ARCHMAT

(ERASMUS MUNDUS MASTER IN ARCHaeological MATerials Science)

ISOTOPIC ANALYSIS (SR) IN THE NECROPOLIS OF CASTEL SOZZIO, VITERBO, ITALY: STUDY OF POPULATIONAL INTERACTIONS

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Abstract

Human mobility corresponds to the natural movement of peoples on a geographical horizon in a defined historical context. Understanding its aspects and dynamics provides a comprehensive picture of how different peoples interact. Oxygen and strontium, like all the natural elements, have a precise isotopic distribution that can be altered by biochemical and environmental factors. What is interesting is that the values of these alterations are characteristic for a given geographical area rather than for another. By examining the concentration of strontium and oxygen and their stable isotopes (atoms with variable mass numbers) inside bones and teeth, it will be possible to obtain information about the population dynamics of the area of interest. Information that will complement a broader study of ancient DNA.

The first excavation of the necropolis of Castel Sozzio begun in 1997 and stopped abruptly the following year. In 2020, investigations were resumed under the direction of Prof. Emanuela Borgia (Dipartimento di Scienze dell'Antichità, Sapienza Università di Roma) and the Soprintendenza Archeologia Belle Arti e Paesaggio per la provincia di Viterbo e l'Etruria meridionale. The research conducted at Castel Sozzio, through the joint application of archaeological and anthropological sciences, is aimed to investigate the late antique phases of the area, which witnesses a continuity of occupation between the 4th-5th centuries AD and the 7th century AD. In this historical phase, the interaction between Romans, Goths and Lombards can be assumed in this area; however, the lack of material evidence and the absence of written documents relating to the necropolis site require specific concerning anthropological material, in order to determine or understand more broadly the dynamics of these contacts.

With this in mind, an isotopic analysis was carried out on 33 samples, of which 26 were human and the remaining 7 constitute the baseline (samples of: animal bones, plants, soil) allowing us to make a comparison and

distinguish local and non-local individuals.

Sex and Age studies have also been carried out on the human samples, showing an heterogeneous composition of the individuals buried in the necropolis.

Sr isotope analyses were conducted by MC-ICP-MS (neptune) at the Department of Chemical and Geological Sciences of the University of Modena. This made it possible to identify two non-local groups within the necropolis that interact with the indigenous substrate.

1. SITE HISTORY

The necropolis of Castel Sozzio is located in a valley south-east of today's Civitella D'Agliano in the province of Viterbo. It is accessible via an east-west road that connects the hilly foothills of the Volsinii mountains with the Tiber valley

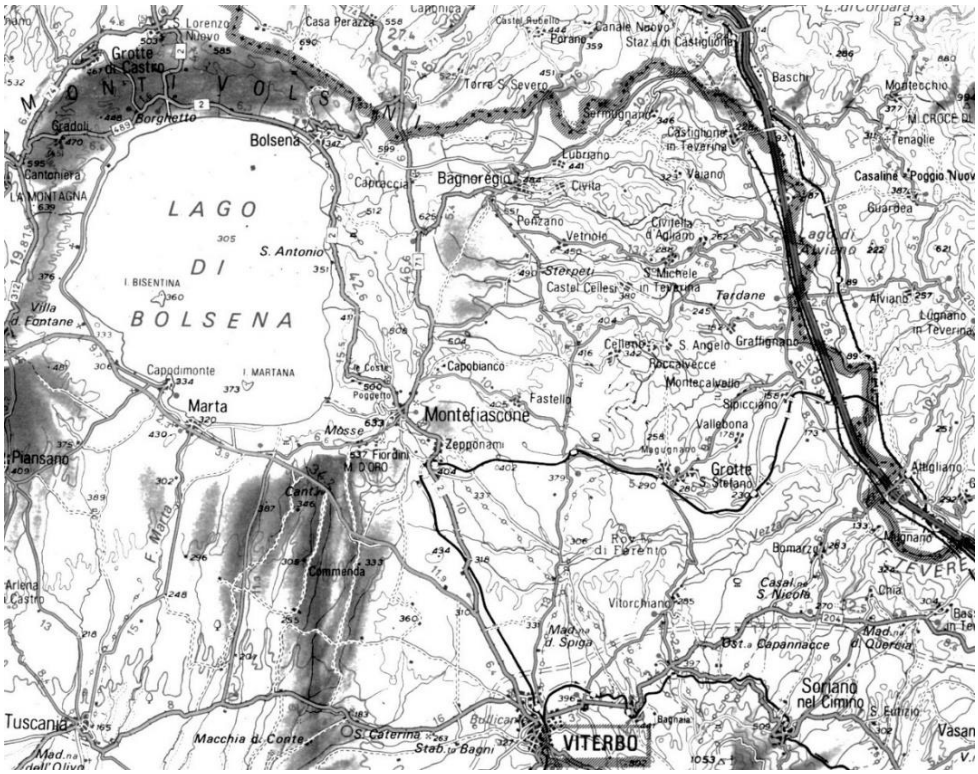

Fig 1: Map of Viterbo demonstrating Lake Bolsena, the Tiber River, and exact location of Civitella D'Agliano. (courtesy Borgia 2021, unpublished material all rights reserved)

The place-name Castel Sozzio refers to the hamlet that stands on high ground along the Barca di Alviano road: it is known in archival sources from at least the mid-fourteenth century and is mentioned in numerous subsequent maps as an important settlement on the right bank of the Rio Chiaro, a tributary of the Tiber.

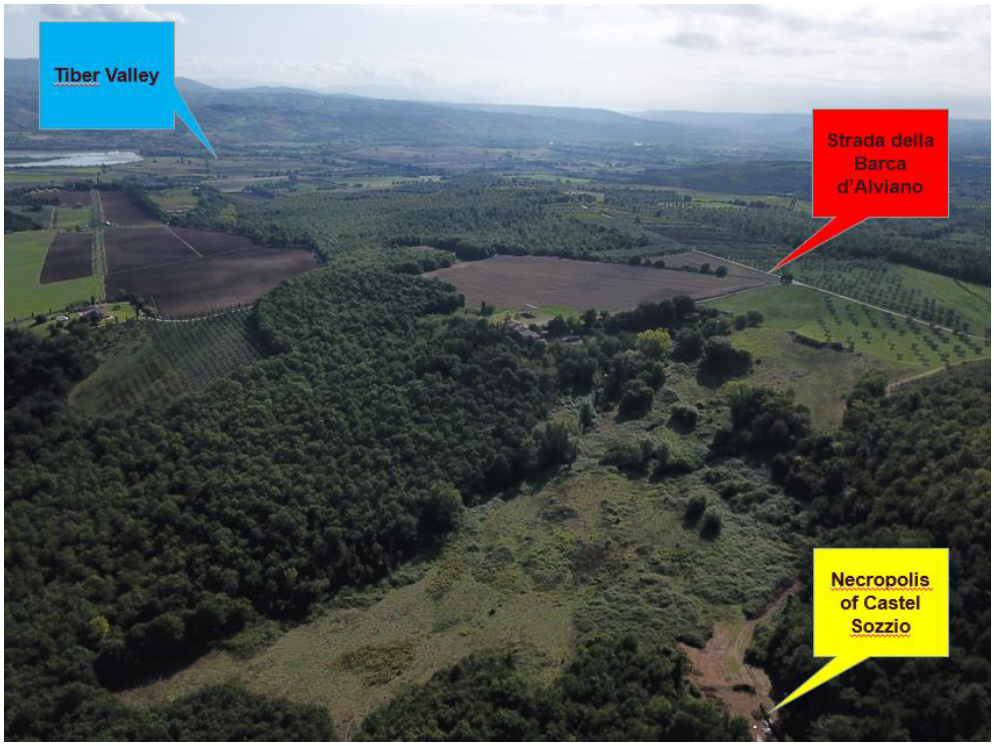

Fig.2 Aerial photo of the area under investigation (courtesy Borgia, E. 2021, unpublished material all rights reserved)

The burial area is of particular importance because it adds to the few sites known in the north of Lazio in a period between late antiquity and the early Middle Ages:

- Selvicola - Ischia di Castro Toiatti – Pontacolone 1985; Incitti 1992]
- Bomarzo e Norchia [Raspi – Serra 1974b, 1976]
- Palombaro - Montalto di Castro [Corsini 1985]
- La Ficonaccia - Pian de'Gangani [Corsini 1985]
- Rossignolo e S. Lucia - Bagnoregio [Cagiano De Azevedo-Schmiedt 1974; Pompei 1994.]

It is currently the only and perhaps the largest known funerary area in the Latium Teverina relating to the late Roman and Early Medieval phases.

A preliminary chronological interpretation dates the necropolis between the 5th and the 6th-7th century AD [Pompei, 1994; Borgia, 2021] maybe with scanty occupation until the 8th century: this was a crucial period for the area,

where the Romans were replaced by the Goths (alternating with the Byzantines) and then by the Lombards.

The first archaeological excavations in the area were undertaken in 1997 by Prof. M. Cecchelli, then professor of Christian archaeology at the Sapienza University of Rome, and although the importance of the site and its uniqueness was clear from the outset, investigations continued only until 1998. Then followed a period of abandonment that ended only with the rediscovery of the site by Prof. E. Borgia (Associate Professor of Classical Archaeology and Archaeology of the Roman Provinces at La Sapienza, University of Rome), who, thanks to the three-year excavation concession granted by the Direzione Generale Archeologia Belle Arti e Paesaggio (Decree 929 of 10/07/2020), resumed the investigation of an area almost unknown from an archaeological point of view.

2. THE ARCHAEOLOGICAL DATA

The reconnaissance activities of the area carried out close to the first excavation campaign led, on the basis of the material collected, to hypothesise the presence of an agricultural settlement from the Roman period [Cecchelli et al. 1997]. The oldest epigraphic documents naming a "loco dicto castro Sotio" subsequently associated with the church of S. Maria "de castro Sotio", of which traces have been lost, are not earlier than 1363. [Pompeii 1995].

The archaeological excavation campaigns of 1997 and 1998 saw the opening of two essays, named α and β, within which investigations were resumed in the 2020 campaign (**Fig. 3; 4**) [Borgia 2021].

Fig. 3: Aerial photograph of site showing both excavated trenches 2020. (Courtesy Borgia, E. 2021, unpublished image, all rights reserved).

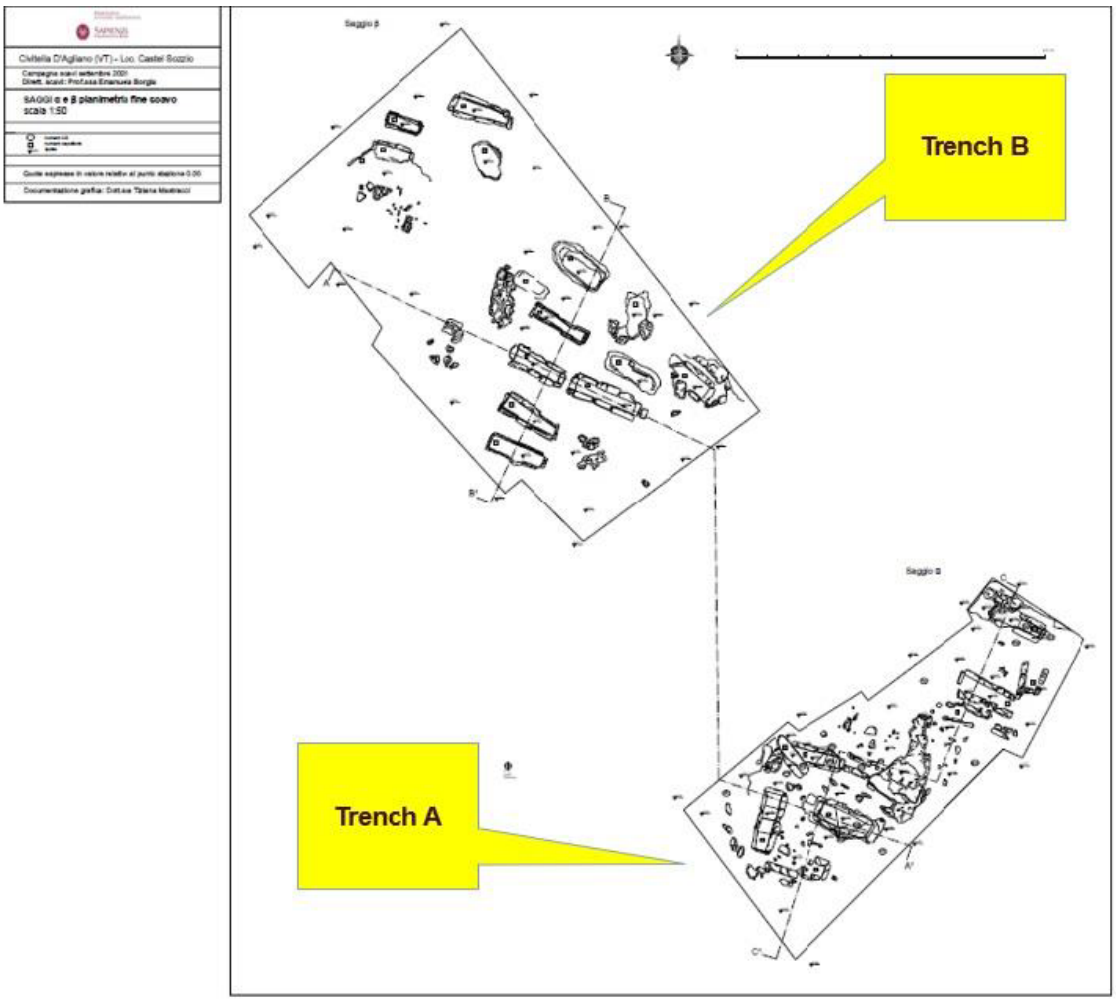

Fig. 4: Archaeological plan of the excavated areas of Castel Sozzio necropolis at the end of the 2020 excavation depicting both Trench Alfa and Trench Beta and the related tombs discovered in 2020. (Courtesy Borgia 2021, unpublished plan, all rights reserved 2021).

At the moment we do not know the settlement area linked to this necropolis, nor its limits, but the necropolis continues both in the area between the two trenches and beyond the limits of the excavation. A total of twenty-seven intact graves were identified until the 2020 excavation campaign, twenty-three of which were excavated, in addition to some burials disturbed by agricultural activities. The tombs are arranged in parallel rows and are cut into the clayey soil or the travertine rocky bank that is outcropping in places: they are almost all oriented west-east, with the head of the deceased to the west, while only a small number appear to be oriented north-south, with the head of the deceased

to the north. However, there does not seem to be a chronological hiatus in the use of the tombs with the two different orientations.

From a typological point of view, most of the tombs are of the lithic box type, with the walls covered with slabs of tuff or grey ignimbrite, the bottom made of beaten clay or cut into the travertine bank and the covering made of three or four slabs of tuff or ignimbrite.

All the tombs of the necropolis investigated so far are extremely poor, completely lacking in grave goods and any element that might help to date them accurately, although tombs 11 and 10 saw the re-use of large white mosaic fragments covered in hydraulic mortar, which constitutes archaeological evidence of the presence of a Roman villa in the immediate vicinity. Thanks to the taphonomic evidence it was possible to demonstrate that most of the burials show multiple reuses with reduction of the bones of the previously deposited burials (**fig. 5**).

Fig 5: Evidence of: reductions and reuse of the burial site (T15); Burials associated with the main one (T.4 sup); Circular pits with individuals deposited in secondary placements (T.4) (courtesy Borgia 2021, unpublished material all rights reserved).

In some cases, circular pits dug into the ground have been identified, generally shallow and arranged around the perimeter of the lithic box burials; these are secondary burials disrupted either by agricultural activities or by the nature of the burials themselves. It can be assumed that these are the result of operations that saw a reuse of the burial with a consequent reduction or partial removal of the previous occupants. Only the outcome of further investigations and the systematic re-examination of the ceramic finds pertaining to the phase of frequentation of the necropolis, in addition to the fundamental data that will emerge from the ongoing analyses of the anthropological remains, will be able to contribute to a clearer definition of the phases of life of the funerary area and the community to which it refers.

As mentioned above, there is evidence to suggest that a Roman villa or even a vicus stood in the immediate vicinity of the necropolis, whose evolution may have followed the process that characterises the numerous manor and country villas that have never been systematically investigated in this area. These complexes, in fact, saw a phase of prosperity in the proto-imperial period, linked to the intensive agricultural exploitation of the territory, followed by a downsizing between the 3rd and 4th centuries, reflecting the economic changes of the Roman Empire. Following the fall of the Western Roman Empire, the area of the Latium Teverina was affected between the end of the 5th and the beginning of the 7th century by the political vicissitudes that characterised this transitional phase.

The advent of the Heruli, later replaced by Theodoric's Ostrogoths in 489, was followed by Justinian's intervention to reconquer the peninsula with the Greek-Gothic War, which ended with the, albeit short-lived, reunification of Italy under the Eastern Roman Empire.

At this time, just to the south-east of the area of Castel Sozzio, ran the so-called Byzantine corridor, an area that could be described as geographically and culturally separated.

Finally, between the six-seventies and the first decades of the seventh century, the area fell, like the whole of central Italy, under the dominion of the

Lombards and became part of the Duchy of Spoleto. This historical complexity shows the importance of the necropolis in question, for which the identification of endogenous or exogenous elements is of fundamental importance.

3. THE ANTHROPOLOGICAL DATA:

A first piece of extremely important information for the interpretation of the site is provided by the taphonomic evidence [Duday, 2006]. These clearly show multiple actions of reopening the tomb aimed at adding new individuals: the space occupied by the previous burials was reduced in order to allow the insertion of a new individual.

The function of the circular pits arranged around the perimeter of the burial is still to be fully understood; what is evident, by the way, is that they are secondary burials arranged around a central burial probably defining a relationship to this one.

At present they have been interpreted as relating to the reuse of the lithic coffin tombs: when the space inside the burial was exhausted the first occupants were reburied outside the coffin but in relation to it.

The analysis of the skeletal remains from the necropolis, taking into account the burials in primary, secondary or reduced position and the bones scattered outside the graves, has allowed us to identify about seventy buried individuals (updated to the 2020 excavation campaign), of which about ten were recognised within a layer where the extremely fragmentary bones are not connected due to the intense previous agricultural activities, to the action of the fauna and to the roots of shrubs and trees grown during the period of abandonment. Although the sample was chosen from a small number, it was possible to determine the gender and age of almost the entire sample (**Table 1**).

TOMB	US	AGE	SEX
T. 1	us 19	6-8 years	
T. 2	us 19	7-8 years	
T. 3	us 104	30-40 years	F
T. 4 Ind.1	us 48	5-7 years	
T. 4 Ind. X4	us 31-32	30-35 years	M
T. 4 Ind. 2	us 64	30-40 years	F
T. 4 Ind. 3	us 46	35-45 years	M
T. 5 (Ind. A)	us 66	20-30 years	M?
T. 5 Ind.1	us. 59	30-50 years	M?
T. 5 Ind.2	us. 59	30-50 years	?
T. 5 Ind.3	us. 59	30-35 years	M
T. 5 Ind.B/5	us. 59	20-40 years	IND.
T. 5 Ind.C/5	us. 59	35-45 years	F
T. 6	us.92	30-35 years	F
T. 6 Ind A	us 99	25-35 years	F
T. 6 Ind B	us.99	30-35 years	F
T. 7 Ind. 1	us.134	25-50 years	M
T.7 Dep. 1	us. 175	NR	NR
T.7 Dep. 2	us. 176	30-40 years	IND.
T.7 Ind. 2	us. 134	30-50	M
Fragments in layer	34	nr	nr
Fragments in layer	18	30-50	NR
T. 8	us.69	25-35 years	M
T. 9 Ind A	us. 121 dep 1	20-50 years	NR
T. 9 Ind B	us. 121 dep 2	Nr.	NR
Various and fragmentary Dep.			
T.10 ind. Inf.	us.159	25-45 years	M
T.10 ind. N.D.	us. 150	NR	NR
T.10 ind. Sup.	us. 156	30-40 years	M
T.11	us. NO	NR	NR
T. 13/19 (Ind. A)	us 162		M
T. 13/19 skull 1 (Ind. B)	no us		
T. 13/19 Skull 2	no us		
T. 13/19 Sporadic bones	no us		
T. 15 primary dep. (skull 5)	us.210	40-50 years	M
T. 15 skull 1	207	30-50 years	M
T. 15 skull 2	207	NR	F
T. 15 skull 3	207	35-45 years	F
T. 15 skull 4	207		M

T. 15 skull 6	207	30-45 years	F
T. 15 skull 7	207	25-30years	F
T. 15 skull 8	207	30-50 years	M
T. 15 skull 9	207	2-5 years	
T. 15 n°12-13-16-17	207		
T. 15 scattered bones	59	nr	nr
T. 16	208		
T. 16	129		
T. 19 NE dep 1	118	40-50	M
T. 19 NO dep 2	118	?	M
T. 20	no us	30-50 years	M
T. ?	169		
T. ? scattered bones	148		
T. 26	191	30-50 years	M
T. 26	190		
T. 26	189	40-45 years	F
T. 27	193	11-15 years	
Mixed	Nr		
T. 24 Ind. D	310	11 - 13 years	
T. 24 Ind. C	312	25-30 years	F
T. 24 Ind.a	306	40-50 years	F
T. 24 Ind.b	306	>50	M
T. 30 Ind.a	325	18-22 years	M
T. 30 Ind.b	325	25-30years	F?
T. 30 Ind.C	325	30-45 years	F?
T. 30 Ind.d	325	2-3 years	
T. 31	329		
T. 31 Ind.b	318	30-50years	M
T. 31 Ind.a	318		F
T. 32	321	25-35years	F

Tab.1: Table summarising the anthropological data relating to the determination of sex and age of individuals

Given the low stratigraphic reliability and the loss of documentation from 1997 and 1998, these 10 extremely fragmentary individuals were kept separate, taking into account only the skeletal remains from a more secure archaeological context, as it is not possible to verify at present whether these remains or parts of them can be attributed to individuals from the layer below.

Of the sixty individuals investigated, twenty-four were male, eighteen were female, five were juvenile/children? (under ten years old) and two were juvenile (between ten and fifteen years old); for the remaining eleven individuals the sex could not be determined due to the fragmentary nature of the remains. (**Chart 1; Chart 2**)

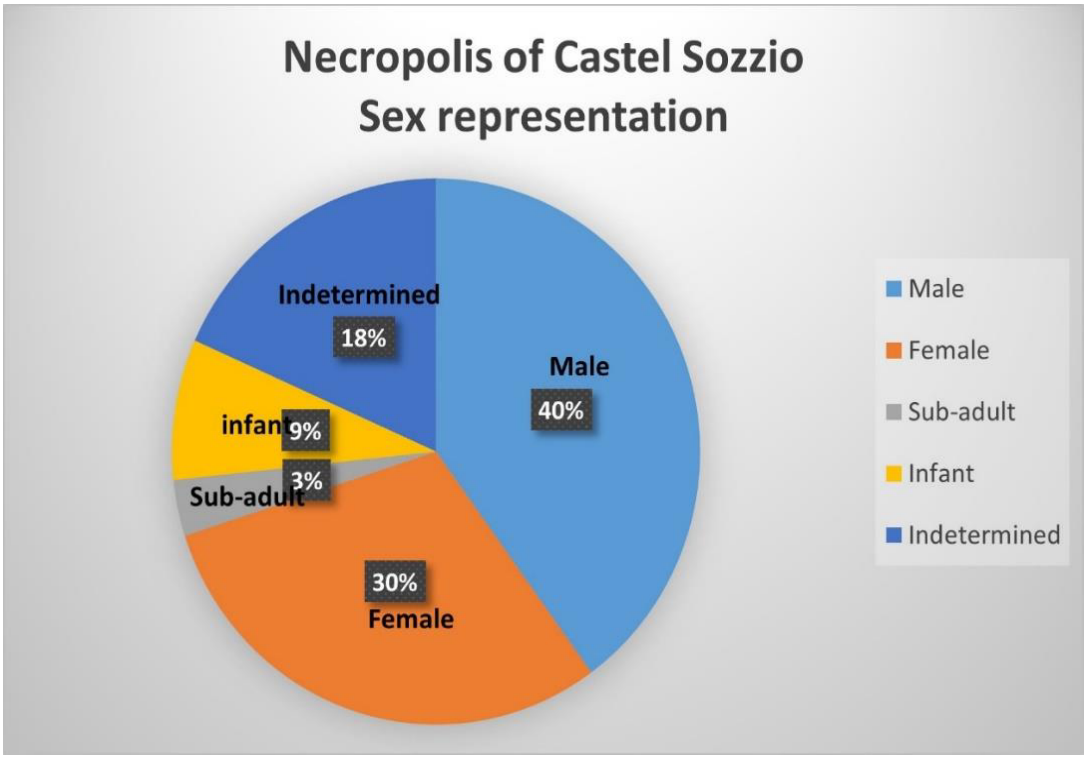

Chart 1: Necropolis of Castel Sozzio sex representation

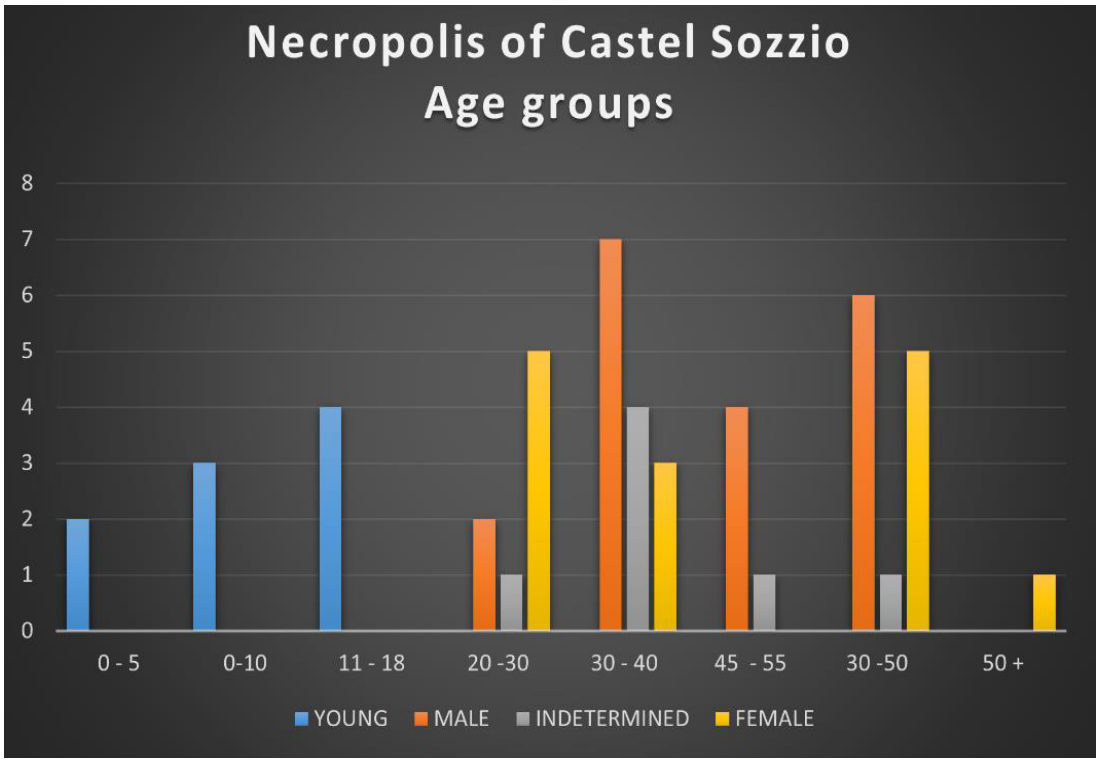

Chart 2: Necropoli of castel Sozzio Age Groups

When comparing the age at death to sex of the individuals, even taking into account the high proportion of indeterminate individuals, it is possible to observe a higher mortality rate for women than for men in the 20-30 age group. The life expectancy of the community of the necropolis of Castel Sozzio is on the whole within the standards of the period. No significant pathologies were detected in the sample, although this is still an initial study.

4. CASTEL SOZZIO: CONSIDERATIONS ABOUT THE MOBILITY STUDIES

Anthropologists at an early stage regarding the interpretation of different societies considered mobility as an extraordinary phase: a variation from a sedentary pattern.

However, considerations about complex social structures that see multiple factors, be they economic, political and ideological or driven by conflict or flight from persecution or attributable to external forces such as climate change [Tsuda et al. 2015; Wendrich and Barnard 2008], have led to new analyses which, on the one hand, sometimes see an exceptionality in the face of the mobility of one or more groups, on the other, an act that is anything but unique, arriving at the understanding that all societies, however static, possess a more or less ample percentage of mobile individuals [Wendrich and Barnard 2008]. One should consider the dynamism of human social organisation and the different modes of production that typically contribute to the structure of an individual community [Anthony 1997; Cabana and Clark 2011; Tsuda et al. 2015; Wendrich and Barnard 2008] and that may determine aspects of uniqueness or affinity.

We need to considerate also the uniqueness aspects of the populations who influence and are influenced by the places which they move through. The same consideration has to be applied to the autochthonous population, where the unicities are not limited merely to the occupied geographic area or to the biological distance.

Markers that are generally given social meaning and further shaped by self-perception, including age, sex, gender, status and ethnicity, must be considered within this analysis (Brettell 2008; Díaz-Andreu et al. 2005; Insoll 2007; Knudson e Stojanowski 2008; Gregoricka 2020).

The collective and the group identity a stratified society that does not recognise itself in a single community should also be considered in order to better explain the motivations and subsequent choices dynamics by the

communities (Baustian et al. 2014; Clark e Wilkie 2006; Fowler 2016; Gregoricka 2020a; Sørensen 2013).

In order to study a reality, even if geographically limited, it is necessary to take into consideration all those aspects linked to the territory in its broadest sense. In order to analyse the interactions that took place in the Teverina area, with specific reference to the site of Castel Sozzio, the process of territorial reorganisation that followed the fall of the Western Roman Empire and the amplification of migratory flows in Italy, often seen exclusively from the point of view of a systemic crisis, must be considered and investigated also as a new structuring of spaces both at the level of settlements and of occupation of the territory by allochthonous populations that went to replace and/or integrate (not always violently) the autochthonous ones that occupied the territory in previous periods.

From this non-catastrophic point of view, but aimed at investigating the reorganisation of the territory by an evolving society, the human occupation of Northern Lazio, in particular of the middle Tiber valley in the critical phase between the end of the Roman Empire and the Early Middle Ages (4th-9th century), represents a case study of great interest in understanding how the settlements and the populations that inhabited them developed new socio-economic models adapting to the new political balances.

The area between Lake Bolsena to the west and the Tiber River to the east, centred around the towns of Civitella D'Agliano, Graffignano and Sipicciano, is a privileged context for the study of this transitional phase, since it is the scene of the interaction of different cultures - Romans, Goths and Lombards - between the 6th and 7th centuries [Borgia (in press) 2022]. The investigations currently underway in the late Roman and early medieval necropolis of Castel Sozzio (Civitella D'Agliano, VT), thanks to a three-year excavation concession from the Direzione Generale Archeologia Belle Arti e Paesaggio (Decree 929 of 10/07/2020, direction Prof. Emanuela Borgia), will make it hopefully possible to better define the type of settlement and occupation of an area that is currently almost unknown from an archaeological point of view.

5.0 ISOTOPIC MOBILITY ANALYSIS

The provenance of foods, artifacts, animals and individuals is a central topic in archaeology, ecology, forensic science and even in social sciences and humanities. A broad range of methods from genetics to inorganic chemistry can be used to disentangle the geographical origin or the movement of goods/people across the landscape, depending on the nature of the material itself [Gregoricka, 2021; Tommasini et al., 2018].

The combined analysis of archaeological, anthropological and isotopic data constitutes a fundamental element in the reconstruction of the dynamics of settlement, mobility and social and cultural interaction, sometimes allowing us to precisely determine the causes and effects of the movement of groups of people.

5.1 METHODOLOGY

Strontium isotope ratio (87Sr/86Sr) analysis of tooth enamel and bone is a technique used in archaeology to identify residential mobility evidence among past populations [Montgomery et al., 2006a, 2014; Wallace et al., 2010; Montgomery and Grimes, 2010; Kador et al., 2014; Sheridan et al., 2013; Gregoricka 2021]. The strontium isotope ratio reflects local bedrock geology [Price et al. 2002]. Strontium passes from the rocks to the soil and the groundwater, and from there to the local plants and animals [Porder et al., 2003; Price et al., 2002]. Therefore, humans, who absorb strontium largely through the food and water that they consume, tend to have 87Sr/86Sr values similar to those available in the local bedrock geology and -more precisely- the local bioavailable 87Sr/86Sr [Price, 2015; Laffoon et al. 2017].

Whereas bone continuously remodels, tooth enamel for most of the permanent human dentition forms in early childhood and does not change after it has formed. Thus, if an individual's enamel 87Sr/86Sr values turns out to be different to his/her bones 87Sr/86Sr values or to the local bioavailable 87Sr/86Sr, changes in his/her place of residence can be demonstrated [Price et al., 2002]. In studies of this kind, archaeological and modern small animals are normally used as a proxy to local

bioavailable 87Sr/86Sr at the investigation site [Price, et al., 2002; Nafplioti, 2012]. The human enamel and bone 87Sr/86Sr values are compared with those of local animals in order to check whether or not these values overlap and to distinguish between locals and non-locals.

Oxygen isotope ratio 18O/16O is often used in conjunction with the study of strontium [Evans et al. 2012] because a stable oxygen ratio becomes part of the food and drinking chain through rainfall, which largely depends on the geography of the place analysed [Lightfoot, O'Connell 2016; Waters-Rist, Palmer 2016]. Hence, the ingested water reflects the local meteoric precipitation, although the relationship is not always so straightforward [Evans et al. 2012; Lightfoot, O'Connell 2016]. Stable oxygen isotopes are also incorporated in tooth enamel and can provide further information about whether or not the individual moved during his/her lifetime, giving further support to the analysis of 87Sr/86Sr. (**fig.6**)

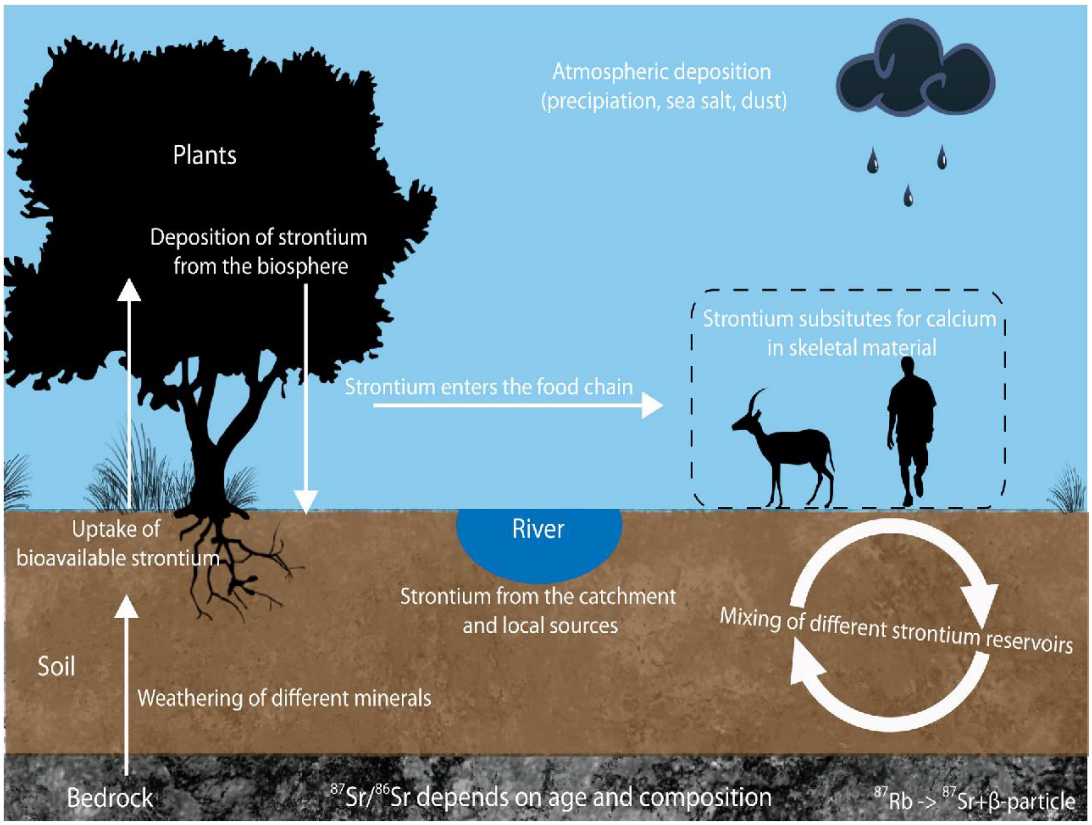

Fig. 6: Strontium isotopes transmitted through the weathering of rocks to form soil, into the biosphere via plants and in drinking water. (M. Willmes 2015)

5.2 SELECTION AND SAMPLING

The first objective was be the creation of a Bioavailable Sr baseline by sampling local fauna from archaeological layers of terrestrial malacofauna and local plants.
As part of this research, 33 samples were selected, of which 31 human samples, 2 animal samples, 2 soil shells samples and 3 grass samples (**Tab.2**)

ID	Tag name	Sample type
CS-1	CS 98 S B T20	M1 inf Dx
CS-2	Cs 98 S. A T15 cr V	M2 inf Dx
CS-3	Cs 98 S A T15 cr VIII	M1 sup Sn
CS-4	Cs 98 S A T15 cr IX	M1 sup Sn
CS-5	Cs 98 S A T15 cr VII	M1 sup Dx
CS-6	Cs 98 S A T15 cr VI	M1 sup Sn
CS-7	Cs 98 S A T15 cr III	M1 sup Dx
CS-8	Cs 98 US 325 T.30 Ind.A	M1 inf Dx
CS-9	Cs 98 US 325 T.30 Ind.B	M1 inf Sn
CS-10	CS 97 US 61 T.4 Ind. 2	M2 sup Dx
CS-11	CS 97 US 46 T.4 Ind. 3	M2 sup Dx
CS-12	CS 97 us 66 T.5	M2 inf Dx
CS-13	CS 97 us 59 T.5	M1 inf Sn
CS-14	CS 20 us 310 T.24	M1 inf Dx
CS-15	CS 20 us 312 T.24	M2 sup Dx
CS-16	CS 20 us 306 T.24	M1 inf Sn
CS-17	CS 99 S.B T.11	M1 sup Sn
CS-18	CS 98 us 163 T.21	M1 sup Dx
CS-19	CS 20 us 321 T.32	M1 Inf Sn
CS-20	CS 98 us T.7 Ind.1	M1 sup Sn
CS-21	CS 98 S.B T.13/19	M1 sup Dx
CS-22	CS 97 us 99 T.6 Ind.A	M1 Inf Sn
CS-23	CS 97 us 92 T.6	M1 Inf Dx
CS-24	CS 97 us 99 T.6 Ind.B	M2 sup Dx
CS-25	CS 98 us 156 T10 Ind Sup.	M1 inf Dx
CS-26	CS 98 us 153 T10 ind Inf	M1 inf Dx

CS-27	CS 98 S.B us 12	M2 Ovicaprid
CS-28	CS 98 S.B us 12	M2 Sus
M-321		Terrestrial Snail
M-328		Terrestrial Snail
P-01		Grass
P-02		Grass
P-03		Grass

Tab.2: Summary table with associated lab sample ID, Tag Name (i.e. burials and reference us) and sample type

The enamel of human teeth M1 and M3 was sampled, according to the protocol (explained in the chapter "Experimental protocol used", *infra*) [Price et al. 2002; Ambrose 2006] applied by the Environmental Archaeology Laboratory Boston University Standardized by K. Wade in 2018.

Subsequently the 87Sr/86Sr ratios were determined by Neptune MC-ICPMS, housed at the Centro Interdipartimentale Grandi Strumenti of the University of Modena and Reggio Emilia. The experimental protocols applied are described in Lugli et al. (2017, 2018). Repeated measures of NBS987 yielded an 87Sr/86Sr value of 0.710237 ± 0.000011 (2 SD; n = 18). All values were normalized to an NBS987 accepted value of 0.710248 (McArthur et al., 2001).

5.3 SAMPLING PROTOCOL

Equipment and sample sterilization ~ 15 mins

Equipment needed: 2 Dremel tools, carbide drill tips, ultrasonicator, lab coat

Disposables needed: DI water, isopropyl alcohol, small and large Kimwipes.

1. Be sure that Dremel tool mounting assembly and body are clean of all dust before beginning. Wipe down with Kimwipes and isopropyl alcohol.

2. Place tooth in distilled/nanopure water bath in ultrasonicator and sonicate until adhering soil falls off. Wash with isopropyl alcohol and allow to dry.

3. Sonicate all carbide and diamond drill bits in isopropyl alcohol to clean, along with any removable parts of Dremel tool mounting assembly.

Enamel exposure ~ 15 min/tooth

Equipment needed: eye protection, 2 Dremel tools, carbide drill tips, baths, ultrasonicator, lab coat, quartz crystal.

Disposables needed: DI water, isopropyl alcohol, gloves, small and large Kimwipes

1. Wearing disposable gloves and eye protection, carefully hold tooth over clean work area.
2. Put down large Kimwipe to catch powder removed for disposal.

2. Use Dremel tool marked “Cleaning” with carbide drill bit to remove outer layer of calculus/plaque over tooth enamel. Do this lightly but thoroughly to expose white enamel

over an area larger than the location where enamel samples will be removed, to minimize contamination.

3. Use Kimwipes and isopropyl alcohol to wipe down the tooth; set aside for enamel

Extraction

4. Use Dremel to drill quartz over same work surface for 10 seconds.

5. Dispose of Kimwipe with dust in trash can; wipe down area with isopropyl alcohol and Kimwipes.

6. Reclean Dremel and bits as described in equipment and sample sterilization before going on to next tooth.

7. Repeat enamel exposure steps until all teeth are cleaned. Clean all teeth prior to extracting enamel.

Enamel extraction ~ 10 min/sample line

Be sure to remove only enamel and no dentine, which will contaminate the sample

Equipment needed: eye protection, 2 Dremel tools, carbide drill tips, baths, ultrasonicator, balance (measuring to 0.0001 g), extra fine Sharpie, lab coat, quartz crystal

Disposables needed: DI water, isopropyl alcohol, 1.5 ml centrifuge tubes, gloves, small and large

Kimwipes, weigh paper, aluminum foil

1. Label a 1.5 ml microcentrifuge tube with Sharpie.
2. Use balance to weigh labeled microcentrifuge tube; record empty weight.
3. Place clean sheet of aluminum foil on work area. Put on new pair of gloves.
4. Tare balance with weighing paper; place weigh paper in center of aluminum foil.
5. Attach clean diamond bit to Dremel tool marked “Enamel”. Holding tooth very close to the center surface of the weigh paper, drill a horizontal line just below the occlusal surface of tooth. Accumulate 10-15 mg of tooth enamel powder on the weigh paper; tilt into labeled microcentrifuge tube. Close tightly.
6. Calibrate balance to zero. Weigh filled tube and record. Calculate and record weight of enamel in the sample.
7. Use Dremel to drill quartz over same work surface for 30 sec.
8. Dispose of weigh paper; recycle aluminum foil. Wipe down work area with alcohol.
9. Reclean Dremel and bits as described in before going on to next tooth.
10. Repeat enamel extraction steps at desired intervals until entire tooth is drilled. Complete single tooth before moving on to next tooth.

Enamel pre-treatment for apatite extraction

This protocol uses pretreatment with bleach (removes organics) and acetic acid

(removes adsorbed carbonates). Always run a full procedural blank (empty tube) through this protocol.

Collagen removal ~ overnight procedure

Equipment needed: eye protection, lab coat, microcentrifuge tube rack, vortexer, pipetters, microcentrifuge tube rack lid

Disposables needed: gloves, 50% bleach solution (in wash bottle), aluminum foil, pipette tips

1. Wear gloves, lab coat, and eye protection. Place sample tubes in microcentrifuge rack and transport into fume hood in wet lab. Turn fume hood on. Do all work in fume hood.
2. Open each tube and fill with ~1.5 ml of 50% bleach solution to remove collagen. Close tube before moving to next sample.
3. Vortex for 3 sec. Open tubes and let them stand overnight in fume hood, covered loosely with a sheet of aluminum foil.

Carbonate removal ~ 9 hrs, + overnight

Equipment needed: eye protection, lab coat, microcentrifuge tube rack, vortexer, pipetters, microcentrifuge, vacuum desiccator, microcentrifuge tube rack lid, ultrasonicator, freezer Disposables needed: gloves, DI water (in bottle), pipette tips, 0.1 M acetic acid

1. Close tubes. Vortex, then place in microcentrifuge for 5 mins at 5000 rpm for (with tube hinge pointing down); decant bleach. If the sample does not readily vortex, place tube in ultrasonicator and sonicate for 10 sec, then vortex for 3 sec.
2. Centrifuge 5 mins at 5000 rpm and decant; add distilled water to fill. Rinse samples 4 times total. *
3. Add 0.1 M Acetic acid (0.1 ml per 1 mg of original sample weight); vortex briefly. Let tube stand open exactly 4 hrs.
4. Vortex, then place tubes in vacuum desiccator and slowly evacuate air until samples achieve a low boil for 5 mins.
5. Return to atmospheric pressure, and repeat twice evacuation and repressurization.

6. Depressurize, then wait 4 hours.

7. Centrifuge tubes for 5 minutes and decant acetic acid. Rinse samples 4 times total. *

Decant and pipette out last bit of liquid, re-centrifuging as needed to keep sample solid. Leave tubes open.

8. Place open tubes in a microcentrifuge tube box in the freezer for 30 mins. Open the vacuum desiccator before taking samples from freezer.

9. Place open tubes immediately in the desiccator. Do not allow samples to melt first. Samples dry in ~ 12-15 hrs.

*To rinse samples: Fill tube with DI water. Vortex for 5 sec. Centrifuge for 2 min at 2500 rpm. Remove supernatant with pipette and discard.

Apatite yields ~ 30 mins

Equipment needed: eye protection, lab coat, analytical pan balance, notebook, computer with Excel

Disposables needed: gloves

1. Close dried centrifuge tubes. Reweigh tubes, which now contain only apatite. Record

weights into lab notebook. Then use spreadsheet to calculate apatite yield from wet chemistry. Enter calculated data in lab notebook.

2. Samples are ready to be taken to TIMS clean lab for strontium extraction.

1 M Acetic acid recipe: 1000 ml H20 + 57.2 ml acetic acid.

0.1 M Acetic acid recipe: 900 ml H20 + 100 ml 1.0 M acetic acid.

5.4 STRONTIUM EXTRACTION

Sample preparation has been carried out in the class 1000 clean laboratory of the Department of Chemical and Geological Sciences of the University of Modena and Reggio Emilia (Italy) under the direction and supervision of Dr F. Lugli.

Our sample should now be washed using MilliQ water and digested using concentrated HNO3. Care should be taken at this stage to avoid possible contamination.

Chemical extraction chromatography was conducted under a class 10 laminar flow hood.

For isolation of Sr ions from solutions, 3 mL polypropylene columns filled with 300 µL of Eichrom Sr-Spec resin (bead size 100-150 µm) (Eichrom Technologies, LLC) were used. Philip Horwitz et al. (1992).

The protocol applied in this study refers to Protocol 1 described in Claudio Argentino et al. (2021). (fig. 7)

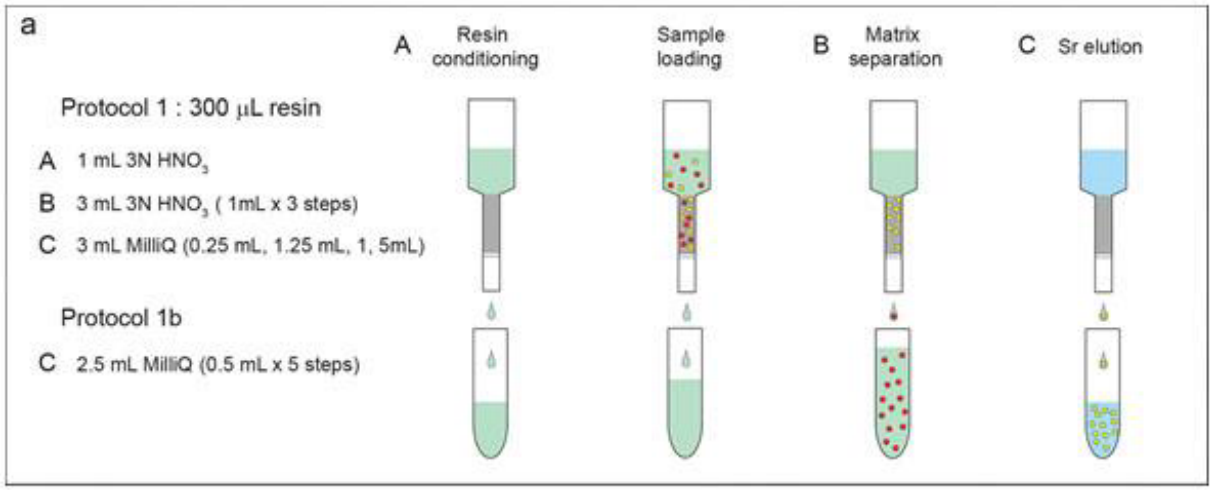

Fig. 7: Extraction chromatography protocols for combined 87Sr/86Sr and δ88/86Sr analyses of pore water by MC-ICP-MS (Argentino et al. 2021).

Protocol 1:

- The resin is pipetted into the column and allowed to settle to avoid the formation of bubbles or voids that would reduce Sr retention.
- The resin is pre-cleaned by rinsing three times with MilliQ® water (column reservoir full) and then conditioned with 1 mL of 3N HNO3.

- Three millilitres of sample solution is then loaded into the column. Matrix elements are subsequently removed by adding 3 mL of 3N HNO3 in three steps (1 mL each).
- The bulk solution passed through the column so far is discarded. Strontium is finally recovered by adding 3 mL of MilliQ® water in three separate phases (0.25, 1.25 and 1.5 mL) and collected directly into a clean 15 mL Falcon® tube.
- To assess whether the volume of MilliQ® and the number of steps in the elution step can influence the final Sr result, an SRM 1640a and a JCt-1 sample were treated with a modified elution procedure according to Deniel and Pin (2001) [Weber et al. 2020], hereafter referred to as Protocol 1b. In protocol 1b, 2.5 mL of MilliQ® is added in five steps (0.5 mL each).
- All final Sr-containing solutions are adjusted to 4% HNO3 for MC-ICP-MS analysis.

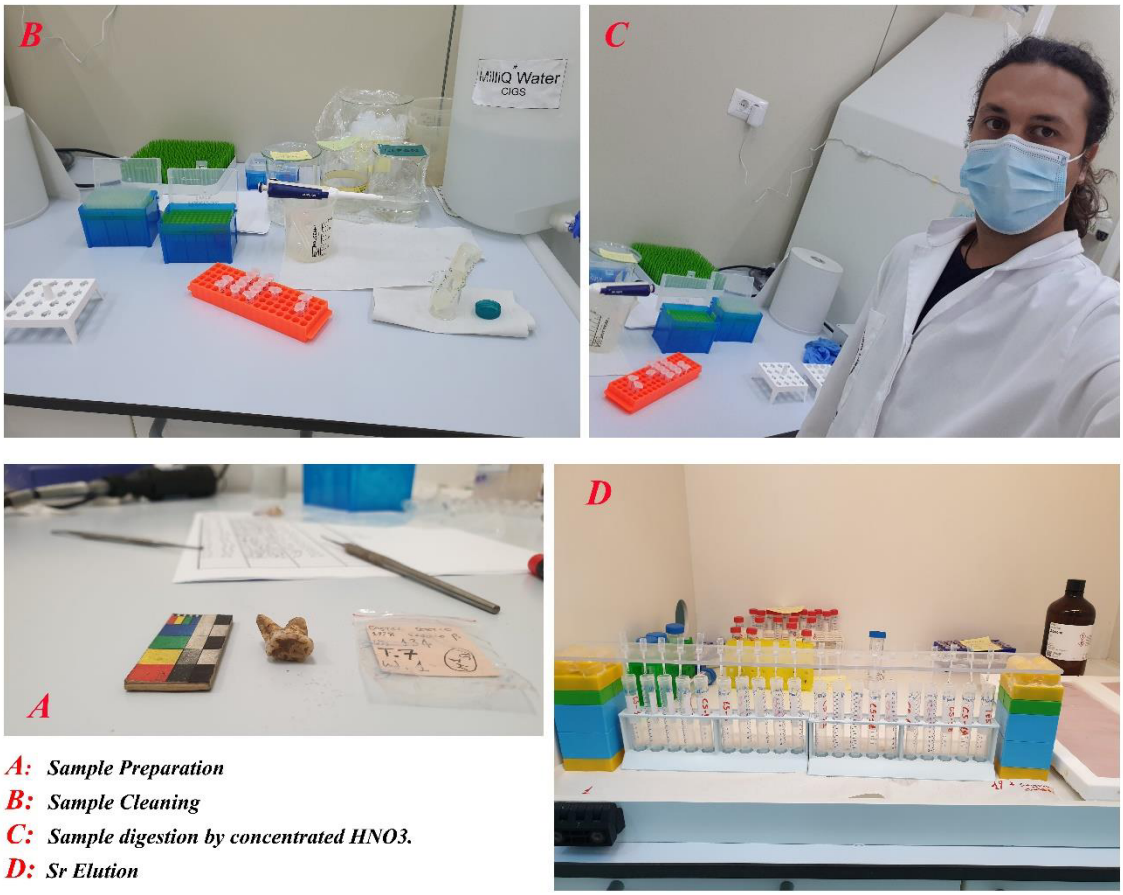

Fig.8: Different steps of sample preparation **A**: Enamel Extraction **B**: Sample Cleaning **C**: Sample digestion by concentrated HNO3 **D:** Sr Elution.

6 DISCUSSION

Regarding the isotopic analyses based on the Strontium isotope ratio (87Sr/86Sr), the difficulty of differentiating human groups that grew up in regions with similar geology has been highlighted [Giostra 2019]. In order to obtain more precise data, it is therefore appropriate to integrate those ones with the Stable oxygen isotopes analysis which provide further information about whether or not the individual moved during his/her lifetime, giving further support to mobility studies.

The data obtained and presented in this research suffer from this problem as they are based solely on the analysis of the Strontium isotope ratio.

In order to correctly reconstruct the encroachment processes of other populations within a local matrix substratum, it is also appropriate to consider a scenario in which not only long-distance movements but also forms of regional mobility, as well as a model of interaction in which the encroaching population is integrated more or less rapidly.

It should therefore be pointed out that without elements of the material culture associated with the burials we cannot recognise these individuals nor define their real degree of integration or social role. An element of support in this sense may be provided by the analyses inherent in the Palaeo-diet.

The creation of a site baseline resulting from the analysis of the soil and grass snail samples allows us to clearly define the native population group, even if we consider that individuals from another group but born in the area show the same reference signal and are therefore local.

Three different groups emerge from the analyses: one relating to local individuals and two others, hereafter referred to as 'Non-Local 1' and 'Non-Local 2', referring to divergent values, clearly indicating groups from two different geographical areas (**Chart.3**)

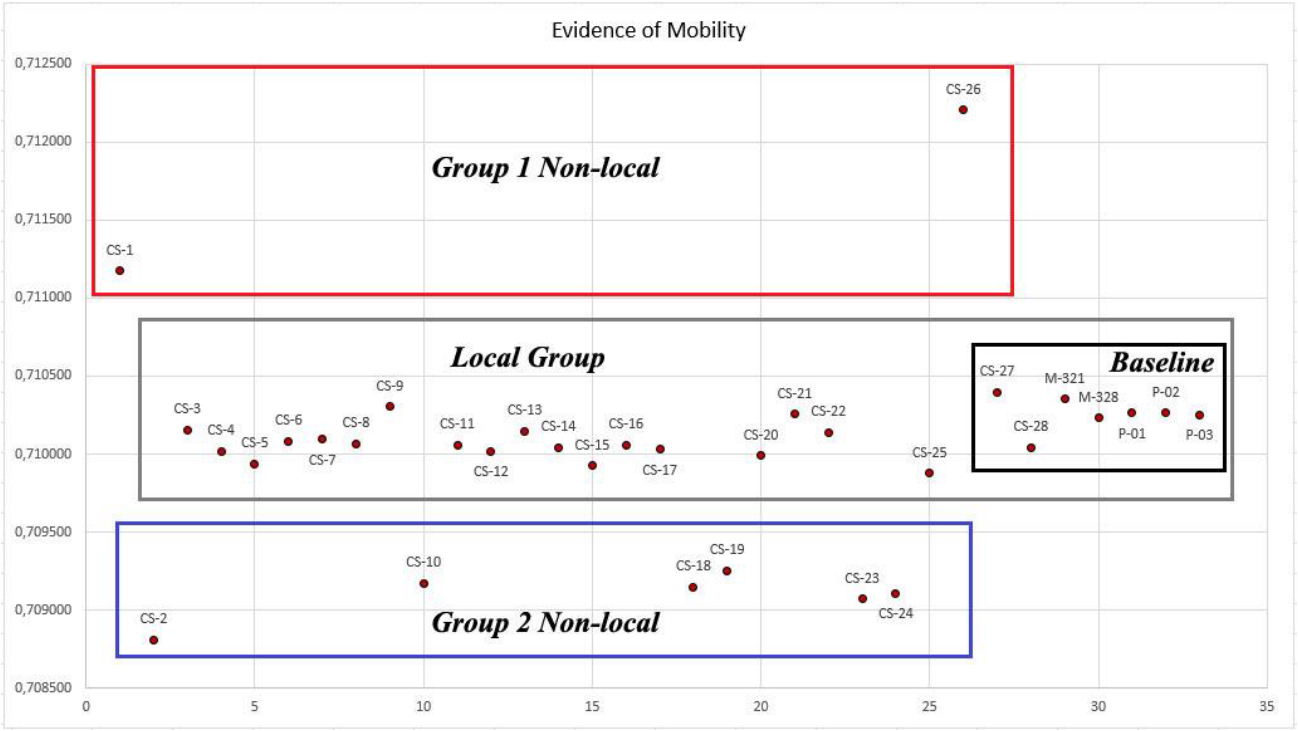

Chart 3: Differences in isotopic values. from the local group (grey), two non-local groups can be clearly distinguished: Group 1 (red) Group 2 (blue)

Matching the data obtained from this analysis with the ones previewed by the Department of Chemical and Geological Sciences of the University of Modena (study conducted by: Department of Cultural Heritage, University of Bologna, Ravenna, Italy, Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy, Lamont-Doherty Earth Observatory, Columbia University, Dipartimento di Storia Culture Civiltà, University of Bologna, Department of Archaeology, Durham University, Max Planck Institute for Evolutionary Anthropology, Department of Human Evolution, Leipzig, Germany) it was possible to identify potential areas of origin of the Non-Local groups.

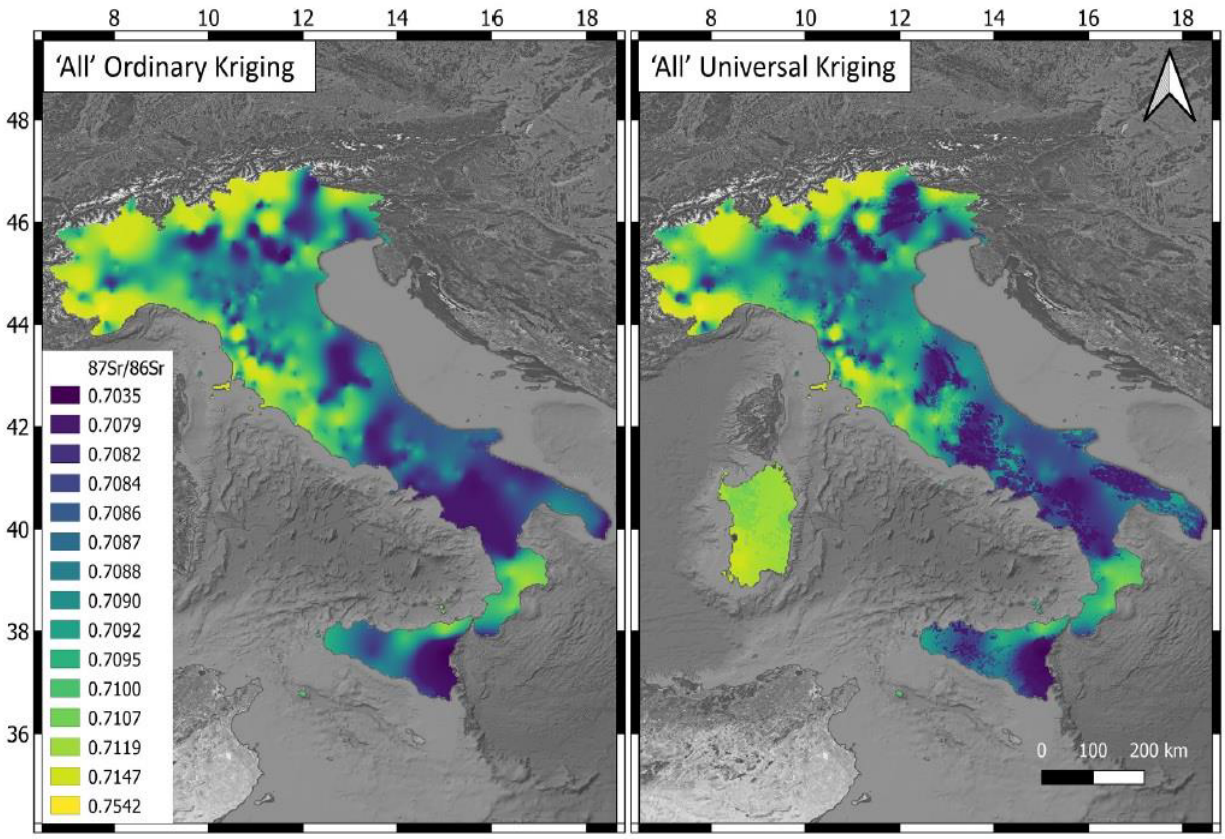

Fig.9 Ordinary and Universal (with external drift) kriging models obtained for the 'all' 87Sr/86Sr dataset. Maps were obtained using SAGA 7.9 and QGIS 3.8. (Lugli et al. in press.)

Pending new isotopic, archaeological, chronological data and DNA study, it is possible on the basis of the data obtained to put forward hypotheses concerning the origin of the groups interacting on the local substrate.

The Non-local Group 1, the values of which range between 0.7112 and 0.7123 (87Sr/86Sr), would appear to come from the central-eastern, north-eastern area of Italy. Although similar levels may refer to Sardinia, it is unlikely that this is the place of origin of the non-local group 1, as there is no archaeological data to support this potential hypothesis for this historical period.

The non-local group 2, whose values range between 0.7079 and 0.7084 (87Sr/86Sr), refers to a population from the central-northern area of the Italian peninsula.

Similar levels may refer to South-occidental Italy, but again there is no evidence to support this hypothesis or to suppose the movement of a large number of people to this

area in this phase.

The results, which can be considered preliminary, therefore allow us to distinguish between the autochthonous population and the two allogenic groups, while also giving us clues as to their possible area of provenance.

On the basis of the results obtained so far, we can hypothesise that the interaction with the local matrix substrate is probably attributable to Gothic and/or Longobard peoples who occupied the areas from which the identified non-local individuals came during the period of necropolis use.

Before being able to formulate an exhaustive hypothesis that will allow us to understand the dynamics of mobility and interaction, it will be necessary to integrate new anthropological archaeological data and future analyses that will see the addition of Stable oxygen isotopes studies to the analyses of 87Sr/86Sr. These should be superimposed on the analysis of the ancient genome (aDNA) and a series of absolute dates (C14) aimed at defining the chronological horizon of the site and determining the phases of interaction between human groups.

7 CONCLUSIONS

This work is based on the joint study of traditional archaeological and anthropological evidence - analysis of the tomb furniture, spatial distribution, burial typology, stratigraphy, etc. - and new technologies - such as isotopic analysis (Sr and O for mobility), genomic analysis (aDNA), and information from the analysis of material culture, archaeological context and bioarchaeological evidence.

This approach will allow us to understand more accurately the biological, social, cultural and commercial interactions on the local matrix substrate and on the encroaching population, making up for the impossibility of identifying the "distance" covered by migratory movements through archaeological data alone.

The data coming from the archaeological studies already published [Cecchelli M.; Pompei M. C.; Riganati F.; 1997] and in progress [Excavation Campaign 2020 and 2021 directed by Prof. E. Borgia: Borgia 2021] such as the structure and the sepulchral typology, the grave goods, were put in relation with the analyses related to the Mobility and the Paleo-diet, conducted in collaboration with the Department of Chemical and Geological Sciences, University of Modena, and the Department of Environmental Biology, Sapienza. A fact that gives food for thought is the high percentage of non-local individuals compared to the sample analysed. If this were to be confirmed, it could constitute further evidence to confirm the theory that both settlement and occupation of the territory by allochthonous populations went to replace and/or integrate (not always violently) the autochthonous ones that occupied the territory in previous periods.

It should also be pointed out that at the present to date there are no signs of malnutrition or stress related to reduced food intake, nor the increase in trauma usually related to violent situations.

We can assume that these two groups from the central-northern area of the

Italian peninsula, identified as Goths or more probably Lombards, overlapped and integrated with the local population, presumably influencing and being influenced by it.

New analysis are in progress thanks to the collaboration with the Dept. of Evolutionary Anthropology (Vienna) and the David Reich Lab: Ancient DNA Biology and Disease (Harvard), to conduct studies related to the reading of the ancient genome [Antonio, Coppa, La Pastina, Pinhasi, Pritchard, et al. 2019; Fernandes, Coppa, La Pastina, Lipson, Pinhasi, Reich, et al. 2020]. Changes in the population components analysed through mobility and genetic studies will allow us to identify the social structure/kinship more precisely; comparison with published data from the archaeological context will, together with the palaeo-diet, provide information to identify the economic and social context more clearly.

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