In search of homelands: using strontium isotopes to identify biological markers of mobility in late prehistoric Portugal

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1. Introduction

The archaeological record of the Late Neolithic through Early Bronze Age in the Estremadura region of Portugal (Fig. 1) provides clear evidence of the rise of a socially-complex, chieftain-scale non-state society (Cardoso, 2007; Gonçalves, 1999; Lillios, 1995). While several settlement sites in this area have been discovered (including Fórnea, Pico Aguda, and Boiaca), the most prominent and well-excavated is the walled fortification of Zambujal (c. 2800–1800 BC), which has long been considered a central location of population aggregation, craft production, and metallurgy in this region until its eventual abandonment during the Bronze Age. While it is assumed that population migration and long distance trade played an important role in the region’s development, little is known about the migration patterns of individuals or groups. Therefore, this study uses strontium isotope ratios (87Sr/86Sr) in dental enamel to distinguish non-local individuals from seven Late Neolithic–Early Bronze Age burial populations related to Late Neolithic and Copper Age settlement sites, in particular Zambujal, near the municipality of Torres Vedras in the Estremadura region (Fig. 1).

2. Strontium isotope ratios and landscapes

In archaeological research, the measurement of radiogenic strontium isotope ratios (87Sr/86Sr) in biological tissues can be used...
to identify human and animal migration patterns (see Beard and Johnson, 2000; Bentley, 2006; Price et al., 2002, 2012). This is possible because the strontium isotope signature of each particular geographic area permeates the landscape and local groundwater and is absorbed into the local plants and residing animals. Strontium is incorporated into teeth and bone through water and food intake. Due to its close chemical affinity, it substitutes for calcium in the mineral component (hydroxyapatite) of hard tissues (Bentley, 2006; Ericson, 1985; Nelson et al., 1986; Schroeder et al., 1972:496). Radiogenic strontium isotopes (\(^{87}\text{Sr}/^{86}\text{Sr}\)) do not fractionate when absorbed into human and animal tissues, and thus an organism’s strontium isotope signature directly reflects the bioavailable strontium in its environmental range. Therefore, animals and humans occupying the same territorial ranges and ingesting only local plants, animals, and water, should bear similar strontium isotope signatures. Conversely, between regions that are geologically distinctive, humans and animals should exhibit differences in strontium isotope ratios according to the local lithology. When significant geologic heterogeneity exists in larger regional landscapes it is possible for humans and/or animals to migrate into areas in which the local bioavailable \(^{87}\text{Sr}/^{86}\text{Sr}\) ratio deviates enough from that of the home range for this difference to be clearly identifiable in analyses of hard tissues. This approach requires geologic diversity over transversable distances, and thus our study area in central Portugal should be amenable to strontium isotope fingerprinting of human migration given the wide range of rock types of different ages present in the region (Fig. 2). However it is important to clarify that this methodology is unable to distinguish between individuals who originate from different locations that share similar bioavailable \(^{87}\text{Sr}/^{86}\text{Sr}\) values, and therefore the number of migrants recognized will provide only a minimum estimate of mobility.

2.1. Geology of the Estremadura

The Estremadura region of southwestern Portugal borders the Atlantic coast and encompasses both the Lisbon and Setúbal peninsulas (Fig. 1). Geologically the region is quite diverse, occupying part of the Lusitanian Basin, a northern Atlantic basin formed during a rifting phase of the late Triassic. This basin, which is mainly composed of Cretaceous and Jurassic sediments with pockets of Triassic sediments in the north, connects to the Alentejo and the Algarve Basins in the southeast, and is delineated in the north and east by the Late Paleozoic Hercynian basement rocks of the Iberian Meseta (Cunha and dos Reis, 1995; Wilson, 1988) (Fig. 2). The landforms of the Lusitanian Basin are geologically younger than other parts of the Iberian Peninsula and are mainly composed of heterogenous lithologies including limestones, sandstones, clays, marls, basalts and volcanic rocks (Azerêdo et al., 2002; Wilson, 1988). In general the Lusitanian Basin lacks many of the igneous granites and metamorphic schists found in the Portuguese interior, although some intrusive massifs of granites are found west of Lisbon near the municipality of Sintra (Sparks and Wadge, 1975). The Lusitanian Basin is further divided by faults into the Northern Lusitanian Basin, the Central Lusitanian Basin, and the Southern Lusitanian Basin (Schneider et al., 2009). In the northeast of the Central Basin the landscape is dominated by Jurassic marine limestone massifs, while in the inland west and south, Jurassic limestones are interspersed with large areas of Cretaceous sandstones and conglomerates (Schneider et al., 2009). Basalts are prevalent in the volcanic complexes around Lisbon and in the southeast of the region the lowlands of the lower Tagus basin are mainly composed of Triassic sandstones. Rivers, including the Almonda and the Nabão originate in the northern highlands and cut through the southeastern Tagus Tertiary Basin where Miocene
sediments include sandstones, clays and conglomerates (Antunes et al., 1999; Marks et al., 1994). Numerous karstic caves systems permeate the landscape. The plentiful caves and alkaline soils of the region provided an excellent environment for the preservation of archaeological and biological materials. The carbonate-dominated Mesozoic sediments of the Lusitanian Basin are expected to have $^{87}\text{Sr}/^{86}\text{Sr}$ close to marine values (0.707–0.710: e.g. Schneider et al., 2009), or slightly higher depending on the contribution of clastic deposits (local water analyses have $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.709–0.711: Voerkelius et al., 2010). Additionally, as this is a coastal region, seawater rainfall and sea spray incorporation into the terrestrial food chain may also lead to $^{87}\text{Sr}/^{86}\text{Sr}$ ranges that are close to seawater values (Bentley, 2006). In contrast, the older Paleozoic Hercynian basement metamorphic and granitic rocks of the interior should generally have more radiogenic values ($^{87}\text{Sr}/^{86}\text{Sr} > 0.713$: e.g. Bea et al., 2003).

3. Materials and methods

3.1. Selected archaeological sites

While the goal of this study was to analyze human skeletal remains related to the settlement complexes associated with the fortified site of Zambujal in order to investigate mobility patterns in this region, human settlements and burial places were geographically separate in the Estremadura region of Portugal during the Late Neolithic and Copper Age. Consequently, with the exception of a small amount of dental remains from three individuals recovered from the ruins of Zambujal itself, all other sampled humans are drawn from contemporaneous burials in the surrounding region that are believed to house the dead of communities related to Zambujal. Human remains were selected from six collective burial sites located within 25 km (15 miles) of Zambujal near the city of Torres Vedras (Fig. 3). The included sites are: (1) The natural cave burial site of Cova da Moura (Belo et al., 1961; Gallay and Spindler, 1970; Silva, 2003; Spindler, 1981); (2) the natural cave burial site of Feteira II (Waterman and Horwath, 2009; Waterman, 2012); (3) the natural cave burial site of Lapa da Rainha II (Kunst and Trindade, 1990; Waterman, 2012); (4) the artificial cave (rock shelter) burial site of Bolores (Kunst and Trindade, 1990; Lillios et al., 2010); (5) the artificial cave (rock cut tomb) site of Cabeço da Arruda I (Silva, 2002, 2003; Trindade and da Veiga Ferreira, 1956); (6) the large burial tholos of Paimogo I (Tholos de Pai Mogo I) (Gallay et al., 1973; Silva, 2002, 2003; Spindler and Gallay, 1972). These sites are diverse in terms of their funerary context, perhaps representing differences in social status by burial type (Waterman, 2012), and span the Late Neolithic through Early Bronze Age (3500–1800 BC), coinciding with the time period in which Zambujal grew, flourished and was eventually abandoned.

3.2. Sampled materials and methods

When evaluating the strontium isotope ratios in hard tissues it is important to make a distinction between bone and dental enamel.
when it comes to strontium uptake. Bone remodels throughout life in response to stress, strain, and calcium homeostasis (Manolagas, 2000), therefore, the strontium isotope signature in bone may change over time if a person moves into a new region or consumes non-local foods. This means that a previous native strontium isotope signature in bone may be altered or eradicated in time making it difficult to distinguish between native and migrant individuals from their bones alone. In contrast, dental enamel does not remodel during life (Simmer and Hu, 2001) and therefore preserves the strontium isotope signal from the time of original dietary uptake during enamel formation. For the permanent 2nd and 3rd molars used in this study, this formation time is during the first 4–16 years of life (AlQahtani et al., 2010). Enamel is also more robust with respect to diagenesis and alteration than bone (e.g. Price et al., 2002), and thus, it can be expected to retain the original bioavailable strontium isotopic signature more consistently.

When seeking to discern the presence of individuals of non-local origin in a burial population, it is first important to define the local bioavailable \(^{87}\text{Sr} / {^{86}\text{Sr}} \) isotope composition. Two established methods to estimate the local \(^{87}\text{Sr} / {^{86}\text{Sr}} \) range for archaeological samples are: 1. using the mean of human dental enamel analyzes ± 2 s.d. (Bentley et al., 2004; Price et al., 2001), or 2. using local faunal remains. In particular, tooth and bone samples taken from animals with very limited geographic ranges (e.g. Oryctolagus [rabbits], Helix [snails], or other small fauna) recovered from the same archaeological sites where the human population sample is drawn provide the best faunal estimate of the local \(^{87}\text{Sr} / {^{86}\text{Sr}} \) range (Bentley et al., 2004; Price et al., 2002). Additionally, Price et al. (2002) propose that samples from larger fauna be included as well, as a clear understanding of the migration ranges of multiple species will help to inform our understanding of human migration patterns. In accordance with these suggested practices, in this study \(^{87}\text{Sr} / {^{86}\text{Sr}} \) isotope ratios derived from large and small fauna (Bos [cattle], Helix [snails], Oryctolagus [rabbits], Ovis/Capra [sheep/goat], and Sus [pigs]) were examined and compared with the human data. As dietary intake influences \(^{87}\text{Sr} / {^{86}\text{Sr}} \) isotope ratios in calcified tissues, it is important to consider dietary practices when examining human strontium ranges. In particular, a high amount of marine foods in the diet can influence \(^{87}\text{Sr} / {^{86}\text{Sr}} \) isotope ratios by bringing them closer to the marine range. As the sampled Zambujal community lived in an estuary environment close to the coast, the influence of marine food input should be considered. However, based upon stable carbon and nitrogen analyses recently completed on these same burial populations, diets appear to be based largely upon terrestrial proteins and C3 plants with little marine input (Waterman, 2012).

For this study, dental remains from 55 humans and 22 animals were selected from the aforementioned sites. Enamel surfaces were first cleaned with acetone and the top layer of enamel was removed to prevent diagenetic contamination (Budd et al., 2000; Price et al., 2002; Wright, 2005). A small amount of enamel (4–10 mg) was then removed for analysis using a Dremel tool and a Dremel 5/64 in. diamond wheel point. Third and second molars were preferentially selected. However, in cases when other molars were not available, 1st molars were used instead. All chemical processing of the enamel samples was carried out in the University of Iowa Department of Earth & Environmental Sciences clean laboratory. Samples were dissolved in 1 mL of 3 M HNO₃, using sonication to aid digestion. Strontium was isolated with Eichrom Sr-spec ion-exchange resins using standard procedures (see Waight et al., 2002). \(^{87}\text{Sr} / {^{86}\text{Sr}} \) ratios were then measured using a Nu Plasma HR multi-collector inductively-coupled-plasma mass-spectrometer (MC-ICP-MS) in the Department of Geology at the University of Illinois at Urbana-Champaign. Samples were introduced to the machine.
using a Nu Instruments DSN-100 desolvator system equipped with a nebulizer with an aspiration rate near 0.1 mL min⁻¹. The samples were alternately run with standards (SRM 987, SCS coral and E&A) using a sample-standard-bracketing measurement protocol wherein standards were run every 3–5 samples (Rehkämper et al., 2004). The ⁸⁸Sr beam intensities for all samples and standards ranged from 4 to 12 V (100 ppb solutions). Masses of ⁸₃Kr to ⁸₈Sr were measured during a single cycle comprised of 2 blocks of 25 scans (5 s integration per scan) with a 40 s baseline determination using ESA-deflected signals. Instrumental mass bias was internally normalized to an ⁸⁶Sr/⁸⁸Sr ratio of 0.11940 and then corrected ratios were normalized to the NIST SRM 987 international standard value of 0.710268 (which had a reproducibility of ±0.000038: 2 s.d., n = 46) to correct for day-to-day variability. The SCS coral standard gave ⁸⁷Sr/⁸⁶Sr of 0.709176 ± 0.000016 (2 s.d., n = 16). No corrections were necessary for Sr introduced as part of sample production as procedural blanks were <100 pg Sr.

4. Results

4.1. Faunal results

The results for all the sampled fauna are presented in Table 1 and Figs. 4 and 5. For the three snail shells recovered from soils excavated at Bolores, the ⁸⁷Sr/⁸⁶Sr isotope ratios ranged from 0.7111221 to 0.7111881 with a mean of 0.711517 ± 0.000335. The low standard deviation in this group likely reflects the extremely limited movement of snails across the landscape, and the fact that the samples were recovered from the same site. The ⁸⁷Sr/⁸⁶Sr isotope ratios for the six rabbit samples ranged from 0.709750 to 0.713280 with a mean of 0.711768 ± 0.001333. All of the rabbits came from the archaeological levels at Bolores, with the exception of one recovered from Zambujal. The range for rabbits was larger than for snails, possibly reflecting a more widespread territorial range. ⁸⁷Sr/⁸⁶Sr isotope ratios were also obtained from the pig samples, one from Zambujal and one from Cova da Moura. Like the rabbit sample from Zambujal, the pig samples from Zambujal had an ⁸⁷Sr/⁸⁶Sr isotope ratio on the lower end (0.708054) of the overall range of all of the surveyed animals. The ⁸⁷Sr/⁸⁶Sr ratios for the seven ovicaprid (sheep/goat) samples ranged from 0.705502 to 0.711643, with a mean value of 0.709212 ± 0.002. All of the tested ovicaprids were acquired from the site of Zambujal. Despite the fact that all of the ovicaprids were acquired from the same site, they have the largest ⁸⁷Sr/⁸⁶Sr ratio range of any of the sampled faunal groups. This most likely is a reflection of the extremely diverse foraging behavior of sheep and goats, and the likelihood that the animals may have been brought in from other areas with different bioavailable strontium signatures. One animal in particular, Z1129, has ⁸⁷Sr/⁸⁶Sr ratios that are significantly divergent from all of the others (0.705501), the lowest ⁸⁷Sr/⁸⁶Sr ratio record for any of the animals. The three bovids that were tested exhibited an ⁸⁷Sr/⁸⁶Sr isotope ratio range of 0.707795–0.711497, with a mean value of 0.709739 ± 0.001858. Once again, despite the fact that all of the sampled bovid mandibles were derived from Zambujal, the ⁸⁷Sr/⁸⁶Sr isotope ratios were quite varied, although not as variable as the ovicaprid values. These variations likely reflect that bovids were also grazing on diverse landscapes, or that some domesticated animals were being traded into the region from neighboring areas. All of the rabbit samples except one came from the site of Bolores. The non-Bolores rabbit sample was from Zambujal and had a much lower ⁸⁷Sr/⁸⁶Sr isotope ratio. The large ranges presented by the animals sampled in this study suggest: 1) more variation in the bioavailable strontium across limited distances than expected, 2) larger animal migration ranges, or 3) long-distance animal trading (Figs. 6 and 7).

Table 1 ⁸⁷Sr/⁸⁶Sr ratios for fauna.

<table>
<thead>
<tr>
<th>Site</th>
<th>Genus</th>
<th>Sample</th>
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4.2. Human results, defined local range and identified migrants

When the human data from the individual sites are considered, the standard deviations were low in comparison to the faunal remains (Table 2). This result was unexpected. In other studies (e.g. Price et al., 2002), humans typically have greater Sr isotope variability in comparison to small fauna. Because of the large ranges found in the animals in comparison with the human samples, the human samples were used to determine the local bioavailable ⁸⁷Sr/⁸⁶Sr isotope ratio range. The mean of the ⁸⁷Sr/⁸⁶Sr isotope ratios for the entire human sample with outliers removed is 0.710115. Thus, the local bioavailable ⁸⁷Sr/⁸⁶Sr composition for the surveyed region (roughly 25 km²) is defined as 0.70900–0.7115 (plus or minus 2σ from the sample mean). Based upon this local range, only two samples from Zambujal were indicated as being potentially migrants.
burials, Cova da Moura and Cabeço da Arruda I, contained sampled individuals whose $^{87}$Sr/$^{86}$Sr ratios were outside the local range (CM 2, CM 95, CM 81, CM 30, and CAI 11).

5. Discussion

For the sampled human population, only 9% (5 out of 55) can be classified as migrants into the region. In most of the sampled burials, no non-local individuals were identified, while the majority of the identified migrants (4 out of 5) come from one site, the large burial cave of Cova da Moura. The only other burial to contain non-local individuals is Cabeço da Arruda I, for which one migrant was identified. In addition to housing 80% of the migrant individuals identified in this study, Cova da Moura is the only burial in which individuals with significantly enriched $^{87}$Sr/$^{86}$Sr ratios were found. In particular two individuals from Cova da Moura had $^{87}$Sr/$^{86}$Sr ratios between 0.714 and 0.721 (CM 95, 0.720730 and CM 30, 0.714383), reflecting childhoods potentially spent in a region with geologically older features. The high proportion of non-local individuals in the Cova da Moura cave suggests that this burial is somehow socially distinct with 4/12 or 30% of the Cova da Moura sampled individuals having spent at least part of their childhoods elsewhere. Cova da Moura is also an exceptional burial in terms of its relative wealth of well-preserved Late Neolithic and Copper Age artifacts, many of which, such as jet, variscite, and ivory objects, are rare or imported from distant locations (Schuhmacher et al., 2009; Thomas, 2011). However, while all of the burial locations selected in this study were used for hundreds of years, radiocarbon dates from Cova da Moura suggest that burials at this location span a larger temporal window with this cave being used for burials for as long as 1000 years (Cunha et al., 2007). Thus, it is possible that the larger percentage of identified migrants is also related to Cova da Moura's relatively long use-life.

General intersite variability of human $^{87}$Sr/$^{86}$Sr ratios is low. The exceptions to this are Cova da Moura, the large cave burial with the greatest number of migrants, and the tholos of Paimogo I, in which human $^{87}$Sr/$^{86}$Sr ratios appear to be slightly elevated in comparison to the other burial populations. In contrast, the sampled fauna (with the exclusion of the snails) display more variability, and all animal groups have higher standard deviations than are found in the human burials (Cova da Moura excluded). When considering the variation in the sampled animals, one of the three bovids, one of the two pigs, and two of the seven ovicaprids can be classified as non-local according to the defined local range. All of these possible migrant animals exhibit $^{87}$Sr/$^{86}$Sr isotope ratios outside the lower end of the local range as defined by the human population. The majority of the fauna examined in this study and all of the fauna with non-local $^{87}$Sr/$^{86}$Sr ratios were recovered from Zambujal. Based upon a cursory investigation of faunal skeletal element distributions from Zambujal while selecting faunal elements to sample, a relative lack of head and lower extremities in comparison with other portions of the skeleton was noted. This may suggest that animals were being butchered elsewhere and that Zambujal may have been the location of final consumption rather than the place where animals were raised and fed. Therefore, variations in the $^{87}$Sr/$^{86}$Sr ratios from the animals recovered from Zambujal may be attributable to animals being brought to Zambujal from other areas, possibly for feasting activities or as tribute.

While rabbits are generally considered to be a good animal to use to approximate the bioavailable range because they have limited territorial movement, in this study all of the rabbits but two exhibited $^{87}$Sr/$^{86}$Sr ratios above the defined human bioavailable range, included three rabbits which can be classified as non-local according to the defined local human range. As part of the Lusitanian Basin, the Estremadura in general is composed of Cretaceous and Jurassic sediments and lacks many of the igneous granites and metamorphic schists found in the Portuguese interior that would be expected to result in elevated $^{87}$Sr/$^{86}$Sr ratios. However, heterogeneous lithological features abound at a local scale in the
Strontium isotope ratios (\(^{87}\)Sr/\(^{86}\)Sr) have been used to identify migrant individuals in archaeological sites in Portugal. Migrants were identified based on higher \(^{87}\)Sr/\(^{86}\)Sr ratios compared to the local population. This approach has been particularly useful in the Estremadura region, where a variety of geological features such as variscite, slate, and amphibolite were used as raw materials in the Neolithic and Copper Age. The higher \(^{87}\)Sr/\(^{86}\)Sr ratios in migrants compared to the local population suggest that they originated from different areas, and in some cases, as far as 200 km away. This study supports the idea of long-distance migration and the integration of different communities in the Neolithic and Copper Age in Portugal.
of the non-local individuals from Cova da Moura had $^{87}$Sr/$^{86}$Sr ratios that could match the older geologic formations of the Alentejo region of Portugal. This would correspond with known exchange patterns, and suggests that both people and goods were moving into the region from the Alentejo and perhaps vice versa. Surprisingly, the $^{87}$Sr/$^{86}$Sr ratios for the fauna were much more heterogenous than for the human data. This was unexpected and suggests that either the fauna (especially larger domesticated animals) had a higher mobility than most of the human populations and/or that the humans largely subsisted on food and water sources from a limited geographic area compared to the fauna. The heterogenous lithological features of the Estremadura may also influence our understanding of the range of faunal variability. Thus, further sampling of small fauna from a larger range of sites would be useful in creating a map of the bioavailable $^{87}$Sr/$^{86}$Sr ratios ranges for the region, allowing us to more clearly trace prehistoric human mobility in the Estremadura. While the methodology used in this study can only identify the minimum number of migrants based upon the geological diversity of natal landscapes, comparison of our results with preliminary work from nearby sites (Boaventura et al., 2010) indicate that within a 20–200 km range, there are significant isotopic variations in bioavailable Sr ($^{87}$Sr/$^{86}$Sr: 0.706–0.708 Estria and Caraveiros; 0.709–0.712 Zambujal; 0.715–0.718 Pêrigões; Fig. 2), demonstrating the utility of Sr isotopes for migration studies in this part of the Iberian Peninsula.

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