# ARLINGTON SPRINGS REVISITED

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## ABSTRACT

In 1959 Phil C. Orr of the Santa Barbara Museum of Natural History discovered two human femora eroding from a depth of 11 m in an arroyo wall at Arlington Canyon, Santa Rosa Island, California. Subsequent radiocarbon dating produced an age of ca. 10,000 yr before present (BP) for associated charcoal and a portion of the bone itself. We have analyzed samples from the Arlington Springs Site, CA-SRI-173, using modern advances in bone chemistry analysis and radiocarbon dating. Two different bone proteins were used for our radiocarbon dating tests – collagen and osteocalcin. These two very different biomolecules produced results that differed by several thousand years from an age of 6,610 ± 60 on formic acid-extracted osteocalcin to the oldest age measurement of 10,960 ± 80 on XAD-decalcified collagen. Dates derived from charcoal and rodent bones from the stratum that yielded the human bones produced age estimates of 10,000 yr to 11,500 yr BP.

**Keywords**: Santa Rosa Island, radiocarbon dating, Pleistocene, paleoindians.

## INTRODUCTION

The Arlington Springs Site on Santa Rosa Island, CA-SRI-173, is one of a few archaeological discoveries that have been radiocarbon-dated to the terminal Pleistocene in insular California (Erlandson 1994; Erlandson et al. 1996a, 1996b; Glassow et al. 1982-1983; Morris and Erlandson 1993). In 1959 Phil C. Orr discovered two human femora partially exposed in sediments 11 m deep in the arroyo wall of Arlington Canyon. In 1960 after convening a team of scholars from several scientific disciplines to examine the archaeological and stratigraphic context of this find, an irregular block of earth (60 cm x 40 cm x 33 cm) was removed containing the bones. Prior to archiving this block of earth in a plaster jacket, a portion of the bone was removed for subsequent dating and chemical analysis (Orr 1962a, 1962b, 1968; Oakley 1963). An age of 10,000 yr BP was estimated for the femora based on radiocarbon dates on charcoal from the stratum yielding the human bones and on one of the femora (Berger and Protsch 1989; Olson and Broecker 1961; Orr 1960). The advent of new techniques of bone protein isolation and AMS radiocarbon dating was the impetus for reevaluating the Arlington Springs discovery. These new data add significantly to our understanding of late Pleistocene human presence on Santa Rosae, the land mass ancestral to the modern Northern Channel Islands.

## METHODS

### Preliminary Studies

In May 1987, two co-authors of this paper (Morris and Johnson) identified Orr's plaster-jacketed block of earth from the Arlington Springs Site in a basement storage area at the Santa Barbara Museum of Natural History. After initial approval by the museum's Collections and Research Committee, several studies were undertaken in 1989 and 1992 with National Park Service (NPS) funding. The first step was to assess the physical condition of the bone. After removing the plaster jacket, an isolated bone fragment was taken for possible radiocarbon dating. This sample was sent to the Laboratory of Isotope Geochemistry-Environmental Isotope Research at the Department of Geosciences, University of Arizona where the amount of original protein was analyzed. The bone was termed "not dateable" because the specimen contained 0.053% total nitrogen and had a glycine depletion ratio of 235 (Long, pers. comm. 1989: AA-No. 3223/A-No. 4968). The possibility remained, however, that bone still within the cast sediment would have better protein preservation.

In December 1993, a partial excavation was undertaken of the Arlington Springs block of earth after Thomas Rockwell of the Department of Geological Sciences, San Diego State University, removed soil samples. A soil sample examined by G. James West of Davis, California contained no pollen within the sediment enclosing the human bones. Fragments of two human femora were exposed during the block's excavation; however, a portion of one femur and a third unidentified bone element were left in situ should

future analyses be warranted. Masks, lab coats, and sterile rubber gloves were worn to ensure that no contamination would occur prior to DNA testing. All soil matrixes were water-screened through 1/16-inch-mesh screen and all residuals were dried and archived.

Phillip Walker of the Department of Anthropology, University of California Santa Barbara, performed histological analyses that revealed no remaining bone tissue structure. Joseph Lorenz of the Department of Anthropology, University of California, Davis determined that DNA had not been preserved. Walker noted that the human bones were probably from a female, because the linea aspera was not well developed on the most intact femur fragment. This femur's subtrochanteric mediolateral diameter was 27.88 mm, and its anteroposterior diameter was 24.19 mm. These measurements fit nicely into the female range using statistics for skeletons from the Channel Islands area that have had sexes established based on pelvic characteristics (Walker, pers. comm. 1999).

### Bone Chemistry Analysis and Radiocarbon Dating

After measuring the best-preserved femur fragment, we sent bone fragments to three different specialists. Each person was to isolate a different chemical phase of the bone. Michael De Niro, Department of Geological Sciences, University of California, Santa Barbara, concluded that the bone proteins had a carbon to nitrogen ratio of less than 40 (C/N ratio < 40), and therefore the bone was too degraded for radiocarbon dating by his enzyme collagenase technique (De Niro, pers. comm. 1994; see De Niro and Weiner 1988). Independent analyses were next conducted separately by Stafford and Ajie, two co-authors of this study. Stafford analyzed the amino acids remaining in the bone and radiocarbon dated different chemical fractions of the preserved bone protein (Stafford 1998; Stafford et al. 1988, 1990, 1991). In contrast, Ajie attempted to extract and radiocarbon date osteocalcin, a non-collagenous bone protein (Ajie et al. 1990, 1991, 1992). Both Stafford's and Ajie's samples were AMS-radiocarbon dated at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Radiocarbon Laboratory.

During diagenesis, bone protein degrades into peptides and amino acids that are lost differentially. The bone mineral carbonate-hydroxyapatite becomes contaminated with exogenous humates that significantly contaminate the sample. To assess protein preservation and remove foreign organic matter, Stafford used two techniques: (1) using quantitative amino acid analyses to evaluate if collagen was preserved, and (2) removing humates with XAD-2 resin (Stafford et al. 1988, 1991). Two pieces from the same bone were processed separately (Samples A and B). Two chemical fractions were dated from Sample A: the HCl-insoluble residue (7830 ± 110 yr CAMS-13055) and XAD-purified hydrolyzate (9180 ± 70 yr CAMS-16814). Sample B was dated using XAD-purified collagen hydrolyzate and yielded an age of 10,960 ± 80 yr (CAMS-16810).

Osteocalcin has been believed to possess important characteristics that make it especially useful for radiocarbon dating. First, osteocalcin is the next most abundant protein in bone, after collagen. Second, osteocalcin is tightly bound to the hydroxyapatite, the major mineral component of bone. In this bound state, the protein is protected from diagenetic processes that would attack collagen.Third, osteocalcin is apparently unique to vertebrate tissues and has not been detected in plants, bacteria, and invertebrates. Due to its limited occurrence in nature, contamination by foreign osteocalcin is improbable. Osteocalcin was extracted using the formic acid procedure of Poser et al. (1980). The bone sample was ground and extracted in 21% formic acid solution. This solution was then transferred to dialysis tubing, molecular weight cutoff 5,000 daltons. The sample was dialyzed for three days against distilled water. The distilled water was changed every twelve hours. At the end of

**Table 1. Comparative amino acid data for Arlington Springs bone sample A.[a]**

| | | Arlington Springs | |
|---|---|---|---|
| **Amino Acid** | **Modern** | **Entire bone before pretreatment (AAA-618)** | **HCl-insoluble residue (AAA-734)** |
| Hydroxyproline | 93 | 0 | 79 |
| Aspartic Acid | 50 | 172 | 57 |
| Threonine | 19 | 24 | 23 |
| Serine | 33 | 41 | 31 |
| Glutamic Acid | 79 | 164 | 81 |
| Proline | 115 | 55 | 102 |
| Glycine | 327 | 270 | 323 |
| Alanine | 113 | 93 | 117 |
| Valine | 20 | 36 | 32 |
| Methionine | 11 | 12 | 9 |
| Isoleucine | 14 | 16 | 14 |
| Leucine | 31 | 37 | 36 |
| Tyrosine | 6 | 11 | 2 |
| Phenylalanine | 14 | 15 | 18 |
| Histidine | 8 | 8 | 4 |
| Hydroxylysine | 8 | 6 | 4 |
| Lysine | 28 | 12 | 27 |
| Arginine | 31 | 28 | 40 |
| Total Residues | 1000 | 1000 | 1000 |
| Total nanomoles (AA/mg bone) | 2170 | 10 | 918 |
| Protein Relative to Modern Bone (%) | 100 | 0.33 | 29.62 |

[a]Values are in residues per thousand amino acids (R/1000).

**Table 2. Radiocarbon dates from the Arlington Springs Site (CA-SRI-173).**

| Material/Provenience | Lab No. | $^{14}$C age (yrs BP) | References | Comments |
|---|---|---|---|---|
| Previously Published Dates: | | | | |
| Charcoal from organic earth in contact with human bone | L-568-A | 10,400 – 2,000 | Orr 1960, 1962a, 1962b | |
| Charcoal from 1 foot away | L-650 | 10,000 – 200 | Olson and Broecker 1961; Orr 1962a, 1962b | |
| Long bone fragment | UCLA-1899 | 10,080 – 810 | Berger and Protsch 1989 | |
| Charcoal from stratum beneath that in which human bone was found | UCLA-748 | 11,300 – 160 | Berger and Libby 1966 | |
| Osteocalcin Analysis (Ajie) | | | | |
| Formic acid procedure | CAMS-14363 | 6,610 – 60 | This report | |
| Collagen Analysis (Stafford) | | | | |
| Femur Fragment | CAMS-13055 | 7,830 – 110 | This report | Untreated HCL-insoluable collagen (Bone Sample A) |
| Femur Fragment | CAMS-16814 | 9,180 – 70 | This report | XAD-decalcified collagen (Bone Sample A) |
| Femur Fragment | CAMS-16810 | 10,960 – 80 | This report | XAD-decalcified collagen (Bone Sample B) |
| Dating of Associated Materials | | | | |
| Charcoal from stratum in which human bone was found | CAMS-13036 | 10,090 – 70 | This report | Collected during 1993 fieldwork by T. Rockwell |
| *Peromyscus nesodytes* mandible from soil matrix around human femora | CAMS-17125 | 11,490 – 70 | This report | Collagen after humates removed |

dialysis, the content was freeze-dried and combusted to generate the $CO_2$ for radiocarbon dating.

To obtain a basis for chronological comparison to the Arlington Springs bone samples, some independently collected materials were submitted for AMS-dating. Thomas Rockwell submitted a charcoal fragment that he collected from the same stratum containing the human femora. He collected this charcoal sample in 1993 during NPS-supported field mapping of the Arlington Springs site stratigraphy. In addition to the associated charcoal sample, bones of the extinct deer mouse *Peromyscus nesodytes* were used for AMS radiocarbon dating. These rodent bones were recovered during the laboratory excavation of the sediment block. The most recent dates on *P. nesodytes* are derived from its association with archaeological deposits at Daisy Cave, San Miguel Island, California (Guthrie 1993, 1998). The rodent *Peromyscus maniculatus* replaced *P. nesodytes* during the early Holocene, apparently when early humans introduced *P. maniculatus* from the mainland (Walker 1980). The sediment block yielded a *P. nesodytes* mandible that was submitted to Stafford for AMS-radiocarbon dating.

## RESULTS

Stafford's analysis of amino acids in the Arlington Springs whole bone sample indicated non-collagenous amino acid composition for total bone (Table 1). As chemical purification of the bone progressed, ages became older as chemical purity increased. The age differences between 7830 ± 110 on decalcified bone vs. 9180 ± 70 BP and 10,960 ± 80 on XAD-purified hydrolysates reflect the XAD resin's ability to remove exogenous humates.

Ajie's examination of the molecular weight profile of osteocalcin in the Arlington Springs bone using electrophoresis and mass spectrometry revealed that this protein was present but degraded. The radiocarbon measurement derived from the formic acid-extracted osteocalcin was 2,570 to 4,350 years younger than dates obtained on purified collagen extracted by Stafford (Table 2). This result reverses the pattern observed in a previous study from another southern California site in which older osteocalcin $^{14}$C dates were obtained from poorly preserved bone in comparison to collagen-derived dates (Ajie et al. 1992).

The radiocarbon date obtained on charcoal, 10,090 ± 70 yr (CAMS-16810) is consistent with previously published dates for the stratum containing the Arlington Springs human bone. The *Peromyscus nesodytes* mandible contained collagen that was better preserved than the human femora. When decalcified, the mandible retained a five-percent pseudomorph whereas the human bone disaggregated into an amorphous residue. The date on the deer mouse collagen, 11,490 ± 70 yr (CAMS-17125), is the oldest radiocarbon measurement associated with the Arlington Springs find (Table 2).

## DISCUSSION

During his initial investigations of the Arlington Springs Site, Phil Orr recognized that the human femora appeared to have been redeposited. The bones apparently had been eroded from their original locus and were incorporated into alluvial sediments of a small stream channel. For this reason it is uncertain if the associated charcoal or deer mouse bones are coeval with the human bone. All fossils could have become mixed through erosion of sediments upstream from where they were found. Despite this caveat, the Arlington Springs locality is an outstanding sedimentary record that encompasses an estimated 20,000 years. Thomas Rockwell and his students at San Diego State University are analyzing it in detail. Numerous charcoal samples have been obtained from stratigraphic layers overlying the stratum in which the human bones were recovered. The complete dating of this geological section should help bracket the chronological placement of the earliest evidence for human presence at this site.

The different radiocarbon measurements on the human bone are an example why no single radiocarbon measurement is definitive for the Arlington Springs human material. The osteocalcin analysis produced a date significantly younger than dates from all other materials. Until recently, it was believed that one of the principal advantages of osteocalcin analysis was that it was less susceptible to contamination in the diagenetic processes that affect collagen. Recent radiocarbon testing of this assumption on a series of bone samples of varying age and degree of collagen preservation has shown that osteocalcin can degrade even more rapidly than collagen and can yield radiocarbon ages that can be older or younger than a specimen's known age (Burky et al. 1998). This problem with osteocalcin-dating was not known at the time of our study. We therefore conclude that the osteocalcin date on the Arlington Springs bone is too young.

With regard to his study based on collagenous material in the Arlington remains, Stafford believes that the difference between the two XAD-hydrolyzate dates (9,180 ± 70 yr and 10,960 ± 80 yr) is due to difference in collagen preservation within the same bone. Sample B contained more collagen than Sample A, apparently because of variation in microdepositional environment. Stafford's opinion is that the age of the Arlington Springs human bone is bracketed between the oldest XAD measurement of 10,960 ± 80 yr and the age measurement on the extinct deer mouse bone, 11,490 ± 70 yr. Redating additional chemical fractions from the other femur would establish if the 10,960 yr age is an absolute minimum age for the collagen. The uppermost age estimate of 11,490 ± 70 yr is considered to be a reliable, maximum value for two reasons. First, the rodent bones were better preserved chemically than were the human bones and contained more collagen; consequently, the rodent bone's radiocarbon age measurement is considered to be accurate. Second, the human remains were apparently reworked into older sediments that would have yielded the rodent bones. Therefore, the rodent bones would be older than the human bones. Also, the difference between the 10,960 ± 80 yr and 11,490 ± 70 yr may be due more to radiocarbon calibration problems than actual age differences (Fiedel 1999).

If the Arlington Springs remains date at least 10,960 ± 80 yr old, this human is slightly earlier than the first confirmed level of human occupation at Daisy Cave on nearby San Miguel Island at 10,390 ± 70 yr (Erlandson et al. 1996a, 1996b). With the recently discovered, nearly complete pygmy mammoth skeleton (*Mammuthus exilis*) now radiocarbon dated to 12,840 ± 410 BP (Agenbroad 1998), the period between the latest evidence for mammoths and the earliest evidence for humans on the Northern Channel Islands appears to be narrowing.

## ACKNOWLEDGMENTS

The authors acknowledge the support of the following individuals who assisted in this study through research assistance and sharing information: Larry Agenbroad, Michael De Niro, Jon Erlandson, Michael Glassow, Rika Kaestle, Joseph Lorenz, Thomas Rockwell, Erv Taylor, Jan Timbrook, Gilbert Unzueta, Jr., Phillip Walker, and G. James West. Stafford's analyses were performed at his Laboratory for AMS Radiocarbon Research, University of Colorado. Nancy Ding and Jennifer Nehring at CU provided considerable laboratory assistance. We thank John Southon and Lynn Ingram at the Lawrence Livermore National Radiocarbon Laboratory for providing the AMS radiocarbon measurements. Channel Islands National Park provided funding for this study.

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