

The Roman Military Community as a Melting Pot: Biomolecular Evidence from the Lower Rhine Limes

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Abstract

Military communities in the Roman borderlands are increasingly understood as socially mixed and dynamic rather than homogeneous military enclaves. This study adds a biomolecular perspective through combined strontium, oxygen and carbon isotope analysis, and ancient DNA of 33 inhumed and 44 cremated individuals buried at *Praetorium Agrippinae* in modern-day Valkenburg (South Holland, the Netherlands). The site contains one of the largest, well excavated Roman-period cemeteries in the Lower Rhine Limes Borderlands, dominated by cremations with a smaller yet substantial inhumation component. Sr-O-C isotope data indicate a large variety of geographical regions of origin among both inhumed and cremated individuals, demonstrating sustained mobility into the settlement throughout its 300-year existence. Ancient DNA analysis establishes heterogeneous ancestry profiles and an absence of close kinship ties, further supporting a diverse population with varied geographic origins. Overall, the biomolecular evidence positions the Roman inhabitants of *Praetorium Agrippinae* as a melting-pot borderland community shaped by movement, demographic diversity, and social differentiation.

Introduction

In recent decades, the concept of *military communities* has transformed how scholars interpret life along the Roman borderlands. Earlier *limes* research, rooted in the traditions of the *Reichs-Limeskommission*, primarily treated forts, *canabae* and *vici* as separate archaeological site types, focusing on architectural and unit histories (Johnson 1983; Breeze 1987). Since the late 1990s, however, studies have reframed the Roman army as a social formation embedded in wider communities rather than an isolated entity. The seminal volume *The Roman Army as a Community* (Haynes 1999a) catalysed this shift by emphasizing households, religion, identity, and interaction between soldiers and civilians. Subsequent work has expanded this perspective through analyses of domestic assemblages, gender, age, and identity within forts and associated settlements (Allison 2013; Greene 2019; Gardner 2016; Collins and McIntosh 2014). As a result, the term *military community* has become standard in Roman borderland archaeology, capturing the social, economic, and familial entanglements that structured life along the *limes*.

Bioarchaeological approaches can directly address the mobility that structured Roman military communities in borderland contexts by combining strontium, oxygen, and carbon isotope analyses with aDNA to reconstruct childhood origins, migration histories, kinship, and population structure. Multi-isotope studies in northern provinces have demonstrated the power of these methods to identify non-local individuals and trace patterns of recruitment and provisioning (Chenery et al. 2010; Madgwick et al. 2019), while integrated isotopic and genomic work increasingly reveals how movement and ancestry shaped life at the empire's margins (Scheib et al. 2024; Depaermentier et al. 2023). Despite this potential, bioarchaeological research explicitly targeting military or fort–vicus assemblages in Roman borderlands remains limited. Reviews emphasize that mobility studies have focused mainly on urban or cemetery contexts, with restricted baselines and fragmented datasets constraining broader applications (Shaw et al. 2016; Eckardt, Müldner, and Lewis 2014). Recent frameworks developed for the Lower Rhine Limes highlight both the feasibility and interpretive power of systematic isotopic sampling in these regions (Kootker, Van der Velde, and Heeren 2022; Kootker and Heeren 2022). Expanding such integrated approaches promises to illuminate how movement and interaction shaped the formation, diversity, and cohesion of Roman military communities in the borderlands.

In Valkenburg (South Holland, the Netherlands) the Roman military base *Praetorium Agrippinae* was excavated 45 years ago and has since remained an archaeological enigma. As one of the most north-western military settlement along the European limes, its location supported a strategic hub function within the Roman military network. The site's wet preservation conditions have resulted in exceptional organic preservation, particularly of wooden, leather, but also metal artefacts. These finds provide insight into the composition of the military community, including artefacts associated with childhood, such as toys and infant feeding vessels (Smits 2006). The presence of women and children is further supported by osteoarchaeological evidence (Smits 2006; Lonnée and Maat 1998; Dierendonck, Hallewas, and Waugh 1993).

Valkenburg site has yielded one of the largest assemblages of both cremated ($n = 501$) and inhumed ($n = 124$) human remains from the Lower Rhine Limes, and the largest in the Roman Netherlands, representing multiple phases of occupation between the first and third centuries CE (Mols and Polak 2020; Polak and Kooistra 2013; Vos and Oosterbaan in prep.). These remains offer rare direct evidence of the people who inhabited and moved through a Roman military landscape that is otherwise known primarily from architectural and artefactual data. Despite variable preservation and contextual resolution, the assemblage constitutes a robust dataset for isotopic and genetic analyses of mobility, ancestry, and kinship in a military borderland setting.

The inhumations can be divided into two groups. The first group consists of neonates and infants (< 1 year, $n = 80$), that were buried in small coffins or baskets and that were occasionally accompanied by small grave goods (Smits 2006). The second group comprises adults (20–70 years, $n = 31$ in total, $n = 18$ males, $n = 4$ females, and $n = 9$ unknown, based on osteology), and children and adolescents (1–20 years, $n = 13$). They were generally buried without grave goods, aside from occasional pottery, and were sometimes buried in a manner indicative of limited standardisation in funerary practice (Smits 2006; Lonnée and Maat 1998). Such burial practices, including prone positions and the interment of multiple individuals within a single grave, are frequently documented in Roman cemeteries (Taylor 2008; Arcini 2009; Milella et al. 2015).

Even though most of the individuals were cremated, cremation was not a uniform practice. The cremation graves in Valkenburg exhibited great variation in funerary practice, mainly reflected in the archaeological record through differences in grave type (Table 1) (Smits 2006). Particularly, *bustum*-type graves ($n = 46$) were frequently recorded. In this burial practice, cremation of one or more individuals was performed directly above the grave, after which the burnt remains collapsed into the underlying pit (Noy 2000; Vos and Oosterbaan in prep.). Although this grave type is often associated with the Roman military and soldiers (Philips and Wilson 2021; Petculescu 2025), its exact origin is still debated (Ota 2007; Noy 2000). Together, the heterogeneity observed in both inhumation and cremation practices raises important questions regarding the composition, social structure, and cultural background of the Valkenburg community. These patterns point to variability in origin, affiliation, and lived experience that cannot be fully resolved through funerary evidence alone and calls for a study capable of accessing individual biographies.

By integrating isotopic (Sr-O-C), elemental, and genomic analyses (McColl et al. 2024), this study applies a bioarchaeological framework to explore how mobility and ancestry shaped the social composition of a Roman military community in the Lower Rhine borderlands. The combined approach allows for a direct examination of population diversity and movement within and beyond the Roman army, contributing new evidence to broader debates on identity, migration, and connectivity in the Roman world. This study illustrates biomolecular evidence for Roman military communities as biologically diverse and highly mobile populations, demonstrating how individual life histories were embedded within and actively (re)shaped the networks that defined the Empire’s borderlands.

Table 1
Variation of burial practises and number of graves found in Roman Valkenburg. Table adapted from Smits (Smits 2006) and supplemented based on Vos & Oosterbaan (Vos and Oosterbaan in prep.).

Typology Vos & Oosterbaan	Typology Waugh	Description Provincial-Roman grave typology	N
H	A	Inhumation	124
		<i>Subtotal inhumation graves</i>	<i>124</i>
F	B	<i>Bustum</i> / Flat graves of the <i>bustum</i> type	64
C	C	<i>Brandgruben</i> -graves	173
E	D	Urn graves with pyre debris	51
D	D*/F	Urn graves/organic urn graves	25
B	E	<i>Brandschüttungs</i> - graves	17
A	G	<i>Knochenester</i> (graves with a concentration of clean cremated remains)	35
G	J	Disturbed/robbed/unknown	96
I	L	Cleared pyre sites	40
		<i>Subtotal cremation graves</i>	<i>501</i>
		Total number of graves	625

Materials & Methods

In total, 33 individuals from inhumation graves and 43 individuals from cremation graves were sampled, resulting in 43 diaphyseal samples and 34 rib samples. The cremated bone samples were selected on the basis of preservation and the level of burning, ensuring that all of the samples were fully calcined. The inhumation sampling resulted in 64 enamel samples for strontium, oxygen and carbon isotope analysis, distributed over 21 first molars (M1), 24 second molars (M2), and 19 third molars (M3). The graves from neonates were excluded from this study.

Sample preparation

The full pre-treatment protocol for strontium isotope analysis and strontium concentration measurements on cremated human remains is outlined in De Coster & Kootker (De Coster and Kootker 2024) and briefly described here. The samples were first mechanically cleaned with a Proxxon diamond coated ball burr to remove the outer layer and any remaining sediments. A second step consists of transferring the samples to glass vials and leaching the samples with circa 1.0 mL (10:1 ratio) 1M acetic acid (CH₃COOH) ultrasonically for 3–10 minutes, followed by two Milli-Q™ rinses, and a 10-minute Milli-Q™ ultrasonic wash (Snoeck et al. 2015; Snoeck et al. 2018). This was repeated until the Milli-Q™ in the glass vials was clear or showed a white cloudy colour. Subsequently, the samples were left on the hotplate at 50°C to dry overnight, powdered and transferred into 1.5 mL capped glass vials for further analysis. Similarly, the full sampling protocol for sampling enamel is outlined in Kootker & De Coster (Kootker and De Coster 2024) and summarized here. First, the outer layer of the enamel was mechanically removed. Next, 1–10 mg of enamel powder was collected in 2 mL glass vials with screw caps for subsampling or pre-weighted, acid-cleaned Eppendorf® centrifuge tubes and weighed, ready for Sr isotope analysis. Approximately 0.3 mg was weighed into exetainer vials for the O-C isotope analysis.

Analytical details

Sr-O isotope composition

The Sr purification process was done in the clean laboratory facility at the Vrije Universiteit Amsterdam. Both cremation and inhumation samples were dissolved in 0.6 mL of 3M HNO₃ and centrifuged. Once dissolved, 0.5 mL was used for column chromatography, while the remaining 0.1 mL was kept for concentration measurements. The columns consisted of in-house made 2 mL pipette-tips columns filled with 80 µL Eichrom Sr resin (100–150 µm mesh) to extract the strontium (De Coster et al. 2024). The purified strontium samples were collected in HCl-HNO₃ cleaned 7 mL Savillex® Teflon vials with conical interior and one drop of 0.5% of H₃PO₄ was added, dried down and nitrated with 14M HNO₃. The blanks were spiked with an ⁸⁴Sr enriched in-house made solution.

The purified Sr samples were redissolved in 2 µL 10% HNO₃ and 1 µl was loaded onto single annealed rhenium filaments with 2 µL TaCl₅. A Thermo Scientific™ Triton Plus™ thermal ionisation mass spectrometer (TIMS) at the Vrije Universiteit Amsterdam was used to determine the isotope compositions

using a static routine. All Sr isotope ratios were corrected for mass fractionation to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$. During this study (2022–2024), the reproducibility of the TIMS was 0.710259 ± 0.000017 for repeated analysis of NIST® SRM® 987 ($n = 267$, 2s, loading size 200 ng). This is comparable to the long-term reproducibility (2017–2024): 0.710256 ± 0.000018 ($n = 696$, 2s). NIST® SRM®1400 and an in-house tooth standard were also continually analysed during this study and resulted in a reproducibility of respectively 0.713108 ± 0.000028 ($n = 20$, 2s) and 0.707864 ± 0.00008 ($n = 25$, 2s).

The column blanks ($n = 17$) contained an average of 28 pg Sr. This amount is considered negligible when compared to the average amount of strontium present in human enamel and cremated bone (Harvig et al. 2014; Kootker, Van der Velde, and Heeren 2022; Snoeck et al. 2015; Snoeck et al. 2018).

Approximately 0.3 mg of enamel sample was collected in exetainer vials and transferred to the stable isotope laboratory at the Vrije Universiteit Amsterdam for carbon-oxygen isotope analysis. There, the samples were flushed with pure helium (5.0). Phosphoric acid (H_3PO_4) was added for 24 hours at constant temperature of 45°C to dissolve the samples. A Thermo Finnigan GasBench II preparation device, interfaced with a Thermo Finnigan Delta+ mass spectrometer was used to analyse the isotopic composition. The measured isotopes were normalised to the Vienna Pee Dee Belemnite (VPDB) scale using an in-house carbonate reference material (VICS) calibrated against NBS19 and LSVEC certified reference materials.

[Sr], [Ca] and [Pb] measurements

Depending on the sample size, aliquots were taken from the remaining 0.1 ml 3M HNO_3 solution and diluted to a total dilution factor of 10,000 in acid-cleaned ICP vials. The strontium ([Sr]), calcium ([Ca]) and lead ([Pb]) concentrations were measured on a ThermoFisher iCAP TQ ICP-MS. Sr ($m/z = 88$) was measured no gas in the collision-reaction cell, while kinetic-energy-discrimination (KED) using helium (He) was applied for Ca ($m/z = 44$). Indium (In) was used as an internal standard to correct for sensitivity drift, and quantification was achieved via external calibration against calibrants gravimetrically prepared from certified single-element standards. Repeated digestions and measurements of the NIST® SRM® 1400 ($n = 12$) yielded recoveries of 100.1–103.2% for Ca and 97.1–98.3% for Sr, with intermediate precision (i.e., repeatability of individual analyses) better than 5% relative standard deviation (1s). All [Sr] from the cremated bone samples were normalised to 40% Ca to account for organic loss using the following equation:

$$[\text{Sr}]_{\text{normalised}} = \frac{[\text{Sr}]_{\text{measured}}}{[\text{Ca}]_{\text{measured}}} \times 40\%$$

DNA Analyses

A total of 30 Valkenburg individual whole-genome sequences (individual information and accession codes available in the Supplementary Table 1) were obtained from McColl *et al.* (McColl et al. 2024). Read alignment and ancient DNA authentication were also performed following the procedures described in McColl *et al.* (McColl et al. 2024). Single-nucleotide polymorphism (SNP) genotype calling was initially conducted using BCFTOOLS v. 1.9 mpileup and call (Danecek et al. 2011), applying a minimum mapping quality threshold of > 30 (parameters: -E -q 30 -Q 30).

Genome imputation

Genomic imputation was performed using the GLIMPSE2 v. 2.0 framework (Rubinacci et al. 2023). High-coverage modern genomes from the 1000 Genomes Project Phase 3 dataset (Siva 2008) were used as the imputation reference panel. Only genomes with coverage above 0.4 were selected for imputation (27 individuals). Following imputation, genotypes were filtered based on posterior genotype probabilities, where individuals were retained if the mean posterior probabilities were ≥ 0.95 (26 individuals). After imputation, variant coordinates were lifted to the hg19 human reference genome (GRCh37 (Auton et al. 2015)) using the liftover tool implemented in CrossMap v. 0.7 (Zhao et al. 2014).

Clustering analyses

PCA was performed by projecting the ancient individuals onto present-day populations using the SNP positions from the Affymetrix Human Origins (HO) array (Patterson et al. 2012). Genotype data were obtained from the Allen Ancient DNA Resource (AADR) v.54.1 (Mallick et al. 2024). A total of 2,207 present-day Eurasian individuals were extracted from this dataset. Imputed genotypes were merged on 519,224 HO SNP sites using the *mergeit* utility in EIGENSOFT v8.0 (Patterson, Price, and Reich 2006; Price et al. 2006). Principal components were estimated using the *smartpca* module of EIGENSOFT, with ancient individuals projected onto the variation of present-day populations (parameter: *Isqproject* = YES; *numoutvec* = 4).

Genealogical relationships

To investigate genealogical relationships through shared IBD fragments (Ringbauer et al. 2024) among contemporary individuals, genomic data for previously sequenced ancient individuals were retrieved from the AADR v.54.1 (Mallick et al. 2024). Additional individuals were obtained from recent publications (Scheib et al. 2024; Antonio et al. 2019). A total of 711 individuals from Europe dating within ± 1000 years of the mean date of the Valkenburg individuals (1800 BP) were selected, and the corresponding alignment files were downloaded from the European Nucleotide Archive (individual information and accession codes available in the Supplementary Table 1). Genotype calling and imputation for these individuals were performed following the procedures described above. For IBD analyses, we restricted the data to the 1240K SNP panel (Haak et al. 2015) as these positions are widely represented among previously published ancient genomes. Only 638 individuals with a $\geq 700,000$ SNPs covered on the 1240K, and mean genotype posterior probability > 0.95 were retained for downstream analyses. IBD estimation was conducted using anclBD v0.7 (Ringbauer et al. 2024).

Phenotype predictions

Pigmentation was inferred using the HlrisPlex-S panel for skin, hair, and eye colour (Chaitanya et al. 2018; Walsh et al. 2013). We applied the probabilistic framework implemented in Phenotypic Inference (Perretti et al. 2025), which estimates trait probabilities using genotype posterior probabilities, thereby improving prediction accuracy for low-coverage genomes. Alignment (BAM) files from 28 individuals (> 0.4X) were used as input to derive genotype likelihoods and obtain HlrisPlex-S probability estimates, performing 1,000 replicate inferences per individual. Trait probabilities for each replicate were calculated using the HlrisPlex-S web server (Chaitanya et al. 2018; Walsh et al. 2013). Final phenotype likelihoods were summarized as percentage support for each predicted trait category based on the HlrisPlex-S posterior distributions.

Results

Sr-O-C isotopes and Sr concentrations [Sr]

Strontium concentrations [Sr], strontium ($^{87}\text{Sr}/^{86}\text{Sr}$), oxygen ($\delta^{18}\text{O}_{\text{PDB}}$), and carbon ($\delta^{13}\text{C}_{\text{VPDB}}$) isotope ratios were measured in enamel from first (M1), second (M2), and third molars (M3), allowing both inter- and intra-individual comparisons. The descriptive statistics of the Sr-O-C isotope data of the inhumations are shown in Table 2 and depicted in Figs. 1 and 2.

The $^{87}\text{Sr}/^{86}\text{Sr}$ span a broad range across all molars (0.70856–0.71285). Means are similar between molar types (M1: 0.71004; M2: 0.70987; M3: 0.70978), with medians clustering around 0.7096–0.7099. When all molars are combined (n = 64), the mean $^{87}\text{Sr}/^{86}\text{Sr}$ is 0.70982, with ratios distributed on both sides of the established local range (Kootker et al. 2016). The $\delta^{18}\text{O}_{\text{PDB}}$ range from – 8.3 to – 3.8‰ across all molars. Means are comparable between molar types (M1: – 6.1‰; M2: – 6.2‰; M3: – 6.2‰), with medians between – 5.7 and – 6.2‰. The combined enamel dataset (n = 59) has a mean $\delta^{18}\text{O}_{\text{PDB}}$ of – 6.1‰, overlapping but also extending beyond the locally reported range (– 6.5 to – 4‰) (Kootker and Heeren 2022; Kootker, Van der Velde, and Heeren 2022). Modern dental enamel show intra-dental variation of approximately 0.0002 in $^{87}\text{Sr}/^{86}\text{Sr}$ (Plomp et al. 2020) and around 2‰ in $\delta^{18}\text{O}_{\text{PDB}}$ (Plomp et al. 2020; Kootker, Van der Velde, and Heeren 2022). Therefore, observed variation must exceed these thresholds to be considered indicative of mobility. In this study, 45% of individuals displayed intra-molar variation greater than 0.0002 in $^{87}\text{Sr}/^{86}\text{Sr}$ (CL002, CL003, CL005, CL006, CL007, CL023, CL036, CL038, CL041, CL043, CL045, CL046, CL048, CL049), and 58% showed variation greater than 2‰ in $\delta^{18}\text{O}_{\text{PDB}}$ (CL002, CL003, CL005, CL006, CL007, CL008, CL015, CL019, CL023, CL024, CL0035, CL036, CL038, CL039, CL041, CL046, CL048, CL049). Notably, 39% of individuals exceeded the mobility thresholds in both isotope systems (CL002, CL003, CL005, CL006, CL007, CL023, CL036, CL038, CL041, CL046, CL048, CL049). The $\delta^{13}\text{C}_{\text{VPDB}}$ show a total range from – 14.9 to – 10.5‰. Means increase slightly from M1 (– 13.0‰) to M3 (– 12.0‰), with corresponding medians of – 12.7, – 12.7, and – 11.8‰ respectively. Interquartile ranges vary between 0.75 and 1.7‰. When combined (n = 59), the $\delta^{13}\text{C}_{\text{VPDB}}$ fall largely within the expected range for C_3 -based terrestrial diets reported for the region (– 14 to – 11‰), though several samples approach the upper limit of this range. Strontium concentrations ([Sr]) vary substantially between individuals and molar types, ranging from 62 to 266 ppm. Mean concentrations increase from M1 (116 ppm) to M2 (135 ppm) and decrease slightly in M3 (126 ppm). The combined dataset (n = 34) has a mean [Sr] concentration of 125 ppm and an interquartile range of 62 ppm, indicating considerable variability in enamel strontium content.

Table 2
Descriptive analyses of the $^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{18}\text{O}_{\text{PDB}}$ and $\delta^{13}\text{C}_{\text{VPDB}}$ measurements of the inhumations of Valkenburg. Key: M1 – first molar; M2 – second molar; M3 third molar.

	$^{87}\text{Sr}/^{86}\text{Sr}$				$\delta^{18}\text{O}_{\text{PDB}}$ (‰)				$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)				[Sr] in ppm			
	M1	M2	M3	All	M1	M2	M3	All	M1	M2	M3	All	M1	M2	M3	All
Min	0.70884	0.70856	0.70869	0.70856	-8.3	-7.9	-7.8	-8.3	-14.9	-14.9	-13.0	-14.9	62	65	81	62
Max	0.71285	0.71198	0.71225	0.71285	-4.6	-3.8	-5.7	-3.8	-11.5	-10.5	-10.9	-10.5	205	266	176	266
Mean	0.71004	0.70987	0.70978	0.70982	-6.1	-6.2	-6.2	-6.1	-13.0	-12.8	-12.0	-12.7	116	135	126	125
Median	0.70987	0.70954	0.70972	0.70955	-5.7	-6.2	-6.2	-6.2	-12.7	-12.7	-11.8	-12.5	109	149	127	121
Q1	0.70907	0.70912	0.70908	0.70907	-6.7	-6.7	-6.5	-6.7	-13.7	-13.8	-12.5	-13.3	98	85	97	90
Q3	0.71073	0.71011	0.71003	0.71021	-5.3	-5.8	-5.7	-5.5	-12.4	-12.0	-11.7	-11.8	128	158	151	152
IQR	0.00166	0.00099	0.00095	0.00113	1.5	1.1	0.9	1.2	1.2	1.7	0.8	1.5	30	73	55	62
n	21	24	19	64	19	24	16	59	19	24	16	59	12	11	11	34
Local baselines (Kootker et al. 2016; Kootker, Van der Velde, and Heeren 2022)	0.7088–0.7092				-6.5 to -4				-14 to -11				NA			

Strontium isotope ratios were measured in calcined bone from diaphyseal and rib samples, alongside normalized strontium concentrations, which can give insights to possible intra-individual mobility. Although turnover rates vary between individuals and are dependent on many variables, generally diaphyseal samples reflect a longer period during life, compared to the ribs that roughly represent the last five years before death (De Coster et al. 2024).

The $^{87}\text{Sr}/^{86}\text{Sr}$ show a limited overall range across all cremated bone samples (0.70838–0.71121). Means are nearly identical between diaphyseal (0.70954) and rib samples (0.70951), with comparable medians (~0.7094). When combined ($n = 80$), the overall mean $^{87}\text{Sr}/^{86}\text{Sr}$ is 0.70953, with ratios spanning both sides of the locally established baseline range (Kootker et al. 2016). Normalized strontium concentrations range from 94 to 227 ppm across all samples. Means are similar between diaphyseal (165 ppm) and rib samples (164 ppm), with medians of 160 and 163 ppm, respectively. In the combined dataset ($n = 65$), the mean normalized strontium concentration is 165 ppm.

Table 3
Descriptive statistics of the $^{87}\text{Sr}/^{86}\text{Sr}$ and normalized [Sr] data of the cremations from Valkenburg Marktveld.

	$^{87}\text{Sr}/^{86}\text{Sr}$			Normalized [Sr] in ppm		
	Diaphysis	Rib	All	Diaphysis	Rib	All
Min	0.70838	0.70853	0.70838	117	94	94
Max	0.71080	0.71121	0.71121	227	227	227
Mean	0.70954	0.70951	0.70953	165	164	165
Median	0.70940	0.70939	0.70940	160	163	162
Q1	0.70918	0.70908	0.70915	145	136	144
Q3	0.70993	0.70971	0.70988	183	186	183
IQR	0.00075	0.00064	0.00073	38	50	39
n	43	34	77	35	30	65

Sample sizes vary due to preservation and analytical constraints. For inhumations, enamel analyses yielded $^{87}\text{Sr}/^{86}\text{Sr}$ for 64 molars (M1: $n = 21$; M2: $n = 24$; M3: $n = 19$), $\delta^{18}\text{O}_{\text{PDB}}$ and $\delta^{13}\text{C}_{\text{VPDB}}$ for 59 samples, and strontium concentrations for 34 samples. For cremations, $^{87}\text{Sr}/^{86}\text{Sr}$ were obtained from 80 calcined bone samples (46 diaphyseal, 34 rib), and normalized strontium concentrations from 65 samples (35 diaphyseal, 30 rib).

DNA traces of Valkenburg

The principal component analyses (PCA) performed on the DNA data in this study support a heterogeneity in genetic ancestries. While most Valkenburg individuals cluster with present-day Western European populations, notable outliers were present (Fig. 4A). A 19–28-year-old male (CL032) grouped closely with present-day Greek populations, while a 13–16-year-old male (CL043) clustered with contemporary Estonian individuals. Overall subtle regional differences were recorded. For instance, 16 Valkenburg individuals fell closest to modern populations from the British Isles, whereas seven clustered nearer to populations from France and Spain.

Sex ratio and lack of genealogical relationships

The molecular sex ratio of the individuals analysed was biased toward males (XY), comprising of 22 males and seven females. Our identical-by-descent (IBD) analyses (Ringbauer et al. 2024), which indirectly informs about genealogical affinities, revealed no detectable relationships up to the fifth degree among the Valkenburg individuals. No evidence of such relationships was found between these individuals and more than 600 previously published contemporaneous genomes from the period 1000 BCE to 1000 CE that were previously accessed across Europe (a full list of individuals used is provided in Supplementary Table 1).

Valkenburg phenotypes

We performed phenotypic reconstructions using the Hirplex-S for the 27 Valkenburg individuals, predicted hair, eye, and skin pigmentation (Chaitanya et al. 2018; Walsh et al. 2013). The adult male clustering within Mediterranean populations (CL032) showed higher probabilities for an intermediate skin tone, together with dark eyes and brown hair. In contrast, the adolescent male clustering within Baltic populations (CL043) was predicted to have pale skin, blue eyes, and blond hair. Individuals within the western European populations generally exhibited likely intermediate skin, brown eyes, and brown hair pigmentation, although additional phenotypes were present, including red hair and pale skin (Fig. 4C).

Discussion

The broad concept of how a military community functioned as a socially and economically fluid entity has relied on historical and material sources. To complement these historically and materially derived characterizations, the isotopic and genetic evidence from Roman Valkenburg offers a biomolecular viewpoint on community composition and mobility.

Along the Lower Rhine Limes, the Roman period is characterized by sustained interaction between incoming populations and regional communities (Verhagen, Joyce, and Groenhuijzen 2019). In their study, McColl et al. (McColl et al. 2024) identified a clear shift from predominantly local ancestry to a more diverse genetic profile during the early Roman period, particularly among individuals from Valkenburg. Similar patterns are reported at other *limes* sites, where ancestry components indicate movements from eastern and northern regions into late Roman settlements in Germania Superior (Blöcher et al. 2025). In contrast, Scheib et al. (Scheib et al. 2024) found that Roman colonization exerted only limited genetic influence on rural populations in Britain. Instead, higher mobility and genetic heterogeneity appear to be concentrated at key nodes of the Roman infrastructural network, especially military and urban centres

(Scheib et al. 2024; Olalde et al. 2023; Antonio et al. 2019), a pattern consistent with the biomolecular evidence from Valkenburg presented in this study that shows pronounced mobility and population heterogeneity within a military-linked settlement.

The Sr-O-C isotopic data from the Valkenburg inhumations display an exceptionally wide range of ratios compared to the relatively narrow isotopic ranges observed in the Netherlands (Kootker et al. 2016; Kootker, Van der Velde, and Heeren 2022; Kootker and Heeren 2022) and surrounding regions (Evans et al. 2023; Willmes et al. 2018; Sengeløv et al. 2025). The observed variability of individuals also demonstrates mobility within an individual's lifetime. While minor isotopic variation can arise from physiological or methodological factors, the extent of variation observed in almost half of individuals is more consistent with changes in geographic residence during enamel formation. Particularly notable is the isotopic variation in both strontium and oxygen isotope systems, with nearly two-fifths of individuals exceeding mobility thresholds in both proxies. This covariation strengthens the interpretation of genuine mobility, as these isotopes reflect different aspects of local environmental signatures, namely the local hydrological cycle and food intake. In contrast, the carbon isotopes show a comparatively restricted distribution that is consistent with broadly similar terrestrial dietary practices across the population. Although some individuals have C isotope ratios towards the upper end of the expected regional range (Kootker, Van der Velde, and Heeren 2022), overall variability remains limited. This suggests that dietary differences were relatively minor and unlikely to be the primary driver of the pronounced isotopic variability observed in the strontium and oxygen systems. This pattern is consistent with a broadly homogeneous diet dominated by C₃ plant resources, such as cereals and vegetables, in which variations in the relative contribution of animal protein would have had only a limited impact on $\delta^{13}\text{C}$ values. The combination of stable carbon isotope signatures with highly variable strontium and oxygen ratios therefore supports the interpretation that geographic mobility, rather than dietary heterogeneity, underpins the observed isotopic diversity within the Valkenburg population. Although the individual isotope ratios observed among the Valkenburg individuals are compatible with a wide range of European regions, the combined Sr-O isotope data allows assessments of potential regions of origin.

One source region frequently suggested in both historical and archaeological interpretations of Valkenburg is the British Isles, owing to the site's geographical proximity and historical accounts of Caligula's early attempt to conquer the islands (Vos et al. 2021; Hoffmann 2013; Wynia 1999; Peddie 2005; Manley 2007). While our demographic results indicate affinities with present-day British populations, this genetic link may have been established well before the Roman Period (Olalde et al. 2023; Cassidy et al. 2025). Previous DNA studies have shown that continental influx of Bell Beaker-related ancestry into the British Isles dates at least from the Copper-Age (Olalde et al. 2023). McColl et al. (McColl et al. 2024) further demonstrate that the major Valkenburg ancestral component likely derives from a North Sea Bell Beaker origin, which offers a plausible explanation for more distal affinities between the Valkenburg individuals and the modern United Kingdom. However, the combined $^{87}\text{Sr}/^{86}\text{Sr}$ and $\delta^{18}\text{O}_{\text{PDB}}$ isotopic data effectively rule out the British Isles as the childhood origin for most of the inhumed individuals. For most individuals, the $\delta^{18}\text{O}_{\text{PDB}}$ values are lower than those typically observed and reported in the United Kingdom, suggesting childhood residence in regions less influenced by a maritime climate. Only six individuals (CL005, CL007, CL008, CL019, CL026, CL035) exhibit Sr-O isotope ratios that are compatible with ranges reported for the United Kingdom (Evans et al. 2023).

Beyond these broader North Sea-related patterns, the genetic data also reveals two outliers. Males CL032 and CL043 clustered close to modern-day Greek and Estonian populations respectively. Ancestry estimates from McColl et al. (McColl et al. 2024) similarly indicated that these two individuals were outliers relative to the major North Sea ancestral component, consistent with our PCA results. CL032 did not have any enamel suitable for isotopic analysis, but isotope analysis was possible for adolescent male CL043. The Sr-O isotope data, however, do not show explicit evidence for mobility during his first 8 years of life. Genetic ancestry does not equate to geographic origin; however, when combined with isotopic evidence, it provides an independent line of evidence for addressing questions of provenance, particularly in the case of such a pronounced outlier. The strontium isotope ratios measured in CL043 (0.71006 and 0.71000 for the first and second molars respectively) are not found in the immediate region of Valkenburg, and the $\delta^{18}\text{O}_{\text{PDB}}$ data are too low for the Netherlands (Kootker, Van der Velde, and Heeren 2022; Kootker and Heeren 2022) and point toward a childhood spent within a large continental landmass, such as Estonia (-7.8‰ and -7.9‰ respectively). Interestingly, the age-at-death was determined to be between 13–16 years old (Lonnée and Maat 1998), which means that the boy moved to Valkenburg between 8 and 16 years old. Based on a recently published strontium isotope baseline map for Estonia (Suni et al. 2025), two possible regions of origins, i.e., coastal and central Estonia (0.7094–0.7147) can be identified. Considering the low $\delta^{18}\text{O}_{\text{PDB}}$ as well, central Estonia would be the most likely region. Taken together, the genetic and isotopic evidence support the plausibility of a childhood in the Estonian region. Assessing whether large-distance mobility is specific to inhumed individuals or characteristic of the total cemetery community requires comparison with the isotopic evidence from cremated remains.

The comparison between inhumation and cremation isotope data is not straightforward, as the two tissues record different life stages: childhood in enamel versus later life in calcined bone (see (De Coster et al. 2024) and references therein). In addition, the contrast between cremated bone, which reflects isotopic signals integrated over periods of skeletal turnover prior to death, and dental enamel from inhumations, which records incremental mineralisation during childhood, result in fundamentally different averaging or mixing intervals. However, with consideration of these crucial differences between the two data sets, important insights are also gained when studying the $^{87}\text{Sr}/^{86}\text{Sr}$ of both the inhumations and cremations at Valkenburg. Mirroring the inhumation data, the cremations exhibit significant variability and includes isotopic ratios that are found across Europe. This indicates that the difference in the social groups using inhumation rather than cremation, cannot be the sole explanation, since both groups appear to consist of mobile individuals. Rather than reflecting fixed distinctions in origin, the coexistence of inhumation and cremation at Valkenburg may point to a funerary landscape shaped by choice, circumstance, and mobility.

From the isotopic perspective presented in this study, the distinction between cremation and inhumation has often been treated as a primary structuring principle within burial analysis, reflecting the fact that cremation constituted the dominant funerary norm in much of the Roman world, including the Netherlands and its surrounding regions (Capuzzo et al. 2020). Against this background, inhumation burials in Valkenburg has frequently been approached as an exceptional practice and interpreted as potentially socially or culturally 'different'. However, this emphasis contrasts with the way other forms of variability within burial practices are generally approached.

Considerable variation exists within both cremation and inhumation burials, including differences in grave form, body position, demography, spatial distribution, chronology, and the presence or absence of grave goods. These forms of variability are rarely assigned the same categorical weight as the cremation–inhumation distinction, but are instead understood as reflecting a range of choices made in the treatment of the deceased. From this perspective, the distinction between cremation and inhumation may not be fundamentally different from other observed variations, such as prone versus supine burial or bustum versus urn cremation.

All of these practices represent decisions made by the community for a deceased individual, the motivations of which, whether social, cultural, practical, or symbolic, are not always archaeologically accessible. While in certain Roman contexts deviations from the cremation norm may indeed carry interpretative weight (Kootker and Heeren 2022), this may not be necessarily the case in military settings like Valkenburg. In the context of a Roman castellum, cremation and inhumation may have functioned as parallel and socially accepted funerary practices rather than as markers of cultural or social distinction.

The explicit separation of cremations and inhumations into distinct analytical groups may therefore overstate the significance of this single aspect of funerary practice, particularly in the absence of clear isotopic, demographic, or contextual evidence supporting such a division, as is the case here. The results presented in this paper suggest that greater caution is warranted in treating inhumation as inherently ‘other’, and that Roman (military) cemeteries may require an analytical approach that places less interpretive weight on funerary rite alone.

An alternative interpretation is that the inhumed individuals represent a structurally lower socio-economic segment of the Valkenburg community (Lonnée and Maat 1998). The outdated idea of non-local inhumation versus local cremation has been disproven both in this study and in other work (Osterholtz 2025; Barberan et al. 2007; Nugent 2017). Other differences could point toward lower status for the inhumations. Almost all skeletons show indicators of heavy physical labour (Lonnée and Maat 1998). Taking into account that these indicators would be less visible in cremations, it remains unsure that this was a specific attribute of the inhumations. Most were buried without grave goods (Smits 2006; Lonnée and Maat 1998). Importantly, the inverse interpretation (‘inhumation as an indicator of elevated social status’) is not supported at Valkenburg, as no richly furnished inhumation graves have been identified at the site. This study also found no evidence of genealogical relationships indicative of kinship among the inhumed individuals, nor between these individuals and more than 600 others sampled across Europe, supporting the interpretation of a burial scenario lacking interpersonal genetic ties. Although it cannot be established with certainty that such factors directly influenced the community’s decision-making, cremation is generally more time-consuming and sometimes more costly than inhumation (McKinley 2006), which may have contributed to the choice of burial for these individuals. The radiocarbon dates (McColl et al. 2024) indicate that they died across different periods and therefore do not represent a single contemporaneous episode or exceptional event, nor is it indicative of a family’s specific preferences since no kinship was found. Instead, their treatment appears to reflect a recurring mortuary practice directed toward a particular segment of the population. When considered together with their demographic profile, minimal grave goods, signs of physical strain, and the cost differential between cremation and inhumation, it is also plausible that these inhumed individuals occupied a lower-status position within the broader Valkenburg community.

These considerations have important implications for how the biomolecular evidence from Valkenburg should be interpreted. Because the majority of the population was cremated, only a small subset of individuals, namely those who received inhumation, preserved DNA and combined Sr-O-C isotope analyses. Consequently, the genomic results and much of the multi-isotope enamel data may reflect either the life histories of individuals occupying a lower socio-economic position within the community, or a subset of the population differentiated by funerary practice rather than by social or biological distinction alone. Moreover, it should be noted that the available cremation dataset itself represents only a limited subset of the total number of cremation graves, further constraining the representativeness of the biomolecular sample. In contrast, the cremated majority can contribute only strontium isotope ratios, which provide information on childhood mobility but not on ancestry or dietary patterns. The strong variability in strontium signatures observed in both groups nonetheless demonstrate that long distance mobility was a widespread phenomenon within the Valkenburg community. However, the finer-grained genetic and isotopic evidence relating to ancestry, diet, and life-stage mobility reflect the experiences of individuals whose mortuary treatment already sets them apart archaeologically. When interpreting the combined biomolecular dataset, it is therefore important to recognize that the inhumed individuals might not constitute a representative cross-section of the Valkenburg population. Rather, they provide insight into one particular segment of a broader and more heterogeneous borderland population. At the same time, they do prove that some level of long-distance connectivity existed and shaped the community at Valkenburg, indicating that individuals with diverse geographic origins were incorporated into the settlement across multiple generations.

Combined, the biomolecular results provide direct evidence for the demographic diversity and mobility that scholars have increasingly recognized in Roman borderland communities (McColl et al. 2024; Blöcher et al. 2025; Greene 2019). Rather than a closed military enclave, Valkenburg appears to have been a place where individuals of different geographic origins, cultural practices and social identities lived in proximity and participated in a shared borderland environment. This picture aligns with recent archaeological work emphasizing the socially mixed character of military settlements, where soldiers, families, labourers, and other non-military individuals formed interdependent communities rather than rigidly separated groups (Allison 2013; Walas 2015; Greene 2019; James 1999; Haynes 1999a). The Valkenburg assemblage offers a tangible biomolecular example of the heterogeneity long suggested in archaeological and historical studies (Haynes 2013; Haynes 1999b), underscoring how borderland populations were fundamentally shaped by movement, interaction, and continual demographic renewal.

Conclusion

The biomolecular evidence from Valkenburg demonstrates that this Roman borderland settlement became a demographic melting pot, shaped by sustained mobility and population heterogeneity. Integrated isotopic and genetic analyses reveal that individuals of diverse geographic origins were incorporated into a shared community along the Lower Rhine Limes, consistent with emerging views of Roman military settlements as socially entangled rather than homogeneous entities.

Strontium and oxygen isotope data indicate that mobility were widespread and occurred across different life stages. Comparable levels of strontium isotope variability in both cremated and inhumed individuals suggest that mobility was not restricted to a particular burial rite. Instead, the coexistence of cremation and inhumation reflects a broader spectrum of funerary choices rather than fixed distinctions in geographic origin. Treating burial rite as a primary analytical division risks overstating its interpretive significance, particularly when other forms of mortuary variability are not assigned equivalent weight. At the same time, the inhumed individuals likely represent a specific segment within this melting pot rather than a separate population. Indicators of heavy physical labour, minimal grave goods, lack of kinship ties, and the relative cost of burial practices point toward social differentiation within the community. While preservation biases limit the representativeness of the biomolecular sample, the strong isotopic variability observed across burial rites nonetheless demonstrates that mobility was a defining feature of the Valkenburg population.

Overall, Valkenburg emerges as a borderland community fundamentally shaped by movement, interaction, and continual demographic renewal, providing a biomolecular case study of the diverse and dynamic character of life in the borderlands of the Roman Empire.

Declarations

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CrEiT authorship contribution statement

MDC - Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft. **FF** - Formal analysis, Writing – original draft. **WV** – Resource, Writing – editing and reviewing. **JO** - Writing – editing and reviewing. **GRD** - Writing – editing and reviewing. **LK** - Conceptualization, Methodology, Data curation, Funding acquisition, Project administration Writing – editing and reviewing.

Data availability

All isotope ratio and elemental concentration data generated and analysed during this study are available in the IsoArch database.

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Figures

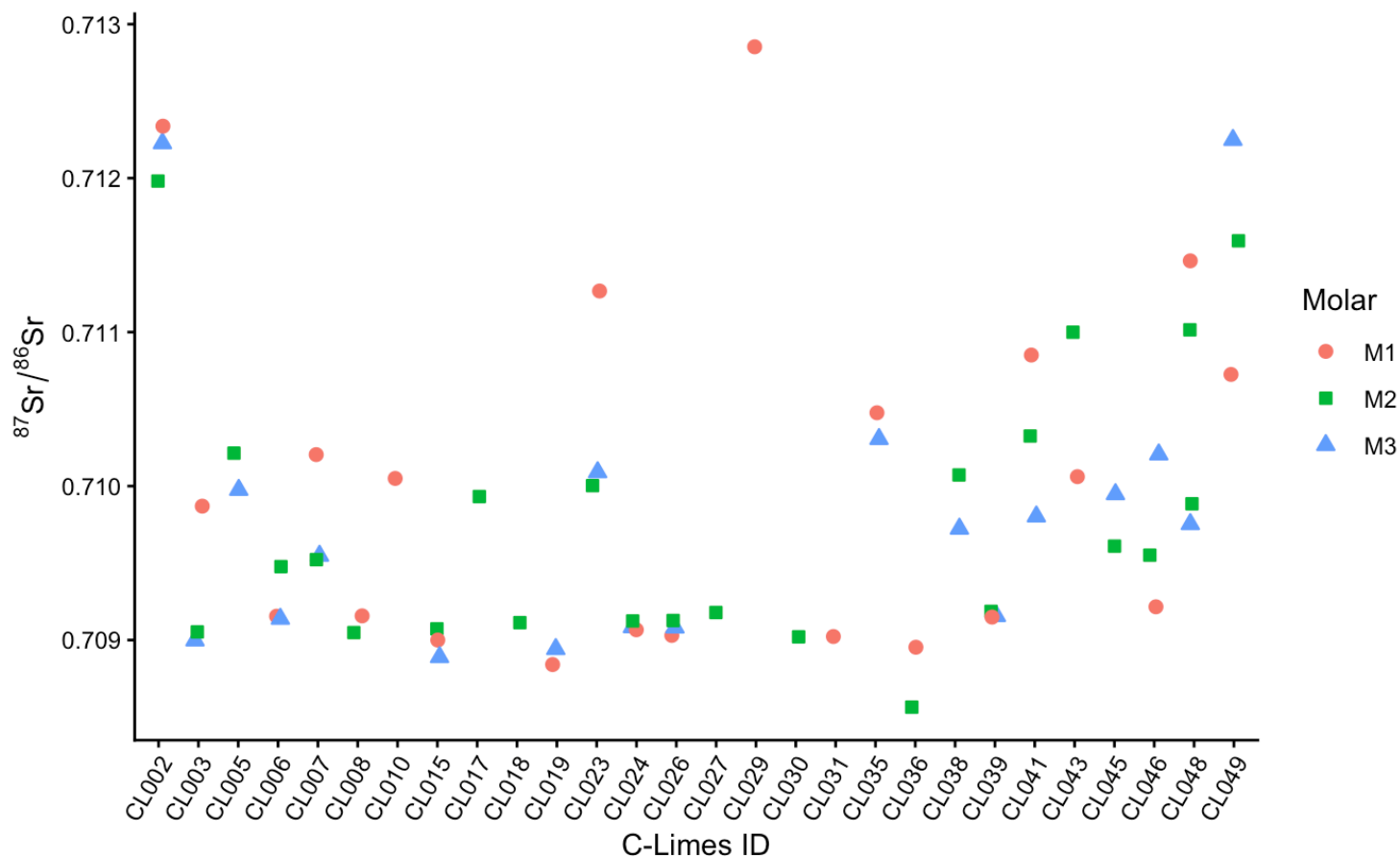


Figure 1

$^{87}\text{Sr}/^{86}\text{Sr}$ of dental enamel samples plotted by individual. Multiple symbols represent different molars (M1-M3) from the same individual.

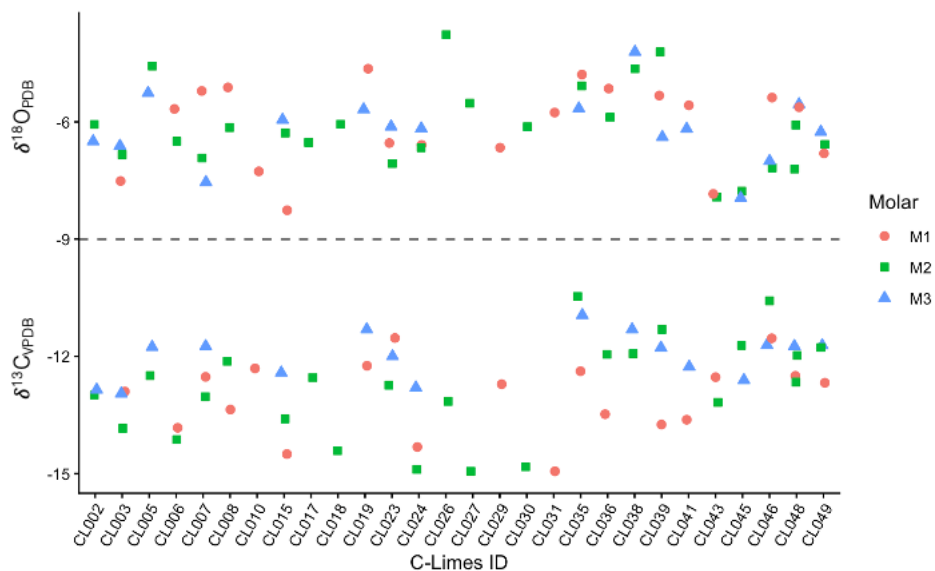


Figure 2

$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ measured in dental enamel, plotted per individual. Colours represent different molars (M1-M3).

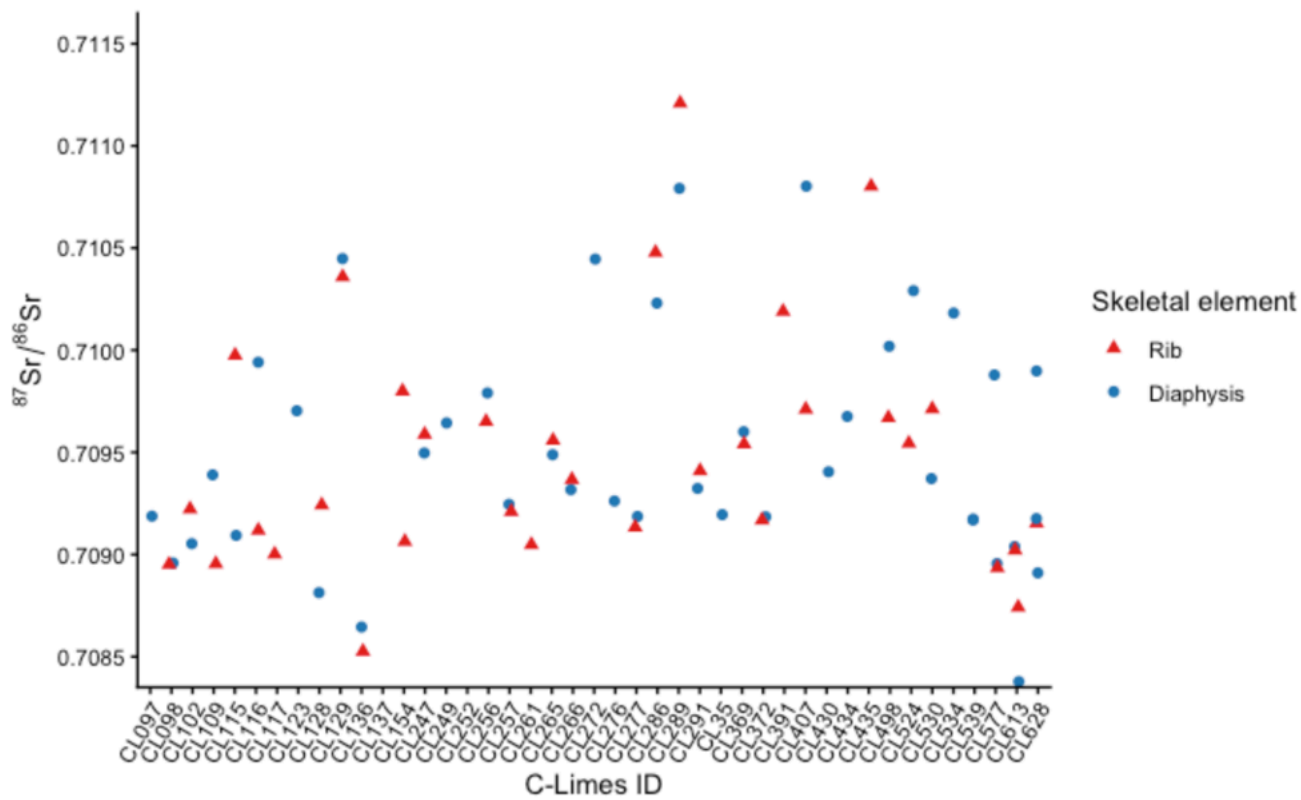


Figure 3

$^{87}\text{Sr}/^{86}\text{Sr}$ of cremated remains plotted per individual.

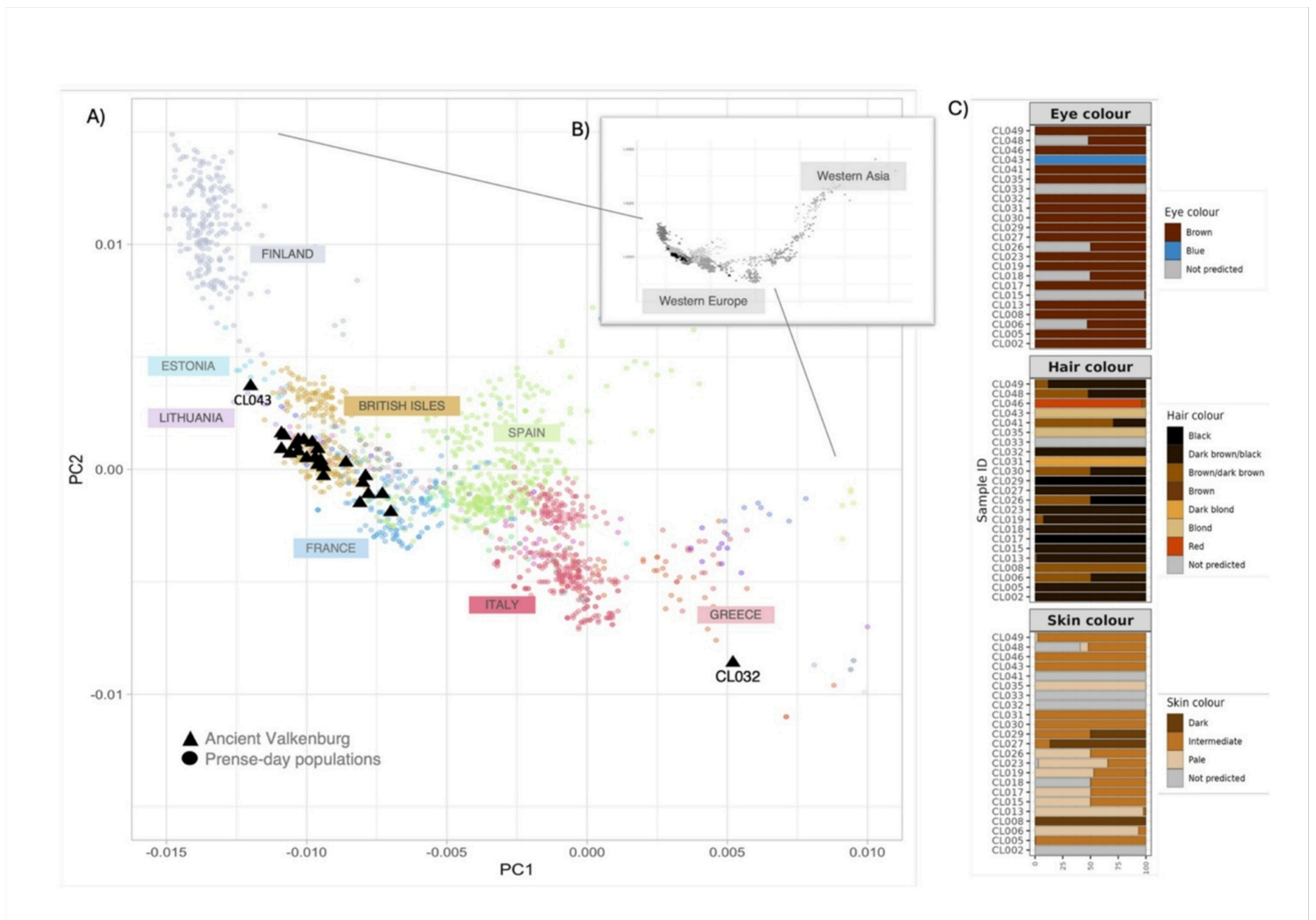


Figure 4

DNA traces of Valkenburg. **(a)** Principal component analysis (PCA) results projecting Roman Valkenburg individuals (black triangles) against present-day Eurasian populations (coloured circles). Circles coloured by country of origin. **(b)** PCA including Roman Valkenburg individuals given the Western Eurasia present-day genetic diversity. **(c)** Pigmentation predictions for hair, eye, and skin using the Hirisplex-S. Horizontal bars denote individuals analysed. Colours represent the percentage of the likely pigmentation given posterior probabilities.

Supplementary Files

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