



## Ancient Roman mitochondrial genomes and isotopes reveal relationships and geographic origins at the local and pan-Mediterranean scales<sup>☆</sup>



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### ARTICLE INFO

#### Keywords:

Roman Italy  
Ancient DNA  
mtDNA diversity  
Isotope analysis  
Bioarchaeology  
Iron age  
Maternal ancestry

### ABSTRACT

Rome initiated several campaigns to expand, conquer, and enslave local Italic populations following the establishment of the republic in 504 BCE. However, the cultural and biological changes resulting from Roman subjugation across Italy remain a topic of intense historical debate. Although important, historic and archaeological lines of evidence fail to track the impact of forced enslavement and enculturation at individual and broader genetic scales and, more generally, offer fewer clues regarding the potential affinities of Roman period Italians to European, Near Eastern, western Asian and North African populations at this time.

In this paper, we present the whole mitochondrial (mtDNA) genomes of 30 Roman period (1st–4th centuries CE) individuals buried in the Vagnari *necropolis* in southern Italy. We integrate the mtDNA data with previously published bioarchaeological and isotope ( $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ ) data for the Vagnari assemblage and compare Roman haplogroup composition to 15 newly sequenced mitochondrial genomes obtained from a pre-Roman Iron Age skeletal assemblage, located in close proximity to Vagnari. Additionally, we contrast our South Italian dataset with a further 332 complete ancient mtDNA genomes from the pan-Mediterranean region, Europe, western Asia and North African regions.

Population pairwise  $\Phi_{\text{ST}}$  values suggest that Roman Italians share closer genetic similarity to Neolithic, Bronze Age, and Armenian Iron Age populations from western and central Europe than with Iron Age Italians, Ptolemaic, and Roman period Egyptians. Vagnari individuals with  $\delta^{18}\text{O}$ ,  $^{87}\text{Sr}/^{86}\text{Sr}$ , and mtDNA data suggest a predominantly local demographic was employed at the site. However, two individuals belong to eastern Eurasian haplogroup D4b1c, indicating that the maternal ancestors of these two individuals migrated to South Italy prior to the 1st century CE. Additionally, we provide the first genetic evidence for possible maternal relatedness in a Roman period skeletal assemblage. Our research highlights the significance of integrating multiple lines of bioarchaeological data to inform interpretations about Roman colonial expansion and its impact on population structure.

### 1. Introduction

The application of ancient DNA (aDNA) and stable isotope analysis has greatly improved our understanding of population-wide demographic changes and disease in past populations (Bos et al., 2016; Duggan et al., 2016; Haak et al., 2015; Schuenemann et al., 2017; Skoglund et al., 2012; Wagner et al., 2014; Wong et al., 2017). A

growing interest in the paleodemography of ancient Mediterranean populations has prompted the biomolecular analysis of skeletal assemblages from this historically complex region (e.g., Bouwman et al., 2008; Chilvers et al., 2008; Lacan et al., 2011; Lazaridis et al., 2017; Olalde et al., 2015). Despite increased interest and a growing number of publicly available aDNA datasets, investigations detailing the genetic makeup of southern Italian pre-Roman and Roman period populations

<sup>☆</sup> Iron Age and Roman mitochondrial consensus sequences were uploaded to GenBank under accession numbers: MG773610–MG773654.

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are absent. Detailed genetic and isotopic analyses of classical Italian remains stand to elucidate important demographic questions inaccessible through artifactual, textual, and epigraphic evidence alone. Traditional approaches to studying the effects of Roman expansion and colonialism relied primarily on literary sources and archaeological evidence (Attema and van Leusen, 2004; de Ligt and Northwood, 2008; Salmon, 1955; Stek and Pelgrom, 2014; Whittaker, 1994). The historical sources focused on their audience of literate, privileged, male, and dominant patriarchs of Rome while marginalizing other members of Roman society, such as women, children, and lower class individuals living in rural areas (Bradley, 1994; Crawford, 2001; Paoli, 1973; Williams, 1997). More recent investigations have concentrated on integrating several lines of aDNA, historic, bioarchaeological, and isotopic data from a broad spectrum of Roman society to inform their interpretations about past mobility and the prevalence of disease (e.g., Killgrove and Montgomery, 2016; Marciniak et al., 2016; Prowse, 2016; Prowse et al., 2010). Although research has focused on how Roman subjugation of the indigenous Italic and Greek colonial populations impacted the demographic composition of Roman Italy, little is known about the biological composition of populations in southern Italy as a result of these military and political conquests. This paper investigates how pre-Roman and Roman genetic diversity changed over time, and the genetic relationships Roman period South Italians had with contemporaneous populations across Europe, western Asia, the Near East, and North Africa, before and during the classical period.

Our investigation combines previously analyzed  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope data (i.e., Emery et al., 2018; Prowse, 2016) and bioarchaeological data ( $n = 43$ ), with whole mitochondrial DNA (mtDNA) sequences obtained from Iron Age Botromagno (7th–4th centuries BCE) ( $n = 15$ ) and Roman period Vagnari (1st–4th centuries CE) ( $n = 30$ ). We compare southern Italian Iron Age and Roman mtDNA haplogroup compositions to ancient mtDNA sequences ( $n = 332$ ) spanning the Upper Paleolithic through the Roman periods and document two cases of possible maternal relations in the Roman *necropolis*. We analyze the population history of pre-Roman and Roman period southern Italy within the context of social and political upheaval during the Roman period at the local and broader Mediterranean scales.

## 2. Iron Age Botromagno and Roman Period Vagnari

### 2.1. Botromagno (7th–4th century BCE)

Following the establishment of the Roman republic in 504 BCE, Rome conquered and expanded its territory into central and southern Italy. Roman armies conquered and acquired territories in central Italy starting in the 5th century BCE, defeating the Samnites over the span of three major wars (343–290 BCE), and expanded to areas of southern Italy by the 3rd century BCE (Cornell, 1995). The Iron Age site at Botromagno is a hilltop settlement located West of the modern town of Gravina in Puglia (Fig. 1). The settlement was home to a prosperous *Peucetian* community (7th–4th century BCE) at a time of intense Greek colonization of southern Italy (Small, 1992).

Historic records indicate that Botromagno (recognized as Roman *Silvium*) was secured by a Roman consular army in 306 BCE (Small, 2002). Southern Italy's Italic and Greek communities were subjected to repeated conflicts between Greece and Rome (Pyrrhic Wars), and Rome and Carthage (Punic Wars). These conflicts coincided with an apparent depopulation of the southern Italian Iron Age sites between the 4th and 3rd centuries BCE, and by the 2nd century BCE with the establishment of Roman colonies (*coloniae*) (Small, 1992).

Early archaeological excavations at Botromagno were conducted on behalf of the British School at Rome between 1965 and 1974 by Joan Taylor (Brooks et al., 1966; Small, 1992; Taylor et al., 1976; Ward-Perkins et al., 1969). Excavations uncovered a substantial number of groticella tombs, associated artifacts, and a modest settlement composed of small house-dwellings, courtyards, and two *necropoli* at the

base of the hill, known locally as Padreterno and Parco San Stefano (Small, 1992). No public spaces or centralized governing quarters were identified, suggesting that the site was likely a dependent rural village (*vicus* or *pagus*) (Small, 2002). Burials were interred with grave goods characteristic of native Apulian pottery traditions with shifting burial customs ranging from Iron Age Italic to Hellenistic in nature. Individuals were interred according to both local and Greek customs, with tomb structures during the 6th century BCE taking the form of pit extensions and sarcophagi. However, the flexed burial positions at Botromagno are consistent with Italic traditions of the Early to Middle southern Italian Iron Age, remaining unchanged until larger, increasingly elaborate *semicamara* tombs were constructed in the 4th century BCE (Small, 1992; Peruzzi, 2016).

### 2.2. Vagnari (1st–4th century CE)

Vagnari is a 1st–4th century CE Roman period site located 14 km northwest of Gravina in Puglia (Fig. 1). The cemetery is located adjacent to a small rural village (*vicus*) and early Roman villa (1st c. BCE–1st c. CE) (San Felice), located on a hill to the South of the cemetery (Small et al., 2000). Excavations at the Roman *necropolis* began in 2002 when subsurface structures were identified as tombs. Since then, over 140 burials have been recovered, many containing a number of grave goods interred with the deceased. Five tomb ‘types’ were identified at Vagnari: “*alla cappuccina*”, cremation, funnel burials for libation offerings, soil burials, and disturbed graves (Brent and Prowse, 2014; Small and Small, 2007). A select number of grave goods recovered to date include fragmentary and intact ceramic pots, oil lamps, coins, bronze vessels, hobnails, glass vessels, bracelets and necklaces, and various iron and bronze items, such as pruning hooks and spearheads (Brent and Prowse, 2014). Current evidence points to a community engaged in agricultural and industrial production likely driven by slaves, lower-class workers, and/or freedmen who lived onsite at the adjacent *vicus*.

Preliminary mtDNA data generated by Prowse et al. (2010) targeting the HVR-1 (hypervariable region) region of the mitochondrial genome, assessed the partial mitochondrial profiles of 10 Vagnari individuals, and identified haplogroups typical of Eurasian populations (haplogroups H, J, K, and T). Two individuals belonged to haplogroups characteristic of African (L) and eastern Eurasia (D) maternal ancestry.

### 2.3. $\delta^{18}\text{O}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ analysis of the Vagnari assemblage

By integrating  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data that are known to vary geographically, it may be possible to determine the geographic region of origin (or long-term residency before death) of human remains by matching tooth  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values with the local  $\delta^{18}\text{O}$  (rainwater) and  $^{87}\text{Sr}/^{86}\text{Sr}$  (geological substrate) baseline variation (Bentley, 2006; Bowen, 2010). As a result, isotopic analysis of archaeological remains has provided a substantial amount of information regarding migration and mobility in the past (e.g., Barberena et al., 2017; Gregoricka and Sheridan, 2017; Marsteller et al., 2017; Pellegrini et al., 2016; Perry et al., 2017; Peschel et al., 2017; Wilhelmson and Price, 2017), as the  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values in teeth reflect the isotopic composition of food and water consumed during childhood (Cheney et al., 2012; Schweissing and Grupe, 2003).

$^{87}\text{Sr}/^{86}\text{Sr}$  values for the Italian peninsula generally increase from southern Italy (< 0.707) to the Alps (> 0.710) along a North-South axis (Emery et al., 2018). Higher  $^{87}\text{Sr}/^{86}\text{Sr}$  values from Italy's northern Alpine region reflect the late Mesozoic and Cenozoic-era granitic and metamorphic rocks that dominate the northern region. Conversely, South Italy is constrained by limestone carbonates and young igneous rock, which are reflected in lower  $^{87}\text{Sr}/^{86}\text{Sr}$  values. Prior  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis of faunal bone and sediment recovered from the region surrounding Vagnari indicate low  $^{87}\text{Sr}/^{86}\text{Sr}$  variability for the site, ranging between 0.708 and 0.709 (Emery et al., 2018; Lugli et al., 2017).

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