs, R. Kaestle, R. Mahli and Glen H. Doran
2002 Serum albumin phenotypes and a preliminary study of the Windover mtDNA haplogroups and their anthropological significance, 395 - 411, In: *Windover: Multidisciplinary Investigations of an Early Archaic Florida Cemetery*, Glen H. Doran (editor and contributor). University Presses of Florida, Gainesville.

Stafford, Thomas W., Jr.
2002 *Biogeochemistry of Fossil Bones used for Chronological and Paleodietary Analyses: Factors Affecting the Accuracy of Radiocarbon Mmeasuremen*ts. Stafford Research Laboratories, Inc. Boulder, Colorado.

Stafford, Thomas W., Jr., P.E. Hare, L. Currie, A.J.T. Jull and D. J. Donahue
1991 Accelerator radiocarbon dating at the molecular level. *Journal of Archaeological Science* 18:35-72.

Stafford, Thomas W., Jr., H. A. Semken, Jr., R. W. Graham, W. F. Klippel, A. Markova, N. G. Smirnov, and J. Southton
1999 First accelerator mass spectrometry 14C dates documenting contemporaneity of nonanalog species in late Pleistocene mammal communities. *Geology* 27:903-906.

Steele, D. G. and C. A. Bramblett
1988 *The Anatomy and Biology of the Human Skeleton*. Texas A & M University Press, College Station, Texas.

Steele, D. G. and J. F. Powell
1993 Paleobiological evidence for the peopling of the Americas: a morphometric view. In *Method and Theory for the Investigating the Peopling of the Americas*, edited by, R. Bonnichesen and D.G. Steele, pp. 141-164. Center for the Study of the First Americans, Corvallis, Or

Stone, A. C. and M. Stoneking
1996 Genetic analyses of an 8000 year-old native American skeleton. *Ancient Biomolecules* 1:83 - 87.

Stone, A. C., G. R. Milner, S. Pääbo, and M. Stoneking
1996 Sex determination of ancient human skeletons using DNA. *American Journal of Physical Anthropology* 99:231-238.

Stone, Anne C. and Mark Stoneking
1993 Ancient DNA from a pre-columbian Amerindian population. *American Journal of Physical Anthropology* 92:463-471.

Stone, Anne C., George R. Milner, Svante Paabo and Mark Stoneking
1996 Sex determination of ancient human skeletons using DNA. *American Journal of Physical Anthropology* 99:231-238.

Suarez, Brian K., Jill D. Crouse, and Dennis H. O'Rourke
1985 Genetic variation in North Amerindian populations: the geography of gene frequency. *American Journal of Physical Anthropology* 67:217-232.

Taylor, A. J. and C. L. Highley
1995 *Archeological Investigations at the Loma Sandia Site (41LK28): A PrehistoricCemetery and Campsite in Live Oak County, Texas*. Studies in Archeology 20, Texas Archeological Research Laboratory, The University of Texas at Austin.

Thomas, K.D.
1993 Molecular biology and archaeology: a prospectus for inter-disciplinary research. *World Archaeology* 25:1-17.

Tieszen, L. L. and T. W. Boutton
1989 Stable carbon isotopes in terrestrial ecosystem research. In*: Stable Isotopes In Ecological Research*. P. W. Rundel, J. R. Ehleringer and K. A. Nagy, editors. Pp. 167-195. Springer-Verlag, New York.

Tommaseo-Ponzetta, M., M. Attimonelli, M. De Robertis, F. Tanzariello and C. Saccone
2002 Mitochondrial DNA variability of West New Guinea populations. *American Journal of Physical Anthropology* 117:49-67.

Torroni, Antonio, Hans-Jurgen Bandelt, Vincent Macaulay, Martin Richards, Fulvio Cruciani, Chiara Rengo, Vincente Martinex-Cabrera, Richard Villems, Toomas Kivisild, Ene Metspalu, Juri Parki, Helle-Viivi Tolk, Kristiina Tambet, Peter Forster, Bernd Karger, Paolo Francalacci, Pavao Rudan, Branka Kaniciejevic, Olga Rikards, Marja-Liisa Savontaus, Kirsi Huoponen, Virpi Laitinen, Satu Koivumaki, Bryan Sykes, Eileen Hickey, Andrea Novellotto, Pedro Maral, Daniele Sellitto, Alfredo Coppa, Nadia Al-Zaheri, A Silvana Santachiara-Benerecetti, Ornella Semino and Rasaria Scozzari
2001 A signal, from Human mtDNA, of Postglacial recolonization in Europe. *American Journal of Human Genetics* 69:844-852.

Trigger, Bruce G.
1990 *A History of Archaeological Thought*. Cambridge University Press.

Turner, Ellen Sue and T. R. Hester
1999 *Stone Artifacts of the Texas Indians* (third edition). Gulf Publishing Company, Houston.

Tuross, Noreen
1993 The other molecules in ancient bone: non-collagenous proteins and DNA. In: *Prehistoric Human Bone: Archaeology at the Molecular Level*, 275-292, by Joseph B. Lambert and Gisela Grupe (editors). Springer-Verlag, New York.

Tuross, N., M. L. Fogel, L. Newsom and G. H. Doran
1994 Subsistence in the Florida Archaic: The stable-Isotope and archaeobotanical evidence from the Windover site. *American Antiquity* 59:288-303.

1997 Organic preservation at Monte Verde. In: *Monte Verde: A Late Pleistocene Settlement in Chile, vol. 2: The Archaeological Context and Interpretation*, edited by T. D. Dillehay, pp. 73–83. Smithsonian Institution Press, Washington, D.C.

Tuross, N. and C. Kohlman
2002 Potential for DNA testing of the human remains from Columbia Park, Kennewick, Washington. National Park Service Archeology and Ethnography Program, www.cr.nps.gov/aad/kennewick/tuross_kohlman.htm.hltp://www.cr.nps.gov/aad/kennewick/tuross_kohlman.htm> .

U. S. Army Corps of Engineers, Galveston District
2002 Draft Proposal, The Buckeye Knoll Site (41VT98). Prepared for parties consulting under Section 106, National Historic Preservation Act.

Varela, Hector Hugo and Jose Alberto Cocilovo
2002 Genetic drift and gene flow in a prehistoric population of the Azapa Valley and Coast, Chile. *American Journal of Physical Anthropology* 118:259-267.

Verano, J. W. and D. H. Ubelaker (editors)
1992 *Disease and Demography in the Americas*. Smithsonian Institution Press, Washington, D.C.

Wada, E.
1980 Nitrogen isotope fractionation an its significance in biogeochemical processes occurring in marine environments. In: *Isotope Marine Chemistry*, E. D. Goldberg, Y. Horibe and K. Saruhaske, editors. Pp. 375-398. Uchida Rokakuho, Tokyo.

Weinstein, Richard A.
1992 *Archaeology and Paleogeography of the Lower Guadalupe River/San Antonio Bay Region: Cultural Resources Investigations Along the Channel to Victoria, Calhoun and Victoria Counties, Texas*. Coastal Environments, Inc. Baton Rouge, Louisiana.

1994 *Archaeologial Investigations along the Lower Lavaca River, Jackson County, Texas: The Channel to Red Bluff Project*. Coastal Environments, Inc. Baton Rouge, Louisiana.

2003 E*arly Archaic through Late Prehistoric Settlement along the Lower Lavaca River: Archaeological Data-Recovery Investigations at the Possum Bluff and Kendrick's Hill Sites, Jackson County, Texas*. Report in preparation, to be submitted to the U.S. Army Corps of Engineers, Galveston District in 2003. Coastal Environments, Inc. Baton Rouge, Louisiana.

APPENDIX
Sample Sizes for Addressing Bioarchaeological Questions at 41VT98:
A Set of Empirical Tests

Robert A. Ricklis

Introduction

As discussed in the foregoing Treatment Plan, certain key bioarchaeological questions concerning the Buckeye Knoll findings can be addressed only through minimally destructive analytical techniques, namely, AMS radiocarbon dating, stable isotope analysis, and DNA analysis. As already noted, these questions are non-trivial, and thus should be approached using a sampling strategy that can provide the basis for reasonable interpretations. This raises the question of what constitutes adequate sample sizes for addressing the stated research questions.

Basically, there are two approaches to determining adequate sample sizes (Pape and Patton 1996:151-152). The first is to imitate sample sizes used in previous studies that have produced generally accepted results. The second is to statistically define the level of accuracy needed to answer a given research question at a predetermined level of confidence.

As for the first approach, sample sizes used to define the temporal boundaries and otherwise analyze prehistoric cemeteries vary considerably, and neither the rationales for sampling sizes nor their statistical reliability are generally explicitly discussed. Non-destructive analyses often include entire recovered burial populations, though the level of detail varies considerably, for a variety of reasons (c.f. Hall 1981; Huebner and Comuzzie 1993; Doran 2002). Sample sizes for the destructive techniques vary greatly and are usually limited by Native American concerns and budgetary constraints. For these reasons, it is difficult to get a clear sense of what comprises a truly adequate sample size based solely on past experience. We can note, however, examples of two cemetery studies that appear to have produced satisfactory results. The Windover Site, an Early Archaic cemetery in Florida (Doran 2002:59), has yielded 14 radiocarbon dates, with 13 (93%) falling between 7100 and 8120 B.P. This clustering led to the conclusion that the cemetery can be confidently placed within a 1,000-year time span. At the Mitchell Ridge site on the upper Texas coast, a sample of 20 radiocarbon dates served to place a total of 51 burials within time periods with sufficient accuracy to permit significant inferences about patterns of cultural change over some 1500 years (Ricklis 1994:463-466). For establishing basic chronological parameters with some confidence, around 15-20 radiocarbon dates appear to provide a useful sample, insofar as the results and interpretations have been accepted by both peer reviewers and in general by the archaeological community.

As shown in Table 1 in the Treatment Plan, most DNA sampling of prehistoric North American sites has involved very small sample sizes. Obviously, little can be learned about the proportional representation of various haplogroups at a specific site from very small samples. At the Windover site, a larger set of 19 samples was sufficient to provide a useful measure of genetic homogeneity of the population, as most individuals were placed within the X haplogroup (Smith et al. 2002).

In the second approach, wherein adequacy of sample size is determined by the statistical level of confidence thought appropriate to the questions being asked, a reliable prediction can be made based upon *a priori* knowledge of the representations of key variables in the total sampling universe (e.g., where previous analyses have been performed on the sampling universe in question [e.g., Cochran 1977:5-10]). For example, it is possible to statistically determine adequate sample size if we wish to know the proportional representation (i.e., percentage) of two variables (such as two haplogroups or the proportional representation of individuals with coastal vs. inland diets) within the available burial population. However, if, as in the present case, we do not know the actual number of variables represented, the prediction of sample-size needed to confidently ascertain relative percentages cannot be reliably made on a mathematical basis.

On the other hand, it is possible to establish *hypothetical models* of the basic patterns or representation of variables within the sampling universe, and then to test the number of sample units (n) required to identify those patterns or representations. In the case of the Buckeye Knoll burials, the sampling universe (N) is inherently limited by the number of burials (N=79) available for study. This places intrinsic limits on the kinds of questions that can be answered. Since the 79 burials presumably represent a far greater number of individuals who actually lived in the region during the Archaic (and are only an undetermined fraction of the burials at the site itself), and do not appear to represent all possible time periods, the full range of biological variability of people(s) who may have occupied the site, or had a sociocultural or biological connection to the individuals buried there, is likely not represented. Realistically, then, our sampling strategies must be designed to identify *basic patterns or key variables* that are likely to be represented within the limitations of N. Rare attributes or extreme outliers within a distribution of attribute variability should not and cannot be expected to necessarily be identifiable. Moreover, accurate percentages or proportional representation of variables such as genetic haplogroups may not be possible, if in fact there are several such variables represented within N; in general, a sample size much larger than 79 is required to obtain relatively small margins of error at high confidence levels (e.g., to determine within a margin of error of +/-5%, at a 95% confidence level, the proportional representation of only two variables, a sample size (n) of over 200 is required (Pape and Patton 1991:156-162). Clearly, the questions that can be asked of the Buckeye Knoll mortuary materials must be realistically framed with this in mind.

The Basic Research Issues

The questions/issues formulated in the attached Treatment Plan are susceptible to elucidation within the constraints of our sampling universe of 79 burials. Specifically, these questions are:

1. What is the temporal range of the Early Archaic cemetery and how tightly do the burials cluster in time? As shown below, this can be answered with confidence using a fraction (approx. 25-40%) of the available burial population.

2. How were the Early Archaic peoples buried at Buckeye Knoll related to other early populations in North America and to later (Late Archaic) people buried at the site? Addressing this question relies significantly on the identification of mitochondrial DNA haplogroups (which can fall into one or more of five groups A, B, C, D, and X). If DNA is adequately preserved (as indicated by positive results in the initial testing) for haplogroup identification, the question that must be addressed is, which and how many haplogroups are represented? The presence/absence and number of the various haplogroups is the basis for genetic comparisons with data from other sites/regions and time periods. As shown below, the presence/absence of the various haplogroups can probably be identified with limited sampling; on the other hand, even analysis of the entire available burial population (N=79) would probably not serve to accurately reveal the proportional representations of three or more such groupings.

3. What were the basic dietary patterns represented in the Buckeye Knoll mortuary population? As noted in the previous pages of this Treatment Plan, this issue can be addressed through the use of stable isotope data. Existing data bases (e.g. Hard et al. 2002) show that three basic dietary patterns are identifiable for the Texas coastal plain and adjacent regions after Middle Archaic times (coastal-marine, prairie-riverine, and inland-central Texas). Also highlighted above is the importance of the Early Archaic bioarchaeological materials from Buckeye Knoll for identifying the time depth of these patterns as well as indicating the range of environmental mosaics exploited during the Early Archaic. In the presentations that follow, it is apparent that the three variables are readily identifiable using a fraction of the available burials from the site.

Procedures

The tests are designed to show what sample sizes can provide redundancy between sampling results on the one hand, and sets of pre-established (i.e., known) hypothetical patterns on the other. The procedures employed here were simple and involved three steps, as follows:

1. A hypothetical pattern or set of variables was established using a sampling universe (N) of 70 (a rounding off of the 73 Early Archaic burials that comprise the most critical focus of interest). These are:

   a. A pattern of temporal distribution of burials in which all 70 are plotted evenly along a hypothetical time line of 1300 years, that is, from 6200 B.P. to 7500 B.P. This distribution effectively represents an idealized, even distribution of the burials through time. Note that the time range of 1300 years is based on the four radiocarbon dates obtained initially, while the actual time span may be longer, it is probably not greatly so, given the discrete stratigraphic context of the cemetery and the apparently distinctive cultural pattern represented by the mortuary artifact assemblage.
   b. A clearly contrasting scenario in which the pattern of temporal distribution of the 70 burials was arbitrarily clustered around 6850 B.P., with 68% of the burials falling within 215 years of that date and the remaining 32% falling into the

remaining time span between 6200 and 7500 B.P. (thus roughly approximating a normal distributional curve around the date of 6850 B.P.) The point here is to determine the number of dated burials required to identify a non-diffuse or clustered temporal range for cemetery use.

c.. An arbitrary scenario in which three variables—which could in effect be either three DNA haplogroups or three dietary patterns—were present in distinctly different percentages in the total sampling universe of 70 burials.

d. A contrasting scenario in which five variables—the five genetic haplogroups-- are present in approximately equal percentages within the sampling universe of 70 burials.

2. A table of numbers was constructed for each of these four scenarios, as follows
   a. In the first table, each number from 1 through 70 had a corresponding radiocarbon date among a total of 70 such dates that were evenly distributed (i.e., separated by 18-year intervals) throughout the above-mentioned 1300-year time span (see Figure 1, top graph).
   b. In the second table, each of the numbers 1 through 70 was assigned to a corresponding radiocarbon date within the pattern wherein the 70 dates were clustered around 6850 B.P. (see Figure 2, top graph).
   c. In the third table, each of the numbers 1-70 corresponded to one of three variables, but in differing proportions (i.e., variable 1 was represented by the percentage of the 70, variable 2 by a smaller percentage, and variable 3 by the smallest percentage (see Figure 3, top graph).
   d. Finally, the fourth table correlated the numbers 1-70 with five different variables (e.g., the five Native American haplogroups), each with about the same percentage (20%) representations within the sampling universe of 70 burials (see Figure 4, top graph).

3. For the four sets of empirical tests that were conducted, numbers 1 through 70 were written on identical cardboard plugs. These were then drawn at random, one at a time, in sets of 10, 20, 30 and 40. That is, in each set of tests, as shown in Figures 1-4, four sets of samples were drawn, one set consisting of 10 randomly drawn numbers, one of 20 randomly drawn numbers, one of 30 and one of 40. In the case of each random drawing, the selected number was correlated to a date or variable on the pertinent table, and this date or variable was used to construct the graphic data shown for each test in Figures 1-4.

   It should be noted that similar empirical tests to those presented here will inevitably produce at least slightly different results, since each set of randomly selected samples will likely differ from those reported here. However, the fact that pattern recognition (as approximate replication of the established pattern used in each test) was obtained with sample sizes of 20-30 strongly suggests that this range in sample size is adequate for identifying basic patterns from which reasonably confident inferences can be made.

Results and Implications

The results of the random selection of samples from the sampling universe of 70 are shown in the accompanying figures. The implications of the results are as follows:

Figure 1: Empirical tests for sample-size reliability where ages of burials are non-clustered. This test assumes an even distribution of burial ages over a 1300-year time span. As may be seen in Figure 1, a random sampling of 10 burials produces a distributional pattern that is ambiguous insofar as there is no clear pattern that indicates whether the true ages of all 70 burials are temporally clustered or diffuse. When the sample size is increased to 20, the diffuse pattern of the burial ages can reasonably be inferred, since the dates span the range between ca. 6200 and 7400 B.P. without any clear clustering. With a sample size of 30, this pattern is clearly expressed and is thus redundant as a representation of the actual, diffuse pattern. Increasing the sample size to 40 provides essentially similar results. In sum, a sample size in the range of 20-30 appears, on the basis of this test, to produce a reasonably accurate reflection of the actual pattern of evenly distributed ages for the 70 burials in the sampling universe.

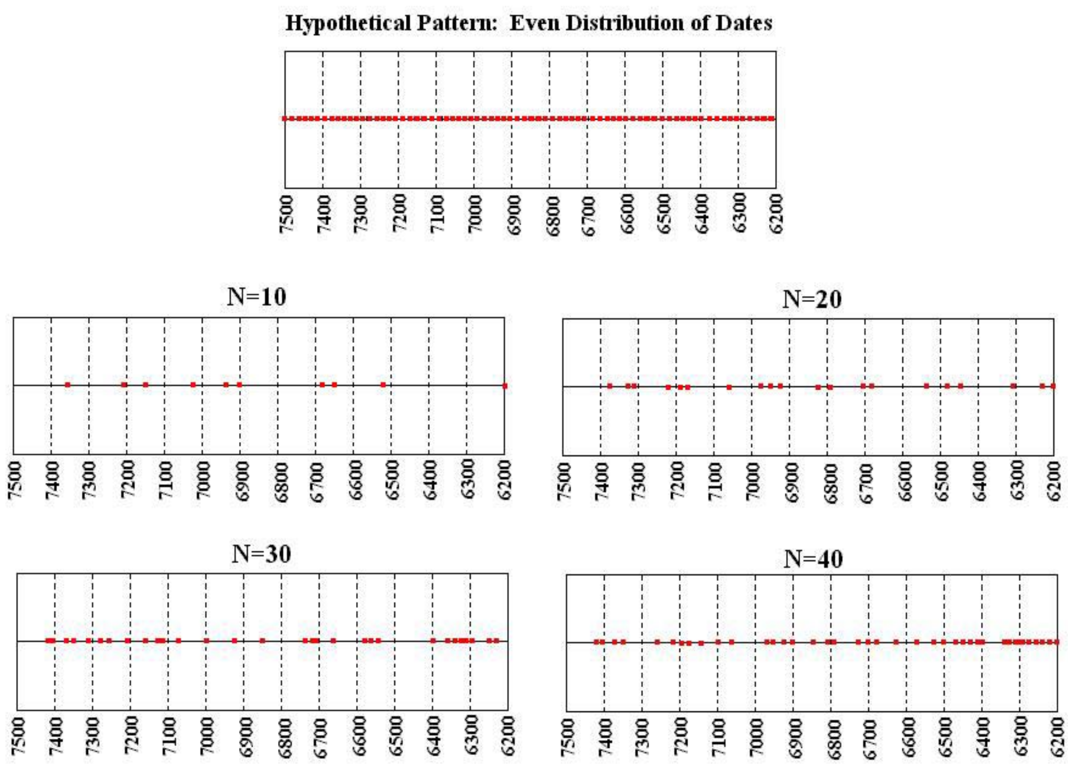


Figure 1. Diagrams showing results of empirical tests for replication of patterning of 70 radiocarbon dates (red dots) that are hypothetically evenly distributed over a 1300-year time span. Top diagram shows the hypothetical distribution, while the other four diagrams show the results of randomly selected samples of 10, 20, 30 and 40 dates.

Figure 2: Shown here are the results of empirical tests for sample-size reliability where the ages of the 70 burials are significantly clustered around 6850 B.P. such as to

roughly approximate a normal (bell-shaped) distribution. As may be seen in the diagrams, a sample size of 10 does suggest the clustered pattern, though it leaves ambiguity in that two outlier dates around 6300 B.P. could easily be inferred to represent a secondary cluster, which is in fact not the case. With a sample size of 20, the actual patterning is more clearly revealed, with most dates falling between 6600 and 7000 B.P. The central clustering is more strongly expressed with a sample of 30, and the fact that a substantial minority (32%) of the burials fall outside that cluster is also apparent. Similar results are apparent when the sample size is increased to 40. In sum, it is apparent that a sample size of 20-30 is sufficient to identify the basic pattern which is that the majority of the burials in the sampling universe fall within about 200 years of 6850 B.P. while a minority are found outside that range.

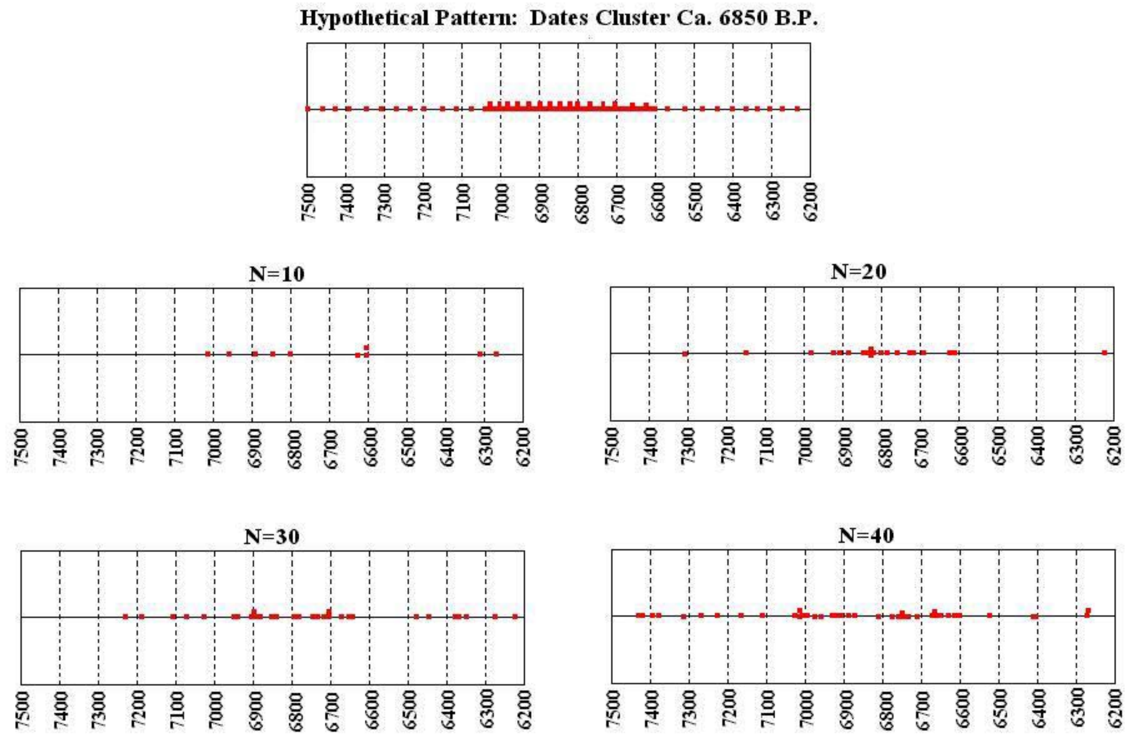


Figure 2. Diagrams showing results of empirical tests for replication of patterning of 70 radiocarbon dates (red dots) that are hypothetically clustered around 6850 B.P. Top diagram shows the hypothetical clustered distribution, while the other four diagrams show the results of randomly selected samples of 10, 20, 30 and 40 dates.

Figure 3: This series of bar graphs suggests that the presence/absence of three variables, and even the proportional representation of those variables, can be identified with a sample of 10-20. In fact, the actual pattern is approximately replicated by all four sample sizes of 10, 20, 30 and 40. While the approximately accurate representation with the sample size of 10 may be a fortuitous outcome in this particular empirical test, the results do suggest that the presence of three variables (e.g., dietary patterns or haplogroups) will be identified with a limited sample size. In fact, a second set of tests [results available upon request] was conducted and produced essentially similar results, although with somewhat more divergence from the actual pattern.

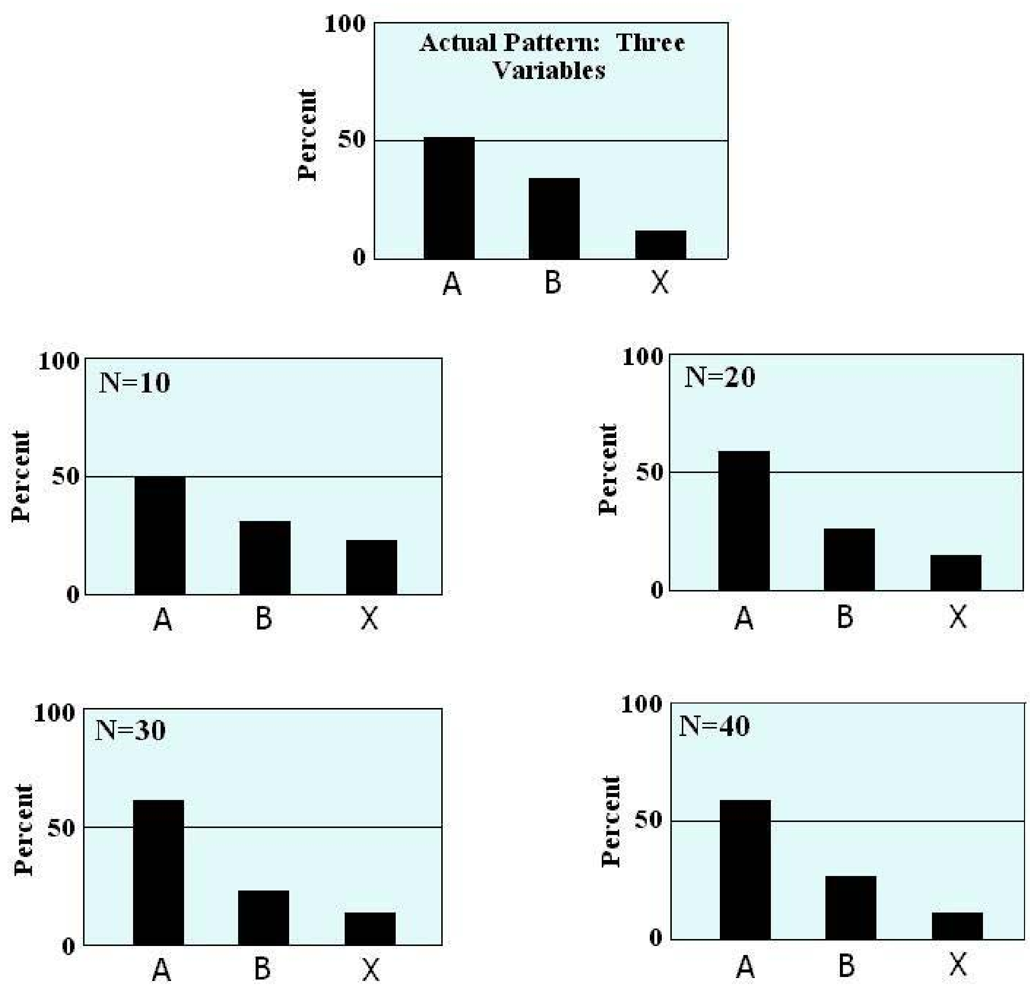
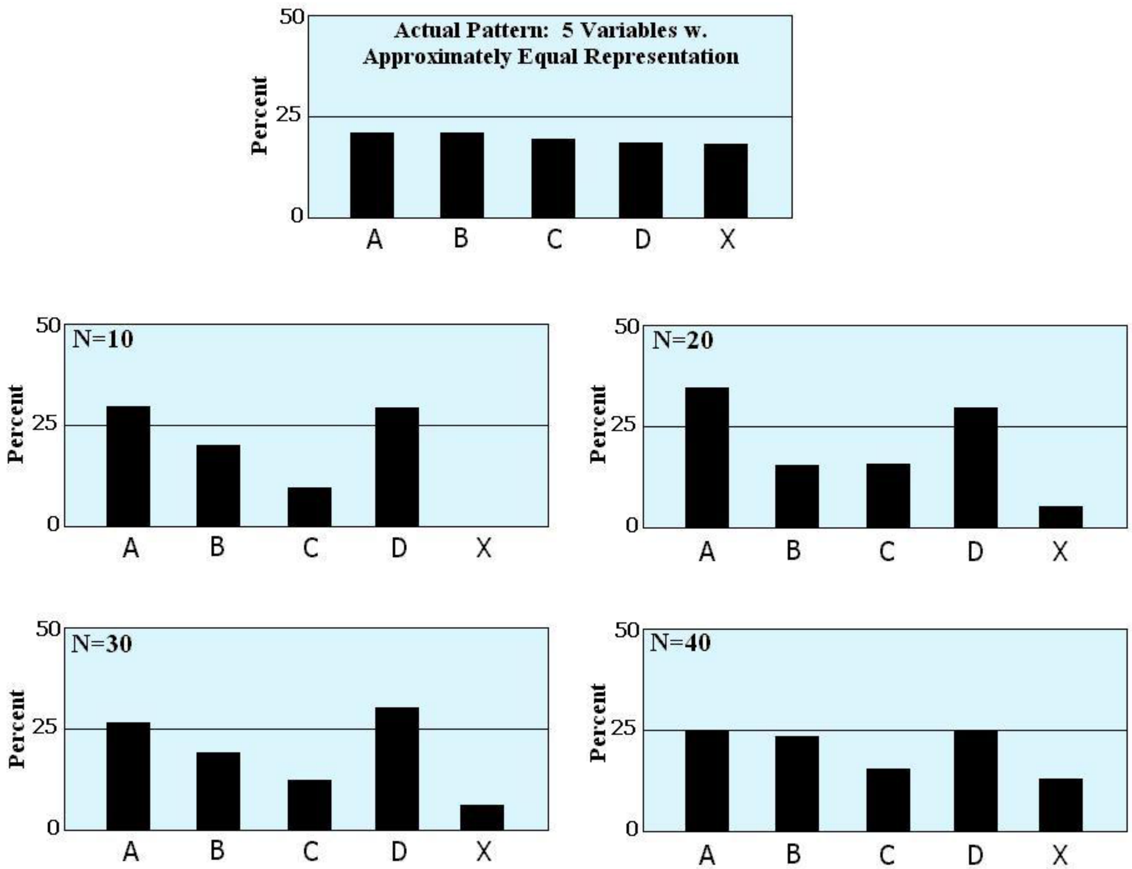


Figure 3. Bar graphs showing results of empirical tests for replication of proportional representation of three variables within a sampling universe of 70 (shown in top graph) using sample sizes of 10, 20, 30 and 40. Note that in the case of 41VT98, the three variables can be either mitochondrial DNA haplogroups (as suggested here by A, B, and X) or three major dietary patterns know for the later Archaic of the region based on stable isotope anlayses.

Figure 4. Here the bar graphs show test results when the actual pattern involves five variables, each of which has approximately equal representation within the sampling universe of 70. In the case of the sample of 10, four of the five variables are identified, while



all are represented by a sampling of 20. As sample size increases, the relative

Figure 4. Bar graphs showing results of empirical tests for replication of proportional representation of five variables within a sampling universe of 70 (shown in top graph) using sample sizes of 10, 20 , 30 and 40. The hypothetical variables represented here are the five mitochondrial DNA haplogroups known for Native American populations.

proportions of the five variables more closely approach the actual representation in the sampling universe of 70. However, even with 40 samples, it is not clear that all five variables are almost equally present. Thus, these empirical tests suggest that in terms of presence/absence of five variables (e.g., five haplogroups), a relative small sample size such as 10 will not necessarily be adequate. The results also suggest that, while all five variables can be recognized with a sample size of 20 or so, even relatively large samples may not accurately reflect that relative proportions or percentages that each of the five variables constitutes in the entire sampling universe.

The latter limitation should be evaluated within the realistic probability that even the entire sampling universe of 70 burials (or precisely 73 in the site's Early Archaic cemetery) is comprised of too small a number to clearly reflect the genetic variability (e.g., representation of haplogroups) of the Early Archaic population associated with the Buckeye Knoll site. Thus, even if all 73 burials were successfully tested for DNA, it is statistically improbable that the proportions of representation of each haplogroup would be revealed accurately.

Thus, it is realistic to limit the DNA inquiry to the basic, and probablistically answerable question of the *heterogeneity vs. homogeneity* of haplogroups within the Buckeye Knoll burials. This accords with the useful results obtained at the Windover site, where 19 DNA samples were sufficient to infer relatively low genetic diversity (Smith et al. 2002). Given the just-discussed improbability that relative proportions can be reliably discerned with N=73, there is no apparent advantage in utilizing all burials for DNA analysis, since even this number of sampling units would not allow us to address additional questions.

Conclusions

Several conclusions can be offered based on these results, as follows:

1. Basic patterning in the AMS dating will take the form of either one or more clusters of dates or a more even, diffuse pattern. The tests suggest that such basic patterns can be identified with a sample size of around 20-30 dates.
2. The presence/absence and approximate proportional representation of three or less variables, such as dietary patterns or DNA haplogroups, can be identified with a sample size of around 20.
3. A higher number of variables (e.g., up to five haplogroups) can be identified in terms of *presence/absence* with a sample size of around 10-20. However, the proportional (percentage) representation of this many variables cannot be determined with confidence with this or even substantially larger sample sizes (as noted above, in a sample with only two variables, a high (e.g.95%) confidence level with a narrow margin of error requires than n is at least 200). The total sampling universe of 79 burials is too small to address this question with statistical confidence.

References Cited

Cochran, W. G.
1977 *Sampling Techniques* (Third Edition). John Wiley and Sons.

Doran, Glen H.
2001 *Windover: Multidisciplinary Investigations of an Early Archaic Florida Cemetery*. University Press of Florida.

Hall, Grant D.
1981 *Allen's Creek: A Study in Cultural Prehistory of the Lower Brazos River Valley, Texas*. Research Report 61, Texas Archeological Survey, The University of Texas at Austin.

Huebner, J. A. and A. G. Comuzzie
1993 *The Archeology and Bioarcheology of Blue Bayou, A Late Archaic and Late Prehistoric Mortuary Locality in Victoria County, Texas*. Studies in Archeology 9, Texas Archeological Research Laboratory, The University of Texas at Austin.

Pape, C. W. and C. V. Patton
1991 *Quick Answers to Quantitative Problems*. Academic Press.

Ricklis, R. A. (editor)
1994 *Aboriginal Life and Culture on the Upper Texas Coast: Archaeology at the Mitchell Ridge Site, 41GV66, Galveston Island*. Coastal Archaeological Research, Inc. Corpus Christi, Texas.

Smith, D. G., B. K. Rofi, F. Kaestle, R. S. Malhi and G. H. Doran
2002 Serum Albumin Phenotypes and a Preliminary Study of the Windover mtDNA Haplogroups and Their Anthropological Significance. In: *Windover: Multidisciplinary Investigations of an Early Archaic Florida Cemetery*, Glen H. Doran, editor, pp. 241-249. University Press of Florida.