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Journal

American journal of physical anthropology, 159(1)

ISSN

0002-9483

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Publication Date

2016

DOI

10.1002/ajpa.22843

Peer reviewed

Isotopic and Genetic Analyses of a Mass Grave in Central California: Implications for Precontact Hunter-Gatherer Warfare

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KEY WORDS human provenancing; hunter-gatherer violence; C N O Sr isotopes; ancient mtDNA; California prehistory

ABSTRACT

Objectives: Analysis of a mass burial of seven males at CA-ALA-554, a prehistoric site in the Amador Valley, CA, was undertaken to determine if the individuals were “locals” or “non-locals,” and how they were genetically related to one another.

Methods: The study includes osteological, genetic (mtDNA), and stable (C, N, O, S) and radiogenic (Sr) isotope analyses of bone and tooth (first and third molars) samples.

Results: Isotopes in first molars, third molars, and bone show they spent the majority of their lives living together. They are not locals to the Amador Valley, but were recently living to the east in the San Joaquin Valley, suggesting intergroup warfare as the cause of death. The men were not maternally related, but represent at least four different matrilineal groups. The men also changed residence as a group between age 16 and adult years.

Conclusions: Isotope data suggest intergroup warfare accounts for the mass burial. Genetic data suggest the raiding party included sets of unrelated men, perhaps from different households. Generalizing from this case and others like it, we hypothesize that competition over territory was a major factor behind ancient warfare in Central California. We present a testable model of demographic expansion, wherein villages in high-population-density areas frequently fissioned, with groups of individuals moving to lower-population-density areas to establish new villages. This model is consistent with previous models of linguistic expansion. *Am J Phys Anthropol* 159:116–125, 2016. © 2015 Wiley Periodicals, Inc.

A romanticized interpretation often depicts hunter-gatherers as peaceful peoples with, at most, very low rates of interpersonal violence when compared with more complex and state-level societies. Recent archaeological and anthropological research has challenged these notions (e.g., Walker, 1989; Keeley, 1996; LeBlanc, 1999; Lambert, 2002; Arkush and Allen, 2006; Allen, 2012; Jones and Allen, 2014). These studies suggest that the level of organization in warfare was often lower among hunter-gatherers, but that per-capita rates of non-lethal violence and homicide were often just as high (but see Fry, 2007 and Fry and Söderberg, 2013 for an alternate perspective).

The ancient hunter-gatherers of California have played an important part in this shift in thinking (Walker, 1989; Jurmain, 1991, 2001; Jurmain and Bellifemine, 1997; Lambert, 1997, 2007; Andrushko et al., 2005, 2010; Jurmain et al., 2009; Bartelink et al., 2013; Eerkens et al., 2014a,b; Schwitalla et al., 2014). Osteological studies show that violence often played a role in mortality. A recent compilation and survey of osteologi-

cal data (Schwitalla et al., 2014) shows that sharp-force trauma appears in over 7% and blunt-force in over 4% of individuals ($n = 6,200$) from Late Holocene Central

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: National Science Foundation (to J.W.E. and E.J.B.); Grant numbers: BCS-1318532 and BCS-1318543; Grant sponsor: UC Davis Forensic Sciences Graduate Program.

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Received 26 December 2014; revised 10 August 2015; accepted 11 August 2015

DOI: 10.1002/ajpa.22843

Published online 2 September 2015 in Wiley Online Library (wileyonlinelibrary.com).

Fig. 1. Map of Central California, showing location of CA-ALA-554 and geographic features discussed in the text.

California (3000 BP—present). Many of these individuals died as a result of injuries incurred during violent encounters, as there is often little or no evidence for healing. Studies also demonstrate a notable increase in the prevalence of such trauma from 5,000 years ago into the historic period (ca. AD 1750). While both males and females suffered from blunt and sharp force trauma, males were affected at a rate that was two to four times higher than females (Schwitalla et al., 2014), consistent with the interpretation that males were typically responsible for raiding and warfare in these societies.

While violence was clearly significant and endemic in Central California, less clear from the evidence is the social nature of such events. Thus, osteological data are often unable to address whether violent events took place as part of intragroup altercations or more organized intergroup warfare. In this article, we turn to stable and radiogenic isotopes and ancient DNA to re-examine a mass burial from CA-ALA-554, located in the Amador Valley, Central California (Fig. 1). Although this represents a single episode of violence, the new archaeometric information reveals important clues about the nature of warfare in ancient Central California.

BACKGROUND

CA-ALA-554 is a precontact site in the city of Pleasanton, California. Planned construction of a shopping center resulted in salvage excavation by a cultural resource management firm (Estes et al., 2012). The excavations documented an extensive site that had been occupied by relatively small-scale but sedentary hunter-gatherers.

The population of this village is estimated to have been between 20 and 60 individuals, though the size may have fluctuated over time. Finds included a range of domestic features (e.g., cooking features, pits, houses), over 200 primary inhumations, and a dense midden deposit that included both formal artifacts (mortars, pestles, projectile points, beads, pendants, smoking pipes), tool production debris (flintknapping waste flakes), and food refuse (animal bones, charred seeds).

Radiocarbon dates, obsidian hydration dates, and temporally diagnostic artifacts indicate the site was occupied between 2500 and 300 cal BP, with most of the material dating to a more narrow window between 1400 and 600 cal BP. This is a time period when the landscape in Central California was rapidly filling up and large numbers of permanently occupied sites are known. Villages were generally well-connected through a regional trading network, as evidenced by the nearly ubiquitous recovery of a range of exotic materials (e.g., obsidian, marine shell beads, marine fish). Diets in the region were heavily focused on plant foods, especially acorn and small seeds (Wohlgemuth, 1996), but supplemented by fishing and hunting of wild game.

During mechanical trenching of the site, an unusual mass burial was discovered and hand-excavated. Designated Burial 85 in the field, the burial included seven individuals lacking grave goods and interred partially on top of one another, all in an extended position with heads oriented towards the NE (Fig. 2). NE orientation is not out of the ordinary, but extension is an unusual, though not altogether absent, burial position during the late prehistoric period in Central Californian sites. Mass

Fig. 2. Digitized representation of Burial 85, showing burial style and relative positioning of the seven individuals.

graves, however, are rare. The burial style, lack of grave goods, and other contextual information (discussed below) suggested the individuals may have met a violent end.

Osteological analyses determined six of the seven individuals to be male, with the seventh (85G) classified a probable male. Estimated ages vary from 18 to 40 years at the time of death. Other than carious lesions and dental hypoplasias associated with 85D and 85F, and tibial periostitis with 85F, the individuals show no evidence of stress or disease. There was, however, evidence for physical trauma among several of the males. Burial 85C displayed a perimortem fracture on the left frontal bone above the left orbit with perimortem fractures radiating throughout the left side of the cranium, and was missing a fragment of the right parietal bone which may represent a second point of impact, as the radiating fractures connect to the area of missing bone. Burial 85D had evidence of a depression on the right frontal bone above the orbital margin and had an obsidian flake near the right ilium that may have been embedded in the soft tissue, and 85F contained a chert biface between the right seventh and eighth ribs that was not embedded in bone, but may have been lodged in soft tissues. The trauma evidence, combined with otherwise healthy males in a mass grave, an unusual burial posture, and a lack of grave goods, is suggestive of an ancient violent event. The remainder of the article assumes this to be the case.

Our analysis is focused, first, on testing the hypothesis that the Burial 85 men at CA-ALA-554 were locals to the site, using stable isotope methods on bone. If violence in Central California occurred primarily within villages (i.e., intragroup), the individuals should display a local isotopic signature. Alternatively, if ancient warfare

was primarily between groups, then the males should display non-local values. Second, we aim to test whether the males from the pit were genetically related. If violence took place at a family level (i.e., within or between families in a village), we expect the males to be closely related. Alternatively, if warfare was conducted between villages or was organized beyond the family level, the males may be unrelated to one another. Together, we use Burial 85 as a case study for understanding the nature of interpersonal conflicts in the region, and speculate on factors that may have contributed to such violent events.

METHODS

Permission to study and sample these individuals was granted by the project's state-appointed Most Likely Descendant (MLD). Sex was mainly estimated in the lab using pelvic morphology, but cranial morphology, postcranial metrics, and overall robustness of the skeleton were used as supporting information when present (Phenice, 1969; Buikstra and Ubelaker, 1994; White and Folkens, 2005). Age-at-death was estimated using cranial, dental, and postcranial characteristics, especially surface morphology of the pubic symphysis, auricular surface of the ilium, but also cranial suture closure, occlusal tooth wear, and antemortem tooth loss when pelvic indicators were not available (Buikstra and Ubelaker, 1994; White and Folkens, 2005).

While five of the seven individuals were relatively complete in their skeletal inventory, a prehistoric house floor was constructed sometime after the mass interment and intruded into the burial pit, resulting in the removal of the cranium and some of the upper postcranial elements of two individuals (85G and 85H). Such disturbances to graves are not uncommon in Central California (e.g., Lillard et al., 1939; Wiberg, 1988), where cemeteries and habitation areas were often one and the same, and features (pits, hearths, houses) were occasionally excavated into existing burial pits. A small piece of bone (maxilla or mandible for burials with crania, and fibulae for those lacking a cranium), and first and third molars, when present, were sampled from each individual to reconstruct the migration history using isotopic data, and establish their maternal relations using ancient mitochondrial DNA (mtDNA). This information was compared with samples from other individuals interred at the site who were buried in more traditional styles and therefore believed to be locals.

For each individual, 1 to 2 g of bone and 0.2 to 0.4 g of enamel were sampled for isotope analysis. Visually, bone preservation appeared to be excellent, both in the field and in the lab, lacking signs of weathering, pitting, or rodent gnawing, and was not crumbly or chalky during cleaning. Our study focuses on interior sections of well-preserved cortical bone, and enamel, minimizing the potential effects of diagenetic changes to isotopic values (Knudson et al., 2005). For bone samples, a Fordham micro-drill equipped with a diamond studded drill bit was used to remove the outer layer, including any sediment or other foreign material adhering to the surface. Each bone sample was then sonicated and washed with deionized water (dH_2O), and separated into three portions, one for carbon, nitrogen, and sulfur analysis of collagen, one for oxygen and carbon isotope analysis of apatite, and one for strontium isotope analysis of apatite, respectively. For tooth samples, small pieces of

intact enamel were removed using the same drill and powdered in an agate mortar and pestle. Tooth enamel apatite samples were separated for oxygen, carbon, and strontium isotope analysis.

To isolate the apatite component, bone and tooth samples were powdered and organics removed by soaking in 1.5% sodium hypochlorite at a ratio of 0.04 ml solution/mg for 24 h (Koch et al., 1997). After centrifugation and discard of the solution, this entire step was repeated, followed by rinsing three times with dH₂O. For the next 24 h secondary carbonate removal was accomplished by placing the samples in 1M acetic acid (at the same ratio of 0.04 ml solution/mg sample) which was replaced after 12 h. The sample was then rinsed three times with dH₂O, dried, and 1 mg samples were analyzed at the UC Davis Stable Isotope Lab on a GVI Optima Stable Isotope Ratio Mass Spectrometer (IRMS). External precision for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}_{\text{apa}}$ values are ± 0.07 and ± 0.04 (1 σ), respectively, based on multiple analyses of the calcite standards NBS-19 and UCD-SM92.

For strontium isotope analyses, 0.05 g of powdered enamel or 0.1 g bone was weighed into a Savillex® PFA container. The samples were treated twice with 15% hydrogen peroxide (H₂O₂) for 24 h with as much of the H₂O₂ pipetted off as possible, and then rinsed with Millipore DI water. For carbonate removal, 2 ml of 1M acetic acid was added for another 24 h. Acetic acid was then pipetted off and the samples were rinsed two more times in Millipore DI water. Sample dissolution was done in 4 ml of 2.5N hydrochloric acid (HCl) in capped Savillex® PFA containers at 120°C for 24 h. The caps were then removed and the dissolved sample solutions dried down. Once ready to start column work, the samples were rehydrated with 800 μl of 8N nitric acid (HNO₃), capped, and placed on the hotplate at 110°C for at least 1 h. The samples were loaded into Teflon micro-columns with Eichrom Sr Spec resin conditioned in 8N. HNO₃. To begin, matrix and other interfering elements were removed (particularly Rb, but also Ba and Pb) by rinsing the sample with 400 μl of 3N HNO₃ five times, and rinsing once with 200 μl of 0.5N HNO₃. Clean catch beakers were placed below the columns and Sr eluted with 400 μl of 0.5N HNO₃ seven times. The samples were then dried, rehydrated with 400 μl of 8N HNO₃ and the rinse procedure repeated to ensure complete purification of Sr. Samples were analyzed on a Nu Plasma (Nu032) Multi Collector-ICP-MS at UC Davis Interdisciplinary Center for Plasma Mass Spectrometry attached to a desolvating nebulizer (DSN-100). Ratios were corrected for mass fractionation to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$. Signals for masses 87 (^{87}Rb) and 84 ($^{84}\text{Kr} + ^{84}\text{Sr}$) were only in the order of a few mV and were monitored to correct for the interference of ^{87}Rb on ^{87}Sr and ^{86}Kr on ^{86}Sr . Bracketing SRM 987 standards were run every three to four samples and the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios of these samples were then normalized to an accepted value of 0.710248 for the NIST SRM 987. Standard BCR-2 was processed with the samples, and produced a normalized $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.705041 ± 0.000013 . This value is within uncertainty of compiled $^{87}\text{Sr}/^{86}\text{Sr}$ value for BCR-2 of 0.705019 ± 0.000016 reported by Weis et al. (2006).

Collagen was extracted by placing approximately 0.5 to 1 g of surficially cleaned, but unpowdered, bone in 0.5M hydrochloric acid (HCl) in a refrigerator set at 5°C. HCl was replaced every few days until the bone was demineralized (when the bone no longer visibly reacted with the HCl and was soft and slightly transparent). Samples were

then rinsed with dH₂O, immersed in 0.125M NaOH for 24 h to remove humic acids, and rinsed again. Water with a pH of 3 was then added to the sample which was placed in an oven set to 70 to 90°C for approximately 24 h to solubilize collagen. The pH3 solution was pipetted into a clean vial and freeze-dried to isolate collagen. $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}$ were measured using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer, and $\delta^{34}\text{S}$ measured using a Elementar vario ISOTOPE cube interfaced to a SerCon 20-22 isotope ratio mass spectrometer. All collagen samples were analyzed at the Stable Isotope Facility, University of California Davis. Carbon isotope ratios ($\delta^{13}\text{C}$) are reported expressed in permil notation (parts per thousand relative to a standard) relative to the Pee Dee Belemnite standard (arbitrarily set at 0‰), nitrogen isotope ratios ($\delta^{15}\text{N}$) are expressed against N₂ in modern atmospheric air (also arbitrarily set to 0‰), and sulfur isotope ratios ($\delta^{34}\text{S}$) are expressed against the Vienna Canyon Diablo Troilite. The long-term standard deviation for samples in the lab is 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$. Two of the collagen samples were also sent to the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility in Woods Hole, MA, for radiocarbon dating.

Finally, ancient DNA (aDNA) extraction and PCR setup were carried out in a clean room aDNA laboratory at the Institute for Genomic Biology at the University of Illinois, Urbana-Champaign, following best practices (Gilbert et al., 2005). All lab personnel have their mtDNA on file and the HVI sequence did not match haplotypes in the personnel database. Reagent blanks were included in extraction and PCR amplification steps, and all results were replicated through two independent extractions. Stationary lab supplies were decontaminated with bleach and/or DNA Away, and disposables were UV-irradiated for 10 min before use. All reagents were certified DNA/DNAse free. Researchers wore disposable laboratory suits, hair nets, and two pairs of nitrile gloves when handling aDNA samples. All post-PCR steps were completed in a separate lab located in the Medical Sciences building at the University of Illinois, Urbana-Champaign.

For aDNA, one tooth was sampled from five individuals, and bone from the remaining two, and multiple DNA extractions, genotyping and DNA sequencing were completed in two rounds of analysis separated by a year. To remove surface contaminants, each tooth or bone fragment was individually soaked in 6% sodium hypochlorite (household bleach) for 5 min, then rinsed three times with DNA-grade water. Samples were air-dried, then UV-irradiated for 10 min on each side. Under a low-flow hood, tooth roots and bone were powdered using a dremel tool at low speed. DNA was extracted from the powder following the protocol outlined by Cui et al. (2013). Two hundred milligrams of powder was incubated overnight at 37°C in a solution containing 300 μl 10% *N*-lauryl sarcosyl, 4 ml 0.5M EDTA, and 100 μl 20 mg/ml proteinase-K (Life Technologies). After centrifugal filtration with a 30K NMWL Ultra-4 filter unit (Amicon®), approximately 150 μl of DNA-containing sample solution was purified using the QIAquick PCR Purification kit (QIAGEN). DNA was eluted twice in 30 μl EB Buffer (QIAGEN) to obtain a final volume of 60 μl . Samples were then purified a second time in the same manner, and DNA extracts were stored at 4°C before PCR amplification.

Mitochondrial DNA was PCR-amplified with overlapping primer sets (Kemp et al., 2007) spanning the first

TABLE 1. Demographic, osteological, ancient DNA, and radiocarbon data on individuals comprising Burial 85 at CA-ALA-554

Burial	Sex	Age	Stature	mtDNA	Haplotype	^{14}C Date
85B	Male	18–23	162.7	D1	223-325-362	1180 ± 20
85C	Male	28–33	160.6	D1	223-325-362	
85D	Male	33–38	166.3	A2	093-111-223-290-319-362	
85E	Male	18–23	159.8	B2	189-217-362	
85F	Male	35–40	159.2	B2	167-189-217	
85G	Prob. Male	30–40	161.8	failed		1170 ± 20
85H	Male	35+	163.7	D1	223-325-362	

Haplotype are reported without the 16,000 designated for the HV1 region.

TABLE 2. Stable and radiogenic isotope values for bone, first molars, and third molars for the Burial 85 individuals from CA-ALA-554

Burial	Bone						M1			M3		
	$\delta^{13}\text{C}_{\text{col}}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	$\delta^{13}\text{C}_{\text{apa}}$	$\delta^{18}\text{O}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$^{87}\text{Sr}/^{86}\text{Sr}$
85B	-21.2	9.8	-1.2	-14.3	-7.1	0.70791	-15.7	-7.3	0.70747	-15.9	-8.2	0.70739
85C	-21.6	10.2	-2.0	-15.5	-7.0	0.70781	-15.3	-6.4	0.70742	-15.3	-7.6	0.70743
85D	-21.3	10.6	-1.9	-15.3	-8.0	0.70792	-15.3	-8.5	0.70741	-15.0	-8.2	0.70749
85E	-21.4	10.1	-2.4	-13.0	-6.5	0.70794	-15.2	-6.6	0.70737	-15.0	-7.7	0.70735
85F	-22.0	10.0		-14.3	-7.0	0.70793	-15.7	-7.4	0.70750	-15.1	-8.5	0.70743
85G	-21.2	10.3	-2.2	-14.4	-7.0	0.70799						
85H	-21.7	10.4	-2.5	-14.7	-6.5	0.70798						

Burials 85G and 85H were missing crania, and hence, teeth. Burial 85F did not produce enough collagen to run $\delta^{34}\text{S}$.

hypervariable region (HVI) of the human mitochondrial genome as well as for coding regions diagnostic of Native American haplogroups. PCR was carried out in 20 μl volumes with 2 μl DNA template, 0.3 μl 20 mM primers, 1 μl Platinum Taq (Invitrogen), 0.2 μl 10 $\times$ buffer, 0.25 μl 50 mM MgCl_2 , and molecular grade water. Thermal cycling conditions followed the manufacturers' protocol with 40 cycles of PCR. PCR-amplified DNA was visualized on a polyacrylamide gel to ensure successful amplification. Amplified DNA was purified with ExoSAP-it and prepared for sequencing, which was completed at the Keck Center, UIUC. DNA sequences were edited and aligned to the revised Cambridge Reference Sequence (rCRS) using Sequencher (Anderson et al., 1981; Andrews et al., 1999). Mitochondrial haplogroups were confirmed by restriction fragment length polymorphism (RFLP) and size separation of coding region markers specific to Native American haplogroups as in Kemp et al. (2007).

RESULTS

Collagen yields from all seven bone fragments were high (>4%) and C/N ratios fell in a narrow range within the range of values expected in well-preserved bone (DeNiro, 1985; see Supplementary Table). Collagen from individuals 85B and 85F produced nearly identical AMS radiocarbon dates of 1180 ± 20 and 1170 ± 20 BP, respectively (Table 1). This supports the interpretation by field archaeologists that all seven individuals were interred at the same time. The median calibrated dates for these two burials are AD 841 and AD 855, respectively, with two-sigma ranges between AD 780 and 940. By contrast, charcoal from the house floor was radiocarbon dated to 940 ± 30 BP (Estes et al., 2012), which has a median calibrated age of AD 1097, with 2-sigma calibrated age range of AD 1010 and 1150. This indicates that the mass burial event took place some 70 to 370 years before the construction of the intrusive house.

Mitochondrial DNA results were obtained on six of the seven individuals (Table 1). All results were consistent across DNA extractions and negative controls were consistently clean. mtDNA shows high diversity with at least four haplotypes present. Of the six individuals, only three could potentially be maternally related (85B, 85C, and 85H). However, even these three share a common haplotype that is consistent with the founding D1 lineage, making it possible that they do not share a direct maternal relationship.

Isotopic data are presented in Table 2. Figure 3 shows plots of bone $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}$ for the seven Burial 85 males against other individuals from the site thought to be locals. For context, the figure also shows ellipses for skeletal populations from other regions in Central California (summarized in Eerkens et al., 2013). Likewise, Figure 4 shows $^{87}\text{Sr}/^{86}\text{Sr}$ against $\delta^{18}\text{O}$ for bone, again relative to a sample of other local individuals from the site (to date, fewer individuals from CA-ALA-554 have been measured for $^{87}\text{Sr}/^{86}\text{Sr}$ than for bone collagen). Recent inter-laboratory comparisons show significant variation in $\delta^{18}\text{O}$ values produced for the same sample of bone (but negligible variation for bone collagen; Pestle et al., 2014). In fact, Pestle et al. (2014) show higher intra-lab variation for $\delta^{18}\text{O}$ than for other isotope ratios, and relate part of this variation to sample treatment methods (see also Garvie-Lok et al., 2004). This may account for some of the spread in $\delta^{18}\text{O}$ values observed within particular sites in Figure 4. We note, however, that all the samples in Figure 4 were run in the same lab using the same chemical treatment method, and for the CA-ALA-554 samples, were run in the same batch on the same day. This should reduce the inter-sample variation for $\delta^{18}\text{O}$, but in the discussion below we focus especially on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $^{87}\text{Sr}/^{86}\text{Sr}$.

Figures 3 and 4 clearly show, first, the relative homogeneity of the Burial 85 males, and second, the distinctive bone isotope values for these individuals relative to the rest of the CA-ALA-554 population. Mann-Whitney U

Fig. 3. $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ for bone collagen, comparing Burial 85 males with other CA-ALA-554 individuals, and showing ranges observed in other regional sites from Central California. Ellipses based on Eerkens et al. (2013), and represent approximate range of empirical data from previous sites.

Fig. 4. $^{87}\text{Sr}/^{86}\text{Sr}$ vs. $\delta^{18}\text{O}$ for bone from Burial 85 compared with other Central California populations previously analyzed by the authors (site trinomials given).

tests comparing the range of isotope values in bone from the Burial 85 men versus other individuals from CA-ALA-554 indicate significant differences ($P < 0.005$) for all five isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$, $\delta^{18}\text{O}$, and $^{87}\text{Sr}/^{86}\text{Sr}$). Because bone reflects diet, and by extension geography, over the previous several years of life (Manolagas, 2000; Hedges et al., 2007), the isotope data suggest the Burial 85 individuals resided together in the same region in the years before their death, but in a different geographic area than CA-ALA-554.

Isotope values from first (M1) and third (M3) molars give additional context to the Burial 85 males, providing life history information on where they lived before their adult years (Table 2; Fig. 4). Figure 5 plots $^{87}\text{Sr}/^{86}\text{Sr}$ values for the M1-M3-bone series for the five burials with crania and teeth. The values from first molars are nearly identical to those from third molars, but are quite different than values from bone. Mann Whitney U-tests comparing the range of values in Burial 85 bone vs. enamel show significant differences for $^{87}\text{Sr}/^{86}\text{Sr}$ ($P < 0.0001$) and $\delta^{13}\text{C}$ ($P = 0.03$), but not for $\delta^{18}\text{O}$ ($P = 0.07$). This

Fig. 5. $^{87}\text{Sr}/^{86}\text{Sr}$ life history information for Burials 85B-85F, showing changes from first and third molars to bone.

suggests the males were born (first molars) and lived their early teenage years (third molars) in one common location, but spent their recent adult years in another common location, and moreover, that both these locales were different than where they were buried, as suggested by the data from other CA-ALA-554 burials.

DISCUSSION

The combined isotopic data reveal unambiguously that the Burial 85 males were not local to CA-ALA-554. This result suggests that the encounter leading the burial of these seven males away from their home was inter-, and not intra-, village in nature. In this respect, the results mimic isotopic data from CA-YOL-117 and CA-SCL-38 (Gardner, unpublished data; Eerkens et al., 2014a,b) where males from mass graves also show distinctly non-local bone isotopic signatures, and are interpreted as victims of violent events. Indeed, similar violent events involving mass graves and/or trophy-taking are known from many other examples in the archaeological record of Central California (Andrushko et al., 2005, 2010; Bartelink et al., 2013; Schmitz et al., 2014). The non-local isotopic values at CA-ALA-554 may explain the perimortem trauma observed in several of the Burial 85 males. We suggest that inter-village warfare or raiding was responsible for their early deaths.

The mtDNA and isotopic data provide additional details regarding the genetic affiliation and geographic origin of these seven males, and by extension, the organization of warfare or raiding parties. The Burial 85 men are composed of at least four different matrilineages, and could not have been, for example, politically aligned full brothers or maternal cousins. It is possible they were related only through the male lineage (e.g., Göhler, 1990), being half-brothers or paternal cousins, but Y-chromosome extraction for these individuals was unsuccessful. It is often the case that mtDNA, found in high copy number, will be preserved, but nuclear DNA (including the Y chromosome) will be too degraded for analysis. In short, the CA-ALA-554 case is consistent with the hypothesis that warfare was organized above the level of the family in ancient California and that warfare involved sets of more distantly related, or perhaps even unrelated, males. This finding contrasts with

at least one largely anecdotal ethnographic account of warfare in California, that suggests that groups of related kin typically comprised raiding parties (McCorkle, 1978; p. 696). Instead, the Burial 85 data are more similar to a recent study of warfare patterns among the Yanomamö, where raids typically include unrelated males from different villages (Macfarlane et al., 2014). Similarly, recent work on hunter-gatherer residence patterns by Hill et al. (2011) found that individuals residing in a single village are commonly unrelated. While it is possible the Burial 85 males came from a single village, the spatial resolution of isotopic analysis is not fine enough to distinguish residence in a single common village, or different but nearby villages. We do not suggest warfare in ancient California necessarily mimics patterns among South American horticulturalists, or any other group, but merely note similarities.

Although we know where they were buried, which was presumably near the location they died, the isotopic data offer additional insights regarding the geographic homeland of the Burial 85 males. In Central California, oxygen isotopes generally vary on a gradient that runs from east to west with more negative $\delta^{18}\text{O}$ values further inland due to the effects of isotope fractionation during precipitation (Kendall and Coplen, 2001). By contrast, Sr isotopes tend to vary with California geography as well, especially on a north-south axis. In the northern Sacramento Valley there are younger volcanic deposits of basalt (i.e., Mount Shasta) that produce lower $^{87}\text{Sr}/^{86}\text{Sr}$ values (~ 0.704), while in the southern Sacramento Valley, the granitic Sierra Nevada Range produces higher $^{87}\text{Sr}/^{86}\text{Sr}$ values (0.709–0.710). As well, carbon and nitrogen isotopes tend to discriminate foragers living in coastal vs. brackish estuarine vs. inland locations in Central California (see Eerkens et al., 2013). Although our database of sulfur isotope analyses in California to date is small, $\delta^{34}\text{S}$ helps discriminate individuals from the Sierra Nevada foothills who have higher values (2 to 7%), and southern San Francisco Bay where there are lower values (–10 to –3%; unpublished data), from those who lived in the Central Valley (–4 to 3%).

In the Burial 85 case, the carbon, sulfur, and oxygen isotope data suggest the seven males were from a location outside of the San Francisco Bay area, to the east of CA-ALA-554, but not as far east as the Sierra Nevada foothills. The Sr isotopes suggest a location to the south of CA-ALA-554, while nitrogen isotopes suggest these individuals were living in a riverine environment with little access to marine resources, including anadromous fishes such as salmon and sturgeon. The sulfur data from bone collagen are also consistent with a location in the Central Valley. Likewise, the $\delta^{13}\text{C}$ data from the teeth suggest the males had never lived near the coast, but spent their entire lives in an inland location. Taking all the isotopic data together, we suggest these individuals lived in the San Joaquin Valley, perhaps along the San Joaquin River or one of its major tributaries. The overall similarity of the four isotope values among the males suggests they were living either in the same village or in close proximity to one another in nearby villages.

The isotope data from the teeth, however, suggest that these males did not spend their entire lives at this location. Although oxygen isotopes from the bone and teeth are similar, Sr isotopes indicate they were born and lived their teenage years together in a location slightly to the north. The slight enrichment in $\delta^{13}\text{C}$ of bone apa-

tite relative to enamel of M1 and M3 teeth, as observed, would be consistent with such a settlement shift, assuming anadromous fish were more abundant further north in the San Joaquin River system (Gobalet, 2004) or in the California Delta. Thus, the isotope data suggest the males made the same residential move after the formation of their third molar enamel (ca. age 14). Movements associated with marriage, displacement due to territorial intrusion by another group, or the fissioning of a larger village due to internal conflict or overcrowding are potential reasons these males could have changed their place of residence in their teenage years. If this was the case, the isotopic evidence suggests they moved together as a unit, as they show the same isotopic shift in their teeth vs. bone (shown in Fig. 5). Given that the youngest individual is estimated to have died between the ages of 18 and 25, and assuming third molar enamel finishes growing at age 14, such a residential move must have happened between 4 and 9 years before the event that brought about their death. We further note that Sr and O isotopes of Burial 85 bone are not intermediary between the CA-ALA-554 locals and the Burial 85 third molars. Thus, diagenetic alteration of bone, from third molar values to the observed values, cannot explain the data observed in bone (see discussion in Bentley, 2006). This supports the interpretation that the bone isotope values are tracking the residence patterns of these individuals after their teenage years, and not post-depositional alteration.

While the proposed residential move did not take the Burial 85 males directly to the Amador Valley, it may have brought them closer to, and in contact with, the people from CA-ALA-554. It is possible, for example, that the Burial 85 males moved from their natal village to a location in the eastern foothills of the Coast Range, in an area between the San Joaquin Valley and the Amador Valley. At this new locale, they may have sought to establish new hunting and gathering territory which could have brought them into conflict with CA-ALA-554 inhabitants who already had claims on those lands. Ethnographic accounts in Central California indicate that inter-group violence often involved territorial disputes and access to natural resources (e.g., hunting grounds, fishing spots, plant gathering resource patches), for revenge, and less commonly, for obtaining marriage partners (James and Graziani, 1975; McCorkle, 1978).

We speculate that the event leading the Burial 85 may have been a byproduct of a village fissioning event. It is estimated that the northern San Joaquin Valley, as well as the California Delta and southern Sacramento Valley, had among the highest population densities in California at the time of contact (Baumhoff, 1963; Cook, 1976; Milliken, 2006; Rosenthal et al., 2007). Increasing population pressure may have led to fissioning of villages within the San Joaquin Valley, and the establishment of new satellite villages in lower population density areas, especially during intermittent periods of drought or reduced food availability. Although beyond the scope of this article, such population movements are in line with predictions from Ideal Free Distribution (IFD) models of settlement growth and territorial expansion, where groups of individuals move from areas with high productivity but also high population density to areas with lower productivity and lower populations (e.g., Winterhalder et al., 2010; Hale, 2010; Coddling and Jones, 2013; Maréan, 2014). Other mass burial contexts from the lower Sacramento Valley and the south San Francisco Bay Area

attest to similar patterns of intragroup violence. There, mass burials showing evidence of violent death have isotopic signatures indicating they were also nonlocal to the area in which they died, and came from areas that are thought to have hosted higher population densities (e.g., Gardner, 2013; Monroe et al., 2013; Eerkens et al., 2014a,b). Taking this hypothetical reconstruction one step further, the genetic data are particularly interesting. As the Burial 85 males are not maternally related, our hypothesis suggests that such fissioning must have occurred across multiple kin lineages, not one or two families that were outcast or voluntarily left a village. This may indicate that establishing new villages was a planned and organized activity, with the new settlers comprising a cohesive social group with a number of unrelated or distantly related individuals (i.e., a viable genetic population). Evaluation of such demographic expansion models will require further exploration with larger datasets.

Finally, we note the devastating effect that the loss of seven seemingly healthy males in their prime must have had on the village that the Burial 85 males were from. Most villages in Central California were small. Though it is difficult to estimate population sizes from archaeological data, ethnographic studies show that villages were typically comprised of less than twelve households, and often as few as two to four (Kroeber, 1925; Baumhoff, 1963; Cook and Heizer, 1965; Bettinger, 2015). Assuming half of the inhabitants in a village are women, and half of the remainder are either children or elderly, seven males likely comprised a significant proportion of the adult male population in such a village. These males, of course, would have been partly responsible for provisioning their families, especially with wild game, and protecting their village from intruders.

CONCLUSIONS

New isotopic and genetic data from a mass burial from CA-ALA-554 provide important context to the event leading to the death and interment of seven healthy males. Our data support the initial interpretation of the excavators (Estes et al., 2012) that the pit reflects an ancient violent event. Our data show that the seven Burial 85 males were not local to the Amador Valley, where they were buried around AD 850. Instead, they seem to have been living in a riverine environment to the east, in or near the San Joaquin Valley. This finding indicates that intergroup rather than intragroup violence accounts for this ancient violent event. mtDNA data indicates the males were not maternally related, but represent at least four different matrilineages. This suggests that intergroup warfare in the region was organized above the level of the family, at higher multifamily or perhaps multivillage levels.

Analyses of teeth provided important contextual life history information on the previous places of residence of the Burial 85 males. Isotopic data suggest that the men did not spend their entire lives in their natal village but rather moved to a second location after their teenage years. Stable isotopes suggest that their natal village was also likely somewhere in the San Joaquin Valley, but perhaps slightly to the north of their most recent residence. After age 13 to 16 when formation of enamel in their third molars was complete, the men moved to the south, closer to, but not in the Amador Valley.

Demographic expansion provides a possible model that could explain this particular case. Such a model predicts that villages in high-density areas can outgrow their resource base and/or ability to maintain peaceful internal social and political climates, resulting in group fissioning and territorial expansion. Groups of maternally unrelated males, and presumably females, then fissioned off from these villages to start new ones nearby in areas with lower population density, where they would need to establish new hunting and gathering territories. Of course, these new foraging territories were often already being used by other people. Such fissioning, then, brought may have brought groups of people into conflict. We hypothesize that the mass burial at CA-ALA-554, and perhaps others previously reported (Gardner, 2013; Eerkens et al., 2014a,b), may have been a byproduct of such territorial expansion.

Although this reconstruction and explanatory model has not yet been evaluated within a broader framework for California, additional cases will provide empirical data from which to test it. As well, such demographic expansion also provides a detailed model that may explain empirical patterns in the distribution of linguistic groups in Central California (Golla, 2011; Codding and Jones, 2013). The distribution of languages suggests that some groups were able to expand their geographic range at the expense of others (Golla, 2011; Codding and Jones, 2013), including the proposed Numic expansion in the Great Basin (Bettinger and Baumhoff, 1982, 1983), the Miwok expansion (Moratto, 1984), and the Patwin intrusion (Golla, 2011).

In any case, the CA-ALA-554 case builds on a growing database of studies that show that hunter-gatherers in Central California did not always live in peace, as commonly romanticized. Instead, like all human societies (Ferguson, 1984; Chagnon, 1988), interpersonal and intergroup conflicts occasionally led to acts of violence, including mass homicide. It is only through the careful archaeometric analysis of these cases that we can begin to untangle this more realistic, nuanced and anthropologically interesting prehistory.

ACKNOWLEDGMENTS

The authors thank TJ and Ramona Garibay (Most Likely Descendant) for supporting the research, and William Self Associates for providing access to the materials. The authors thank Andy Engilis of the UC Davis Museum of Wildlife Fisheries Biology for access to the freeze dryer, Joy Matthews (Stable Isotope Facility, UC Davis) for assistance with the stable isotope work, Fred Tulleners for his support, and Robert Bettinger for discussions regarding the data.

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