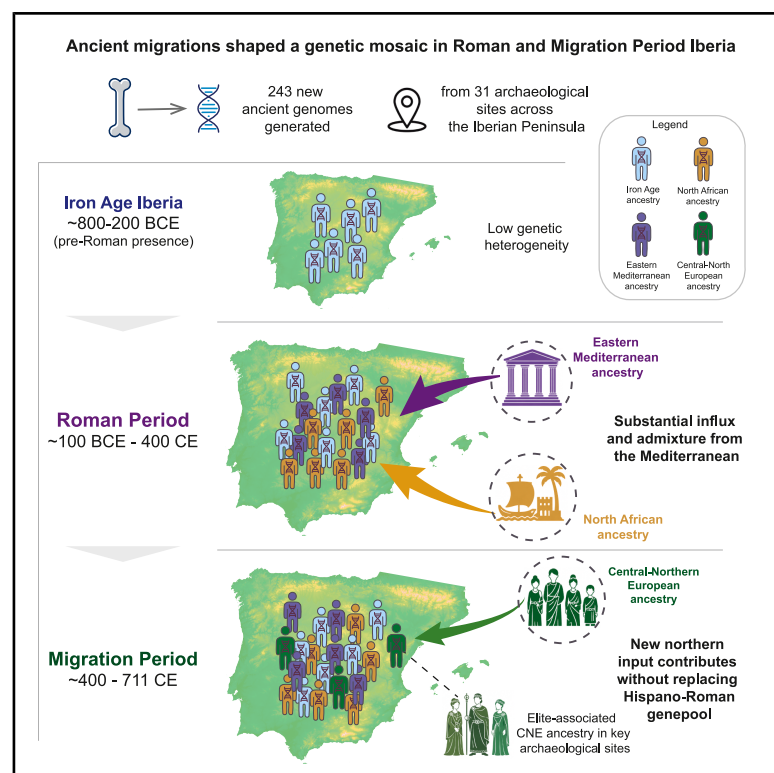


Contrasting demographic impacts of Roman colonization and early medieval migrations in the Iberian Peninsula

Graphical abstract



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In brief

Roman and post-Roman Iberia was transformed by extensive mobility across the Mediterranean, with large-scale inputs from Eastern Mediterranean and North African populations reshaping its genetic landscape. During the migration period, Central-Northern European ancestry arrived largely through elite-associated groups that intermarried with local populations but did not replace the highly diverse population that had formed under Roman rule.

Highlights

- We generated 245 ancient genomes revealing Roman and early medieval Iberian diversity
- Roman rule transformed Iberia into a genetically diverse population mosaic
- Eastern Mediterranean and North African ancestry spread widely in Iberia
- Migration period elites contributed ancestry without major population replacement

Article

Contrasting demographic impacts of Roman colonization and early medieval migrations in the Iberian Peninsula

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SUMMARY

It has been unclear how Roman and later Germanic political control shaped the demography of Iberia and how it differed from other parts of Europe. We report genetic data from 243 ancient individuals dating to 100–800 CE, analyzed alongside 58 previously published individuals. In Roman times, we document an influx of people with ancestry from the Eastern Mediterranean and an influx of North Africans, especially in the center and south. Beginning in the 5th century, Germanic and Alan (Sarmatian) groups assumed political control. Although we identify individuals with Central-Northern European-associated ancestry, including siblings buried ~725 km apart, the Hispano-Roman population remained continuous in Iberia. These demographic patterns contrast sharply with those in Britain, where major demographic change followed Rome's collapse, and with those in the central Mediterranean, where both periods were associated with profound transformations. Together, these findings highlight regional variation in admixture and continuity across post-Roman Europe.

INTRODUCTION

Rome became a Mediterranean power by the 3rd century BCE. At its peak, it controlled the entire Mediterranean coastline and

significant territories on three continents.¹ One of Rome's earliest major conquests was the Iberian Peninsula. Because of its unique geographic position next to Africa, on the main connection between the Atlantic and Mediterranean, and its

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rich natural resources, the peninsula held long-standing strategic importance. It attracted earlier civilizations, including the Phoenicians, Greeks, and Carthaginians, who had established trading colonies on its eastern shores.²

The Romans arrived in Iberia in the late 3rd century BCE during the Second Punic War, while the last independent tribes in the northwest submitted to Roman rule during Augustus's Cantabrian Wars.³ The Romans heavily exploited the peninsula's resources, including especially metal and minerals, as well as agricultural products and manpower.¹ Roman control weakened during the migration period of the 5th century CE,^{4,5} with Alans (Sarmatians), Vandals, and Suebi arriving by 409 CE. The Suebi established a kingdom in 411 CE in the northwest, which lasted until 585 CE,^{4,5} while the Romans recovered some control over the peninsula in 429 CE after the Vandals and Alans crossed the Strait of Gibraltar into North Africa. The Visigothic kingdom, established by 507 CE, ruled until the Muslim conquests of the 8th century,⁶ and it exerted an

enduring influence on the Hispanic kingdoms' identity during the medieval period.

The era of Roman control profoundly changed linguistic, cultural, religious, and legal landscapes.¹ With the exception of the Proto-Basque language, all the Paleohispanic languages became extinct by the first century CE⁷ and were replaced by Latin, whose daughter languages are still widely spoken in the region. *Hispania* became a core province within the Roman Empire, producing several Roman emperors, including Trajan, Hadrian, and Theodosius I. Germanic kingdoms during late antiquity (ca. 5th–8th centuries CE) sometimes portrayed themselves as the inheritors of Roman rule, and aspects of Roman administration and governance continued to function under the new rulers; for instance, the Visigothic kingdom created a hybrid legal system deeply influenced by Roman law.⁶

Whether the profound cultural and political transformations associated with Roman rule and the migration period were accompanied by major population shifts remains debated.

Ancient DNA studies in the city of Rome,⁸ the central Italian region,⁹ the British Isles,¹⁰ and the Danubian Balkans¹¹ have revealed substantial mobility from the Eastern Mediterranean (where most major urban centers were located) during the imperial period.^{8,9,11} By contrast, Iberia remains sparsely sampled for this period,^{12,13} preventing a systematic genetic analysis of how these processes unfolded at the western edge of the Roman world.¹² While historical estimates suggest that Germanic groups arrived in relatively limited numbers in the peninsula (estimates have been proposed for ca. 100,000 Visigoths and 20,000 Vandals, Alans, and Suebi,¹⁴ compared with a proposed local Iberian population of ca. 4 million^{15,16}), their demographic impact has not been evaluated using genomic data.

Here, we present new genome-wide data from 243 ancient individuals spanning four regions of the Iberian Peninsula. This dataset allows us to access population structure and mobility in Iberia between the Roman and late antique periods, quantifying the demographic imprint of these transformative centuries.

RESULTS

Data generation

We generated genome-wide data from 243 ancient individuals (Table S1A; Data S1, section 2) through in-solution hybridization enrichment with either the “1240k” panel of ~1.2 million single-nucleotide polymorphisms (SNPs)^{17,18} or the “Twist” panel, which targets ~1.4 million SNPs.¹⁹

The newly reported individuals were excavated from 31 archaeological sites (totaling 33 necropolises) (Figure 1A) dating from the 2nd to the 8th century CE (Figure 1B) and located in four main areas: the southeastern coastal region of the peninsula, the northeastern region around the ancient provincial capital of *Tarraco* and the Ebro River basin, the central Iberian plateau, and the ancient provincial capital of *Augusta Emerita* in the southwestern interior (Figure 1A). The sites span regions with varying degrees of Romanization and diverse social contexts, including rural/urban, coastal/interior, and cultural affiliations such as Hispano-Roman or Germanic. We highlight 30 individuals from Corralón de Los Blanes at the Roman colony and provincial capital *Augusta Emerita* (Figure 1A, location 20), including 5 Roman-period and 25 Suebian or post-Suebian individuals, when the site briefly served as the Suebian capital (440 CE). We also analyzed 36 individuals from the late antique site of Pla de l’Horta (Figure 1A, location 6) with possible Germanic material influences²⁰ and 32 dated to the Roman period from Cortijo Coracho (Figure 1A, location 5), an early Christian basilica site in Iberia.²¹

For genome-wide analysis, we retained 212 newly reported individuals who yielded more than 15,000 SNPs with overlapping sequencing data and without signals of contamination: cytosine deamination at the terminal nucleotide above 3%; a ratio of Y to combined X + Y chromosome sequences below 0.03 or above 0.35; for male individuals with sufficient coverage, an X chromosome contamination estimate below 1.1% in the lower bound of the 95% confidence interval; and an upper-bound rate for the 95% confidence interval for the rate to the consensus mitochondrial sequence above 5%. We generated 35 new radiocarbon dates and retrieved an additional 32 from the literature.

A turn to extreme ancestry heterogeneity in the Roman period

To qualitatively assess the genetic affinities of Roman and late antique populations, we performed principal component analysis (PCA). We projected the newly reported ancient individuals onto the two main principal components (PC1 and PC2) computed based on patterns of genetic variation among 1,036 present-day West-Eurasian (WE) individuals (Figures 1C–1F) genotyped on the Affymetrix Human Origins array.

Populations of Iberia became highly genetically heterogeneous in the Roman period, as shown by their broad dispersal in PCAs (Figures 1C–1F). Their high ancestry heterogeneity contrasts with that of the previous Iberian Iron Age (IA) populations ($n = 24$)^{13,22–25} (Figures 1C–1F, blue polygon, and 2A; Table S1B). Although IA genomes from Iberia are limited due to the predominance of cremation practices (see [Limitations of the study](#)), we included all available high-quality individuals from this period ($n = 24$) (Table S1B; Data S1, section 5.1), spanning non-Indo-European-speaking east/south regions, Indo-European-speaking north/west regions, and contact zones²⁶ (Figures 1A, blue asterisks, and S7; Data S1), capturing broad IA genetic diversity. Previous studies indicate genomic continuity with late Bronze Age populations, with perhaps an increased Central European signal in Indo-European-speaking areas.^{13,25} The increased heterogeneity in IA versus Roman and late antique populations (Figures 2A and 2B) is statistically significant. We computed Euclidean distances of individuals (>20,000 SNPs, 1240k panel) to their group centroid in PC1-PC2 WE space (Figures 2A and 2B) and applied a Fligner-Killeen test for homogeneity of variances, revealing significant differences in dispersion ($p = 3.18 \times 10^{-5}$), indicating that the spread of genetic variation differs between IA and Roman samples. We further compared distances of Roman-period individuals to IA versus Roman centroids and observed a significant difference (Wilcoxon test, $p = 3.21 \times 10^{-6}$), consistent with an ancestry shift after Roman colonization examined in subsequent sections. To test continuity, we modeled the Roman population using the IA population as the sole ancestry source (Data S1, section 5); the model showed an extremely poor fit ($p = 1.49 \times 10^{-179}$), rejecting direct genetic continuity.

The increase in genetic heterogeneity remains evident when the analysis is performed regionally and extended to include late antique individuals. Specifically, three of the four sampled regions (the Ebro River basin, southeast Iberia, and the central Iberian plateau) show statistically significant increases in heterogeneity when comparing pre-Roman to Roman and late antique individuals ($p = 1.43 \times 10^{-3}$, 5.11×10^{-4} , and 9.48×10^{-5} , respectively) (Figure 1A). At *Augusta Emerita*, although the trend points to increased dispersion in PCA space relative to the IA, the p value remains just above the nominal significance threshold ($p = 0.064$), likely reflecting limited sample size.

Ancestry modeling using qpAdm quantifies this shift. During the Roman period, only 6 out of 66 individuals (excluding close relatives) can be modeled using Iberian IA populations as the sole ancestry source (Table S3A). However, IA Iberian ancestry is present in admixed form in most individuals, and even in late antiquity, we still find 11 individuals (out of 190 post-Roman Iberian

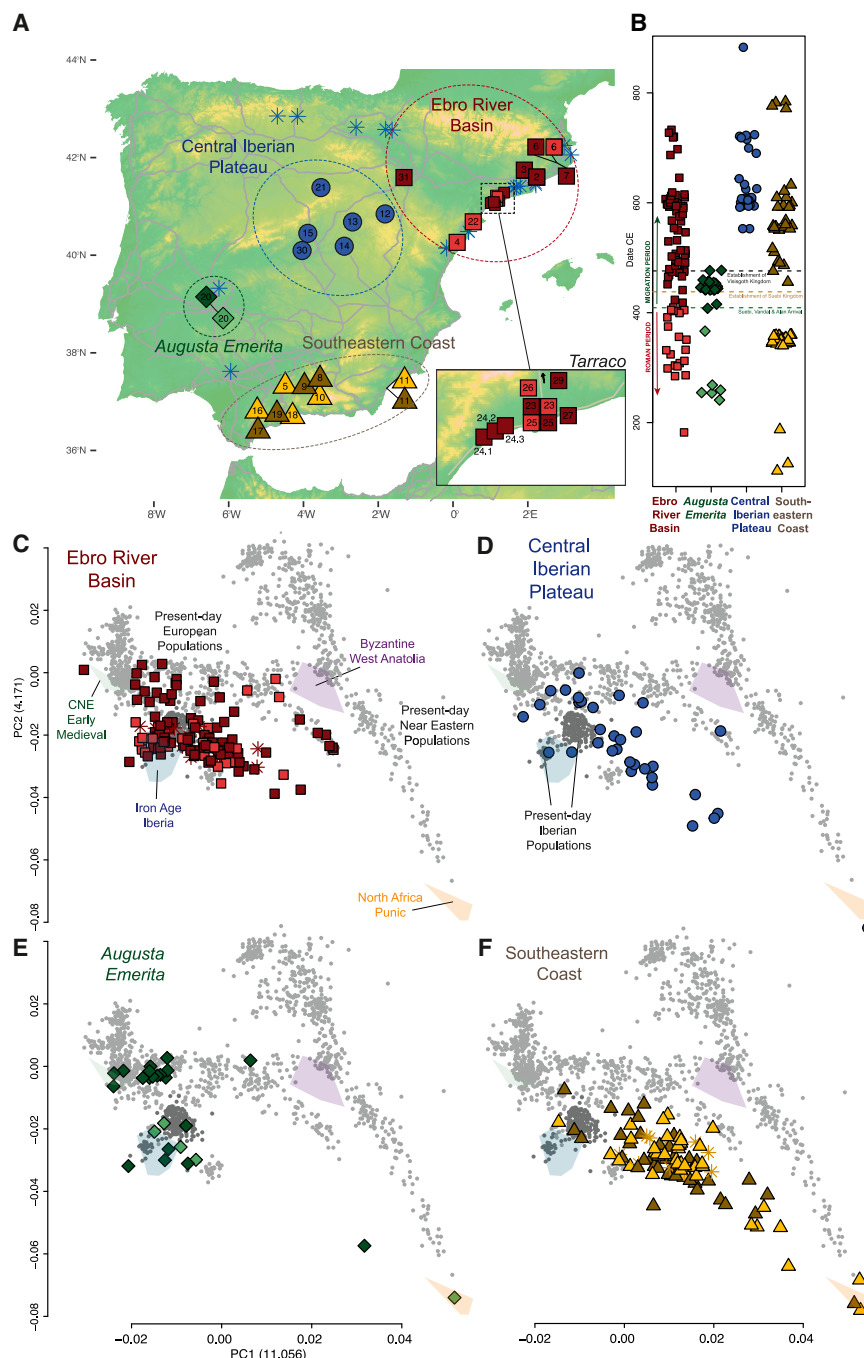


Figure 1. Outline of the newly reported ancient Iberian samples in this study

(A) Geographic distribution of archaeological sites analyzed in this study, grouped into four regional subdivisions: the Ebro River basin (red dotted line), the central Iberian plateau (blue dotted line), the southwestern city of *Augusta Emerita* (green dotted line), and the southeastern coast (yellow dotted line). Site numbers correspond to their entries in [Data S1](#). The main Roman roads are shown in gray. Archaeological sites of the published IA Iberian populations ([Table S1B](#)) used as a local source in *f*-statistics analyses ([Data S1](#), section 5) are marked with cyan asterisks.

(B) Chronological distribution of all individuals analyzed, dated by archaeological context or direct radiocarbon dating and grouped by region. Key historical events linked to Germanic migrations are marked: the arrival of the Alans, Suebi, and Vandals (green dotted line); the establishment of the Suebian kingdom (orange); and the formation of the Visigothic kingdom (black).

(C–F) PCA of West-Eurasian (WE) genetic diversity showing newly reported individuals projected onto a reference panel, color-coded by region of burial: (C) northeast Iberia and the Ebro River basin (red shades), (D) central Iberian plateau (blue), (E) *Augusta Emerita* (green), and (F) southeast Iberia (yellow). Previously published Andalusian-period individuals (8th–15th centuries CE) are marked with asterisks.¹³ Polygons indicate PCA positions of ancient reference populations ([Table S1B](#)) used in *f*-statistics analysis ([Data S1](#), section 5): blue, Iberia IA; purple, Roman and Byzantine individuals from West Anatolia; yellow, North African Punics; green, CNE individuals from the early medieval period. Present-day populations are shown as light gray circles, with present-day Iberians in darker gray.

See [Data S1](#) and [Figure S4B](#) for full PCA context.

group diversity across time in these regions. The central Iberian plateau could not be evaluated for temporal change due to the absence of Roman-period samples. However, comparing all Roman-period individuals from other regions with those from the central plateau shows no significant difference in genetic variance ($p = 0.79$). Thus, although overall genetic heterogeneity remains elevated relative to the IA and does not

genomes) whose genomes are consistent with deriving all their ancestry from local Iberian populations ([Table S3A](#)).

Population structure during late antiquity is remarkably similar to that of Roman Iberia, with comparable ancestry heterogeneity ([Figures 2B and 2C](#)). Euclidean distances to group-specific centroids show no significant difference in genetic variance between the Roman and the late antique periods in the Ebro River basin, southeast Iberia, and *Augusta Emerita* ($p = 0.30$, 0.79 , and 0.09 , respectively), suggesting similar intra-

differ significantly between the Roman and late antique periods, we observe a significant shift in population PCA position. Specifically, late antique individuals are significantly closer to their own centroid than to the Roman centroid (Wilcoxon test, $p = 7.49 \times 10^{-3}$), indicating a directional change in ancestry composition rather than changes in within-group variance. This pattern is consistent with the introduction of additional ancestry components during late antiquity into an already heterogeneous Roman population.

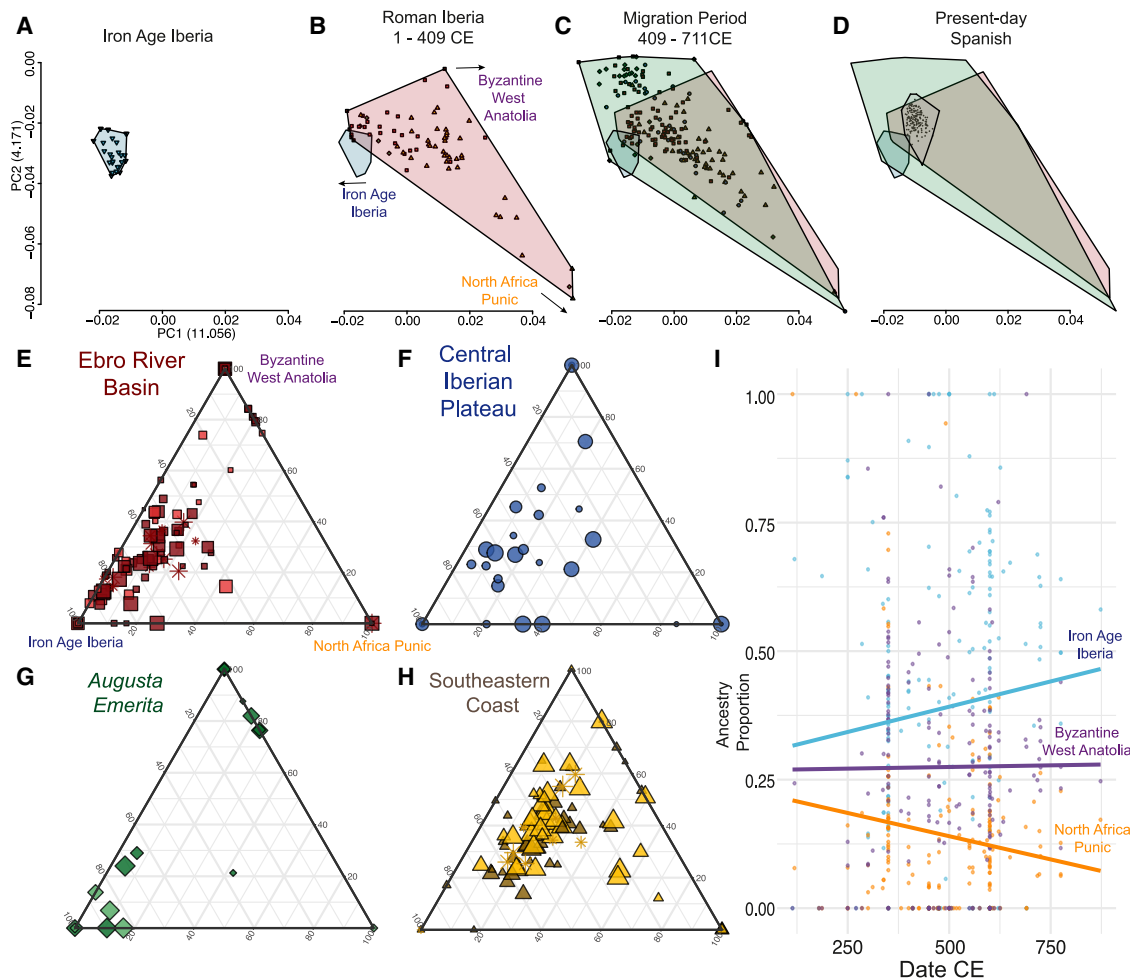


Figure 2. Shifts in ancestry in the Iberian Peninsula caused by Roman colonization and impact across time

(A–D) Evolution of ancient and modern individuals projected onto the PCA of West-Eurasian (WE) genetic variation: (A) IA, (B) Roman period, (C) migration period, and (D) present-day individuals tagged “Spanish” in the Human Origins database.

(E–H) Ancestry composition of late antique and early medieval individuals across the four main Iberian regions, modeled as proportions of three components: IA Iberia (blue labels in the bottom-left corner), West Anatolia Roman-Byzantine (purple label in the top), and North Africa Punic (orange labels in the bottom-right corner). (E) Northeast Iberia and the Ebro River basin (red), (F) central Iberian plateau (blue), (G) *Augusta Emerita* (green), and (H) southeast Iberia (yellow). Shape size reflects the genomic coverage of each individual (number of 1240k SNPs recovered; Table S1A).

(I) Temporal evolution of ancestry proportions among newly reported individuals. Each point represents one individual’s proportion of IA Iberia (blue), West Anatolia Roman-Byzantine (purple), and North Africa Punic (orange) ancestry. Lines indicate best-fit temporal trends.

Eastern Mediterranean ancestry as a major contributor during the Roman period

Ancestry analysis of Roman-period individuals reveals significant ancient Eastern Mediterranean-related ancestry presence (Figures 2E–2H, purple polygon) modeled using Roman and Byzantine West Anatolian groups,²⁷ which occupy an intermediate position in the WE PCA between present-day European and Near Eastern populations (Data S1; Figure S4B). This signal corresponds to an ancestry characteristic of the ancient Eastern Mediterranean peoples, particularly from the Western Anatolian coast, and is associated with major Roman imperial centers such as Ephesus, Pergamon, Smyrna, or Antioch. Overall, 55 out of 66 (83%) Roman-period Iberian individuals carry this ancestry, consistently observed across all three regions with Roman sam-

ples. In *Augusta Emerita*, proportions range from none to 13.8% (mean: $4.12\% \pm 2.6\%$); in the Ebro River basin, the mean is $28.9\% \pm 7.3\%$, detected as early as the 1st century CE at *Emporiae*¹³; and in the southeastern coastal region, the mean is $34.6\% \pm 6.1\%$ (Table S3A). This ancestry signal correlates with the appearance of new Y chromosome lineages such as J-L210, J-L26, and J-Z2177 (Table S1A), absent in Bronze and IA Iberia but common in the ancient Eastern Mediterranean²⁷ and present-day Near East.²⁸

These patterns are consistent with those observed elsewhere in the Roman Empire, including the imperial core in Rome and central Italy^{8,9} and the Danubian region,¹¹ all showing marked shifts toward Eastern Mediterranean ancestry. While Iberia similarly received this input, individuals typically carry it in admixed form,

in contrast to the fully Eastern Mediterranean-derived individuals observed in Italy and at the Danubian-Balkan frontier. This difference may partly reflect a gap in our transect for the Republican and early imperial periods (~200 BCE–200 CE), when mobility may have peaked but cremation was common, limiting DNA recovery. As a result, our data cannot distinguish whether this ancestry reflects direct migration from the eastern provinces, movement via another region, especially the imperial core, or a combination of both. Further integration of mobility proxies, particularly mobility-related isotopes, could help clarify these scenarios.

The latest individual modeled as unadmixed Eastern Mediterranean (I20275) was buried at the Les Goges site (Ebro River basin, northeastern Iberia) and dates from the late 7th century CE, suggesting that some migratory links between Iberia and the wider Mediterranean persisted after the decline of Roman rule. This later connectivity was plausibly mediated by the eastern Roman (Byzantine) Empire, which remained influential in Iberian culture and economy, ruling the southeastern coast down to ~624 CE, or even by the expanding Umayyad Caliphate.

Disproportionate Eastern Mediterranean ancestry relative to Italic ancestry

Italica was the first Roman settlement established in the Iberian Peninsula, founded by Scipio in 206 BCE to accommodate veterans of the Second Punic War and named after his Italic troops.²⁹ Historical sources^{29,30} and epigraphic evidence³¹ indicate that *coloniae* such as *Italica* and *Augusta Emerita* hosted substantial migration from the Italian Peninsula. However, among the 269 newly reported and previously published individuals from Roman and late antique Iberia with sufficient aDNA preservation for genome-wide analysis, only eight show clear genetic ancestry from the Italian Peninsula or the central Mediterranean region (Tables S1A, S1B, and S3A; information compiled in Table S6). Individual I26172, recovered from *Malaca* and dated to 126–232 CE, derived all her ancestry from a Central Mediterranean source³² (Data S1, section 5.3). She was an elderly woman buried with exotic grave goods, including a Levantine-style jug and multiple oil lamps.³³ Similarly, individual I37213 from Cortijo Coracho Basilica necropolis (4th century CE) carried admixed ancestry, with ~30% traced to a central Italian source (Table S3A; information compiled in Table S6). These are the only two Roman-period individuals in the dataset with such ancestry. The remaining six individuals with Central Mediterranean or central Italian-related ancestry³⁰ derive from post-Roman contexts (Table S3A; information compiled in Table S6).

A similar pattern has been observed in other heavily Romanized regions of the empire. For instance, genomic analyses of the Roman Balkans revealed negligible demographic impact from Italian ancestry.¹¹ Even in Rome and central Italy, Eastern Mediterranean ancestry predominated during the imperial period.^{8,9} Therefore, the limited Italic ancestry detected in our transect reflects a wider phenomenon, whereby Eastern Mediterranean populations became the main demographic force behind the increased mobility associated with imperial rule in these regions.

Large-scale gene flow originating from North Africa

In addition to Eastern Mediterranean influence, a North African genetic signal becomes prominent in the Roman period, re-

flected by a shift toward PCA space occupied by ancient North Africans (Figures 1C–1F, yellow polygon). As a surrogate for this signal, we use ancient individuals with fully North African ancestry excavated in Punic contexts³² ($n = 4$) (Figures 1C–1F, yellow polygon; Table S1B; Data S1, section 5.1).

Previous work¹³ detected North African ancestry in four Roman-period individuals from one site in Granada (southeastern coast region). We now document this signal in 33 of 34 Roman-period individuals, with an average ancestry of $26.9\% \pm 2.5\%$ (Table S3A; information compiled in Table S6). These individuals derive from three urban sites (coastal ancient *Malaca* and *Baria* and inland *Acinipo*) and one rural inland site, Cortijo Coracho Basilica, near present-day Lucena (Córdoba). This ancestry is also present in nearly all individuals from the seven late antique sites from this area, with the highest proportions observed at coastal sites such as *Baria*, where one individual (I18199; 432–551 CE) derives 94.7% of his ancestry from North African sources, and Arroyo Vaquero near the Strait of Gibraltar.

North African ancestry is not restricted to southeastern Iberia but is also detected elsewhere at lower proportions (Figures 2E–2H). In the central Iberian plateau, it is present at six late antique sites, ranging from 0% to 85% (average across sites: $15.5\% \pm 2.9\%$); only 7 of 31 individuals lack any North African signal (Tables S1A and S3A; information compiled in Table S6). At *Augusta Emerita*, out of 5 Roman-period individuals, one female (I23773) is entirely North African, and two others also carry this ancestry. In northeast Iberia, North African ancestry is present in eight sites and in about two-thirds of Roman-period individuals ($n = 17$ of 27), ranging from 0% to 25% (average: $6.6\% \pm 2.2\%$) (Table S3A; information compiled in Table S6), and more intensively around *Tarraco*. As in *Augusta Emerita*, this hints that the signal was linked to mobility attracted to the provincial capitals.

Supporting the autosomal results, we find a significant increase in uniparental lineages of likely African origin³⁴ across the four study regions, including mitochondrial haplogroups L1, L2, and L3 and the North African-specific U6 (15 of 298 analyzed individuals in our transect versus two out of 442 individuals before the Roman period^{13,35}; chi-square test, $p = 7.17 \times 10^{-5}$), or Y chromosome haplogroup E1b (8 of 130 analyzed males in our transect versus 2 out of 189 pre-Roman males, excluding close relatives; chi-square test, $p = 0.013$) (Table S1A), which reaches peak frequencies in present-day northwest Africa.³⁶ These lineages coexist with others associated with pre-Roman populations, such as R1b-P312, supporting the admixed nature of the Roman Iberian population.

Compared with other imperial regions, North African gene flow into Iberia was significantly more intense and widespread, appearing in a large portion of individuals. On the Danubian frontier, only 3 of 132 individuals harbor North African ancestry, and one carried East African ancestry.¹¹ Similarly, only 2 out of 48 individuals in the greater Rome region during the imperial period show substantial North African ancestry,⁸ despite the capital's intensive shipping links with North Africa and its demographic attraction.

While North African ancestry is absent in all available IA individuals from indigenous contexts, some individuals from Phoenician/Punic coastal colonies ($n = 5$ of 27) do display it.^{13,32} However, even when pooling both indigenous and colonial

contexts as a pre-Roman baseline ($n = 51$) (Data S1, section 5.1; Table S1B), the Roman and late antique periods display significantly higher North African ancestry ($p = 1.85 \times 10^{-6}$). The Roman period thus marks the first widespread, large-scale North African gene flow into Iberia.

Where Christianity entered the Iberian Peninsula remains debated,⁶ with some scholars³⁷ proposing its introduction via North Africa. We present genomic data from 31 individuals excavated at the Christian Basilica del Cortijo Coracho near Lucena (southeastern Iberia), dated between the 4th and 5th centuries CE. With one exception, all individuals with sufficient genomic data ($n = 30$) carried North African ancestry (ranging 6%–76%; average proportion: $25.0\% \pm 2.4\%$) (Table S3A; information compiled in Table S6). Additionally, we reconstructed a four-generation family network of 16 individuals (Figure S2), mostly modeled with the three-way admixture profile typical of southern Hispano-Roman populations, combining local Iberian, Eastern Mediterranean Roman, and North African ancestries. Since over half of the sampled individuals belong to this extended kin group, with North African ancestry ranging from 0% to 32% (average: $19.2\% \pm 2.3\%$), the community appears tightly knit and highly integrated (Table S3A; information compiled in Table S6). This provides a snapshot of a nascent Christian community with substantial North African ancestry, hinting that this large-scale input might have involved cultural diffusion influencing the religious landscape of late antique Iberia. Our data thus provide genetic evidence for sustained links between Iberia and North Africa during this period, complementing historical evidence for rapid Christian spread through Eastern Mediterranean diasporas and western imperial routes, particularly along shipping routes between Carthage and Iberia.³⁸ Carthage played a pivotal role in translating Christian traditions into Latin, well before Rome shifted from Greek to Latin liturgy in the 3rd–4th centuries.³⁸ In this light, the high North African genetic signal in this Christian community may reflect migration that contributed to Iberian cultural change via demic transmission.

Overall, Roman-period populations in Iberia can be characterized as a genetic mosaic formed through the admixture between local IA groups and migrants from the Eastern Mediterranean and North Africa. The high heterogeneity and the presence of unadmixed individuals indicate a sustained migratory process rather than a single event (Data S1, sections 2 and 5; Tables S1A and S3A; information compiled in Table S6), resulting in a population structure transformed relative to the IA and shaping the demographic landscape of Iberian groups entering the migration period.

Population-level emergence of Central-Northern European ancestry during the migration period

During the migration period, northern and eastern European groups moved into southern Europe, often replacing Roman imperial control with regional kingdoms under the rule of Germanic kings. In Iberia, this included the arrival of the Suebi, Vandals, Alans, and Visigoths,¹⁴ each with distinct origins, migration routes, and interactions with other populations along the way.^{11,39,40} Their presence is traced through Central-Northern European (CNE)-like ancestry in autosomal ancestry and uniparental markers largely absent in earlier Iberian populations.

Our genetic evidence reveals the sporadic presence of a CNE-related ancestry in the Iberian Peninsula prior to the migration period, much like other areas such as the Danubian frontier.¹¹ Previous studies show that individuals from northeastern Iberia already carry detectable CNE ancestry (Data S1, section 5.2), indicating sporadic contact with populations beyond the northern limits of Iberia before the large-scale migrations of the 5th century CE.¹³ In total, 6 of 66 Roman-period individuals carry CNE-like ancestry, corresponding to an average of $2.9\% \pm 0.7\%$ (Table S3A; information compiled in Table S6). Four individuals come from *Tarraco* ($n = 2$, I19795 and I19798; with 68.5% and 14.1% CNE-like ancestry, respectively) and *Emporiae* ($n = 2$, I10866 and I8475; with 41.3% and 16.6% CNE-like ancestry, respectively), major provincial hubs with strong trade connections to southern Gaul^{41,42}; only individual I10866 is directly radiocarbon dated (44 BCE–61 CE). The remaining two individuals (I37208 and I35942) with this ancestry were recovered from Coracho Basilica (Table S3A) and dated by archaeological context. Out of these six individuals, five lack direct radiocarbon dates, so their attribution to the pre-migration period remains uncertain, and some may instead belong to the early migration period stages; caution is therefore warranted in interpreting them as firmly pre-migration.^{11,34}

The scenario shifts during the migration and Visigothic periods, when 53 of 164 individuals (32%) across Iberia exhibit CNE-like ancestry, with an increased average of $22.8\% \pm 2.6\%$. Comparing Roman ($n = 66$) and migration-period ($n = 164$) individuals using a Wilcoxon rank-sum test shows a significantly higher median CNE-like ancestry in the migration period ($p = 4.39 \times 10^{-5}$; means: 0.03 ± 0.007 versus 0.228 ± 0.026). This is corroborated by a Kolmogorov-Smirnov test indicating distributional differences ($p = 1.41 \times 10^{-3}$). We also observe a significantly higher frequency of individuals carrying CNE-like ancestry in the migration versus the Roman period (chi-square, $p = 1.52 \times 10^{-3}$).

Archaeological contexts strongly associated with CNE ancestry

We do not detect fully unadmixed CNE ancestry until individual I22103, a high-status woman securely dated to the early 5th century CE from the roadside site of Hostalot-Ildum (Ebro River basin), marking the earliest clear archaeological context associated with unadmixed migration period ancestry in Iberia. From the early 5th century CE onward, CNE ancestry becomes more pronounced at some sites, consistent with the arrival and settlement of Germanic groups during the migration period (Figures 3C–3F). Germanic groups' appearance in Iberia is well-documented through historical and archaeological evidence and is reflected in newly founded settlements and cultural elements. To investigate this process, we sampled several archaeological sites with cultural elements associated with Germanic influences.

The site of Pla de l'Horta in the northeast Mediterranean region consists of a Roman suburban villa⁴³ that was reoccupied in post-Roman times.^{20,44} Some of the grave findings from this later phase have been associated with Germanic cultural elements.⁴⁴ Among 26 individuals with high-quality data dating from the 5th century onward, we detect 4 individuals (two males

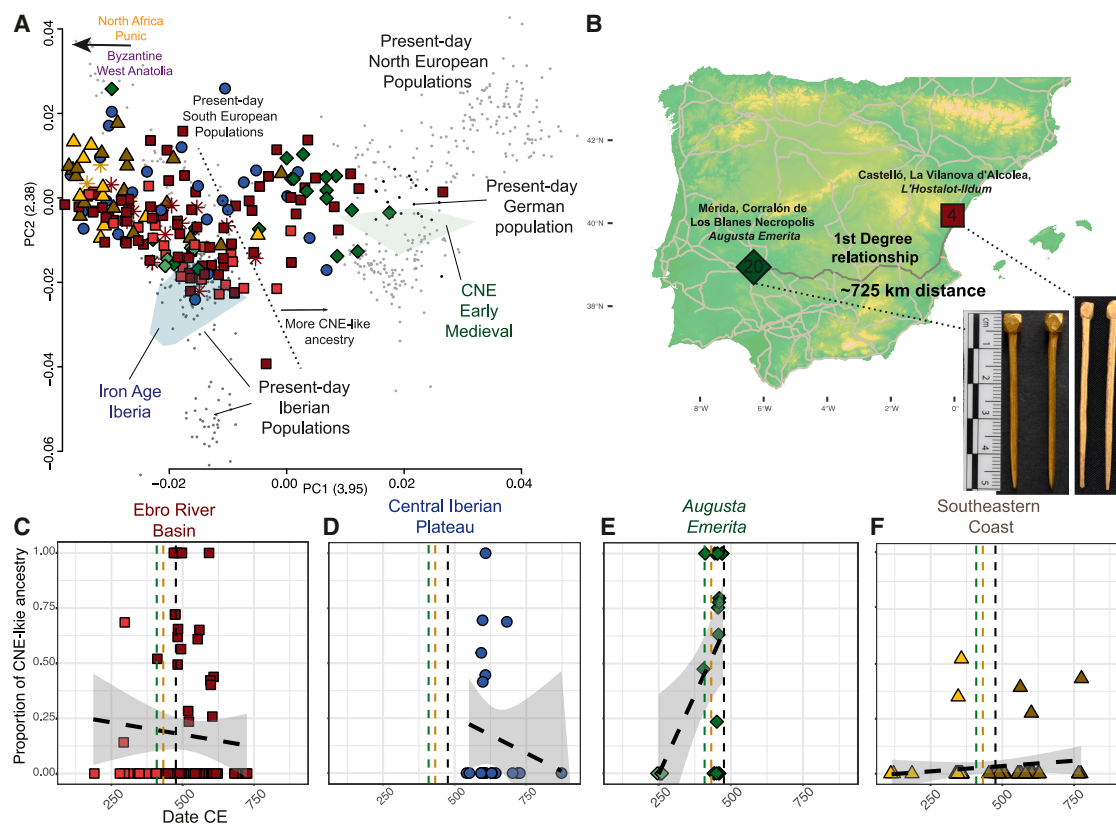


Figure 3. Long-distance mobility and Germanic ancestry in the Iberian Peninsula during the period of Germanic rule

(A) Projection of ancient samples on the PCA of present-day European genetic variation.

(B) Map showing Vilanova d'Alcolea (northeast Iberia) and Corralón de Los Blanes (*Augusta Emerita*, western Iberia), where two first-degree relatives (siblings) were identified. The shortest route between the two sites, following the Roman road network, is indicated (~725 km). Also shown are Germanic-associated gold pins of Danubian-Pontic typology recovered at both sites, highlighting shared cultural influences (scale bar included).

(C–F) Temporal evolution of CNE-like ancestry across the four Iberian regions: (C) northeast Iberia and the Ebro River basin (red), (D) central Iberian plateau (blue), (E) *Augusta Emerita* (green), and (F) southeast Iberia (yellow). Dashed lines mark key historical events: the arrival of the Alans, Suebi, and Vandals (green dotted line); the establishment of the Suebian kingdom (orange); and the rise of the Visigothic kingdom (black). These events frame the observed increase and regional variability of Germanic ancestry through time.

and two females) deriving 100% of their ancestry from CNE-like sources and a further 9 individuals with 44%–72% of this ancestry (Table S3A; information compiled in Table S6). Previous work suggests that Goths who had inhabited the Dniro River plains (3rd–4th centuries CE) interbred with neighboring steppe peoples (such as Sarmatians) before entering the Roman Balkan Peninsula in the 4th century.¹¹ However, we do not detect Sarmatian-related ancestry among the 9 admixed individuals, suggesting that some migration period groups were not affected by this process. The remaining 16 individuals show no Germanic-related ancestry, bearing instead varying proportions of the three ancestries present in earlier Hispano-Roman populations. This suggests that arriving Germanic groups coexisted with local populations in Iberia, even in contexts with Germanic-associated archaeological material.

We also present data from 30 newly reported individuals from the Los Blanes archaeological site at ancient *Augusta Emerita*, the capital of *Lusitania*—5 from the Roman period and 25 from the first generations of Germanic rule (Table S1A). Among the latter are three women archaeologically associated⁴⁵ with the

brief and poorly documented Suebian expansion into *Augusta Emerita*. They died at a very young age, perhaps contemporaneously, and were buried with funerary assemblages that included gold and silver objects originating from Pontic-Danubian-Elbe regions.⁴⁵ Their demise might be tied to the political upheavals in Iberia's tumultuous 5th and early 6th centuries, marked by conflict between the Suebian and Visigothic kingdoms.⁴⁶ The necropolis displays cultural and chronological links with the newcomer Vandals and Suebians—who crossed Gaul within 2 years, implying a different population history from the Goths, who had entered the empire earlier through the Danubian Limes¹¹—and with the Visigoths.

Among the 25 individuals with enough aDNA preservation for genome-wide analysis (Roman period, $n = 5$; migration period, $n = 20$), we find a clear presence of CNE-like ancestry during the migration period: 8 of 20 individuals derive 100% of their ancestry from CNE-related sources and 7 carry 24%–80%. However, unlike other migration period groups,¹¹ we detect no Steppe-like (Sarmatian) signal, notwithstanding historical information that the Sarmatian-related Alans accompanied the

Vandals and Suebi entering Iberia in the early 5th century.^{46,47} The remaining five migration-period individuals lack any CNE-like signal, showing limited initial permeation of the newcomers' ancestry into the local gene pool, even in a settlement with rich Germanic-associated grave goods,⁴⁵ and suggesting local population continuity following the arrival of migrant groups. This continuity is further reflected by two individuals with predominantly North African ancestry at *Augusta Emerita*, one from the Roman period and one from the migration period, as well as an unadmixed Eastern Mediterranean individual from the migration period.

CNE ancestry across social contexts

Germanic migrations associated with the genesis of the Visigothic kingdom were characterized primarily by elite dominance and admixture with pre-existing populations, rather than large-scale replacement. To assess the distribution of CNE-like ancestry across social contexts, we examined its association with burial material culture and inferred social status. Among 52 graves from necropolises featuring Germanic-associated material culture (Pla de l'Horta, Hostalot-*Idum*, and Los Blanes at *Augusta Emerita*), 29 individuals (56%) exhibited CNE-like ancestry, several with markers of elevated social status (Data S1, section 2).

Including all of the analyzed migration-period individuals, we detect 18 of 164 with 100% CNE-like ancestry and 35 admixed individuals carrying varying amounts of this ancestry, alongside 118 individuals lacking it, who belonged to the same burial communities (Table S3A; information compiled in Table S6). The coexistence of Hispano-Roman individuals with Germanic-like ancestry supports a scenario of gradual admixture rather than large-scale population replacement. Indeed, 16 individuals harbor both CNE-like and Iberian IA-like ancestry, including one (I23288) from the Los Blanes necropolis and three (I12033, I20392, and I13572) from the Visigothic period phase of the Pla de l'Horta necropolis, sites with Ponto-Caspian-style archaeological findings (Data S1, section 2). This indicates intermarriage between the Germanic migrants and the local Hispano-Roman population. Although substantial and clearly detectable, the demographic impact of these migrant groups does not suggest a population-wide replacement.

The Germanic newcomers had embraced Arian Christianity, a doctrine initially favored by some Christian Roman emperors but later condemned as heretical. This may have discouraged unions between Hispano-Romans and Goths until Visigothic King Reccared's conversion to the Nicene (Catholic) faith in 589 CE, after which intermarriage between Visigoths and locals was officially permitted. Using median date estimates, individuals dating before Reccared's reign show the highest proportion of unadmixed CNE-related ancestry at 17% (15 of 90 individuals with CNE-related ancestry), compared with 3 of 82 individuals median-dated after 589 CE (chi-squared, $p = 0.012$). However, the presence of admixed individuals median-dated before 589 CE (8 of 90 individuals, versus 15 of 82 individuals after 589 CE; chi-square test, $p = 0.114$) (Tables S1A and S3A; information compiled in Table S6; Data S1, sections 2 and 5) suggests that legal restrictions did not fully prevent the newcomers' integration into the broader population.

To mitigate bias from the overrepresentation of recent migrants at these sites, we conducted additional analyses excluding necropolises associated with Germanic material culture. We find that 23 out of 114 individuals in this subset carry CNE-like ancestry, with a mean proportion of $60.0\% \pm 11.1\%$ among carriers. In the full subset, the overall average CNE-like ancestry is $12.1\% \pm 2.2\%$, indicating notable admixture without widespread population turnover. Although significant ($p = 0.035$), the demographic impact of Migration-period migrations remains lower than those documented during the period of Roman control of Iberia.

Decline and dilution of CNE ancestry after late antiquity

Following Reccared's reign and until the fall of the Visigothic kingdom in 711 CE, Germanic ancestry likely became increasingly diluted through admixture with the larger local population. Nevertheless, traces of this ancestry persisted for generations. Analyzing one newly reported and 25 previously published Andalusian-period individuals (8th–15th centuries),¹³ we find 6 individuals with CNE-like ancestry, ranging from 28% to 80%. However, from the 12th century onward, only two of 10 individuals (I12647 and I12648; Balansiya Taifa)¹³ retain this genetic signal, and only in admixed form (Tables S1B and S3A; information compiled in Table S6).

PCA space examination suggests a discrepancy between early medieval and present-day Iberian population structure (Figures 1C–1F, 2A–2D, S4A, and S4B). Among present-day Iberians in our available data, the largest group consists of individuals genotyped on the Human Origins array classified as Spanish.HO.⁴⁸ Comparing the distribution of distances to group-specific centroids in PCA space between present-day and late antiquity Iberians shows a significant reduction in within-group genetic dispersion in the present-day population ($p = 2.2 \times 10^{-16}$; we excluded present-day Basques in this comparison due to their unique population history^{13,49}) (Figures 2C and 2D; Data S1, section 5.5). We considered two non-mutually exclusive explanations for this heterogeneity reduction, observed in present-day Iberians relative to early medieval populations. First, if late antiquity heterogeneity was primarily driven by immigration from diverse external sources, a subsequent reduction in such inputs would reduce dispersion over time (Figures 2A–2D). Under this scenario, present-day populations would still be modeled using the same sources as early medieval individuals, but with reduced variance in ancestry proportions. Alternatively, later demographic processes may have reshaped population structure through additional ancestry sources not represented in our dataset, producing an apparent more genetically homogeneous subset of the earlier population and leading to both reduced PCA dispersion and poorer model fit using exclusively late antique sources.

To test for continuity or discontinuity, we modeled present-day Iberian populations geotagged by region from the 1000 Genomes Project^{50,51} (IGSR: IBS) to assess the persistence of CNE-like ancestry in the modern gene pool. However, none of the models yielded a statistically acceptable fit (Data S1, section 5.5; Table S4A). Notably, models that included a CNE source consistently produced p values closer to significance, suggesting some persistence. These results indicate that the genetic

landscape of present-day Iberia was shaped not by new large-scale migrations, but by internal population movements and resettlement during the medieval and Renaissance periods. Historical sources indicate substantial demographic disruption during the medieval period, including urban population decline associated with prolonged conflict between northern Christian kingdoms and Andalusí polities.⁵² These processes were accompanied by large-scale resettlement, whereby territories conquered by northern kingdoms were repopulated primarily from northern Iberian populations.^{52–54} However, northern Iberia is currently underrepresented in both our dataset and the published archaeogenomic record, limiting our ability to capture the full ancestral variation of present-day populations and contributing to reduced fit (Data S1, section 5.5). This sampling gap suggests that present-day Iberians may partly derive from unsampled sources, consistent with reduced genetic dispersion relative to late antique populations (Figures 2A–2D).

Close first-generation immigrant relatives separated by large geographical distance

We identify two siblings excavated ~725 kilometers apart, representing the largest geographical separation between first-degree relatives in the archaeogenetic record to date (the next being two Copper Age individuals from southern England separated by 5 km) (Tables S2A and S2C; Data S1, section 4). The brother (I23273) was buried at Los Blanes necropolis (*Augusta Emerita*) and dated to 364–535 CE. His sister (I22103) died between 257–409 CE and was buried at a roadside hospitality center on the *Via Augusta* at Hostalot-Ildum (Ebro River basin)⁵⁵ in a high-status burial with grave goods including a glass vase and two gold pins of Danubian-Pontic tradition,^{55,56} similar to those at Los Blanes (Figure 3B). Both siblings, likely first-generation immigrants, were modeled as having 100% CNE-related ancestry, and the brother carried the typical CNE I1 Y chromosome lineage. This finding highlights the extreme mobility of Germanic groups during late antiquity and elites' connectivity within the peninsula, enabled by the continued operation of Roman infrastructure. The sister's burial at an imperial roadside hostel suggests she died while traveling along the Roman highway infrastructure linking the main late cities of *Augusta Emerita* and *Tarraco*. According to ORBIS (<https://orbis.stanford.edu/>), such a journey would take ~24 days on foot, or ~14 days on horseback for high-status individuals (Figure 3B). While the exact migration path remains unclear, the sister may have died during an initial southward migration, while her brother continued to *Augusta Emerita*, where he was eventually buried; mobility-related isotopic analysis could clarify this. Their relationship, chronology, and locations illuminate previously undocumented pathways of the early migration period groups into Iberia. Her burial along the *cursus publicus* suggests that this Roman communication system was still operational in northeastern Spain during the usurper emperor Maximus' rule (5th century CE), hinting at his involvement early in the Suebi migration.⁵⁷

DISCUSSION

Unlike the colonies established by previous civilizations such as the Greeks and Phoenicians/Punic people,^{13,32} Roman rule

brought widespread inflows of Eastern Mediterranean and North African ancestry into Iberia. Documentary, archaeological, and epigraphic evidence attest to the arrival of Roman colonists who settled in regions such as the Guadalquivir valley starting from the early days of Rome's presence in Iberia^{58,59}; our data demonstrate that they precipitated a large-scale demographic change across the peninsula that persisted deep into the later periods. Migrants to these regions created a society of heterogeneous ancestry interconnected with the broader Mediterranean. Interestingly, most studies to date have documented genetic influence from Eastern Mediterranean groups on individuals in central regions of the Roman Empire, such as Italy and the Balkans,^{8,9,11} an influence we now also demonstrate for the western Mediterranean. Our results further prove that North African ancestry was not restricted to one site but was widespread in southeastern Iberia during the Roman and late antique periods and even reached other regions further north. These results imply a large-scale demographic influx of both women and men from northwest Africa that, under Roman control, brought the two shores of the Strait of Gibraltar genetically closer than ever before. Future archaeogenetic studies in Roman North Africa are necessary to determine whether the mobility between Iberia and North Africa was bidirectional.

The genomic analysis of late antique sites allowed us to identify groups with clear CNE ancestral origins and to document a case of a close familial connection over hundreds of kilometers that seems to pinpoint a route of the Suebian migration into Iberia. The demographic impact of migrations during this period has been genomically investigated in other regions of the Western Roman Empire, such as Great Britain,¹⁰ the Balkans,¹¹ and the Italian Peninsula.^{8,9} In England, the potential Anglo-Saxon contribution was estimated to be under 50% in central-south England and most likely in the range of 10%–40% from the analysis of modern genomes.⁶⁰ This estimate rises to 76% on average when analyzing early medieval burials, mainly from eastern England.¹⁰ Both figures point to a large-scale demographic input from continental North Sea regions. In the modern Balkans, the potential contribution of the Slavic migrations has now been estimated to range from 40% to 60% in places such as modern Serbia to 20% in mainland Greece.¹¹ In the Iberian Peninsula, despite the clear presence of individuals with CNE-related ancestry at specific sites and signs of admixture with the local population, their demographic impact was relatively limited compared with other European regions. The characteristic ancestry mixture and diversity typical of Hispano-Roman society remained in place, and, although admixture occurred, the overall genetic landscape did not see radical change (between 12% and 22%, depending on region and social context).

Present-day Iberian populations show considerably less genetic heterogeneity than the groups analyzed in this work, indicating that further reshaping of the gene pool occurred in later medieval or post-medieval times. The Iberian Peninsula's complex and dynamic history, shaped by Romanization, Visigothic rule, Islamic conquests, and subsequent conquest by the northern Christian kingdoms, presents a unique genetic landscape still to be fully revealed. Future work focusing on medieval and post-medieval genomes will be essential to finally link the ancient with the modern inhabitants of the peninsula.

Limitations of the study

Our study is an example of how dense regional sampling can elucidate both general and fine-scale demographic processes within a focused period. However, a major drawback is the lack of dense sampling for the IA and Roman Republican periods, largely due to cremation as the dominant burial practice. The ancient DNA record is also sparse in vital regions such as northern Iberia and North Africa. A second limitation is the high heterogeneity of the Hispano-Roman population in the late Roman and migration periods, which prevents clustering of the population for *f*-statistics tests and thus reduces statistical power. Our observation of high ancestry heterogeneity within and across sites suggests that the ancestry of some regions we did not sample likely differs subtly from those we did; further insights would come from future sampling of these areas. Finally, the absence of clear genetic continuity between the late antique period and the present-day implies major demographic change during medieval or post-medieval times. The limited number of individuals from these periods in our dataset precludes meaningful inferences about these critical periods.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Pablo Carrión (pablo_carrion@mpg.eva.de).

Materials availability

This study did not generate any new, unique reagents.

Data and code availability

- All data needed to evaluate the conclusions in the paper are present in the paper and/or the [supplemental information](#).
- Newly reported ancient sequencing data have been deposited at the European Nucleotide Archive (ENA) and will be publicly available upon publication with the following accession number ENA: PRJEB96929.
- Pseudo-haploid genotypes for the newly reported ancient individuals have been deposited at <https://reich.hms.harvard.edu/datasets>.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

P.C., I.O., C.L.-F., and D.R. conceived and designed the study. P.C. wrote the initial manuscript draft. P.C., I.O., C.L.-F., M.M., K.H., and D.R. wrote the manuscript and compiled supplementary materials with the input of all other co-authors. J.M.J.-A., N.C., D.V., J.M.V., D.B.O., M.B.-Á., J.H.-M., R.O.-R., C.C., M.I.G.-C., J.A.Q.-C., J.R., J.S.-P., I.N.-L., M.A.C.-P., I.A., V.V.-M., J.M., R.C., M.L.C., J.F.R.-P., M.D.-G., P.C.-C., T.P.-R., E.V.-C., S.V.-Á., J.B., J.S., J.V., A.C.-C., V.M.-E., F.R.-C., V.G.-E., A.R., M.M., E.V., M.S., A. Costa, P.G.-B., P.C.B., P.U.-E., A. Cebrià, T.G.-E., B.C.-G., P.P.-A., C.V.-I., J.M.-P., S.S.-S., J.M.C.-A., S.L.-C., and P.C. assembled archaeological material and prepared the site descriptions. M.I.G.-C. supervised and M.I.G.-C. and P.C. performed the isotopic analysis. N.R. performed laboratory work. S.M. performed bioinformatic data processing. P.C. performed population genetic analyses. V.V.-M. gave input on genetic analysis design. P.C. and I.O. analyzed data. I.O., C.L.-F., and D.R. secured funding.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE MANUSCRIPT PREPARATION PROCESS

During the preparation of this work, the authors used Grammarly to assist with spelling, grammar, and tone; ChatGPT to reduce redundancy and enhance clarity in some sections; and DeepL to assist in translating archaeological references and materials into appropriate English terminology. After using these tools, the authors carefully reviewed and edited the content as needed and take full responsibility for the content of the publication.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

- Mattingly, D.J. (2013). *Imperialism, Power, and Identity: Experiencing the Roman Empire* (Princeton University Press). <https://doi.org/10.2307/j.ctt4cgd4k>.
- Hodos, T. (2020). *The Archaeology of the Mediterranean Iron Age: a Globalising World c.1100–600 BCE* (Cambridge University Press). <https://doi.org/10.1017/9780511979316>.
- Richardson, J.S. (2004). *Hispaniae: Spain and the Development of Roman Imperialism, 218–82 BC* (Cambridge University Press).
- Harper, K. (2017). *The Fate of Rome: Climate, Disease, and the End of an Empire* (Princeton University Press). <https://doi.org/10.2307/j.ctv9b2txr>.
- Heather, P. (2010). *Empires and Barbarians: Migration, Development and the Birth of Europe* (Pan Macmillan).
- Martínez-Jiménez, J., Sastre de Diego, I., and Tejerizo García, C. (2017). *The Iberian Peninsula between 300 and 850: an Archaeological Perspective* (Amsterdam University Press).
- Díaz, B., Estarán, M.J., Simón, I., Sinner, A., and Velaza, J. (2019). Writing, colonization, and Latinization in the Iberian peninsula. In *Palaeohispanic Languages and Epigraphies*, A.G. Sinner and J. Velaza, eds. (Oxford University Press), pp. 396–416.
- Antonio, M.L., Gao, Z., Moots, H.M., Lucci, M., Candilio, F., Sawyer, S., Oberreiter, V., Calderon, D., Devitofranceschi, K., Aikens, R.C., et al. (2019). Ancient Rome: A genetic crossroads of Europe and the Mediterranean. *Science* 366, 708–714. <https://doi.org/10.1126/science.aay6826>.
- Posth, C., Zaro, V., Spyrou, M.A., Vai, S., Gneccchi-Ruscione, G.A., Modi, A., Peltzer, A., Mötsch, A., Nägele, K., Vågane, Å.J., et al. (2021). The origin and legacy of the Etruscans through a 2000-year archeogenomic time transect. *Sci. Adv.* 7, eabi7673. <https://doi.org/10.1126/sciadv.abi7673>.
- Gretzinger, J., Sayer, D., Justeau, P., Altena, E., Pala, M., Dulias, K., Edwards, C.J., Jodoin, S., Lacher, L., Sabin, S., et al. (2022). The Anglo-Saxon migration and the formation of the early English gene pool. *Nature* 610, 112–119. <https://doi.org/10.1038/s41586-022-05247-2>.
- Olalde, I., Carrión, P., Mikić, I., Rohland, N., Mallick, S., Lazaridis, I., Mah, M., Korać, M., Golubović, S., Petković, S., et al. (2023). A genetic history of the Balkans from Roman frontier to Slavic migrations. *Cell* 186, 5472–5485.e9. <https://doi.org/10.1016/j.cell.2023.10.018>.
- Roca-Rada, X., Davidson, R., Williams, M.P., Villalba-Mouco, V., Carvalho, A.F., Ravishankar, S., Collen, E., Haarkötter, C., Taufik, L., Cuesta-Aguirre, D.R., et al. (2025). The genetic history of Portugal over the past 5,000 years. *Genome Biol.* 26, 248. <https://doi.org/10.1186/s13059-025-03707-2>.
- Olalde, I., Mallick, S., Patterson, N., Rohland, N., Villalba-Mouco, V., Silva, M., Dulias, K., Edwards, C.J., Gandini, F., Pala, M., et al. (2019). The genomic history of the Iberian Peninsula over the past 8000 years. *Science* 363, 1230–1234. <https://doi.org/10.1126/science.aav4040>.
- Collins, R. (2008). *Visigothic Spain, 409–711* (John Wiley & Sons).
- Monfort, C.C. (2014). Nuevas tendencias y datos sobre la demografía romana en la Península Ibérica. *BSAA Arqueología*, 53–82.
- Robles, F.B. (2026). *Demografía de la esclavitud en Hispania romana. Lucentum*, 83–100.
- Fu, Q., Hajdinjak, M., Moldovan, O.T., Constantin, S., Mallick, S., Skoglund, P., Patterson, N., Rohland, N., Lazaridis, I., Nickel, B., et al. (2015). An early modern human from Romania with a recent Neanderthal ancestor. *Nature* 524, 216–219. <https://doi.org/10.1038/nature14558>.
- Mathieson, I., Lazaridis, I., Rohland, N., Mallick, S., Patterson, N., Roodenberg, S.A., Harney, E., Stewardson, K., Fernandes, D., Novak, M., et al. (2015). Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* 528, 499–503. <https://doi.org/10.1038/nature16152>.
- Rohland, N., Mallick, S., Mah, M., Maier, R.M., Patterson, N.J., and Reich, D. (2022). Three assays for in-solution enrichment of ancient human DNA at more than a million SNPs. *Genome Res.* 32, 2068–2078. <https://doi.org/10.1101/gr.276728.122>.
- Agustí, B., and Llinàs-Pol, J. (2012). Pla de l'Horta i les Goges: dues necròpolis d'època visigoda a l'entorn de Gerunda. In *Arqueologia Funerària al Nord-Est Peninsular:(Segles VI-XII) (Museo de Arqueologia de Cataluña/ Museo d'Arqueologia de Catalunya)*, pp. 317–330.
- Diéguez-Ramírez, J.P. (2016). Estudio bioantropológico comparado de tres necrópolis históricas excavadas en el término municipal de Lucena (Córdoba).
- Domínguez-Solera, S.D., Torres-Martínez, J.F., Carnicero, S., Olalde, Í., Reich, D., Mallick, S., and Rohland, N. (2025). The human skull from the siege of La Loma (Santibáñez de la Peña, Palencia, Spain). *J. Roman Archaeol.* 38, 645–660. <https://doi.org/10.1017/S1047759425100512>.
- Rohrlich, A.B., Rivollat, M., de-Miguel-Ibáñez, P., Nordfors, U., Liira, A.-M., Teixeira, J.C., Roca-Rada, X., Armendáriz-Martija, J., Boyadzhiev, K., Boyadzhiev, Y., et al. (2024). Cases of trisomy 21 and trisomy 18 among historic and prehistoric individuals discovered from ancient DNA. *Nat. Commun.* 15, 1294. <https://doi.org/10.1038/s41467-024-45438-1>.
- Olalde, Í., Rohland, N., Mallick, S., and Reich, D. (2024). Análisis de ADN de los restos infantiles de La Ayuela. In *La Casa de la Ayuela en el tiempo del Tesoro de Aliseda: Excavaciones preventivas de la Ayuela (2009) y la Estación (2018) en el tramo Cáceres-Mérida de la línea de Alta V. de Extremadura*, A. Rodríguez-Díaz, D.M. Duque-Espino, and I. Pavón-Soldevila, eds. (de Economía, C. Ciencia y Agenda Digital), pp. 592–593.
- Patterson, N., Isakov, M., Booth, T., Büster, L., Fischer, C.-E., Olalde, I., Ringbauer, H., Akbari, A., Cheronet, O., Bleasdale, M., et al. (2022). Large-scale migration into Britain during the Middle to Late Bronze Age. *Nature* 607, 588–594. <https://doi.org/10.1038/s41586-021-04287-4>.
- Sinner, A.G., and Velaza, J. (2019). *Palaeohispanic Languages and Epigraphies* (Oxford University Press).
- Lazaridis, I., Alpaslan-Roodenberg, S., Acar, A., Açıkkol, A., Agelarakis, A., Aghikyan, L., Akyüz, U., Andreeva, D., Andrijašević, G., Antonović, D., et al. (2022). The genetic history of the Southern Arc: A bridge between West Asia and Europe. *Science* 377, eabm4247. <https://doi.org/10.1126/science.abm4247>.
- El-Sibai, M., Platt, D.E., Haber, M., Xue, Y., Youhanna, S.C., Wells, R.S., Izaabel, H., Sanyoura, M.F., Harmanani, H., Bonab, M.A., et al. (2009). Geographical structure of the Y-chromosomal genetic landscape of the Levant: A coastal-inland contrast. *Ann. Hum. Genet.* 73, 568–581. <https://doi.org/10.1111/j.1469-1809.2009.00538.x>.
- Knapp, R.C. (1977). *Aspects of the Roman Experience in Iberia, 206–100 BC* (Universidad, DL).
- Gosner, L.R., and Hayne, J. (2024). Local experiences of connectivity and mobility in the ancient west-central Mediterranean (Equinox).
- Darder, M. (1988). Epigrafía hispánica de época romano-republicana. *Fa-ventia* 10, 191–193.
- Ringbauer, H., Salman-Minkov, A., Regev, D., Olalde, I., Peled, T., Sineo, L., Falsone, G., Van Dommelen, P., Mitnik, A., Lazaridis, I., et al. (2025). Punic people were genetically diverse with almost no Levantine ancestors. *Nature* 643, 139–147. <https://doi.org/10.1038/s41586-025-08913-3>.
- López-Chamizo, S. (2025). Caring for the dead, understanding the living: bioarchaeology of care in a 2nd–3rd century CE burial from Roman Malaca. *J. Archaeol. Sci.: Rep.* 66, 105254. <https://doi.org/10.1016/j.jasrep.2025.105254>.
- Gonder, M.K., Mortensen, H.M., Reed, F.A., De Sousa, A., and Tishkoff, S.A. (2007). Whole-mtDNA genome sequence analysis of ancient African lineages. *Mol. Biol. Evol.* 24, 757–768. <https://doi.org/10.1093/molbev/msl209>.
- González-Fortes, G., Tassi, F., Trucchi, E., Henneberger, K., Pajmans, J.L.A., Díez-Del-Molino, D., Schroeder, H., Susca, R.R., Barroso-Ruiz, C., Bermudez, F.J., et al. (2019). A western route of prehistoric human migration from Africa into the Iberian Peninsula. *Proc. Biol. Sci.* 286, 20182288. <https://doi.org/10.1098/rspb.2018.2288>.
- Cruciani, F., La Fratta, R., Santolamazza, P., Sellitto, D., Pascone, R., Moral, P., Watson, E., Guida, V., Colomb, E.B., Zaharova, B., et al.

- (2004). Phylogeographic analysis of haplogroup E3b (E-M215) Y chromosomes reveals multiple migratory events within and out of Africa. *Am. J. Hum. Genet.* 74, 1014–1022. <https://doi.org/10.1086/386294>.
37. Blázquez-Martínez, J.M. (2003). El mediterráneo y España en la Antigüedad: Historia, Religión y Arte (Ediciones Cátedra).
38. Frend, W.H.C. (1984). *The Rise of Christianity* (Fortress Press).
39. Heather, P. (1996). *The Goths* (Blackwell Publishing (Blackwell)).
40. Papac, L., Ernée, M., Dobeš, M., Langová, M., Rohrlach, A.B., Aron, F., Neumann, G.U., Spyrou, M.A., Rohland, N., Velemínský, P., et al. (2021). Dynamic changes in genomic and social structures in third millennium BCE central Europe. *Sci. Adv.* 7, eabi6941. <https://doi.org/10.1126/sciadv.abi6941>.
41. Sanchez, C. (2022). Trading Networks in Transalpine Gaul before and after the Conquest of 125 BC. In *Rome and the North-Western Mediterranean: Integration and Connectivity*, T.Ñ. Del Hoyo, J. Principal, and M. Dobson, eds. (Oxbow Books), pp. 81–96. <https://doi.org/10.2307/j.ctv371cnww.12>.
42. Colominas, L., and Edwards, C.J. (2017). Livestock trade during the Early Roman Period: first clues from the trading post of Empúries (Catalonia). *Int. J. Osteoarchaeol.* 27, 167–179. <https://doi.org/10.1002/oa.2527>.
43. Costa, A., Palahí, L., Nolla, J.M., and Codina, D.V. (2019). La Vil·la romana del Pla de l'Horta (Sarrià de Ter) i el subúrbium de Gerunda com espai residencial i productiu (Museu d'Arqueologia de Catalunya).
44. Llinàs Pol, J., Tarrés Farrés, A., Montalbán Martínez, C., Frigola Triola, J., Merino Serra, J., and Agustí Farjas, B. (2008). Pla de l'Horta (Sarrià de Ter, Girona): una necrópolis con inhumaciones visigodas en la Tarracense oriental. *Arch. Esp. Arqueol.* 81, 289–304. <https://doi.org/10.3989/aespa.2008.v81.53>.
45. Heras-Mora, F.J., and Olmedo-Gragera, A.B. (2018). In *Novedades en la necrópolis tardorromana de Mérida. Las princesas bárbaras*. *Anas*, pp. 201–208.
46. Castritius H. (2005). Sweben. In: *Reallexikon der germanischen Altumskunde*. Hoops J., Beck H., eds. p. 202–210. https://www.degruyterbrill.com/database/gao/entry/RGA_5595/html?query=%28%28sweben%29%29.
47. Alemany, A. (2000). *Sources on the Alans: a critical compilation* (Brill). <https://doi.org/10.1163/9789004491212>.
48. Mallick, S., Micco, A., Mah, M., Ringbauer, H., Lazaridis, I., Olalde, I., Patterson, N., and Reich, D. (2024). The Allen Ancient DNA Resource (AADR) a curated compendium of ancient human genomes. *Sci. Data* 11, 182. <https://doi.org/10.1038/s41597-024-03031-7>.
49. Flores-Bello, A., Bauduer, F., Salaberria, J., Oyharçabal, B., Calafell, F., Bertranpetit, J., Quintana-Murci, L., and Comas, D. (2021). Genetic origins, singularity, and heterogeneity of Basques. *Curr. Biol.* 31, 2167–2177.e4. <https://doi.org/10.1016/j.cub.2021.03.010>.
50. Clarke, L., Zheng-Bradley, X., Smith, R., Kulesha, E., Xiao, C., Toneva, I., Vaughan, B., Preuss, D., Leinonen, R., Shumway, M., et al. (2012). The 1000 Genomes Project: data management and community access. *Nat. Methods* 9, 459–462. <https://doi.org/10.1038/nmeth.1974>.
51. 1000 Genomes Project Consortium, Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., and et al. (2015). A global reference for human genetic variation. *Nature* 526, 68–74. <https://doi.org/10.1038/nature15393>.
52. Cuberes, D., and González-Val, R. (2017). The effect of the Spanish Reconquest on Iberian cities. *Ann. Reg. Sci.* 58, 375–416. <https://doi.org/10.1007/s00168-017-0810-0>.
53. Henriques, A. (2017). *The Reconquista and its legacy, 1000–1348*. In *An agrarian history of Portugal, 1000–2000* (Brill), pp. 11–44.
54. MacKay, A. (1977). The Problems and Opportunities of Reconquest, c. 1200–1350. In *Spain in the Middle Ages* (Springer), pp. 58–78. https://doi.org/10.1007/978-1-349-15793-8_4.
55. García-Borja, P., Martín, M.B., Bardoll, P.C., Carrión, P., López, V.C., Briansó, M.E., Lalueza-Fox, C., Olalde, I., Broch, J.E.P., and Berlanga, G.P. (2021). La inhumación tardoantigua del Hostalot-Ildum (Vilanova d'Alcolea, Castelló). In *Nuevas Aportaciones. Quaderns de Prehistòria i Arqueologia de Castelló*, pp. 165–188.
56. Kazanski, M. (1989). La diffusion de la mode danubienne en Gaule (fin du IVe siècle-début du VIe siècle): essai d'interprétation historique. *Antiquités Natl.* 21, 59–74.
57. Kulikowski, M. (2011). *Late Roman Spain and Its Cities* (Johns Hopkins University Press). <https://doi.org/10.1353/book.338>.
58. Abulafia, D. (2011). *The Great Sea: a Human History of the Mediterranean* (Oxford University Press). <https://doi.org/10.1093/oso/9780195323344.001.0001>.
59. Alvar-Ezquerro, J. (2007). *Entre Fenicios y Visigodos: la Historia Antigua de la Península Ibérica* (La Esfera de los Libros).
60. Leslie, S., Winney, B., Hellenthal, G., Davison, D., Boumertit, A., Day, T., Hutnik, K., Royrvik, E.C., Cunliffe, B., and Wellcome Trust Case Control Consortium 2, and et al. (2015). The fine-scale genetic structure of the British population. *Nature* 519, 309–314. <https://doi.org/10.1038/nature14230>.
61. Reimer, P.J., Austin, W.E.N., Bard, E., Bayliss, A., Blackwell, P.G., Bronk Ramsey, C.B., Butzin, M., Cheng, H., Edwards, R.L., Friedrich, M., et al. (2020). The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* 62, 725–757. <https://doi.org/10.1017/RDC.2020.41>.
62. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
63. Daley, T., and Smith, A.D. (2013). Predicting the molecular complexity of sequencing libraries. *Nat. Methods* 10, 325–327. <https://doi.org/10.1038/nmeth.2375>.
64. Fu, Q., Posth, C., Hajdinjak, M., Petr, M., Mallick, S., Fernandes, D., Furtwängler, A., Haak, W., Meyer, M., Mittnik, A., et al. (2016). The genetic history of Ice Age Europe. *Nature* 534, 200–205. <https://doi.org/10.1038/nature17993>.
65. Korneliussen, T.S., Albrechtsen, A., and Nielsen, R. (2014). ANGSD: analysis of next generation sequencing data. *BMC Bioinform.* 15, 356. <https://doi.org/10.1186/s12859-014-0356-4>.
66. Schönherr, S., Weissensteiner, H., Kronenberg, F., and Forer, L. (2023). Haplogrep 3 - An interactive haplogroup classification and analysis platform. *Nucleic Acids Res.* 51, W263–W268. <https://doi.org/10.1093/nar/gkad284>.
67. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup (2009). The sequence alignment/map format and SAMtools. *Bioinformatics* 25, 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
68. Alaçamlı, E., Naidoo, T., Güler, M.N., Sağlıcan, E., Aktürk, Ş., Mapelli, I., Vural, K.B., Somel, M., Malmström, H., and Günther, T. (2024). READv2: Advanced and user-friendly detection of biological relatedness in archaeogenomics. *Genome Biol.* 25, 216. <https://doi.org/10.1186/s13059-024-03350-3>.
69. Popli, D., Peyrégne, S., and Peter, B.M. (2023). KIN: a method to infer relatedness from low-coverage ancient DNA. *Genome Biol.* 24, 10. <https://doi.org/10.1186/s13059-023-02847-7>.
70. Rubinacci, S., Ribeiro, D.M., Hofmeister, R.J., and Delaneau, O. (2021). Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nat. Genet.* 53, 120–126. <https://doi.org/10.1038/s41588-020-00756-0>.
71. Ringbauer, H., Huang, Y., Akbari, A., Mallick, S., Olalde, I., Patterson, N., and Reich, D. (2024). Accurate detection of identity-by-descent segments in human ancient DNA. *Nat. Genet.* 56, 143–151. <https://doi.org/10.1038/s41588-023-01582-w>.
72. Huang, Y., and Ringbauer, H. (2022). hapCon: estimating contamination of ancient genomes by copying from reference haplotypes. *Bioinformatics* 38, 3768–3777. <https://doi.org/10.1093/bioinformatics/btac390>.

73. Ringbauer, H., Novembre, J., and Steinrücken, M. (2021). Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nat. Commun.* 12, 5425. <https://doi.org/10.1038/s41467-021-25289-w>.
74. Patterson, N., Price, A.L., and Reich, D. (2006). Population structure and eigenanalysis. *PLOS Genet.* 2, e190. <https://doi.org/10.1371/journal.pgen.0020190>.
75. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., and Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* 38, 904–909. <https://doi.org/10.1038/ng1847>.
76. Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., and Reich, D. (2012). Ancient admixture in human history. *Genetics* 192, 1065–1093. <https://doi.org/10.1534/genetics.112.145037>.
77. Narasimhan, V.M., Patterson, N., Moorjani, P., Rohland, N., Bernardos, R., Mallick, S., Lazaridis, I., Nakatsuka, N., Olalde, I., Lipson, M., et al. (2019). The formation of human populations in South and Central Asia. *Science* 365, eaat7487. <https://doi.org/10.1126/science.aat7487>.
78. Adler, C.J., Haak, W., Donlon, D., and Cooper, A.; Genographic Consortium (2011). Survival and recovery of DNA from ancient teeth and bones. *J. Archaeol. Sci.* 38, 956–964. <https://doi.org/10.1016/j.jas.2010.11.010>.
79. Pinhasi, R., Fernandes, D.M., Sirak, K., and Cheronet, O. (2019). Isolating the human cochlea to generate bone powder for ancient DNA analysis. *Nat. Protoc.* 14, 1194–1205. <https://doi.org/10.1038/s41596-019-0137-7>.
80. Rohland, N., Glocke, I., Aximu-Petri, A., and Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat. Protoc.* 13, 2447–2461. <https://doi.org/10.1038/s41596-018-0050-5>.
81. Dabney, J., Meyer, M., and Pääbo, S. (2013). Ancient DNA damage. *Cold Spring Harb. Perspect. Biol.* 5, a012567. <https://doi.org/10.1101/cshperspect.a012567>.
82. Korlević, P., Gerber, T., Gansauge, M.-T., Hajdinjak, M., Nagel, S., Aximu-Petri, A., and Meyer, M. (2015). Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. *BioTechniques* 59, 87–93. <https://doi.org/10.2144/000114320>.
83. Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., et al. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. USA* 110, 15758–15763. <https://doi.org/10.1073/pnas.1314445110>.
84. Briggs, A.W., Stenzel, U., Meyer, M., Krause, J., Kircher, M., and Pääbo, S. (2009). Removal of deaminated cytosines and detection of in vivo methylation in ancient DNA. *Nucleic Acids Res.* 38, e87. <https://doi.org/10.1093/nar/gkp1163>.
85. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., and Reich, D. (2015). Partial uracil – DNA – glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond., B Biol. Sci.* 370, 20130624. <https://doi.org/10.1098/rstb.2013.0624>.
86. Kircher, M., Sawyer, S., and Meyer, M. (2012). Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* 40, e3. <https://doi.org/10.1093/nar/gkr771>.
87. Fowler, C., Olalde, I., Cummings, V., Armit, I., Büster, L., Cuthbert, S., Rohland, N., Cheronet, O., Pinhasi, R., and Reich, D. (2022). A high-resolution picture of kinship practices in an Early Neolithic tomb. *Nature* 601, 584–587. <https://doi.org/10.1038/s41586-021-04241-4>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
243 newly reported ancient individuals	This paper	Table S1A
Chemicals, peptides, and recombinant proteins		
2× HI-RPM hybridization buffer	Agilent Technologies	5190-0403
Herculase II Fusion DNA Polymerase	Agilent Technologies	600679
Pfu Turbo Cx Hotstart DNA Polymerase	Agilent Technologies	600412
50% PEG 8000	Anatrace	OPTIMIZE-82 100 ML
0.5 M EDTA pH 8.0	BioExpress	E177
Sera-Mag™ SpeedBead Carboxylate-Modified [E3] Magnetic Particles	Cytiva Life Sciences	6.51521E+13
0.05 M HNO3 (Optima)	Fisher Chemical	Cat# A467-2
6 M HCL (TraceMetal Grade)	Fisher Chemical	Cat# A508-4
7 M HNO3 (Optima)	Fisher Chemical	Cat# A467-2
Acetic Acid, Glacial (TraceMetal Grade)	Fisher Chemical	Cat# A507-P212
Silica Magnetic Beads	G-Biosciences	786–916
0.1 M CH3COOH (GR ACS Grade)	Millipore Sigma	Cat# AX0073-6
2% Sodium Hypochlorite Solution	Millipore Sigma	Cat# XX0637-76
30% H2O2 (GR ACS Grade)	Millipore Sigma	Cat# HX0635-2
Bst DNA Polymerase2.0, large frag.	New England Biolabs	M0537
UGI	New England Biolabs	M0281
USER enzyme	New England Biolabs	M5505
Buffer PB	QIAGEN	19066
Buffer PE concentrate	QIAGEN	19065
1 M NaOH	Sigma	71463
1 M Tris-HCl pH 8.0	Sigma Aldrich	AM9856
20% SDS	Sigma Aldrich	5030
3 M Sodium Acetate (pH 5.2)	Sigma Aldrich	S7899
5 M NaCl	Sigma Aldrich	S5150
Ethanol	Sigma Aldrich	E7023
Guanidine hydrochloride	Sigma Aldrich	G3272
Isopropanol	Sigma Aldrich	650447
PEG-8000	Sigma Aldrich	89510
Proteinase K	Sigma Aldrich	P6556
Tween-20	Sigma Aldrich	P9416
Water	Sigma Aldrich	W4502
10× Buffer Tango	Thermo Fisher Scientific	BY5
50× Denhardt's solution	Thermo Fisher Scientific	750018
AccuPrime Pfx Polymerase (2.5 U/ul)	Thermo Fisher Scientific	12344032
ATP	Thermo Fisher Scientific	R0441
dNTP Mix	Thermo Fisher Scientific	R1121
Dyna MyOne Streptavidin C1 beads	Thermo Fisher Scientific	65002
GeneAmp 10× PCR Gold Buffer	Thermo Fisher Scientific	4379874
Human Cot-I DNA	Thermo Fisher Scientific	15279011
Klenow Fragment (10 U/μL)	Thermo Fisher Scientific	EP0052

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Maxima Probe qPCR 2xMM	Thermo Fisher Scientific	K0233
Maxima SYBR Green kit	Thermo Fisher Scientific	K0251
Maxima SYBR Green kit	Thermo Fisher Scientific	K0253
Salmon sperm DNA	Thermo Fisher Scientific	15632-011
SSC Buffer (20×)	Thermo Fisher Scientific	AM9770
T4 DNA Ligase	Thermo Fisher Scientific	EL0012
T4 DNA Ligase, HC (30U/μL)	Thermo Fisher Scientific	EL0013
T4 DNA Polymerase	Thermo Fisher Scientific	EP0062
T4 Polynucleotide Kinase	Thermo Fisher Scientific	EK0032

Critical commercial assays

HiSeq X Ten Reagent Kit v2.5	Illumina	FC-501-2521
NextSeq 500/550 High Output Kit v2.5	Illumina	Cat# 20024906
Twist Human Ancient DNA Panel	Twist Bioscience	SKU: 107479

Deposited data

Sequencing data from 243 newly reported ancient individuals	This paper	ENA: PRJEB96929
Isotopic data of 35 newly reported ancient individuals	This paper	Table S5A
Genotype data from 243 newly reported ancient individuals	This paper	https://reich.hms.harvard.edu/datasets

Software and algorithms

OxCal v4.4.4	Reimer et al. ⁶¹	https://c14.arch.ox.ac.uk/oxcal.html
SeqPrep 1.1	St. John, J. (https://github.com/jstjohn/SeqPrep)	https://github.com/jstjohn/SeqPrep
Bwa 0.6.1	Li and Durbin ⁶²	https://github.com/lh3/BWA
Preseq	Daley and Smith ⁶³	https://github.com/smithlabcode/preseq
ContamMix-1.0.10	Fu et al. ⁶⁴	https://anaconda.org/channels/bioconda/packages/contammix/overview
ANGSD	Korneliussen et al. ⁶⁵	https://github.com/ANGSD/angsd
HaploGrep3	Schönherr et al. ⁶⁶	https://haplogrep.i-med.ac.at/
bcftools	Li et al. ⁶⁷	https://github.com/samtools/bcftools
SAMTools	Li et al. ⁶⁷	https://github.com/samtools/bcftools
READv2	Alaçamlı et al. ⁶⁸	https://github.com/GuntherLab/READv2
KIN	Popli et al. ⁶⁹	https://github.com/DivyaranPopli/Kinship_Inference
GLIMPSE2	Rubinacci et al. ⁷⁰	https://github.com/odelaneau/GLIMPSE
ancIBD	Ringbauer et al. ⁷¹	https://github.com/hringbauer/ancIBD
hapRoh/hapCon	Huang and Ringbauer ⁷² ; Ringbauer et al. ⁷³	https://github.com/hringbauer/hapROH
EIGENSOFT (version 7.2.1).	Price et al. ⁷⁴ ; Patterson et al. ⁷⁵	https://github.com/DReichLab/EIG
ADMIXTOOLS v.6.0	Patterson et al. ⁷⁶	https://github.com/dReichLab/AdmixTools
DATES	Narasimhan et al. ⁷⁷	https://github.com/priyamoorjani/DATES

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ancient Individuals

This study analyzed skeletal remains from 255 ancient individuals (Data S1, section 2) excavated from 31 archaeological sites across the Iberian Peninsula (present-day Spain) spanning approximately 100–800 CE, encompassing the Roman Imperial and Late Antique/Early Medieval periods. The sampled individuals represent a broad geographic distribution across four major regions of Iberia (eastern, central, southern, and northwestern) and derive from a wide range of archaeological contexts, including urban

cemeteries, rural settlements, *villae*, basilicas, and funerary necropolises. Archaeological dating was based on stratigraphy, associated material culture, numismatic evidence, and, where available, direct AMS radiocarbon dating.

243 individuals yielded authentic good-quality ancient DNA and constitute the genetically characterized subset (Table S1A).

Both genetic males ($n = 122$) and females ($n = 121$) are represented. Biological sex was determined genetically and compared, when available, with osteological assessments. Individuals comprise predominantly adults ($n = 158$), although infants ($n = 7$), juveniles ($n = 10$) and subadults ($n = 12$) are also included where preservation permitted genomic analysis. Anthropological assessment was not possible for 68 individuals because of insufficient skeletal preservation.

Detailed archaeological descriptions, osteological information, sampled skeletal elements, calibrated dates, genetic sex, mitochondrial and Y-chromosome haplogroups, sequencing statistics, contamination estimates, and ancestry assignments for every individual are provided in Data S1, section 2, and Table S1A.

Sampling Permission and Research Oversight

All archaeological material analyzed in this study was sampled with the permission and under the supervision of the institutions responsible for the curation and protection of the remains. Sampling procedures and subsequent analyses were conducted in collaboration with the relevant archaeological institutions, curators, and researchers responsible for each collection, who provided oversight of the research and contributed their expertise and contextual information.

The institutions granting permissions and overseeing access to the archaeological material include: Archaeological Museum of Badajoz (Badajoz, Spain); Ayuntamiento de Estepona (Estepona, Spain); Ayuntamiento de Pízarra (Málaga, Spain); Institut Català de Paleoecologia Humana i Evolució Social (Tarragona, Spain); Museo Arqueológico Nacional (Madrid, Spain); Museo Arqueológico y Etnológico de Lucena (Lucena, Spain); Museo Arqueológico de Ronda (Ronda, Málaga, Spain); Museo de Guadalajara (Guadalajara, Spain); Museo de Zaragoza (Zaragoza, Spain); Universidad Autónoma de Madrid (Madrid, Spain); Universidad Complutense de Madrid (Madrid, Spain); Ministerio de Cultura (Madrid, Spain); Universidad Isabel I (Burgos, Spain); Universidad de Granada (Granada, Spain); Universidad de Málaga (Málaga, Spain); Universitat Autònoma de Barcelona (Barcelona, Spain); Universitat Rovira i Virgili (Tarragona, Spain); Universitat de Barcelona (Barcelona, Spain); Universitat de Girona (Girona, Spain); and University of the Basque Country UPV/EHU (Vitoria-Gasteiz, Spain). The responsible contact person(s) for each archaeological site and associated collection are provided in the corresponding entries for each archaeological site in Data S1, section 2.

Study design and data inclusion criteria: Sample size was determined by the archaeological material available for intrusive/destructive sampling and the preservation of endogenous DNA rather than by a prospective statistical power calculation.

Formal randomization was not applicable because sample selection was constrained by the archaeological material available for intrusive/destructive sampling, preservation, chronology, geographic coverage, and institutional permissions. Nevertheless, the sampling strategy aimed to capture the available diversity of archaeological contexts represented during the study period across different regions and archaeological site types of the Iberian Peninsula.

Ancient DNA authenticity and data robustness were evaluated using multiple independent quality-control procedures and criteria, described below.

As an initial step, exploratory data analysis (EDA) techniques were applied without incorporating archaeological, chronological, geographic, or other contextual information associated with the individuals. For further downstream analyses blinding was not applicable because their interpretation depended necessarily on archaeological, chronological, geographic, and contextual information associated with each individual.

For population-level statistical comparisons, first-degree relatives were excluded to minimize non-independence arising from the sampling of closely related individuals from the same archaeological contexts. Analyses specifically examining kinship or familial structure retained related individuals. The corresponding sample sizes are reported for each analysis.

Additional analysis-specific inclusion and exclusion criteria are described in the corresponding STAR Methods sections.

METHOD DETAILS

Ancient DNA Extraction

Of the selected 255 ancient individuals from the Iberian Peninsula (Data S1, section 2), 243 yielded authentic ancient DNA (Table S1A). All laboratory work prior to PCR amplification was performed in dedicated ancient DNA clean-room facilities physically isolated from post-PCR laboratories. To minimize exogenous DNA contamination, the external surfaces of teeth and long bones were mechanically removed. Bone and tooth powder was collected from the cleaned interior using low-speed drilling to minimize heat-induced DNA damage.⁷⁸ When available, cochleae were isolated from the petrous portion of the temporal bone by sandblasting followed by milling.⁷⁹

Approximately 30–100 mg of bone or tooth powder was incubated in lysis buffer containing EDTA, SDS, Tween, and proteinase K to facilitate demineralization, cell lysis, and protein digestion. DNA was purified using silica-based extraction methods⁸⁰ employing silica magnetic beads (Sera-Mag™ SpeedBead Carboxylate-Modified Magnetic Particles) following either manual or automated extraction protocols, with guanidine hydrochloride, sodium acetate, sodium chloride, ethanol, and isopropanol used as components of the binding and washing procedures⁸¹.

For manual extractions, DNA binding was performed using Dabney Binding Buffer^{81–83} consisting of guanidine hydrochloride, isopropanol, and buffering salts that promote adsorption of short DNA fragments to silica. DNA-bound magnetic beads were washed using Buffer PB, Buffer PE, and ethanol to remove salts and inhibitors before DNA was eluted in molecular-grade water. Automated extractions followed the same silica-binding chemistry adapted for robotic liquid handling platforms.

Genomic Library Preparation

Double-stranded DNA libraries were prepared from ancient DNA extracts following previously established protocols.^{84,85} Between 6.2 and 8.4 mg of powder corresponding to the DNA extract was used as input for library preparation. DNA extracts were converted into double-stranded indexed libraries using truncated Illumina-compatible adapters. Adapter ligation was performed using T4 DNA ligase, T4 Polynucleotide Kinase, and T4 DNA Polymerase, with ATP, PEG 8000 and the corresponding T4 reaction buffers ([key resources table](#)).

DNA damage-derived uracil residues were partially removed through partial uracil-DNA-glycosylase (UDG) treatment prior to blunt-end repair. Libraries were treated with USER enzyme and uracil-DNA-glycosylase inhibitor (UGI) to remove a proportion of uracil residues while retaining terminal damage patterns used for ancient DNA authentication. Adapter fill-in reactions were performed using Bst DNA Polymerase 2.0 (large fragment) and Klenow Fragment where required by the library preparation workflow.

Libraries were amplified by PCR using indexed primers and high-fidelity polymerases. Depending on library requirements and amplification strategy, PCR reactions were performed using Hercules II Fusion DNA Polymerase, Pfu Turbo Cx Hotstart DNA Polymerase, or AccuPrime Pfx DNA Polymerase. PCR reactions were prepared using commercially available PCR buffers and nucleotide mixtures, including GeneAmp 10× PCR Gold Buffer, 10× Buffer Tango, and dNTP Mix.

Library amplification success and concentration were assessed by quantitative PCR using Maxima SYBR Green qPCR Master Mix and Maxima Probe qPCR Master Mix. Amplified libraries were subsequently pooled for downstream in-solution hybridization capture.

In-solution Hybridization Capture of Genomic Libraries

Indexed libraries were enriched for human DNA using in-solution hybridization capture targeting 1,233,013 SNPs (“1240k capture”) or 1,352,535 SNPs (“Twist” BioSciences), as well as the mitochondrial genome. Hybridization reactions used 2× HI-RPM hybridization buffer, human Cot-I DNA, salmon sperm DNA, and 50× Denhardt’s solution to facilitate probe hybridization and suppress nonspecific binding.^{17,19}

Libraries enriched with the 1240k reagent underwent two successive rounds of hybridization capture to maximize target enrichment,¹⁷ whereas libraries prepared using the Twist Human Ancient DNA Panel underwent a single round of capture¹⁹. Captured DNA was recovered using Dyna MyOne Streptavidin C1 magnetic beads and washed using SSC buffer and related wash reagents.

High-throughput sequencing

Enriched DNA libraries were pooled according to library concentration and sequenced on an Illumina HiSeq X Ten instrument using the HiSeq X Ten Reagent Kit v2.5, generating 2 × 101 bp paired-end reads, with 2 × 7 cycles used to read the two index sequences.⁸⁶ Libraries were also sequenced on an Illumina NextSeq 500 instrument using the NextSeq 500/550 High Output Kit v2.5, generating 2 × 76 bp paired-end reads and 2 × 7 cycles for the two index reads. Multiple sequencing lanes were used where required to approach library sequencing saturation.

Direct AMS ¹⁴C Dating

We generated 35 new radiocarbon dates ([Tables S1A](#) and [S5A](#)) selected from the newly reported individuals from bone or tooth specimens.

The radiocarbon dating was performed at specialized facilities at the Pennsylvania State accelerator mass spectrometry radiocarbon laboratory (PSUAMS identifier in [Tables S1A](#) and [S5A](#)); CIRAM (France) radiocarbon laboratory (CIRAM identifier in [Tables S1A](#) and [S5A](#)); and the University of Georgia Center for Applied Isotope Studies (UGAMS identifier in [Tables S1A](#) and [S5A](#)). Sample preparation and AMS measurement followed the established protocols of each facility. Bone or tooth samples were decontaminated and subjected to collagen extraction and purification before preparation for AMS measurement; pretreatment procedures included acid-based and oxidizing treatments. Radiocarbon (¹⁴C/¹²C) and stable carbon isotope (¹³C/¹²C) ratios were measured as part of the AMS procedure together with appropriate blanks and standards.

For newly dated individuals, the same skeletal element used for ancient DNA analysis was sampled for radiocarbon dating. Where available, previously published radiocarbon dates and dates recovered from the archaeological literature are also reported in [Table S1A](#). Radiocarbon ages were calibrated using OxCal v4.4.4 and the IntCal20 calibration curve.⁶¹

Radiocarbon dating was used to refine the chronology of individuals for whom archaeological dating was uncertain, to independently assess samples associated with potentially unexpected genetic affinities, and to provide chronological coverage across the sampled archaeological contexts ([Data S1](#), section 2).

Stable Isotope Analysis

Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope measurements were generated for newly reported individuals using analyses conducted at the Pennsylvania State University Accelerator Mass Spectrometry facility.

Isotopic measurements were obtained from bone or tooth samples associated with the individuals included in this study (STAR Methods Direct AMS ^{14}C Dating; Table S5A).

To contextualize the isotopic composition of the newly reported individuals, we compiled comparative $\delta^{13}\text{C}/\delta^{15}\text{N}$ data from published archaeological studies covering the Iberian Peninsula from the Iron Age period through the Early Middle Ages including C3 cereals, C4 plants, legumes, domestic herbivores and omnivores, and marine fish (Data S1, section 3). We plotted individual isotopic values against these reference ranges to evaluate broad dietary patterns and subsistence strategies at the archaeological sites investigated (Figure S1; Data S1, section 3).

Raw Genetic Data Bioinformatic Processing

Bioinformatic processing of the raw sequencing data and the downstream analyses described below were performed using high-performance computing (HPC) resources at Harvard University and Universitat Pompeu Fabra (UPF).

Reads for each sample were extracted from raw sequence data according to sample-specific indices introduced during wet-lab processing, allowing one mismatch (<https://github.com/dReichLab/adna-workflow>). Adapters were trimmed and paired-end reads were merged into single-ended sequences requiring a 15-base pair overlap (allowing one mismatch) using a modified version of SeqPrep 1.1 (<https://github.com/jstjohn/SeqPrep>; <https://github.com/dReichLab/adna-workflow>), which selected the highest quality base in the merged region. Unmerged reads were discarded prior to alignment to both the human reference genome (hg19) and the RSRS mitochondrial reference genome using the ‘aln’ and ‘samse’ commands in bwa⁶² (version 0.6.1). PCR duplicates were removed based on alignment coordinates and read orientation. Libraries were sequenced to saturation across multiple lanes when necessary, and library complexity was assessed using *preseq*,⁶³ with merging performed where appropriate.

Computational pipelines are available on GitHub (<https://github.com/dReichLab/ADNA-Tools>; <https://github.com/dReichLab/adna-workflow>).

Authentication of ancient DNA genomic data

Authenticity of ancient DNA was assessed by:

- 1) Terminal nucleotide deamination: libraries with terminal deamination rates below 3% were excluded.
- 2) X-to-Y chromosome reads ratio: libraries were characterized as potentially contaminated if their X-to-Y chromosome reads ratio was below 0.03 or above 0.35, and excluded from processing and analyses.
- 3) Mitochondrial contamination with *contamMix*⁶⁴: Libraries with an upper bound threshold of the 95% confidence interval exceeding 5% were excluded.
- 4) X-chromosome contamination in males with sufficient coverage using *ANGSD*⁶⁵: Libraries with a threshold of lower bound of the 95% confidence interval $>1.1\%$ were excluded from analyses.

Genotype files were produced by calling pseudo-haploid positions in the “1240k” SNP panel, retaining libraries with $\geq 15,000$ SNPs for confident whole-genome analysis (<https://github.com/dReichLab/adna-workflow>).

QUANTIFICATION AND STATISTICAL ANALYSIS

Mitochondrial and Y-Chromosome Haplogroup Assignment

Mitochondrial haplogroups were determined using reads mapped to the mitochondrial reference genome, retaining reads with mapping quality (MAPQ) ≥ 30 and base quality ≥ 30 . A consensus sequence was generated using *bcftools* and *SAMTools*⁶⁷ under a majority-rule approach with a minimum coverage of two. Haplogroups were assigned using *HaploGrep3*⁶⁶ based on Phylotree (mtDNA tree build 17, Forensic Update 1.2) (Table S1A).

Y-chromosome lineages in male individuals were determined by annotating the path of derived mutations²⁷ following the YFull v8.09 nomenclature. The haplogroup corresponding to the most derived mutation was additionally reported using International Society of Genetic Genealogy (ISOGG, version 15.73) nomenclature. Low-coverage assignments were manually inspected by visualizing alignments at haplogroup-informative positions using IGV.

Kinship Inference

*READv2*⁶⁸ was applied to infer relationships up to the second degree, including in low-coverage individuals. To ensure independence from within-population diversity, we normalized the proportion of non-matching alleles (P0) prior to classification, using default window size and median pairwise P0 settings. Pairwise comparisons were performed across the 1) full dataset and 2) within broad chronological (Roman and post-Roman) and geographic (Northern and Southern Iberia) subsets to account for ancestry heterogeneity. We considered pairs with $>2,000$ overlapping SNPs as reliable for inference.

*KIN*⁶⁹ was used to determine the specific nature of first-degree relationships, including the Vilanova–Blanes pair, which was inferred to represent full siblings. To control for ancestry heterogeneity, we repeated analyses including individuals with similar

ancestry profiles. We further assessed robustness by down-sampling individuals from the Hazleton North pedigree⁸⁷ to comparable coverage levels, confirming accurate first-degree relationship classification at the coverage observed in our dataset.

For higher-coverage individuals (>0.75x coverage for in-solution hybridization capture data⁷¹) we additionally computed shared IBD segments using imputed genome-wide data.

Imputed genomes were generated using GLIMPSE2⁷⁰ with default parameters, using haplotypes in the 1000 Genome Project as the reference panel⁵⁰; as described by Ringbauer et al.⁷¹

We called Identical by Descent (IBD) segments between pairs of individuals using anclBD,⁷¹ using default parameters and genotype likelihoods of the 1240k panel positions as described by Ringbauer et al.⁷¹

Aneuploidy and Runs of Homozygosity Analysis

We calculated possible aneuploidies by computing the mean coverage at the 1240k SNP array of each chromosome divided by the mean coverage at 1240k array autosomal SNPs. Assuming a normal ploidy this ratio should be around 1 for the autosomes.

For the sex chromosomes: values of 1 for females and 0.5 for males at the X chromosome were assumed if no aneuploidies are present, and 0 for females and 0.5 for males for the Y chromosome if no aneuploidies are present. We identify no apparent aneuploidies in any of the newly reported individuals.

Runs of homozygosity (ROHs)

We assessed the possible presence of runs of homozygosity (ROHs) in the newly reported ancient individuals by applying the hap-ROH software (<https://github.com/hringbauer/hapROH>), with default parameters and thresholds as described in Ringbauer et al.^{72,73} We do not detect significant ROHs in any of the newly reported individuals of the dataset.

Principal Component Analysis (PCA)

We conducted Principal Component Analysis (PCA) with the “smartpca” tool in EIGENSOFT^{74,75} (v7.2.1). Ancient individuals were mapped onto present-day individuals’ calculated principal components using “lsqproject:YES” and “shrinkmode:YES”.

We computed 5 PCAs for the current analysis:

1) West Eurasian PCA (Figures 1C–1F; Data S1; Figure S4; higher PCs can be assessed in Data S1 and Figure S5), where PCs were computed on the HO dataset using 1,036 present-day West Eurasian individuals genotyped on the Human Origins array.⁷⁴ This PCA illustrates the genetic affinities of our samples to European, Levantine, and Middle Eastern populations.

2) North-Africa West Eurasia PCA (Data S1; Figure S3A), where PCs were computed using 1314 present-day West Eurasian and North African present-day individuals genotyped on the Human Origins array.⁷⁴

3) Sub-Saharan African PCA (Data S1; Figure S3B), where the PCs were computed using 1722 West Eurasian, North African and sub-Saharan African present-day individuals genotyped on the Human Origins array.⁷⁴ From this analysis, we infer that none of the newly reported individuals carries detectable Sub-Saharan African ancestry. This observation is in line with the f-statistics model exploration (see Data S1, section 5).

4) European HO PCA (Data S1; Figure S3C) where the PCs were computed using 883 present-day South and North European individuals genotyped on the Human Origins array.⁷⁴

5) 1000G PCA (Data S1; Figure S8C), where the PCs were computed using 309 individuals with European ancestry sequenced for the 1000 genome project. The population tags (in 1000G^{50,51}) were TSI, IBS and CEU.

Ancestry modeling of ancient individuals

Individual-level ancestry was inferred using qpAdm for 269 Roman and Late Antiquity individuals (newly reported and previously published) that had passed previously explained filters using qpAdm (ADMIXTOOLS⁷⁶ v6.0) on 1,233,013 SNPs shared between 1240k and Twist capture panels. We used allsnps: YES to maximize SNP overlap and, where possible, avoided genomic data deriving from shotgun sequencing technologies to minimize capture bias effects.

A set of diverse Bronze and Iron Age populations was used as outgroups spanning ancient Africa, Europe, and the Near East to differentiate Iberian, Eastern Mediterranean, North African, Central European and Steppe-related ancestry (Populations tagged: “mota”, “Germany_EBA_Unetice.AG”, “Greece_Mediterranean_BA”, “Iberia_EBA_Argar”, “Kazakhstan_Sarmatian_IA”, “Morocco_Iberomaurusian.AG” and “Turkey_Alalakh_MLBA.AG” in Table S1B; and described in depth in Data S1, section 5.1).

Models were tested iteratively for three-way, four-way, and nested models to resolve contributions from Iberian, Eastern Mediterranean, North African, Central Mediterranean, Central/Northern European, and more potential proxy sources from Late Antiquity chronologies. (Populations tagged: “CNE_EarlyMedieval”, “Iberia_IA”, “Italy_Lazio_Viterbo_Etruscan”, “Italy_Sicily_Punic_Roman”, “Netherlands_MBA_IA”, “North_Africa_Punic”, “Steppe_IA” and “WestAnatolia_Roman_Byzantine” in Table S1B; and described in depth in Data S1, section 5.)

To assess robustness in low-coverage individuals, and validate assignment among alternative European proxies (local Iberian, Central Mediterranean and Central/Northern European), we implemented two robustness checks:

1) Targeted f_4 affinity tests ($Z > |3|$ threshold) comparing each individual’s shared drift to the three alternative European sources (Data S1, section 5.4).

2) qpAdm source–outgroup rotation to assess model stability under proxy substitution (Data S1, section 5.4).

Ancestry modeling of present-day populations

We modeled present-day Iberian populations from the 1000 Genomes Project^{50,51} using the same qpAdm frameworks described previously for ancient individuals (STAR Methods “Ancestry Modeling of ancient individuals”, Data S1, sections 5.1–5.3). Individuals were grouped by administrative region as defined in the 1000 Genomes dataset (Table S4A; Data S1, section 5.5).

We evaluated model performance under different SNP filtering strategies. Specifically, we 1) pruned SNPs known to exhibit strong capture-related bias, retaining 620,466 SNPs,¹⁹ and 2) generated a randomly down-sampled dataset of equal size to control for loss of statistical power (Table S4A).

To further characterize genetic affinities between present-day Iberians and ancient or modern populations sourced from different geographical regions, focusing mostly in European and African populations (Tables S4B and S4C), we computed outgroup f_3 statistics using ADMIXTOOLS⁷⁶ (v.6.0). Tests were performed on the full 1240k SNP dataset (unpruned) to maximize overlap. To minimize bias, we restricted interpretation to tests with >600,000 overlapping SNPs.

Admixture Dating (DATES)

We applied DATES⁷⁷ (https://github.com/MoorjaniLab/DATES_v4010) to admixed individuals modeled with Iberian, Eastern Mediterranean, North African, and Central-Northern European sources (Tables S3A and S5B) to estimate admixture timing for each of the admixture events; grouping all admixed individuals (Table S3A) to test as a meta-population (Table S5B; individual models are commented in Data S1, section 5.6). We ran the tool with parameters: binsize = 0.001, maxdist = 1, jackknife=Yes, qbin=10, runfit=Yes, affit=Yes, and lovaft of 0.45; with a random seed.