Multiproxy evidence highlights exceptional heterogeneous social status in the Middle Neolithic in Europe

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Abstract

The contribution of multidisciplinary approaches to the study of the lifestyles of ancient populations is now well established in the literature. Mobility, nutrition and physical activity are indicators that, when considered together, can document social organisations such as patrilocality, gender practices or social status. Our study is part of this dynamic scientific work and focuses on the Middle Neolithic human remains from the site of Pontcharaud (excavation 1986; 4300-3900 BCE; Auvergne, France). It aims to reconstruct patterns of behaviour, life history, and social organisation, particularly in light of the site's unique funerary features, which remain a subject of debate. New data from bioanthropological investigations (72 individuals), stable isotope measurements (bone collagen $\delta^{34}S$ from 55 individuals; $^{87}Sr/^{86}Sr$ from teeth enamel on 17 individuals) and genetic sex estimation (2 subadult individuals) were collated and integrated with contextual information, including funerary treatments, previously published isotopic data ($\delta^{13}C$, $\delta^{15}N$), and archaeological records. Stable isotope data suggest that some females were exposed to more variable environments compared to males. Strontium and sulphur isotope ratios also indicate differences in resource exploitation (water, plants, and animals from different origin or growth conditions) and suggest a different provenance for at least two females, one of whom was buried in a stone coffin. The seven adult and immature male individuals from the site's only multiple grave yielded distinct stable isotope results (C, N, S and, to a lesser extent, Sr), suggesting different origins and/or animal protein intake, compared to individuals buried in single or double graves. Our multidisciplinary data reveal important variability in diet, provenance, and organisation within the Pontcharaud funerary group. We propose that the seven males from the multiple gathering may have been involved in a shared activity that exposed them to the same environment—perhaps as individuals engaged in a common economic or social practice.

Keywords: Isotopes, Mobility, Diet, Funerary treatment, Economy, Social structuration

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The authors declare that the data supporting the findings of this study are all available within the article and its supporting information (SI) files.

Main text (2500-6000 words)

Introduction

Multidisciplinary investigations aimed at reconstructing past human behaviours have proven particularly effective in understanding the variability of mobility and dietary patterns during Prehistory as well as in exploring gender roles and social status (e.g. Knipper et al 2017; Goude et al. 2019; Mittnik et al. 2019; Cassidy et al. 2020; Masclans Latorre et al. 2021a). Several studies on Neolithic sites across Europe have demonstrated differences in mobility patterns between females and males throughout their life. The trend of a patrilocal system associated with female exogamy is frequently proposed, supported by genetic evidence of exogenous origins and a broader range of strontium (Sr) values recorded in females (e.g. Rivollat et al. 2023; Rey et al. 2019; Bentley et al. 2012; Bickle and Hoffmann 2007; Goude et al. 2019; 2020a). However, early farmers' mobility is not solely restricted to females; rather, it was part of a more complex social organisation within agropastoralist communities. This complexity is increasingly evidenced by the growing dataset of strontium and sulphur (S) isotope analyses (e.g. Rivollat et al. 2023; Bentley 2013; Hofmann 2020).

Differences in access to food resources between individuals, particularly between males and females, have been well documented in Neolithic Europe through carbon (C), nitrogen (N) and sulphur stable isotope analysis on bone collagen (e.g. Goude et al. 2020a). More recently, analysis of dentine collagen has also revealed behavioural variation over the course of an individual's life (e.g. Goude et al. 2020b), which may be related to "social ages" (e.g. Rey et al. 2021) and possibly to the sexual division of labour across age categories (e.g. Villotte, Knusel 2014). For example, an isotopic study based on sequential tooth sampling at the Neolithic site of Gurgy (Yonne, Paris basin, France) explored the possibility of documenting sex-based roles during childhood and adolescence. It highlighted isotopic shifts (C, N, and S) between females and males, first around the age of 9, and then again at approximately 14, mirroring modifications in funerary treatment for the same age categories (Le Roy 2015; Rey et al. 2021). In parallel, sexual division of labour among European Neolithic groups can be inferred from multiple lines of evidence, including: stone tool morphology and use wear in association with the grave goods of the deceased in burials (Masclans Latorre et al. 2021a), high humeral rigidity in females suggesting intense manual labour (Macintosh et al. 2017), lateralized limb use in males possibly linked to throwing behaviours (Villotte, Knusel 2014), and high-intensity upper limb activity in males, possibly related to the practice of archery (Thomas 2014). Other authors have proposed that, rather than biological sex, social status – particularly the social status of exogenous individuals – may also play a significant role in the division of labour. This argument is supported by Goude et al. (2019) who compared multi-element stable isotope data (C, N, S, Sr) and grave goods associated with the deceased (e.g. flint stone, lamb foetuses buried with the deceased).

Beyond the mobility arguments, patrilocal patterns observed in European communities during the Neolithic and throughout the Bronze Age (e.g. Knipper et al. 2017) highlight the importance of both biological and possibly social kinship. The recent data from Gurgy (Yonne valley, Paris basin, France) is particularly eloquent (Rivollat et al. 2023), as a body of genetic and isotopic data points to a paternal rooting in the management of the territory, funeral ritual, and perhaps the family in the broadest sense. The role of sex and social status in the social organisation of Prehistoric communities is also demonstrated by the study of Mittnik et al. (2019), which compares genomic and isotopic data (Bronze Age, Germany), as well as the study of Cassidy et al. (2020), which shows restrictive reproductive access linked to the social elite and megalithic use in Neolithic Ireland. This brief synthesis of scenarios from Prehistoric Western Europe highlights the complexity and diversity of social organisation patterns regulated by at least sex and social status, but also perhaps by age and other factors that can arise in the course of an individual's life (starvation, economy collapse, environmental constraints, interpersonal violence, etc.).

In this context, the Middle Neolithic funerary site of Pontcharaud (Auvergne, Massif Central, France) raises new questions. The Pontcharaud necropolis is characterised by the diversity of funerary deposits and practices. There is no bias toward a particular age or sex for the deceased in the mainly individual and double graves. However, its originality lies in the presence of a multiple gathering[1] of seven individuals buried simultaneously. This deposit is exceptional for the Middle Neolithic period in France, and its signification, as well as the social status of this particular funerary group, is still debated (e.g. Testard 2004; Chambon and Leclerc 2007; Boulestin 2008; Schmitt and Saliba Serre 2014), with interpretations ranging from warriors, accompanying dead, to sacrificed people. At the site level, a previous study including stable isotope (carbon and nitrogen; summary in table 1 and SI 1) and osteological records (Goude et al. 2013) pinpointed dietary differentiation among two categories of individuals: adult females and the seven individuals found in the multiple gathering. Using a multidisciplinary approach that includes osteobiography, biogeochemistry, ancient DNA, and a revised reading of archaeological records, we aim to focus on this special osteological series to explore the role of interrelated biological sex and social status in the formation of this community.

Materials and Methods

Pontcharaud 2 site: a unique archaeological discovery

The first excavation of the Pontcharaud site (figure 1) was conducted in 1986 under the supervision of G. Loison (1998), uncovering 56 archaeological funerary structures (Schmitt et Saliba-Serre 2014)

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[1] Multiple gathering is defined by Boulestin and Courtaud (2022) : if at least two people have been deposited in a unitary use, one will speak of gathering *(rassemblement*) and a gathering is termed ‘multiple’ (*multiple*) whenever it is possible to demonstrate that all the individuals have been gathered at the same time.

containing 72 individuals, dated to the Middle Neolithic chronocultural period (4300-3900 cal. BCE; Goude et al. 2013). In 2019 and 2020, a new excavation was conducted adjacent to the former funerary occupation (Delrieu 2022), discovering 50 new burials (Prodeo et al. 2024). The bioarchaeological investigation of this new site is still ongoing[2]. Besides, in 2000, another excavation in the same locality yielded an additional ten Neolithic burials, which shared similarities in funerary treatment (site of Brézet in Clermont-Ferrand Inrap Excavation, Vernet 2002). Thus, the history of fieldwork in this area suggests the potential for division into sectors, indicating that the material investigated in the present paper, centred on the 1986 remains, may only represent a specific part of the burial ground use by the same pòpulation.

Among the structures excavated in 1986, individuals were found in different burial types, predominantly within wooden coffins (a well-documented funerary practice in this area; Saintot et al. 2016), except for one individual discovered within a stone coffin (individual 12). Overall, grave goods associated with the deceased are infrequent (Loison 1998; Civetta et al. 2009). Most burials contain either one or two deceased. However, one deposit in particular stands out as a multiple gathering (figure 2). This unique structure contains a total of seven individuals (individuals 31 to 37) who were buried simultaneously. Six of them were deposited in a prone position, oriented along a north-south axis and facing northward, except for one skeleton iIndividual 36) lying southward. This individual (36), located at the eastern extremity of the pit, was lying on his back, slightly rotated onto his right side. The first five bodies deposited (33 to 37), in the north part of the pit, were aligned in a row, with their heads oriented along an east-west axis. The last two (31, 32) were shifted to the south. The bodies were initially covered with earth, followed by two limestone slabs of large dimensions and smaller stones covered the deposit, in direct contact with the human remains. Notably, individual 37 was found with a flint arrowhead in an upper thoracic vertebra, while individual 33 had received a blow to the head (Billard 2010). Both traumas strongly suggest a violent death.

The osteological collection

A set of bioanthropological data (sex, age, stature, and pathologies) has been reported in various previous publications (Civetta et al. 2009; Billard 2004; 2010; Schmitt and Saliba-Serre 2014). A previous study (Goude et al. 2013) analyzed 58 human and seven animal bones, providing carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope compositions of bone collagen. This research highlighted (1) a diet with significant animal protein intake for several individuals of both sexes, (2) a higher variability of nitrogen isotope ratio values among females, and (3) a distinct isotopic cluster corresponding to the individuals from the multiple gathering (figure 3; SI 1). These initial biochemical data allowed us to

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[2] Osteological studies by I. Thomson and collaborators https://journals.openedition.org/archeopages/14238?lang=fr

question the role of females and/or individuals with specific social statuses. The findings aligned with other datasets indicating a high level of biomechanical stress recorded in young adults (high spinal loads), possible sexual division of physical activities (based on long bone morphology), and varying geographic origins among females (suggested by cranial shape) (Civetta 2009; Billard 2010). These preliminary elements of discussion, combined with the well-preserved osteological collection of Pontcharaud, encouraged us to pursue further bioarchaeological investigations using a multidisciplinary approach. This included new isotopic analyses (S and Sr) to explore mobility patterns and social structures. Additionally, as a first step, palaeogenomic analyses were conducted on two subadult individuals from the multiple deposit to estimate their sex.

Methods

All the technical and laboratory information is available in Supplementary Information 2.

Osteological records

A control study was conducted on the 72 individuals to confirm the sex and age at death (SI 1). Depending on growth stage, different methods were applied to estimate the age at death of subadult individuals. For children under one year of age, stature was estimated from equations derived from regressions proposed by Sellier (in Schmitt and Georges 2005) based on diaphyseal length. For individuals older than one year, multiple methods were applied. Dental age proved to be a more reliable indicator than bone age and was therefore preferred. Tooth eruption and mineralization were assessed using Ubelaker's reference (1978). When teeth were absent or when the mineralization of permanent teeth was complete, age was estimated based on the fusion of secondary ossification points (Birkner 1980). For adults, age-at-death estimation was based on the state of fusion of later-fusing secondary centers of ossification, following Owings Webb and Suchey (1985) and Albert and Maples (1995), the surface characteristics of the pubic symphyseal face, and the morphology of the sacroiliac surface of the os coxae, following Schmitt's method (Schmitt, 2002, 2005). For sex assessment, the morphology and morphometrics of the os coxae were employed as the primary means of diagnosis, following Brůžek (2002) and Brůžek et al. (2017). For 9 individuals whose os coxae were not well preserved, we applied a secondary sex diagnosis based on discriminant function analysis, a demonstrably reliable method (Murail et al. 1999).

The presence of external auditory exostosis (EAE), an asymptomatic bony growth caused by irritation of the periosteum in the external meatus (Di Bartolomeo, 1979), was assessed following the criteria of Standen et al. (1997) and Crowe et al. (2009). Repeated exposure to cold water is the main factor triggering this irritation, and a high frequency of EAE in an archeological sample can be considered a strong indicator of activities related to aquatic resource exploitation (for a review, see Villotte & Knüsel, 2016). The presence or absence of EAEs was recorded for 45 individuals (34 adults and 11 subadults under 15 years of age). In most cases (82.2%), preservation allowed for bilateral assessment.

Enthesopathy at the humerus was recorded following Villotte' scoring system (2006, see also Villotte, Castex et al., 2010). Data were collected for four entheses on each humerus, for all "adults. However, results are discussed only for sexed individuals whose age at death was estimated to be under 50 years, considering the relative strong influence of age on the apparition of entheseal changes in the skeleton (e.g., Villotte & Santos, 2023). Fractured limbs were not scored, and individuals with systemic pathologies were excluded from the study (see Villotte, Castex, et al., 2010 for exclusion criteria). The final sub-sample consists of 15 females and 7 males. Skeletal markers associated with regular posture (namely squatting and kneeling) were recorded. For squatting, the presence or absence of lateral facets on the talus was assessed following Barnett (1954, stage F) and Boulle (2001, lateral pressure facet) for 35 adults (in most cases (82.9%), both tali were examined bilaterally). For kneeling, given the variability of skeletal changes associated with this posture (Ubelaker, 1979; Villotte, 2024), only changes on the dorsal aspect of the distal part of the diaphysis of the first metatarsal were recorded. Only major, clearly visible changes were considered. Nineteen individuals were included in this analysis, with bilateral preservation of the first metatarsals in only five cases. These osteological data are integrated as part of our holistic approach to the study of the living conditions of human groups. These bone indicators are combined with other information to discuss the impact of physical activities and potential mobility on access to and need for food resources (Masclans Latorre et al. 2021b).

Isotopic analyses

The protocols used for sample preparation and the equipment used for $\delta^{34}S$ and $^{87}Sr/^{86}Sr$ analysis is included in SI 2. Stable isotope analysis of sulphur ($\delta^{34}S$; figure 4) was performed on individuals previously analysed for carbon and nitrogen isotopes, as well as on the limited number of available animal remains (n=7) recovered from the burials (*Bos* sp., caprines and *Sus* sp.; Goude et al. 2013). Specifically, 55 human bone samples selected based on the best collagen preservation from the previous study (biological descriptions and data available in SI 1) were analysed to infer protein intake during the last years of life (SI 2) (e.g. Szulc et al., 2000 ; Valentin 2003). Sulphur isotope analysis aids in identifying food intake and assessing individual mobility (Nehlich, 2015). The spatial distribution of the $\delta^{34}S$ values for mainland France, as reported by Bataille et al. (2021), ranges from approximately 4‰ to 8‰ for the Auvergne region. This range will serve as a reference for interpreting our data. Radiogenic strontium isotope ratios ($^{87}Sr/^{86}Sr$) were measured in the tooth enamel of 17 individuals, selected based on the availability and preservation of dental material, as well as the prior CN data (considering individuals detected as isotopic outliers or based on their archaeological context) (SI 1). When possible, two teeth per individual were sampled: for 11 individuals, both an M3 and an M2/P4 were sampled, while for the remaining 6 individuals, only an M2/P4 was sampled. This sampling strategy enables the assessment of potential changes in geological substrate and/or sources of supply (water, plant, animal) between two life stages: childhood and early adulthood (SI 2). One of the challenges in the targeted region is the highly variable lithology of the Massif Central (SI 2), which results in significant variations in recorded $^{87}Sr/^{86}Sr$ values. Similar environmental complexities have been observed in Neolithic contexts, such as at Gurgy (Rey et al. 2021; Rivollat et al. 2023). Recent $^{87}Sr/^{86}Sr$ values published by Willmes et al. (2018) and Holt et al. (2021) for France indicate that within a 20km radius, and identically within a 50 km radius, from the site, $^{87}Sr/^{86}Sr$ can range widely, from 0.704 to 0.721 (fig. SI2 – 1). Additionally, geological investigations on granitic samples located at 75 km east of the site provide $^{87}Sr/^{86}Sr$ values between 0.721 and 0.723 (Briot 2002). Ideally, we would have to include archaeological microfauna teeth to establish a relevant local baseline, but there is none available at the site and it was not possible to do a broader own bioavailable map for the region by using modern plant as recommended by several authors (ex. Britton et al. 2020; Chevallier et al. 2025). .Given this context the current lack of site-specific mapping, the intra-individual sampling approach proposed here will open the discussion on the variation in lands traversed and used during an individual's lifetime.

Palaeogenomic analyses

Palaeogenomic analysis was conducted on both subadult individuals from the multiple gathering (individuals 34 and 35) to assess their genetic sex, which could not be assessed through traditional osteological methods (SI 2). Petrous bones were specifically targeted due to their superior DNA preservation (Gamba et al., 2014; Pinhasi et al., 2015), and the sampling was carried out directly at the osteological deposit of Les Milles at Aix-en-Provence, France. Details regarding DNA isolation, double-stranded libraries construction with partial UDG treatment, and the use of unique index pairs are provided in SI 2. A shallow shotgun sequencing of the libraries was performed to evaluate the preservation of endogenous DNA and, if a sufficient number of human sequences were retrieved, to estimate the genetic sex of the individuals. The endogenous DNA content and main characteristics of the DNA libraries were determined using EAGER v2.4.5 (see SI 2 for details). We evaluated the sex of the individuals using two independent methods developed by Skoglund et al. (2013) and Mittnik et al. (2016) (SI2).

Results

Osteological records

A total of 23 subadults and 38 adults (>15 years) were identified, with both sexes present (17 males and 19 females). An overall underrepresentation of children was observed. The specific simultaneous deposit of seven individuals contains exclusively adult males, along with the two subadults analysed for aDNA analysis in this study. Endogenous DNA was successfully obtained from both individuals 34 and 35 (with respectively 23,47 and 1,28% of endogenous DNA; see Table 2 in SI 2). The retrieved human sequences exhibited characteristics typical of degraded ancient DNA, including a noticeable percentage of deamination at the 3' and 5' extremities of fragments, and short fragment lengths (Table 2 in SI 2). Genetic sex estimation classified both subadults as males, based on Ry coefficients (0,0038 and 0,0142, respectively; Skoglund et al. 2013), which indicated genotypes "consistent with XY but not XX". However, it is important to note that the reliability of the result for individual 35 is lower due to the low coverage of the library (Skoglund et al 2013, Buonasera et al 2020). Nonetheless, the Y ratio calculated using Mittnik et al.'s (2016) method (0,53 and 0,78, respectively) further confirmed the assignment of XY genotypes for both individuals.

None of the studied individuals exhibit EAEs. This finding aligns with previous research indicating a very low frequency of EAE in European Neolithic groups, possibly due to a moderate reliance on the aquatic environment or a seasonal/temporary access to lakeside environments during life (Villotte, Stefanović, & Knüsel, 2014).

Enthesopathies of the humerus are relatively rare among the young and mature adult individuals (i.e., those under 50 years old) from Pontcharaud (see table 1 in SI 2). Only 3 males (out of 7) and 3 females (out of 15) display lesions, all of which are minor. This is expected, as major enthesopathic changes are predominantly observed in older individuals or those with systemic diseases, and are likely unrelated to biomechanical factors (Villotte, Polet, Colard, & Santos, 2022; Villotte & Santos, 2023). Most affected individuals exhibit only a single enthesopathy, except for female subject 13, who presents two lesions (one at the right lesser tubercle, and the other at the left lateral epicondyle). Given the very low number of lesions and the overall small sample size, any inferences regarding a potential sexual division of labor in this community remain limited. One can note, however, that two females (subjects 12 and 69, out of 8 examined) present a lesion on the right lateral epicondyle, whereas no males do. Additionally, only one individual (male subject 1) displays a lesion at the right medial epicondyle, more precisely at the attachment area of the medical collateral ligament. Lesions in this area have been associated with activities implying a throwing motion (such as spear throwing or the use of axes and adzes, see Polet et al. 2019; Villotte et al. 2010; Villotte & Knüsel, 2014).

None of the individuals display true squatting facets, as defined by Barnett (1954). However, if one adopts Boulle's (2001) perspective that the lateral pressure facet is an osteological feature associated with ankle hyperdorsiflexion, it is highly likely that squatting was a common practice within this community. Indeed, 94.3% of the individuals exhibit such changes, with only two exceptions (male subjects 32 and 39). This high frequency aligns with observations in other Neolithic groups (Villotte, unpublished data). In contrast, kneeling facets are rare in the sample. Only one individual (male subject 39, who notably lacks any pressure facet) displays one clear articular extension on the dorsal aspect of the distal part of the left first metatarsal shaft (the right metatarsal was not preserved). This may be associated with the fact that this individual displays a tibiofibular ankylosis at the right side, maybe related to a traumatic event.

Burial Nb	Sex	Age category	$\delta^{13}C$ vs. PDB (‰)	$\delta^{15}N$ vs. Air (‰)	$\delta^{34}S$ vs. CDT (‰)	$^{87}Sr/^{86}Sr$ enamel/Childhood	$^{87}Sr/^{86}Sr$ enamel/Adolescence
3	F	>60 yr	-19.9	10.0	6.30		
4	F	20-49 yr	-20.3	11.6	5.3		
5	M	>30 yr	-19.8	10.3	7.9		
7		>40 yr	-20.1	10.6	8.8		
8		NA	-20.2	10.6	9.3		
9	F	20-29 yr	-20.2	9.4	15.4	0.710213161	0.710110231
10	M	20-49 yr	-19.7	10.4	10.6		
12	F	20-29 yr	-20.3	9.6	10.2	0.714077411	0.708570854
13	F	20-39 yr	-19.9	10.5	8.3		
14	F	>40 yr	-20.0	10.2	7.3		
15	M	>40 yr	-19.9	9.9	9.4		
16	M	20-49 yr	-19.8	10.4	10.6		
18	M	40-59 yr	-19.8	10.3	6.2		
23	F	20-29 yr	-20.4	10.7			
26	M	40-59 yr	-20.2	11.8	6.8		
27	M	20-39 yr	-19.8	10.6	8.8	0.711569206	0.711603592
29	F	20-29 yr	-19.9	10.0	5.1	0.71089182	0.711305131
30	F	20-39 yr	-20.1	8.7	6.9	0.711130363	0.710944735
31	M	40-59 yr	-20.4	9.4	10.4	0.71008511	0.709490971
32	M	20-29 yr	-20.9	9.7	11.2	0.708686067	
33	M	>30 yr	-20.7	9.4	11.3	0.709797635	
36	M	40-59 yr	-20.5	9.7	11.7	0.710601041	0.7094357
37	M	20-49 yr	-20.8	9.3	11.2	0.711256671	
38	F	NA	-20.2	9.8	6.7		
39	M	20-49 yr	-20.4	11.3	5.0		
40	F	20-49 yr	-20.2	5.3	7.0		
41	F	40-59 yr	-19.8	10.3	6.4		
45	M	>40 yr	-20.2	10.5	5.2	0.711571341	0.711756647
51	F	40-39 yr	-20.3	9.8	3.6	0.71112485	
52	F	20-29 yr	-20.7	11.3	7.3	0.710537298	0.71288097
53	F	20-29 yr	-20.3	9.9	6.0	0.710300926	0.71061283
55	F	20-39 yr	-20.3	9.7	5.6		
60	M	>40 yr	-20.1	10.5	5.6	0.710960294	0.71110326
61	F	20-49 yr	-20.4	10.6	5.9		
62	F	NA	-20.7	13.0	4.5		
63	M	20-49 yr	-20.1	10.1	5.1		

66	M	>40 yr	-19.9	10.6	6.5		
69	F	20-39 yr	-20.2	9.9	7.0		
50		15-17 yr	-20.1	9.5	5.2		
2HS		6-12 months	-19.4	11.0	4.2		
20		2-4 yr	-19.9	11.1	4.4		
22		4-5 yr	-20.5	10.0	5.0		
24		1-2 yr	-19.8	12.0	5.2		
25		3-5 yr	-19.7	11.9			
26bis		2-3 yr	-19.8	10.3	7.3		
34	M	7,5-12,5 yr	-20.9	9.7	8.3		
35	M	12-15 yr	-20.7	9.9	9.7	0.710229219	
42		5-9 yr	-19.7	9.5	5.6		
43		5-6 yr	-20.7	11.4	5.2		
46		6-8 yr	-20.1	10.4	7.4		
54		1-3 yr	-18.9	12.7	5.1		
56bis		5-11 yr	-20.7	10.9	7.0		
57		5-11 yr	-20.4	11.0	5.2		
58		16-19 yr	-20.3	10.3	5.1		
59		7-11 yr	-20.4	9.4		0.71066622	
64		14-17	-20.1	10.0	6.5		
65		0-1 ans	-19.7	12.1	4.2		
67		5-9 ans	-20.2	8.6			
68		16-19 yr	-20.1	9.8			
70		immature	-19.9	10.2	5.3		
71		immature	-19.4	12.2	4.4		

Isotopic data

All collagen samples analysed for sulphur isotope ratios provided enough S content (>0.15%; between 0.5 and 0.26% in this study; Nehlich and Richards 2009) supporting the reliability of the data interpretation (SI 1; table 1). The δ^{34}S values of humans range from 3.6 to 15.4‰ (range = 11.8‰), while animal data span from 3.7 to 12.7‰ (range = 9.0‰), indicating that both groups exploited territories of similar isotopic variability (Figures 4a and 4b). Notably, the δ^{34}S values observed in humans and animals exceed the expected range proposed by Bataille et al. (2021).. In humans, the most pronounced outliers include two women and subjects from the multiple gathering individuals (figure 4b). However, the spatial distribution of these values (*ibid.*) may not fully reflect local isotopic diversity, which is influenced by the significant geological variations in this area. Animal remains at Pontcharaud are scarce within the funerary deposits, and their isotopic diversity could reflect a wide range of geographical origin and trade. The selection of some animal remains associated with the humans in the burial is probably more linked to the diversity of human life histories (pastoralism? exogamy?) and, thus, a wide territory that is difficult to locate.

The $^{87}Sr/^{86}Sr$ data of human enamel range from 0.708 to 0.714 (SI 1). The 22 modern plant samples from the IRHUM database (Willmes et al. 2014) used for bioavailable Sr mapping show a $^{87}Sr/^{86}Sr$ range from 0.704 to 0.722. We also propose a range of values that would allow to identify individuals who have frequented different environments compared with the rest of the group by using the mean ± 2 SD of all the data recorded on the enamel of human teeth (SI 1). The modern plants-soil samples (Willmes et al. 2018; Holt et al. 2021) and mineral water (Négrel et al. 1997) from the area of the site (Limagne basin and surrounding volcanic terrains) show $^{87}Sr/^{86}Sr$ within the same variability (ca. 0.704 to 0.721). All archaeological human teeth strontium isotope ratio values fall within the broad range present nearby, whether from close range or within 20km to 50km radius of the site (Figure 5a and Figure 5b). Furthermore, when using the human enamel $^{87}Sr/^{86}Sr$ to provide a possible "local" range (ie. mean ± 2SD), the range recorded is between 0.708 and 0.713 (SI 1) and encompasses almost all human data. In this context, it is difficult to discuss on local *versus* non-local origin, notably due to the interlocking geological substrates of sedimentary and volcanic origin surrounding the site. The female 12 is, however, an exception and shows great variability in the environments used between childhood and adolescence (SI 1 and Figures 5a and 5b).

Discussion

The results of this new study provide new insights to discuss the diversity of life histories encountered in the Pontcharaud necropolis. This variability can be perceived at different levels, through various parameters, and allows for the distinction of specific groups outlined hereafter, such as the social status of specific individuals, both females and males, and their role within this Neolithic group.

Have females and males different life histories?

The new sulphur stable isotope data confirm previous findings based on carbon and nitrogen isotopic records (Goude et al. 2013; summary in table 1 and SI 1), and a Kruskal-Wallis test indicates significant difference (p-value = 0.003) between the female, the male and the individuals buried in the multiple gathering. The description of the data also indicates that, in the context of similar funeral practices, some females were exposed to more variable environments (range of $\delta^{34}S$: 11.2‰) compared to males (range of $\delta^{34}S$ 5.6‰; excluding the individual of the multiple gathering; figures 4a and 4b). However, it can be emphasized that this variability is mainly driven by the presence of a particular female with a high $\delta^{34}S$ (burial 9, 15.4‰; figure 4a). Even if more subtle, $^{87}Sr/^{86}Sr$ also tends to provide evidence of sex-based differences in mobility or provenance during early life (i.e., from ca 2.5 to 14 years old). Figures 5a and b indeed show that the female from burial 12 and, to a lesser extent, the female from burial 52 (at the limit of the possible local range), acquired their food resources in different environments and/or consumed resources that were not reared or farmed under the same geological conditions between childhood and early adolescence. Also, it should be noted that females with no $^{87}Sr/^{86}Sr$ inter-age variability have slightly lower values (from 0.7101 to 0.7113) than males (excluding the specific multiple: 0.7109 to 0.7117). At Pontcharaud, adults of both sexes show greater $\delta^{34}S$ variability than juveniles (range of $\delta^{34}S$: 3.2‰) buried here, who were probably less mobile, and particularly greater than infants under the age of 3 years ($\delta^{34}S$ from 2 to 5.2‰; figure 4a), whose bone isotopic composition included maternal milk. An extended territoriality or origin is not exclusive to females or adults. In a way, the case of male 39 could testify to this extended territoriality and diversity of physical activities. This individual shows the lowest $\delta^{34}S$ among males, one of the highest $\delta^{15}N$ values in the group, and the unique kneeling facet associated with traumatic lesions on the right lower limb. The mobility of people has already been proposed for other Middle Neolithic sites in relation to sex, age and perhaps social status in France at Le Vigneau (Touraine, North-western France; Goude et al. 2019), and Gurgy (Rey et al. 2019; 2021; Rivollat et al. 2023), in the Mediterranean (Goude et al. 2020b) and in Central Europe (Bentley et al. 2002; Price 2006). In any case, the interpretation of the data (for both adults and subadults of both sexes) is often difficult to link with specific patterns of activity or social organisation, as they may be related to various forms of individual behaviour. In such cases, the combination of isotopic data and activity indicators is crucial to disentangle life histories.

Can we identify females with specific life histories?

When comparing the isotopic dataset, a few females clearly distinguish themselves from the general adult group (excluding the individuals from the multiple gathering) and may have had a distinct life history.

Female 12 shows the highest difference in $^{87}Sr/^{86}Sr$ values between childhood and adolescence (to some extent, same applies to female 52). Female 12 also has an isolated lesion on the lateral epicondyle, possibly due to an intense mechanical stress on the right arm, and is buried in the unique stone coffin of the site (Loison 1998). She has been given specific funeral treatment according to a social status yet to be defined and/or a specific geographical origin (figures 6 and 7).

In contrast, female 52 does not present any specific biological or archaeological features that could help explain the difference in strontium ratios recorded between childhood and early adolescence. However, this female is one of the individuals with the highest $\delta^{15}N$, suggesting a potential status and/or mobility related to specific protein intake.

In the case of female 53, the low mobility hypothesis is supported by the isotopic signals, which indicate no significant change in the geological landscape between childhood and adolescence (figure 5). She exploited resources with the same environmental conditions (water, geology) during the part of her life targeted by our analyses and shares more similar $^{87}Sr/^{86}Sr$ values with all the males from the multiple gathering than the other males..

Finally, for female 9, the scenario is more complex. Sulphur data clearly indicate a different origin compared to the rest of the human group during the last years before death (bone remodelling estimation). However, previous carbon and nitrogen isotopic ratios did not distinguish this individual from the group (Goude et al. 2013), and the strontium ratios suggest a similar origin to most of the other females during her childhood and early adolescence (figures 6 and 7). This individual may have spent a significant part of her life outside the main Pontcharaud group's living territory, but within a geological area where strontium and other environmental parameters are similar.

Like female 12, female 69 shows a lesion related to intense mechanical stress on the right arm. No strontium data is available for this individual, and the C, N and S isotopic ratios do not distinguish her from the rest of the group. The lack of automatic convergence between biogeochemical information and traumatic lesions leads us to believe that there is great diversity in the life histories of these females, compatible with the hypothesis of a diversity of origins and/or food access of several of them.

Who are the males from the specific multiple gathering?

The palaeogenomic study demonstrated that the two subadults (34 and 35) from the multiple gathering were also males, making this burial exclusively composed of male individuals. From a biomolecular perspective, the seven males from the multiple gathering share common characteristics, setting them apart from the other individuals buried in the necropolis. The study of discrete traits across the entire osteological series failed to distinguish them (Schmitt and Saliba-Serre 2014, Billard 2004), and enthesopathy lesions do not show any clusters or patterns among the men found at the Pontcharaud site. The five adult males provided different $\delta^{34}S$, which were higher (10.4 to 11.2‰) than most of the other adults of both sexes (figures 4a, 4b, 6 and 7). This is consistent with previous findings based on carbon and nitrogen isotope ratios, which differentiated them from the rest of the group. Their respective carbon and nitrogen isotopic ranges were extremely homogeneous (mean -20.7‰ ± 0.2 for $\delta^{13}C$ and mean 9.5‰ ± 0.2 for $\delta^{15}N$) and statistically lower (Wilcoxon signed-rank tests, $p < 0,001$ for C and $p = 0,003$ for N) than those measured for other Pontcharaud males, particularly for nitrogen (figure 3; Goude et al. 2013). This previous result indicated different access to protein intake (animal protein level and/or exploitation of different ecological environments). The $\delta^{34}S$ values of the two subadults from the multiple gathering are also higher (8.3-9.7‰) than those of the other juveniles at the site (4.2-7.3‰) (figures 4a and 4b), but lower than the values recorded for the adults from the same deposit. This could indicate a possible ongoing change in the environment. Regarding $^{87}Sr/^{86}Sr$ data, the individuals from the multiple gathering show a wider intra-life range of values (0.708 to 0.711) compared to the other males (0.710 – 0.711). They possibly spent their childhood in areas with a broader bioavailable Sr range (0.708-0.711 vs. 0.710-0.711 for other males). During adolescence, the males from the multiple gathering were located in a different place ($^{87}Sr/^{86}Sr$ of M3: 0.709) compared to the other males ($^{87}Sr/^{86}Sr$ of M3: 0.711). The immature individual from the multiple gathering (indiv. 35, ca. 12-15 years of age) shows a similar $^{87}Sr/^{86}Sr$ value (0.710) to the immature buried in a single deposit (indiv. 59, ca. 7-11 years of age). This value is within the range of female data, but lower compared to the other males; this could suggest the exploitation of the same environment by children, regardless of the mortuary treatment. When combining strontium and sulphur results (figures 6 and 7), the adults can be considered as a distinct cluster with a different life history in terms of mobility/provenance and environment exploited. In short, the biogeochemical dataset of these seven individuals confirmed that they shared similar environment, somewhat different from most of the other Pontcharaud individuals. Adult 32 shows the lowest $^{87}Sr/^{86}Sr$ during childhood (ie. here between 4.5 and 8 years old), similar to female 12 during adolescence (ie. here between 8.5 and 17.5 years old). The youngest of the two subadults (individual 34; 7.5-12.5 years old) may show mobility in progress, with bone $\delta^{34}S$ almost reaching the values of the other subadult and adult individuals (figure 4). Social ages have already been discussed based on isotopic and funerary data (cf. Le Roy 2015; Rey et al. 2021). For example, at Gurgy (Middle Neolithic, Yonne Valley, France), both the 8-9 and 14-15 age groups (the ages of the two subadults here) appear to be possible transitional ages with mobility and/or dietary modifications for both sexes. To conclude regarding isotope data, the seven members of this group shared a common lifestyle and possibly engaged in similar economic activities dedicated to a specific group of males within the community.

From its discovery, the multiple gathering has seemed to constitute a particular group, as its characteristics indicate mortuary gestures that deviate from the current funerary practices observed in the necropolis and the chrono-cultural context. The simultaneity of the multiple gathering being clearly established, two hypotheses are retained to explain such an event according to B. Boulestin's hypothesis (Boulestin 2008): either the dead are victims of a small or large mortality crisis (epidemics, famine, or interpersonal violence), or they represent an event related to the accompanying dead. The accompanying dead are individuals who kill themselves or are killed in connection with the death of a person holding a major and public social role (Boulestin 2022). This practice is documented for the Neolithic period in France, specifically in the 5th and 4th millennia (Lefranc and Chenal 2019; Schmitt 2023). This assumption for the present site was supported by Testart (2004) and Boulestin (2008). The superposition of the cadavers and the prone position of the six individuals, particularly unconventional for this area and the chronological period, contrast with individual 36, who is lying on his back at the eastern extremity of the pit. He could be the accompanied individual; however, his position is also unconventional.

The second hypothesis of a mortality crisis should also be examined. Given that the deposit consists exclusively of males, interpersonal violence becomes a more plausible scenario compared to famine or epidemic, as both of the latter would likely affect the entire population without selective targeting based on sex. Furthemore, individuals 33 and 37 likely died as a result of their injuries. Conflict and violence during the Neolithic in Northwestern Europe are well documented nowadays (Fibiger et al. 2023), and multiple gatherings related to remains from wartime acts are documented at this period across various sites in France (Lefranc and Chenal 2019). Regarding war activities, no healed wounds were observed, except for a mended small injury on the right ulna of individual 33. The use of archery in Neolithic populations has been demonstrated through osteological data, using specifically the frequencies of upper limb enthesopathies (Thomas 2014; Ryan-Despraz et al. 2023). In the studied group, no evidence of archery was noted, but this activity cannot be excluded. Moreover, isotopic data show that this particular group had access to a different diet. However, the carbon and nitrogen stable isotope data show a possible lower trophic level than the other analyzed individuals, while an equivalent or even higher consumption of animal proteins could be expected for warriors, which would result in a higher trophic level (Clauzel et al. 2022). Therefore this hypothesis seems unconvincing but cannot be completely rejected. The seven males could also have been the victims of a massacre[3] (found together at the same moment, in the wrong place?) while practicing their common activity. Activities related to pastoralism and transhumance to high-altitude environments are not the preferred hypothesis for this group, due to strontium isotope values that appear to be little affected by the use of volcanic soils (characteristic of high-altitude environments in this region). Several multiple gatherings interpreted as massacres related to interpersonal violence have been discovered in Europe (e.g. Alt et al. 2020; Meyer et al. 2018; Novak et al. 2019; Schroeder et al. 2019). In these cases, the corpus was composed of both males and females, and the individuals were not included in a cemetery.

Whether Pontcharaud individuals died in conflict as warriors or in a massacre, their bodies were recovered where they died and buried in the community cemetery with funeral rituals specific to the circumstances of their death and/or their social category.

Conclusions and perspectives

The site of Pontcharaud is a funerary complex and may reflect different expressions of the deceased according to their life histories. It has been highlighted that the use of funerary places in Prehistory can

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[3] « killing by one group of people by another group of people, regardless of whether the victims are armed or not, regardless of age or sex, race […] » (Dwyer and Ryan 2012).

be dedicated to a group with a strong biological, cultural or social affinity (e.g., male/female lineage, nuclear families, high or low social status; e.g., Bentley 2013; Mittnik et al. 2019; Rivollat et al. 2023), or even possible alloparenting relationships (Esparza et al., 2017). The Pontcharaud site sheds light on a new bioarchaeological dataset, allowing the identification of very specific practices that make it exceptional in several respects. The new isotopic study strengthens previous observation (Goude et al. 2013) and indicates more variability in environment exploited and/or diet among the females' cluster. The hypothesis of diverse origins compared to males is possible but deserves further investigation (in particular with aDNA). The seven males from the multiple gathering deposit share isotopic similarities among themselves, and dissimilarities with other males and females of the necropolis. Perhaps their gathering responds to their involvement in a particular activity For now, the previous hypothesis of them being warriors whose cadavers were recovered from the battlefield is not completely supported by osteological and isotopic data, although interpersonal violence towards the group is not discarded. Moreover, the reason for this particular gathering still fits two other plausible scenarios: accompanying dead or victims of a massacre. Further aDNA analyses of other buried individuals, as well as the investigation of new parts of the necropolis (currently in progress), will certainly allow for a better interpretation of this specific situation. This study also demonstrated the relevance of a multidisciplinary approach, including a reasoned use of fragile osteological material and a careful assessment of the gain/risk in the selection of samples and methods chosen. This approach has made it possible to highlight an exceptional variability within this funerary complex and will certainly provide relevant methodological support for future studies.

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Table caption - main text

Table 1. Summary of the anthropological data and isotopic results (humans) of Pontcharaud (details for both humans and animals in SI 1).

Figure captions - main text

Figure 1. Location of the site of Pontcharaud and other archaeological sites mentioned in the text. Pictures of two different burial types: a stone coffin on the left and wooden coffins on the right. Photos INRAP/G. Loison.

Figure 2. Photo and drawing of the individuals from the multiple gathering deposit (INRAP/G. Loison modified by A. Schmitt).

Figure 3. A-Carbon and nitrogen stable isotope data from bone collagen of human and some animal remains (Goude et al. 2013). B-Boxplots of nitrogen stable isotope composition distribution for females, males and individuals of the multiple gathering deposit.

Figure 4a. Sulphur isotope composition of bone collagen from females, males, immatures, individuals from the multiple gathering deposit, and animals.

Figure 4b. Boxplots (median, 25th -75th percentiles, minimum, maximum, and outliers) of $\delta^{34}S$ in bone collagen from the different human groups and animals at Pontcharaud, and the range of values expected for individuals from the Auvergne region, as proposed by Bataille et al. (2021).

Figure 5a. Strontium isotope composition from crown enamel of some females, males, and individuals from the multiple gathering deposit.

Figure 5b. Boxplots (median, 25th -75th percentiles, minimum, maximum, and outliers) of $^{87}Sr/^{86}Sr$ in dental enamel from the different human groups at Pontcharaud, mineral waters in the area of the site, and the expected range of $^{87}Sr/^{86}Sr$ for individuals from the Limagne basin and volcanic terrains near the site, as proposed by Holt et al. (2018).

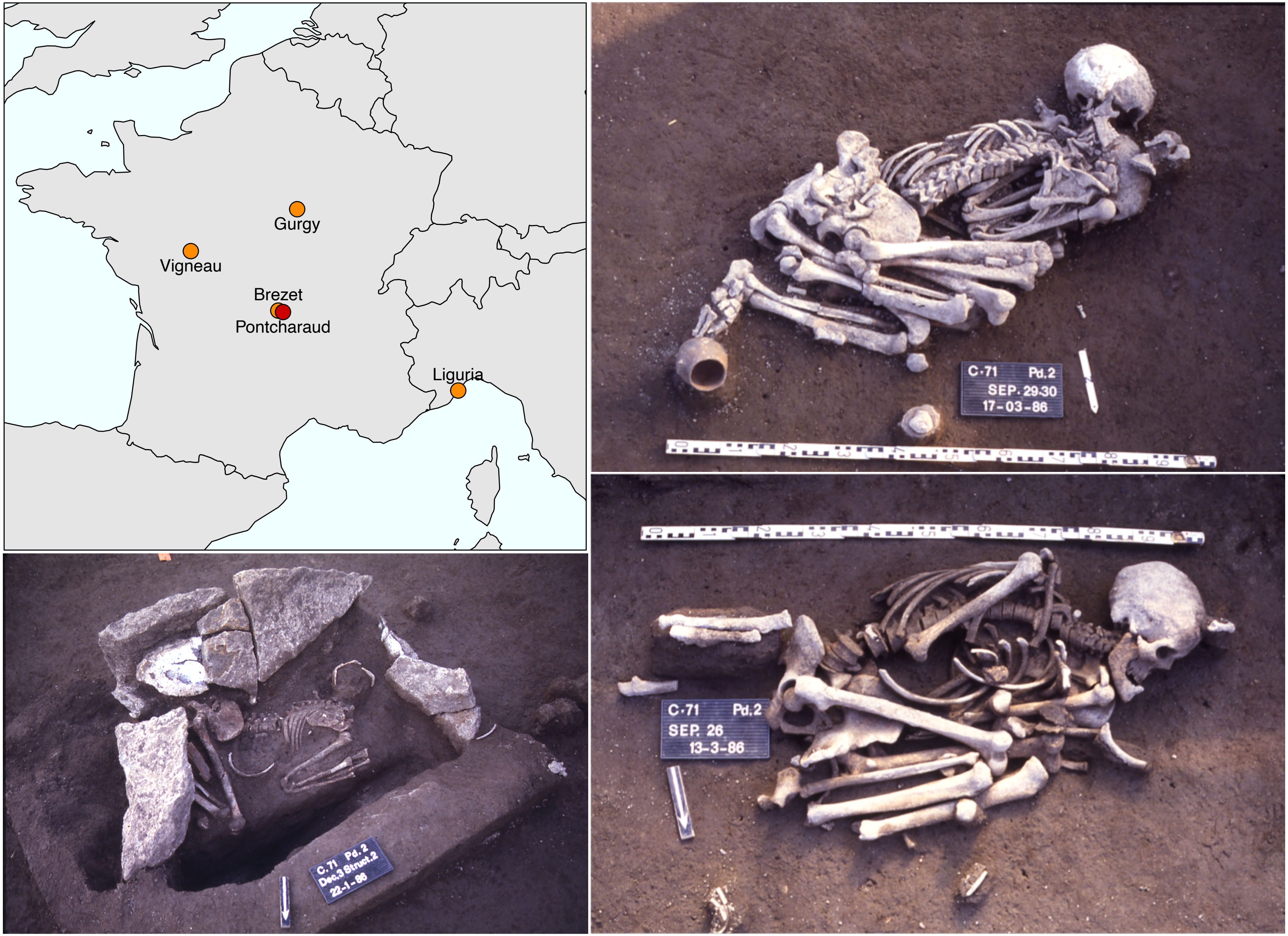
Figure 6. Comparison between sulphur isotope composition of bone collagen and strontium isotope composition from crown enamel grown during childhood for some individuals.

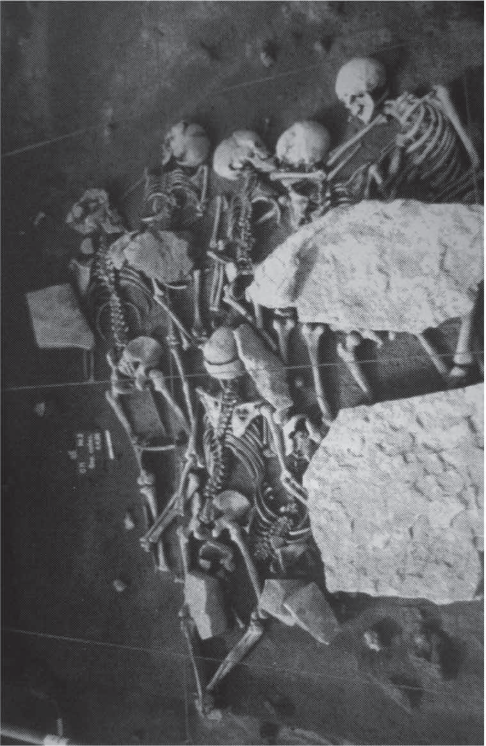
Figure 7. Comparison between sulphur isotope composition of bone collagen and strontium isotope composition from crown enamel grown during early adolescence for some individuals.

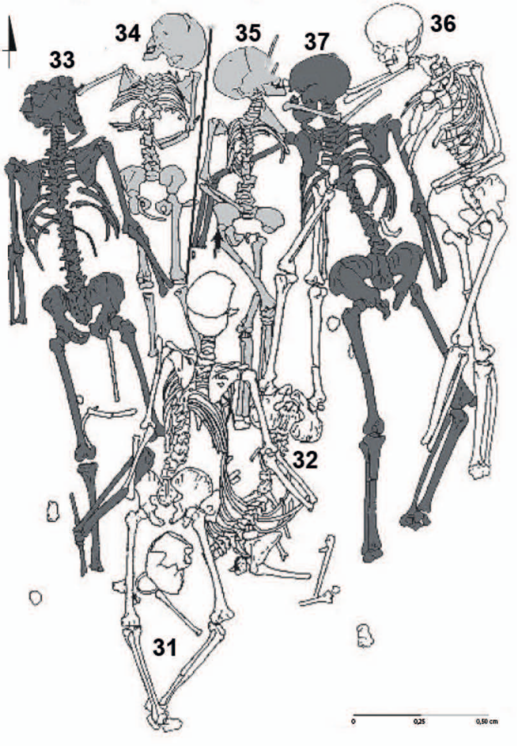
Supplementary Information captions

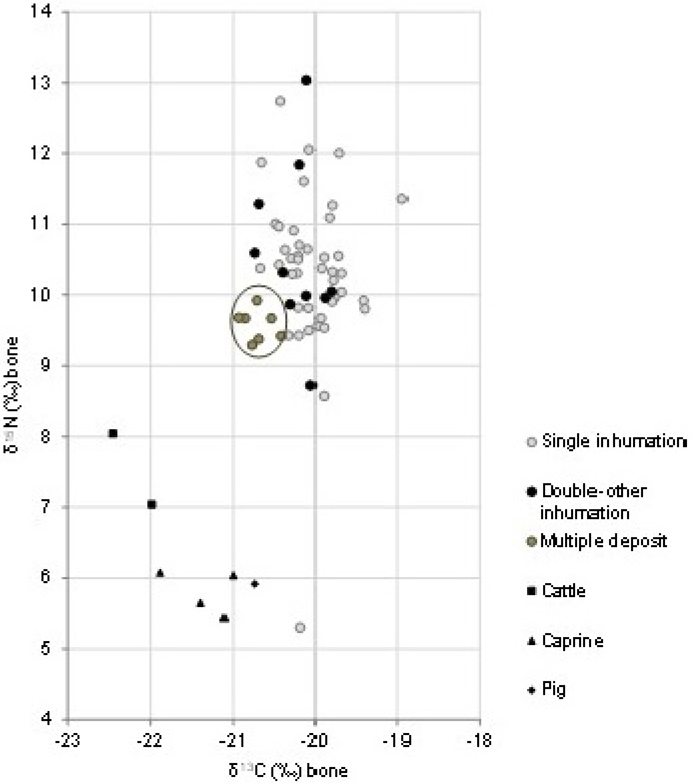
SI 1. Anthropological data and isotopic results (humans and animals) of Pontcharaud (Middle Neolithic, Clermont-Ferrand, Auvergne, France)

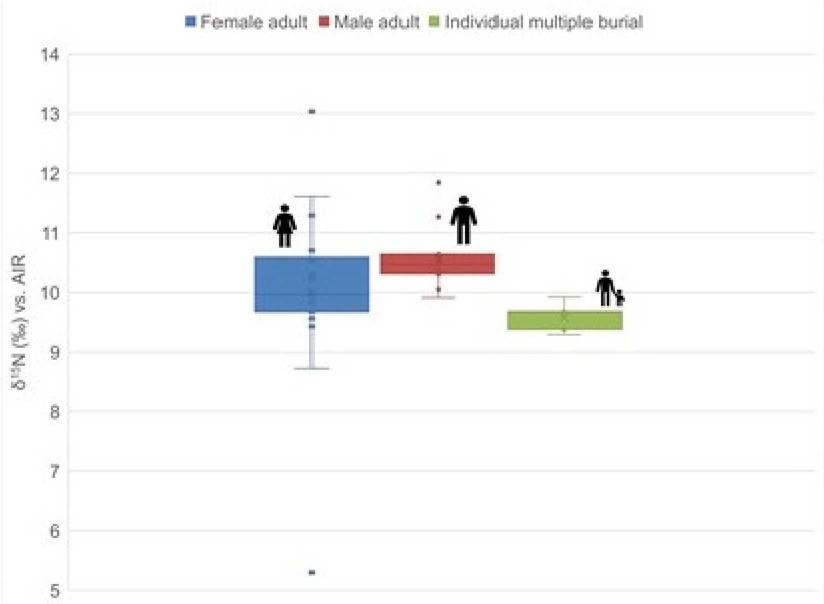
SI 2. Methodological and technical information

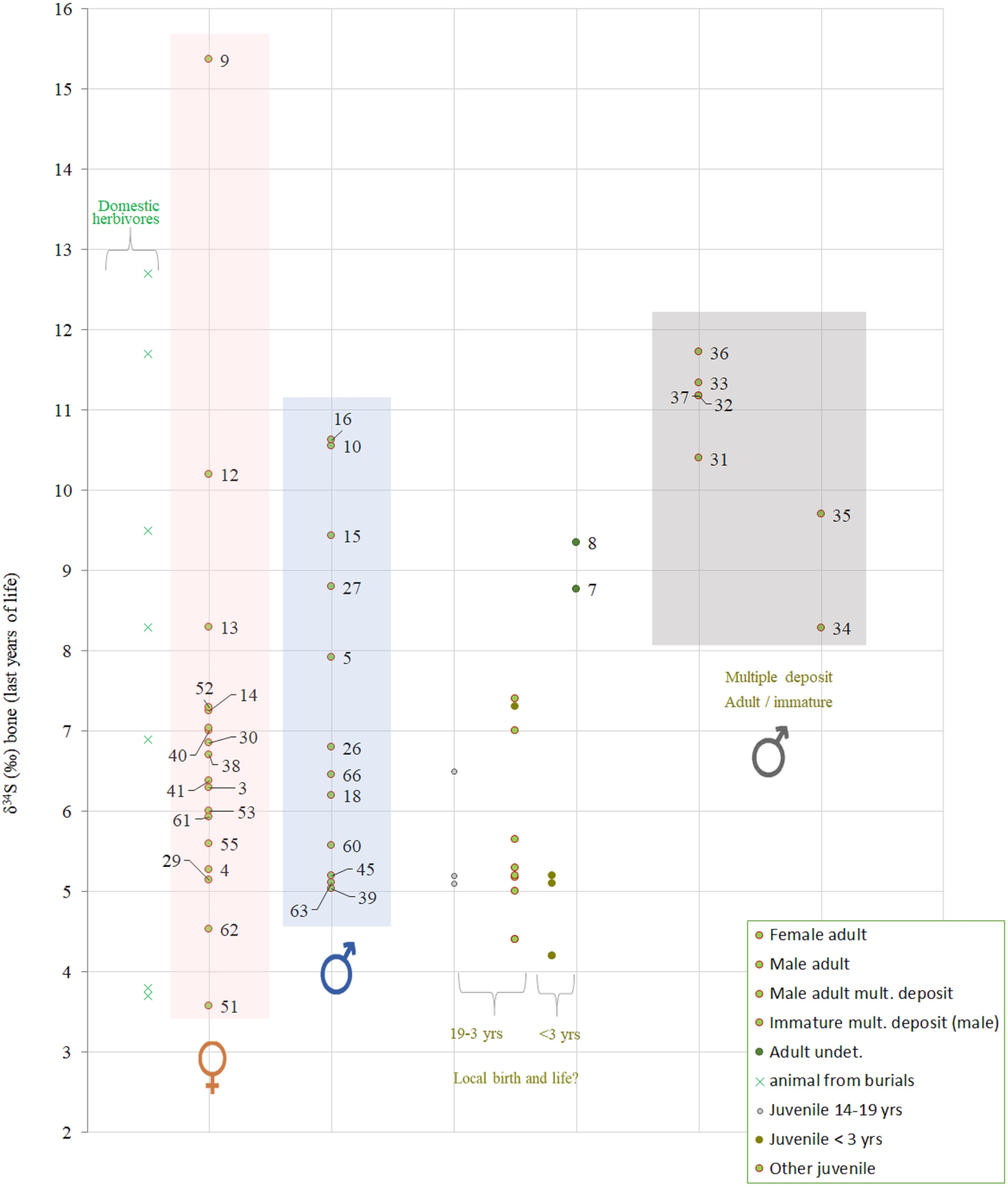

Gurgy
Vigneau
Brezet
Pontcharaud
Liguria
C·71 Pd.2
SEP. 29.30
17-03-86
C·71 Pd.2
SEP. 26
13-3-86
C.71 Pd.2
Dec.3 Struct.2
22-1-86

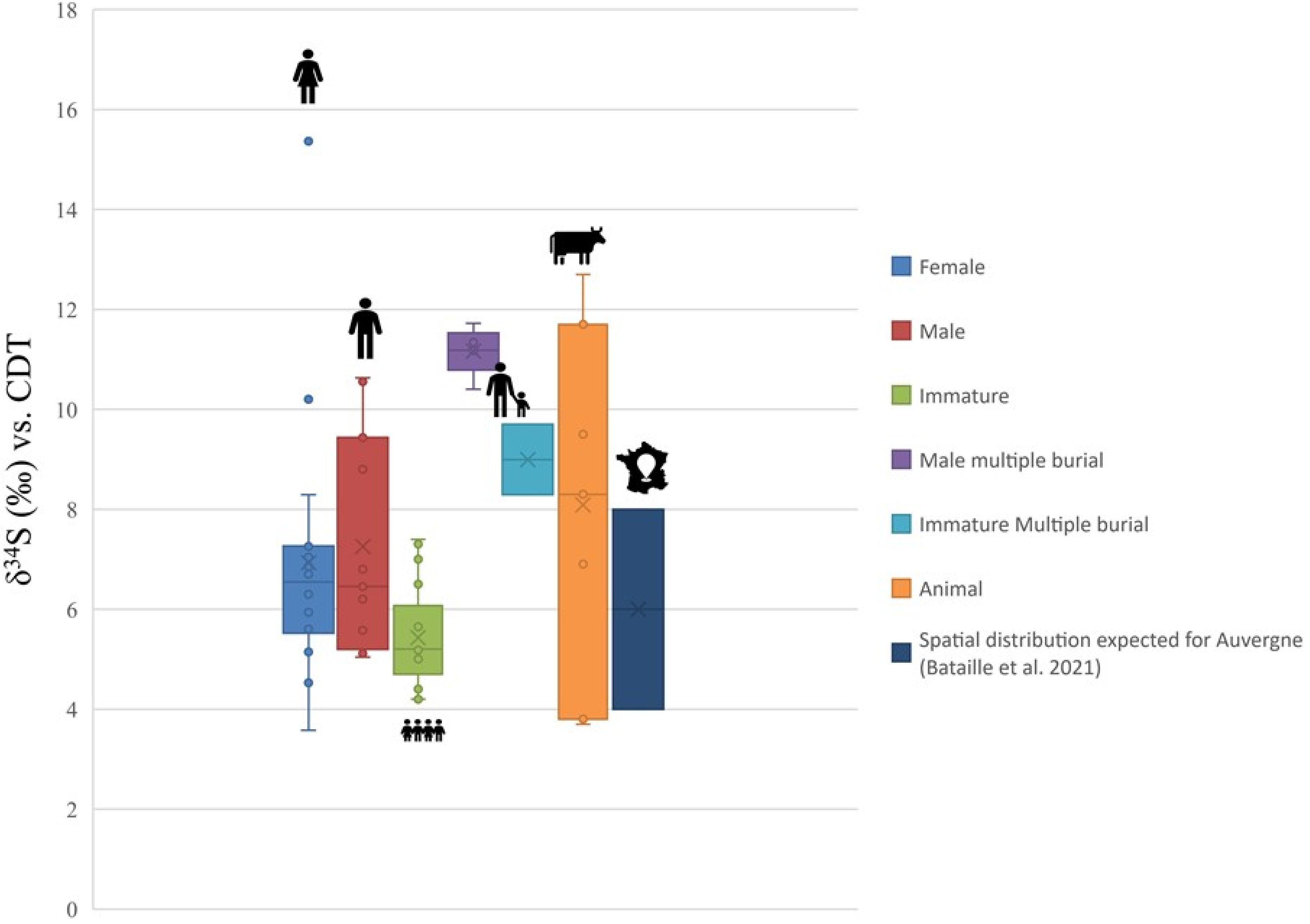



34
35
36
33
37
32
31
0
0,25
0,50 cm


14
13
12
11
10
9
8
7
6
5
4
-23
-22
-21
-20
-19
-18
δ¹⁵N (‰) bone
δ¹³C (‰) bone
● Single inhumation
● Double-other inhumation
● Multiple deposit
■ Cattle
▲ Caprine
◆ Pig


Female adult
Male adult
Individual multiple burial
14
13
12
11
10
9
8
7
6
5
δ¹⁵N (‰) vs. AIR


δ34S (‰) bone (last years of life)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
Domestic herbivores
9
12
13
52
14
30
40
38
41
3
53
61
55
29
4
62
51
♀
16
10
15
27
5
26
66
18
60
45
39
63
♂
6.5
19-3 yrs
<3 yrs
Local birth and life?
8
7
36
33
37
32
31
35
34
Multiple deposit
Adult / immature
♂
Female adult
Male adult
Male adult mult. deposit
Immature mult. deposit (male)
Adult undet.
animal from burials
Juvenile 14-19 yrs
Juvenile < 3 yrs
Other juvenile


18
16
14
12
10
8
6
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2
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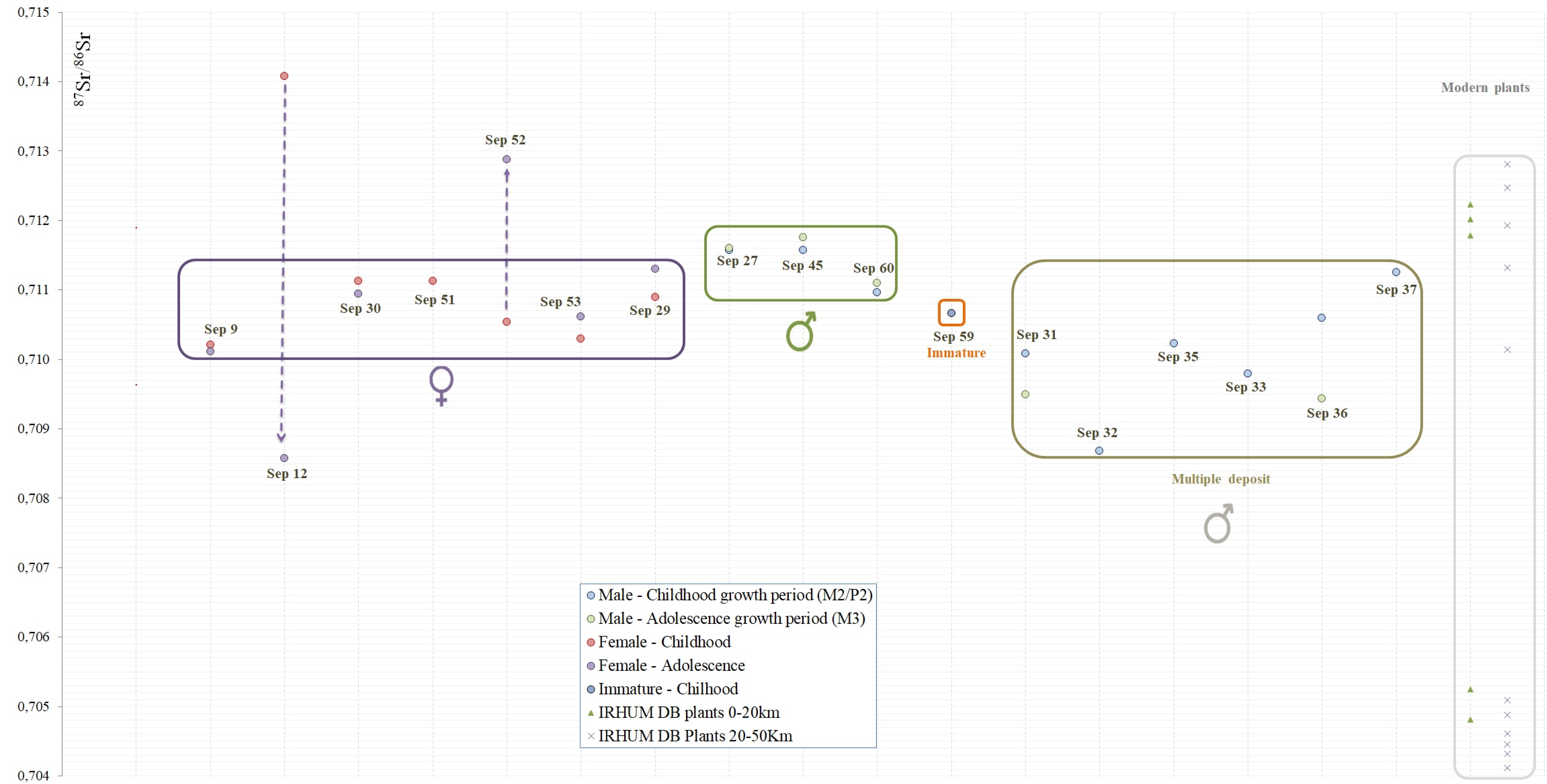
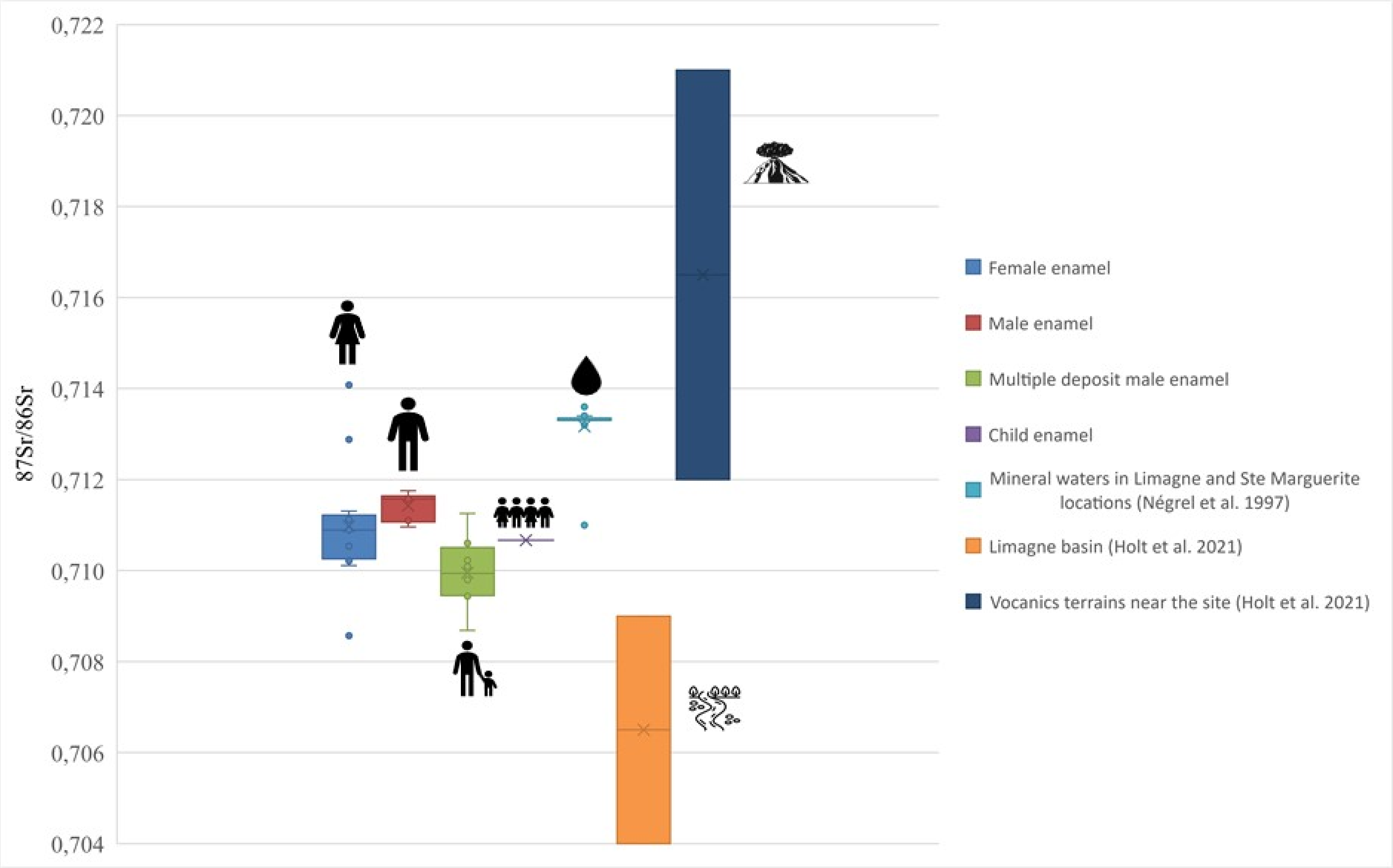
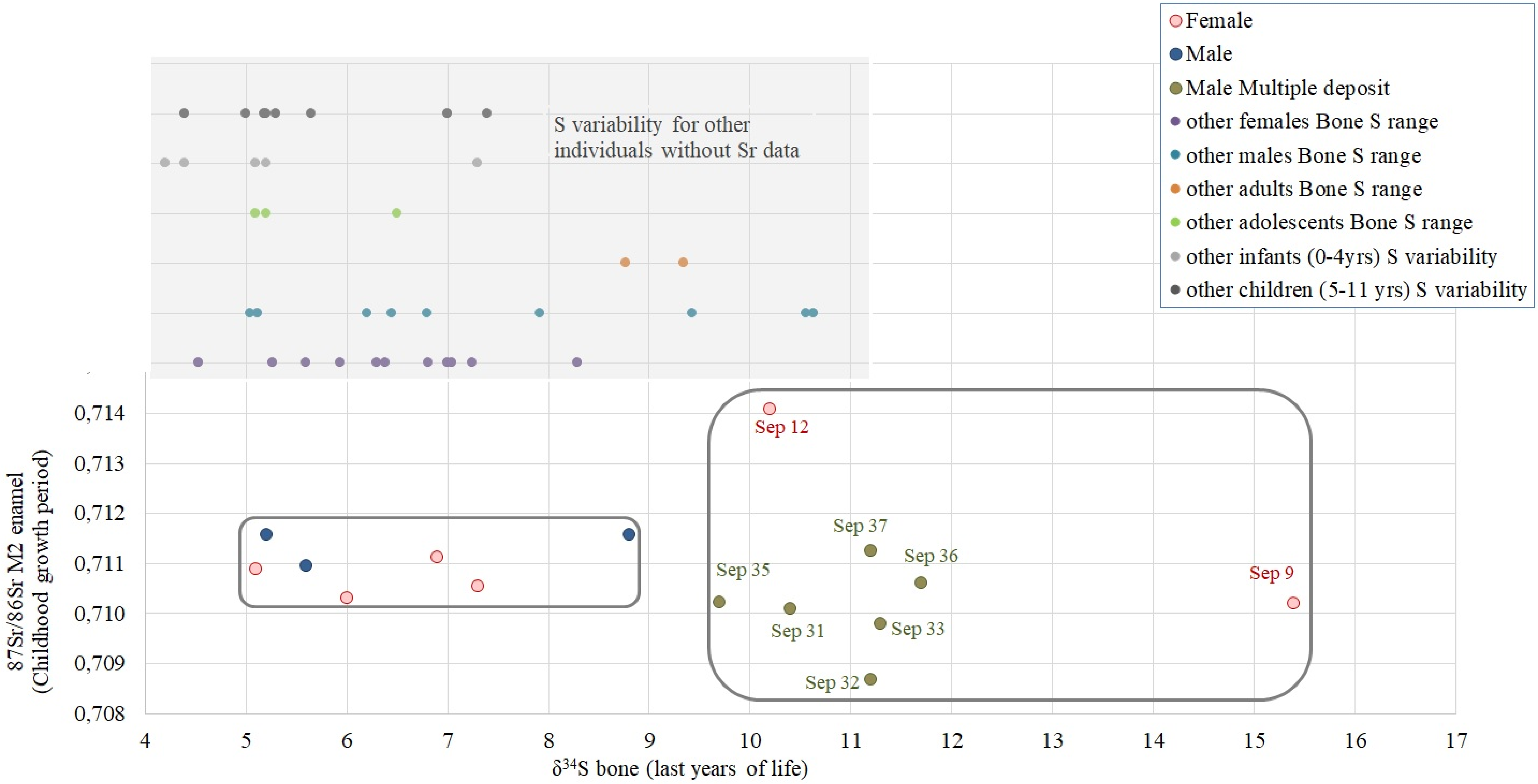
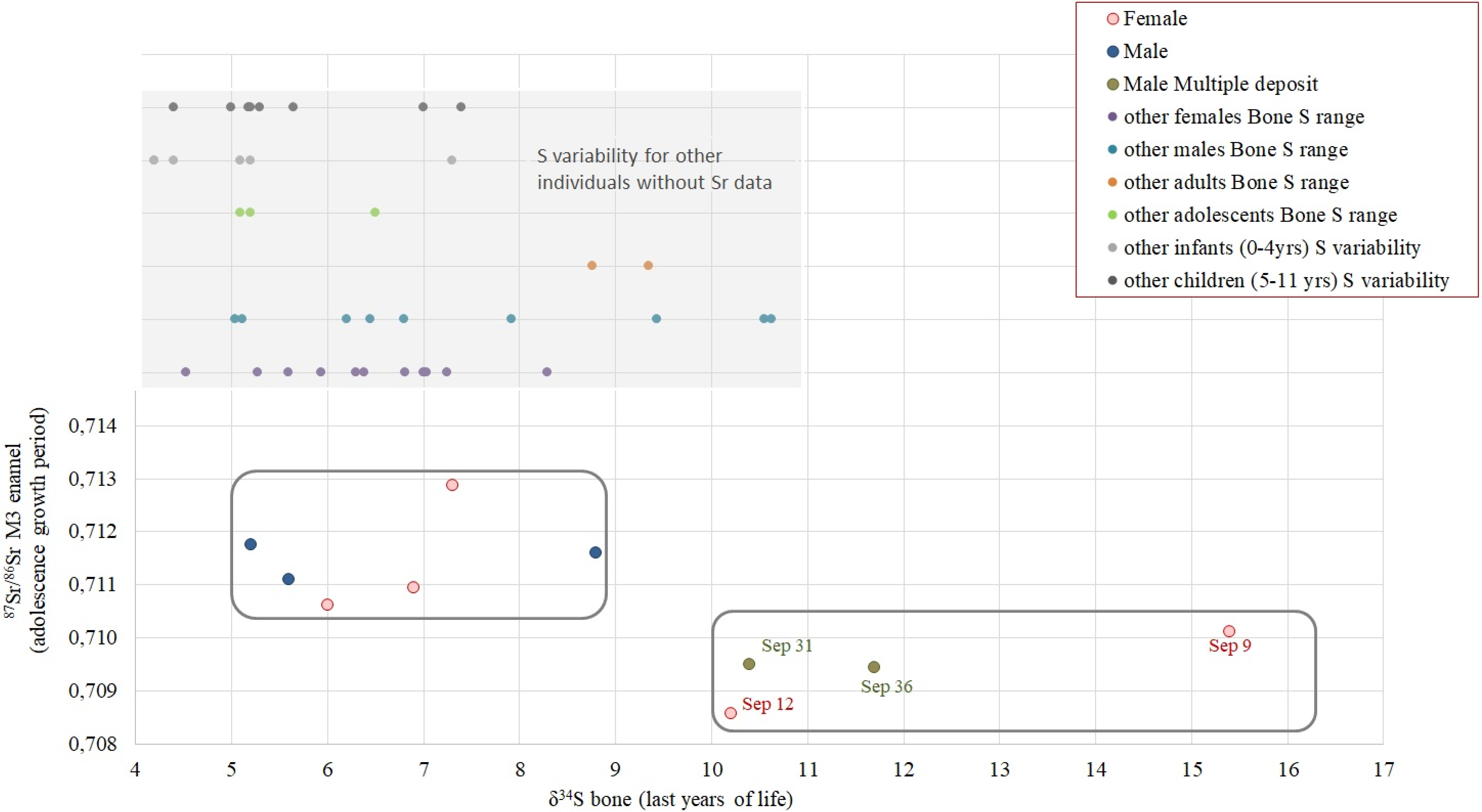
δ34S (‰) vs. CDT
Female
Male
Immature
Male multiple burial
Immature Multiple burial
Animal
Spatial distribution expected for Auvergne (Bataille et al. 2021)


0,715
0,714
0,713
0,712
0,711
0,710
0,709
0,708
0,707
0,706
0,705
0,704
$^{87}Sr/^{86}Sr$
Sep 52
Sep 9
Sep 30
Sep 51
Sep 53
Sep 29
Sep 12
♀
Sep 27
Sep 45
Sep 60
♂
Sep 59
Immature
Sep 31
Sep 35
Sep 33
Sep 37
Sep 36
Sep 32
Multiple deposit
♂
Modern plants
Male - Childhood growth period (M2/P2)
Male - Adolescence growth period (M3)
Female - Childhood
Female - Adolescence
Immature - Chilhood
IRHUM DB plants 0-20km
IRHUM DB Plants 20-50Km


0,722
0,720
0,718
0,716
0,714
0,712
0,710
0,708
0,706
0,704
87Sr/86Sr
Female enamel
Male enamel
Multiple deposit male enamel
Child enamel
Mineral waters in Limagne and Ste Marguerite locations (Négrel et al. 1997)
Limagne basin (Holt et al. 2021)
Vocanics terrains near the site (Holt et al. 2021)


Female
Male
Male Multiple deposit
other females Bone S range
other males Bone S range
other adults Bone S range
other adolescents Bone S range
other infants (0-4yrs) S variability
other children (5-11 yrs) S variability
S variability for other individuals without Sr data
Sep 12
Sep 37
Sep 36
Sep 35
Sep 9
Sep 31
Sep 33
Sep 32
87Sr/86Sr M2 enamel (Childhood growth period)
0,714
0,713
0,712
0,711
0,710
0,709
0,708
4
5
6
7
8
9
10
11
12
13
14
15
16
17
δ^{34}S bone (last years of life)


Female
Male
Male Multiple deposit
other females Bone S range
other males Bone S range
other adults Bone S range
other adolescents Bone S range
other infants (0-4yrs) S variability
other children (5-11 yrs) S variability
S variability for other individuals without Sr data
Sep 31
Sep 36
Sep 12
Sep 9
0,714
0,713
0,712
0,711
0,710
0,709
0,708
^{87}Sr/^{86}Sr M3 enamel (adolescence growth period)
4
5
6
7
8
9
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12
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14
15
16
17
δ^{34}S bone (last years of life)

Supplementary Information 2 – Methodological and technical information

Osteological analyses

S. Villotte, A. Schmitt

		Females		Males	
		Stage A	Stage B	Stage A	Stage B
Right side	Lesser tubercle	13	1	3	0
	Greater tubercle	12	0	4	0
	Lateral epicondyle	6	2	5	0
	Medial epicondyle	11	0	4	1
Left side	Lesser tubercle	11	0	6	0
	Greater tubercle	8	0	5	1
	Lateral epicondyle	9	1	3	1
	Medial epicondyle	10	0	4	0

Table 1. Frequencies of stages A (no lesions) and B (minor changes) for the entheses of the humerus according to side and the sex.

Biogeochemical analyses

G. Goude, D. C. Salazar-Garcia

Theoretical concepts

Sulphur isotope ratios are widely used additionally to carbon and nitrogen isotope ratios as it can be measured in the same protein fraction (collagen) and even sometimes together when analytical equipment allows it. Although δ^{34}S is more commonly used as a geographical marker (values differ depending on proximity to the sea, wind regime, and inland geological and fluviatile landscape composition; e.g., Nehlich 2015; Zazzo et al. 2011), it also helps to detect the consumption of freshwater fish and marine food when significant in the diet (e.g Nehlich 2009 ; Drucker et al. 2018). Recently, this proxy proved to be powerful to highlight mobility during infancy in Prehistoric communities (Goude et al. 2020b) as complementary to strontium isotope analysis. When combined with carbon and nitrogen isotope data, it helps to discuss the impact of mobility *versus* food choice in the collagen isotopic composition (e.g., Goude and Fontugne 2016).

Code de champ modifié

Code de champ modifié

Code de champ modifié

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As highly mineralized (ca. 96% of apatite), teeth are more resistant than bones and usually preferred for biogeochemical investigations of the mineral fraction (Budd et al., 2000; Hoppe et al., 2003). Importantly also, strontium isotopes do not show isotopic fractionation when incorporated through the foodweb (Bentley, 2006). This, together with the fact that enamel has no turnover during life (Nanci, 2007), allows to obtain locality information from a frozen time of its childhood and compare it to later stages of life. ^{87}Sr/^{86}Sr ratios depend on geology and show different values among different geologies, varying according to the bedrock age and the amount of ^{87}Rb. The heavier strontium isotope (^{87}Sr) is an unstable isotope that is formed from the radioactive decay of 87Rb, while the lighter (86Sr) is stable (Bentley, 2006). Different weather conditions like sea spray effect (Bentley, 2006), or the input of varied materials coming from the different environments like dust precipitation or alluvial deposition could influence and modify the estimated bedrock values (Sjögren et al., 2016). This is why bioavailable Sr values to be compared to the study individuals are useful and should be measured ideally from the surrounding areas of the study (Price et al., 2006). The bioavailable Sr background values for this study were selected from the IRHUM database of modern soils and plants (Willmes et al., 2014). Specifically, 22 points located in a radius of 50km around the site were chosen and reported together with the values from the archaeological material in Figure 5.

When possible we sampled for this study two teeth per individual from 11 individuals. Both an M3 and an M2/P4 were selected. From 6 individuals only an M2/P4 was sampled. This sampling strategy allows assessing if changes in geological substrate (SI 2) occurred between two periods of life estimated by using Alqhatani et al. (2010) and Liversidge (2008) crown growth pattern data: childhood (M2/P4:

median age formation between 2.5 and 8 years old for crown) and adolescence (M3: median age formation between 8.5 and 14 years old) (AlQhatani et al. 2010).

Geological particularity of the Massif Central

The Pontcharaud site is located in the Limagne sedimentary basin, bordered by various geological terrains of volcanic origin: to the west, the Plateau des Dômes and the Chaîne des Puys, and to the east, the Monts du Forez (BRGM 2003; (fig. SI2-1 left). Analyses carried out on various mineral water springs by Négrel et al. (1997) in the Limagne sedimentary basin and 25 km south of Clermont-Ferrand show a variation in $^{87}Sr/^{86}Sr$ between 0.7110 and 0.7136. Data proposed in the literature by Willmes et al. (2018) and Holt et al. (2021) indicate $^{87}Sr/^{86}Sr$ values of plants and soils globally between 0.704 and 0.709 for sedimentary areas of the Limagne basin where the site is located, and globally between 0.712 and 0.721 for terrains of volcanic origin (fig. SI2-1 right).

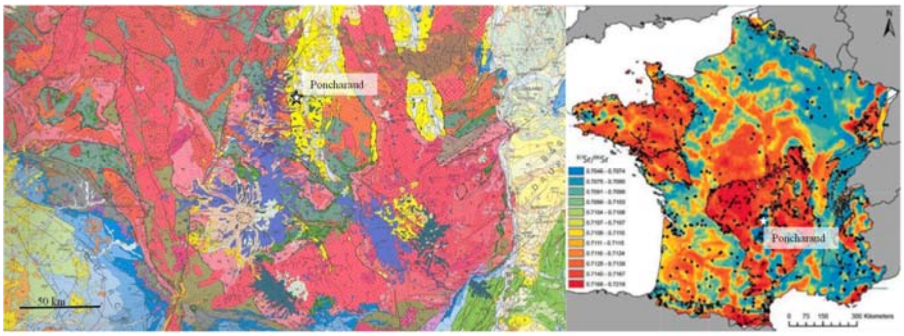

Figure SI2 – 1. Left: geological map of the BRGM (2003) with the location of the archaeological site (white star). Right: contour map using strontium ($^{87}Sr/^{86}Sr$) isotope data made by Holt et al. (2021) and Willmes et al. (2018), with the location of the archaeological site (white star).

Collagen extraction and sulphur stable isotope measurement

Collagen extraction was performed on chunk bones by using a modified protocol from Richards and Hedges (1999). Samples are demineralised in 0.5 M HCl at 5°C, then soaked in NaOH overnight at room temperature and solubilised with HCl pH 3 at 70°C during 48h. Solubilized collagen is filtered with EzeeFilter® device, frozen at -65°C in glass vials and then freeze-dried during 2 days. Elemental composition and stable isotope ratios of sulphur are measured by EA-IRMS (Europa Scientific elemental analyser coupled to Europa Scientific 20-20 IRMS; Iso-Analytical, Crewe, UK). Laboratory standards used are calibrated against IAEA international standard for all measurements; measurement error is 0.2‰.

Enamel preparation and strontium analysis

Sample preparation and analysis were carried out directly in the Clean Labs and Isotope Facilities of the University of Cape Town, South Africa. Prior to analysis, a chunk of enamel of ca. 20mg from each tooth was cut in a longitudinal way to reflect all mineralization stages of the dental piece. These pieces of enamel were cleaned by abrasion and dentine was removed completely, rinsed and ultrasonicated for 20 minutes in MilliQ water. The diamond drill bits used for this process were cleaned with ethanol and ultrasonicated in MilliQ water between samples to avoid cross-contamination (Budd et al., 2000). A subsample of each enamel piece was taken for Sr concentration in order to check for diagenesis. Following this, in a Clean Lab, the remaining chunks of cleaned enamel samples were digested with 2mL bi-distilled 65% HNO3 in a closed Teflon beaker placed on a hotplate at 140 °C for an hour. After this, digested samples were dried and redissolved in 1.5 mL of bi-distilled 2M HNO3 before being centrifuged at 4000 rpm for 20 minutes. The resulting supernatant was then collected for strontium separation chemistry. At this last step, a subsample from each sample was used to calculate the concentration with ^{88}Sr intensity (V) regression equation built with SRM987 standard from NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA). The isolation of strontium as carried out with 200µl of Eichrom Sr.Spec resin loaded in Bio-Spin Disposable Chromatography Bio-Rad Columns following the method described in Pin et al. (1994). The strontium fraction separated this way for each sample was then dried down dissolved in 2 ml 0.2% bi-distilled HNO3 and diluted to 200 ppb Sr concentrations for the isotope analysis. A NuPlasma HR multicollector inductively-coupled-plasma mass spectrometer (MC-ICP-MS) was used to measure the ^{87}Sr/^{86}Sr ratios. Sample analyses were referenced to bracketing analyses of SRM987, using a ^{87}Sr/^{86}Sr reference value of 0.710255 from NIST. All strontium isotope data were corrected for isobaric rubidium interference at 87 amu using the measured signal for ^{85}Rb and the natural ^{85}Rb/^{87}Rb ratio. The instrumental mass fractionation was corrected using the measured ^{87}Sr/^{86}Sr ratio and the exponential law, as well as a true 86Sr/88Sr value of 0.1194. Results of repeated analyses of an in-house carbonate standard processed and measured with the batches of samples in this study are in agreement with long-term results for this in-house standard (^{87}Sr/^{86}Sr; 0.708915; 2 sigma 0.000047; n=125). Furthermore, one blank was added for every two batches in order to double check the cleanliness of the sample preparation.

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Palaeogenomic analyses

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Material

Samples targeted

Authorisation to perform destructive sampling for palaeogenomic analyses was granted by the DRAC Auvergne Rhône Alpes. Two subadults individuals originating from the Pontcharaud exceptional multiple gathering (individuals 34 and 35) were targeted in the present study in document their genetic sex.

Methods

DNA isolation

Sampling procedures were conducted in non-optimal conditions, in the Dépôt SRA d'Aix Les Milles. While work surfaces and tools could be decontaminated with diluted bleach, no samples decontamination steps could be followed, nor could the ambient air be monitored. Only petrous bones were targeted for palaeogenomic analyses according to their higher rate of DNA preservation (Gamba et al., 2014; Pinhasi et al., 2015). A layer of bone surface was abraded around the sampled area before low-speed drilling into the cochlea to retrieve between 20 and 100mg of bone powder.

Bone powder samples were then processed at the aDNA Laboratory of PACEA, University of Bordeaux.

DNA isolation and library construction

DNA was extracted from powder following the protocol described in Velsko et al. (2019). A total of 20mL of DNA template was used to build double-stranded libraries with unique index pairs (Aron et al., 2019; Stahl et al., 2020). We applied a partial UDG (half) protocol to remove most of the ancient DNA damage while preserving the characteristic damage pattern in the terminal nucleotides (Rohland et al., 2015).

We then performed shallow shotgun sequencing to permit assessments of DNA preservation and authenticity in the indexed libraries. Libraries were sequenced on an Illumina NextSeq 500 (2x75bp reads) at Institut de Recherches Biomédicales des Armées (Paris, France).

Bioinformatic analyses

Read processing, alignment and postmortem damage

Raw sequence data were processed using EAGER (Peltzer et al., 2016) including quality checking of FASTQ files with FastQC, and clipping and merging of paired-end reads with Clip&Merge. Autosomal and sex chromosomal alignments were performed against the Human Reference Genome hs37d5 using BWA version 0.7.12 (with a lower read-length cut-off of 30 base pairs with MAPQ R 30 and disabling of seeding with -l 1000 being specified). Duplicate reads with the same orientation and same start and end positions were removed using DeDup v0.12.1. MapDamage v.2.0.6 was used to observe characteristic aDNA damage patterns, before trimming two bases at the ends of each read to remove residual deaminations with BamUtil (https://genome.sph.umich.edu/wiki/BamUtil:_trimBam). A summary of results statistics is given in Table 2.

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Sample	Number of sequences obtained before merging (R1+R2)	Numbre of sequences after merging	Number of duplicates removed	Number of sequences mapped on human nuclear reference genome hs37d5	% of endogenous DNA	Mean coverage nuclear human genome	Number of sequences mapped on human mitochondrial reference genome rCRS	% deamination 1st Base 3'	% deamination 1st Base 5'	Average human sequences length
34	1 814 502	634 055	2 174	182 724	23,474	0,0033	67	0,0761	0,0799	56,59
35	2 108 978	701 373	99	9 308	1,279	0,0001	5	0,1014	0,1016	49,98

Table 2- Principal characteristics of the DNA libraries obtained for Pontacharaud individuals 34 and 35, determined using EAGER v2.4.5 (R1 and R2: reads obtained through paired-end sequencing; merging: merging of R1+R2 complementary reads; deamination: DNA sequences modification characteristic of ancient DNA).

Sex determination

Genetic sex was calculated using two different methods. The first one estimates a coefficient Ry representing the fraction of reads mapping to Y chromosome out of all reads mapping to either X or Y chromosomes and attributes a XX genotype if $Ry \leq 0{,}016$ while assigning a XY genotype if $Ry \geq 0{,}075$ (Skoglund et al., 2013). The authors recommend a minimum of 100,000 chromosome reads mapped on the reference genome. The second method calculates the coverage of each of the gonosomes relatively to the coverage of the autosomes (Mittnik et al., 2016). A threshold of Y ratio at ≤ 0,04 is set for a XX genotype and at ≥ 0,4 for XY.

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