

## ANTHROPOLOGY

# The origin and legacy of the Etruscans through a 2000-year archeogenomic time transect

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The origin, development, and legacy of the enigmatic Etruscan civilization from the central region of the Italian peninsula known as Etruria have been debated for centuries. Here we report a genomic time transect of 82 individuals spanning almost two millennia (800 BCE to 1000 CE) across Etruria and southern Italy. During the Iron Age, we detect a component of Indo-European-associated steppe ancestry and the lack of recent Anatolian-related admixture among the putative non-Indo-European-speaking Etruscans. Despite comprising diverse individuals of central European, northern African, and Near Eastern ancestry, the local gene pool is largely maintained across the first millennium BCE. This drastically changes during the Roman Imperial period where we report an abrupt population-wide shift to ~50% admixture with eastern Mediterranean ancestry. Last, we identify northern European components appearing in central Italy during the Early Middle Ages, which thus formed the genetic landscape of present-day Italian populations.

## INTRODUCTION

The Etruscan civilization occupied a large area of central Italy during the Iron Age, including the modern-day regions of Tuscany, Lazio, and Umbria, with local expansions into neighboring Italian regions throughout its existence (Fig. 1A). This culture is renowned for its outstanding skills in metallurgy, its sophisticated cultural representations, and its extinct language, a non-Indo-European language not yet fully understood (1, 2). Given the peculiarities distinguishing this culture from its contemporary neighbors, the geographical origins of populations associated with the Etruscan civilization have long been a topic of intense debate as far back as ancient times with two main competing hypotheses. The first proposes an Anatolian/Aegean origin as indicated by the ancient Greek writers Herodotus and Hellanicus of Lesbos. This hypothesis is supported by the presence of Ancient Greek cultural elements in Etruria during the so-called Orientalizing Period, between the eighth and sixth century BCE. The second advocates for an autochthonous development as described in the first century BCE by the historian Dionysius of Halicarnassus (3, 4). According to this hypothesis, the Etruscan population originated locally from people associated with the late Bronze Age (Proto-)Villanovan culture around 900 years BCE. While the current consensus among archeologists favors the latter hypothesis (1, 5, 6), the persistence of a probable non-Indo-European language isolate surrounded by Italic Indo-European-speaking groups (such as the Latins) is an intriguing and still unexplained phenomenon that requires further archeological, historical linguistic, and genetic investigations.

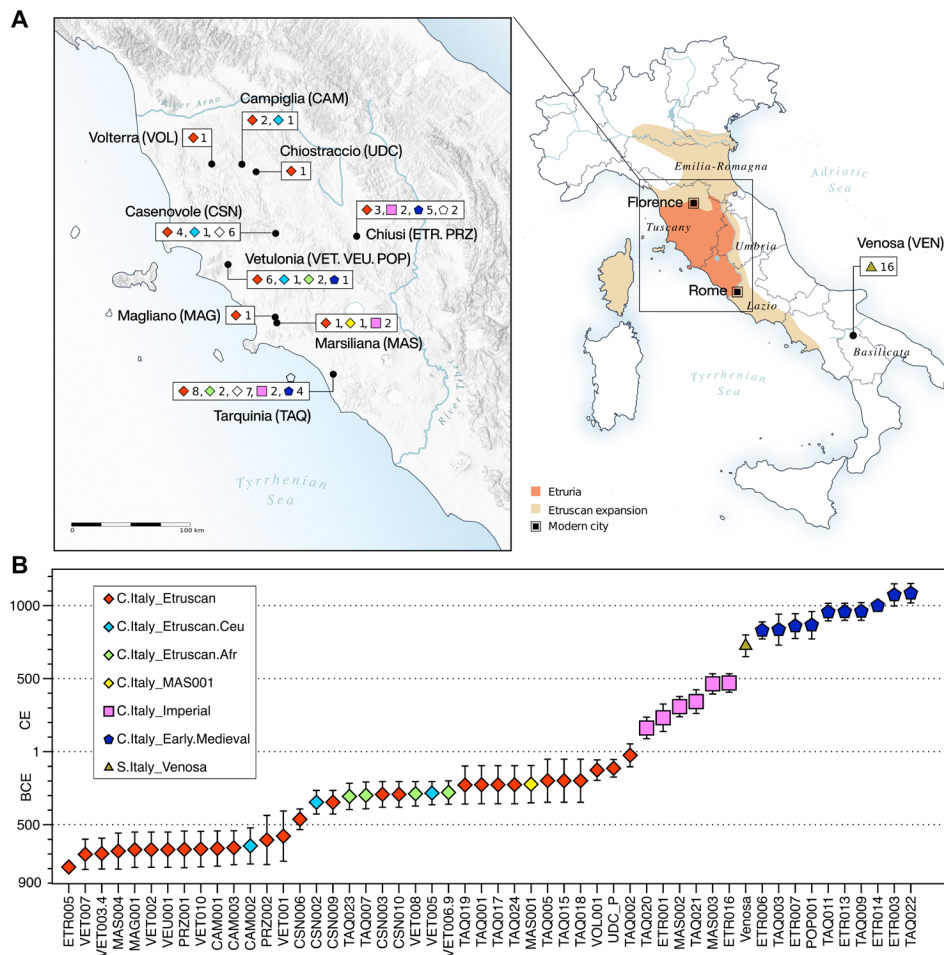
After more than four centuries of extensive regional development, in the fourth century BCE, the Etruscan civilization began to be assimilated into the Roman Republic through a series of Roman-Etruscan Wars, which ended in 264 BCE. Despite this period of change, Etruscan cultural and religious traditions endured through the subsequent centuries, even following the incorporation of Etruria into

the Regio VII territory of the Roman Empire after 27 BCE. During the Migration Period and after the collapse of the Western Roman Empire in the fifth century CE, this region was briefly incorporated into the Eastern Roman Empire. Subsequently, in the Early Medieval

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**Fig. 1. Geographic and temporal distribution of the newly produced dataset.** (A) Geographic map of the Italian peninsula (right) including a zoom-in (left) indicating the maximum extension of Etruscan territories and the location and number of individuals for each archeological site newly analyzed here (not filled symbols refer to individuals with no certain date). (B) Mean radiocarbon dates and 2-sigma intervals for each dated individual or samples genetically related to those individuals (four radiocarbon dates for S.Italy\_Venosa are grouped, reporting their lowest and highest 2-sigma values) (table S1, A and B).

period, large parts of the Italian peninsula were conquered by Germanic-related groups known as the Longobards (or Lombards). Established in the second half of the sixth century CE, the Longobard Kingdom and Duchies ruled in Italy for more than two centuries. They were succeeded in the north by the Carolingian Empire (774 CE), which later developed into the Holy Roman Empire.

The analysis of ancient DNA (aDNA) from individuals that lived during the above-mentioned periods could provide a direct means to investigate whether these historically documented events were associated with detectable changes in population-level ancestry patterns resulting from migration or high rates of mobility and the extent to which these shifts have influenced the contemporary genetic landscape of Italians. The potential contribution of genetic analysis to the study of Etruscan history has been discussed and explored over the past decade (7, 8). Present-day Tuscan mitochondrial DNAs (mtDNAs) demonstrate a relationship with current Anatolian populations that has been interpreted as evidence for a recent Near Eastern origin of the Etruscans (9, 10). By contrast, early studies on mtDNA from Etruscan-associated individuals did not find any evidence of

genetic continuity between Etruscans and present-day populations from the same region, except for some isolated locations in Tuscany (11–13). Ancient genomes from Italy are very limited, with only sparse data available from the Neolithic to the Roman Republic period across the entire Italian mainland (fig. S1) (14–18). Individuals from the ancient city of Rome and its surroundings during the Iron Age and Roman Republic (900 to 27 BCE) harbored the predominant genomic components that characterize most Europeans from the Bronze Age onward (15, 17, 19, 20). In addition, three individuals were found to carry recent genetic influences from Africa and the Near East, a further demonstration of Rome’s wide connections across the Mediterranean as far back as the Iron Age. Unexpectedly, almost all individuals from the later, Imperial period in the vicinity of the Empire’s capital carried large proportions of eastern Mediterranean ancestry, which was later reduced during the Late Antique and Early Medieval periods (17). However, the extent to which these changes are representative of the processes that occurred across the rest of the Italian peninsula remains to be clarified from individuals outside the megacity of Rome and its ancient metropolitan area.

In this study, we investigate the genetic history of Etruscan-associated individuals and subsequent groups in Etruria through an ancient genomic time transect of 70 individuals. These derive from 12 archeological sites, accompanied by 46 newly produced direct radiocarbon dates spanning almost two millennia, from 800 BCE to 1000 CE (Fig. 1B). In addition, we generated genome-wide data from 16 individuals radiocarbon dated to the eighth century CE from the “thermae of Venosa” archeological site in Basilicata (the date refers to the midpoint of the calibrated radiocarbon dates; table S1) to compare the genetic makeup of individuals in the Early Middle Ages in central and southern Italy (Fig. 1). Together, these data resolve key questions regarding the genetic origins of Etruscan-related groups and their relationships with other contemporary and later populations. In addition, we evaluate the genetic impact of historical events such as the establishment of the Roman Empire in Etruria, characterize the genetic makeup of Early Medieval individuals across central and southern portions of the Italian peninsula, and reveal the level of genetic continuity between these past cultures and present-day populations.

## RESULTS

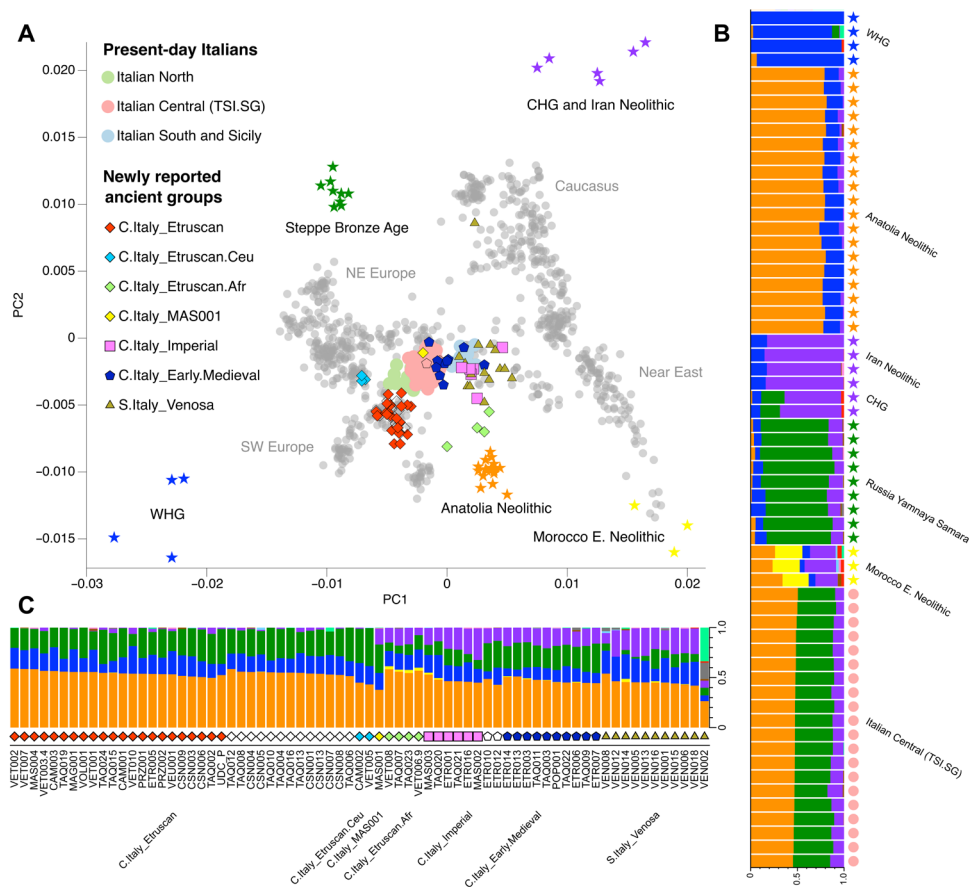
We extracted DNA from the petrous portion of the temporal bone and tooth samples of 86 individuals, created double-stranded DNA libraries, and used shotgun or an in-solution capture approach to retrieve the entire mitochondrial genome and up to ~1.24 million single-nucleotide polymorphisms (SNPs) across the human genome from each individual (table S1A and Materials and Methods). Following criteria for authentication, we restricted all downstream analyses to specimens with low nuclear and mtDNA contamination, typical aDNA damage patterns, and SNP counts above 31,000 (table S1A). These quality controls resulted in a final sample set of 82 individuals that were grouped on the basis of their radiocarbon dates and genetic affinities into three time intervals: 48 individuals from 800 to 1 BCE (Iron Age and Roman Republic), 6 individuals from 1 to 500 CE (Imperial period), and 28 individuals from 500 to 1000 CE (12 from central Italy and 16 from southern Italy). While we would have preferred to mirror conventional historical chronologies (Etruscan to ~300 BCE and Republican to 27 BCE), neither the large radiocarbon dating intervals nor the genetic results warrant a division of the dataset in this way (Fig. 2B and table S2F).

Of individuals associated with the first time interval, the vast majority (40 of 48) form a genetic cluster here named “C.Italy\_Etruscan” that overlaps with present-day Spanish individuals in a principal components analysis (PCA) built with West Eurasian populations from the Human Origins dataset (Fig. 2A) (21). Across this temporal interval (800 to 1 BCE), three groups of PCA outliers are identified, i.e., four individuals shifted toward northern African populations (C.Italy\_Etruscan.Afr), three individuals shifted toward central European populations (C.Italy\_Etruscan.Ceu), and one individual shifted toward Near Eastern populations (C.Italy\_Etruscan\_MAS001) (table S1A). To further inspect the genetic clustering of the central and southern Italian populations studied, we performed unsupervised ADMIXTURE on 71 individuals (Fig. 2, B and C) after the exclusion of genetically related individuals (table S1B and fig. S2). C.Italy\_Etruscan individuals harbor the three genetic ancestries associated with Anatolian Neolithic farmers, European hunter-gatherers, and Bronze Age pastoralists from the Pontic-Caspian Steppe. C.Italy\_Etruscan.Ceu carries a higher proportion of “steppe-related ancestry,” while

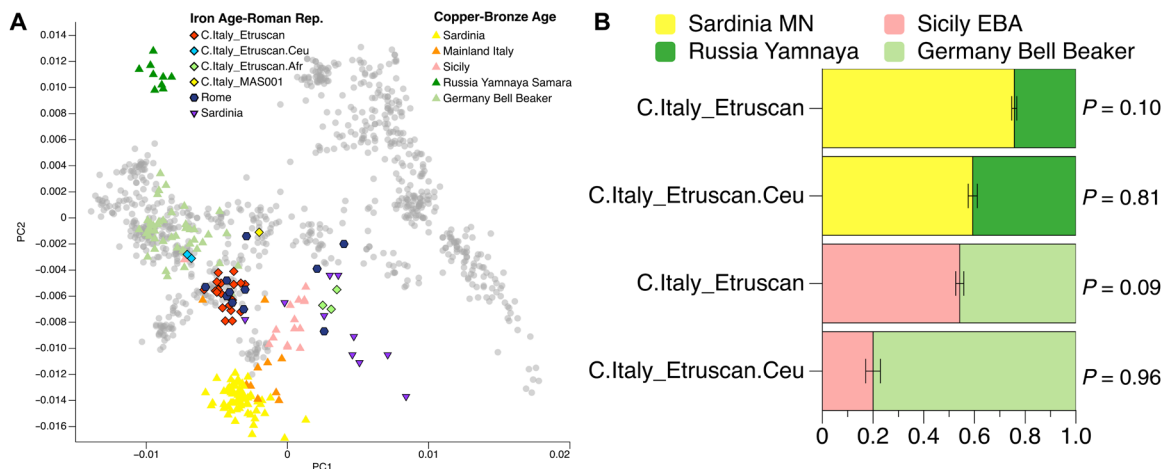
C.Italy\_MAS001 shows a genetic component maximized in Iranian Neolithic farmers. The latter is also present in C.Italy\_Etruscan.Afr individuals alongside an ancestry component identified in an Early Neolithic Moroccan group.

Since the spread of the steppe-related ancestry into Europe during the Late Neolithic and Early Bronze Age has been linked to the diffusion of Indo-European languages (15, 19) and is consistent with linguistic evidence for a “Steppe” homeland (22, 23), we attempted to formally estimate the proportion of steppe-related ancestry in Etruscan individuals who are putatively associated with the non-Indo-European Etruscan language. We first grouped 21 dated and genetically unrelated individuals from the C.Italy\_Etruscan cluster (Fig. 3A) and modeled them with qpAdm ( $P > 0.05$ ) as a mixture of steppe-related ancestry, represented by Bronze Age pastoralists from Samara in western Russia (Yamnaya), and Neolithic or Copper Age populations from Italy (table S4B). This analysis demonstrated around 25% ancestry from such a distal steppe-related source, which reached around 50% when comparative populations were reduced to those more proximate in time and space than the Yamnaya, e.g., central European Bell Beakers (Fig. 3B). Moreover, C.Italy\_Etruscan can be modeled successfully as having derived its entire ancestry from other European populations such as the earlier Bell Beaker group from northern Italy and Iron Age populations from southern Europe (Iberia, Croatia, and Greece) (table S4A). PCA reveals a complete overlap between Iron Age and Roman Republic individuals from Tuscany and Lazio, including the ancient city of Rome (17), indicating that substantial levels of steppe-related ancestry were widespread and homogenized in the multilingual context known to include both Indo-European (i.e., Italic and Celtic) and non-Indo-European (i.e., Etruscan) speakers across central Italy by the Iron Age.

The other contemporaneous ancestry groups identified in this study, albeit represented by small numbers of individuals, add detail and complexity to this picture. Two C.Italy\_Etruscan.Ceu unrelated individuals (VET005 and CAM002) are characterized by a higher proportion of Yamnaya-related ancestry (40%) and are consistent with deriving from those Chalcolithic or Bronze Age European populations from both central and southern Europe that carried a high fraction of steppe-related ancestry (Fig. 3, A and B, and table S4, A and B). This signal is confirmed by the  $f_4$ -statistic  $f_4(\text{Onge}, \text{Test}; \text{C.Italy_Etruscan.Ceu}, \text{C.Italy_Etruscan})$  that is significantly negative ( $z$  score  $> |3|$ ) when the *Test* population comprises eastern hunter-gatherer (EHG) individuals representing about half of the Yamnaya-related ancestry (table S2C) (19). This indicates higher affinity of EHG to C.Italy\_Etruscan.Ceu than to the main C.Italy\_Etruscan cluster, while the opposite is observed when the *Test* population is restricted to southern European Neolithic groups without steppe-related ancestry. We then tested whether C.Italy\_Etruscan.Ceu carries a signature of admixture with local ancestry. Statistics of the form  $f_3(\text{C.Italy_Etruscan}, \text{Test}; \text{C.Italy_Etruscan.Ceu})$  do not reveal evidence of C.Italy\_Etruscan.Ceu resulting from admixture between C.Italy\_Etruscan and any of the 255 ancient *Test* populations in our dataset (table S3A). Moreover, while it is possible to model C.Italy\_Etruscan.Ceu in qpAdm as a mixture between the Etruscan-related individuals and central European Bell Beakers (Fig. 3B), the model still holds when C.Italy\_Etruscan is moved to the reference set and is replaced by Neolithic populations, consistent with an incoming northern ancestry that did not admix locally (table S4C) (24). Last, we estimated the admixture dates for two C.Italy\_Etruscan.Ceu individuals using the software



**Fig. 2. Genetic map and clustering of ancient and present-day populations.** (A) PCA inferred from genomic variation of 60 West Eurasian populations genotyped on the Human Origins array (gray dots with geographic regions labeled in gray) including present-day Italians (circles without outline) onto which we projected the newly reported ancient individuals (symbols with outline, not filled for nonradiocarbon-dated individuals) and comparative ancient populations (stars without outline). (B) Unsupervised admixture ( $K = 11$ ) of comparative ancient individuals and 20 present-day central Italians (TSI.SG). (C) Unsupervised admixture ( $K = 11$ ) of newly reported ancient individuals excluding genetically related individuals. WHG and CHG refer to Western and Caucasus hunter-gatherers, while SW and NE Europe refer to southwestern and northeastern Europe, respectively.



**Fig. 3. Genetic placement and modeling of Iron Age and Roman Republic individuals.** (A) PCA of Italian individuals from the Iron Age and Roman Republic (symbols with outline) plus Copper and Bronze Age individuals from Italy and other source populations (symbols without outline) used in qpAdm. Gray dots represent present-day West Eurasian individuals (see Fig. 2A). (B) Proportion of steppe-related ancestry in C.Italy\_Etruscan and C.Italy\_Etruscan.Ceu as estimated with qpAdm (table S4B). MN and EBA refer to Middle Neolithic and Early Bronze Age, respectively.



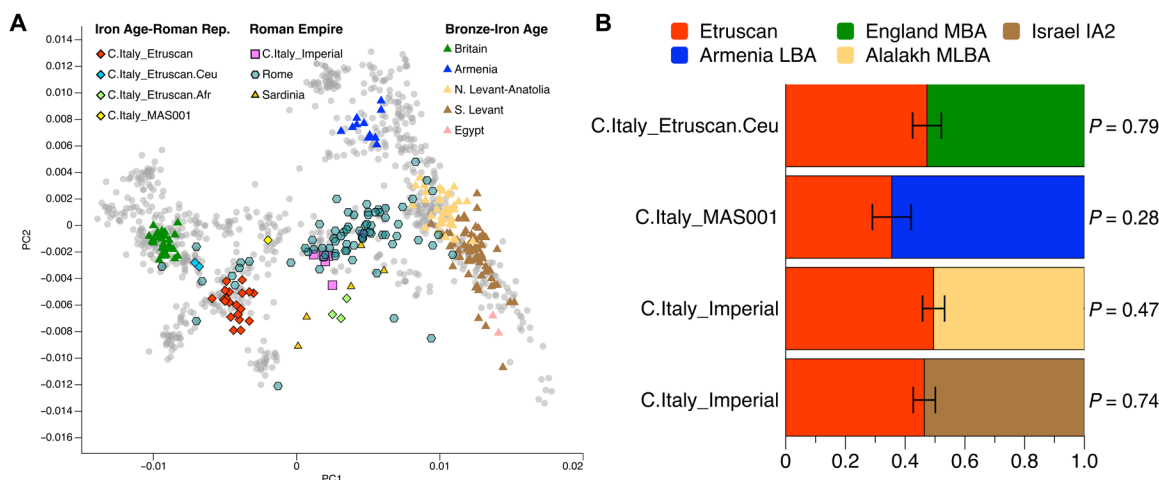
DATES (25). Individual VET005 resulted in an unfeasible date (negative value), whereas CAM002 provided an admixture date of  $19.7 \pm 8.6$  generations, which corresponds to  $572 \pm 249$  years before the individual's date (seventh century BCE). These results suggest that the two tested C.Italy\_Etruscan.Ceu individuals represent an incoming ancestry that is not the result of recent local admixture. Those individuals originate from two different archeological sites and are radiocarbon dated to the seventh and third century BCE, respectively. Therefore, rather than persisting across several centuries, this distinct ancestry profile might have arrived independently from northern regions into central Italy multiple times.

The three individuals grouped in C.Italy\_Etruscan.Afr—after exclusion of a genetic outlier (Materials and Methods, Fig. 4A, and table S2A)—cannot be modeled as a mixture between Neolithic-related and Bronze Age-related European ancestries (table S4B). These individuals are dated to around 300 BCE and were excavated at two archeological sites separated by a distance greater than 100 km (Tarquinia and Vetulonia). Statistics in the form  $f_3$ (C.Italy\_Etruscan, X; C.Italy\_Etruscan.Afr) and  $f_4$ (Onge, X, C.Italy\_Etruscan, C.Italy\_Etruscan.Afr) show evidence of C.Italy\_Etruscan.Afr being a mixture of Etruscans and ancient or modern-day individuals (as a proxy) carrying high proportions of north African or sub-Saharan ancestries (tables S2, B and C, and S3, A and B). Using qpAdm, all admixture models including C.Italy\_Etruscan as one of the sources are rejected (table S4D). However, given the limited availability of genomes from northern Africa with comparable ages (26), we caution that ancestry proportions might be more correctly estimated as additional data from this region become available.

Contrary to previously reported findings from Bronze Age Sicily and Iron Age Sardinia (27, 28), we do not find evidence for Iranian-related ancestry in individuals from central Italy older than 2000 years (fig. S3). We were able to model C.Italy\_Etruscan and C.Italy\_Etruscan.Ceu as a mixture between three distal sources [Anatolia\_Neolithic, Western hunter-gatherers (WHG), and Yamnaya\_Samara] even when Neolithic Iranian individuals were placed in the reference set of qpAdm (table S4H). This suggests that

the genetic history of Sicilians and Sardinians during the Bronze and Iron Ages was substantially different from that of populations on the Italian mainland, as confirmed by the distinctive spheres of interaction observed in the archeological record (29). The C.Italy\_Etruscan\_MAS001 individual represents a single exception in our dataset showing a shift in PCA space toward Near Eastern populations ~200 BCE (Fig. 4A). While  $f$ -statistics do not significantly reject ancestry continuity with the C.Italy\_Etruscan cluster (table S2C), an admixture model between Neolithic- and steppe-related ancestries does not fit the genetic profile of this individual (table S4B). Instead, C.Italy\_Etruscan\_MAS001 can be modeled as a mixture between the C.Italy\_Etruscan cluster and populations from the Caucasus, such as Bronze Age Armenians (Fig. 4B), indicating the sporadic presence of Iranian-related ancestry in Etruria at least by the second century BCE.

During the first half of the first millennium CE, we observe a marked shift in PCA space of all studied individuals toward the Near Eastern cline (Fig. 4A), distributed across the genetic space occupied by present-day southeastern European populations. We grouped nonoutlier individuals dating between 1 and 500 CE into the “C.Italy\_Imperial” cluster (table S2A). Formal  $f_4$ -tests reveal its higher affinity than C.Italy\_Etruscan to ancient groups from Iran, Africa, and the Near East (table S2C). We then used qpAdm to quantify this group's ancestry components, where C.Italy\_Imperial was modeled as a mixture of the sources C.Italy\_Etruscan and 158 published European and Near Eastern genomes from the Bronze and Iron Ages. As a result, the models that were found to fit the data best are those with a 38 to 59% contribution from Levantine or Anatolian populations into the local/preexisting C.Italy\_Etruscan gene pool (Fig. 4B and table S4D). Substantial gene flow from the eastern Mediterranean was also reported in ancient individuals from Rome dated to the Imperial period (17). Despite our limited number of data points from the first five centuries CE, the new results suggest that the contribution of nonlocal ancestry in Rome was larger than in Etruria (Fig. 4A). However, this large-scale genetic impact of incoming groups during the Imperial period was not only limited to the metropolitan



**Fig. 4. Genetic placement and modeling of Iron Age and Imperial period individuals.** (A) PCA of Italian individuals from the Iron Age to the Imperial period (symbols with outline) plus Bronze and Iron Age individuals (symbols without outline) used as source populations in qpAdm. Gray dots represent present-day west Eurasian individuals (see Fig. 2A). (B) Proportion of C.Italy\_Etruscan ancestry in Iron Age outlier groups and in C.Italy\_Imperial as estimated with qpAdm. For each test, the reported non-C.Italy\_Etruscan population is the one providing a proportion that is closest to the median among all fitting models (table S4D). MBA, MLBA and LBA refer to Middle, Middle-Late and Late Bronze Age, respectively, and IA refers to Iron Age.

area around Rome but also extended into the neighboring and more distant regions considered here.

Regarding the last temporal interval of our ancient genomic transect (500 to 1000 CE), we observe that individuals grouped in the “C.Italy\_Early.Medieval” cluster are generally shifted toward central European groups compared to C.Italy\_Imperial and largely overlap with present-day populations from central Italy (TSI.SG) (Fig. 5A) (30). Using  $f_4$ -tests, we can show that this transition is confirmed by a reduced affinity of C.Italy\_Early.Medieval toward eastern Mediterranean populations compared to C.Italy\_Imperial (table S2D). Moreover, the C.Italy\_Early.Medieval cluster can be modeled successfully in qpAdm as a mixture between the preceding C.Italy\_Imperial group and Late Antique or Medieval groups from northern and eastern Europe (among the 59 populations tested) in estimated proportions of 60 to 90% and 10 to 40%, respectively (table S4E). Notably, among the best supported models are those that feature individuals associated with Longobard cemeteries from Hungary and northern Italy (31). If we specifically restrict the analyses to those Longobard-related individuals carrying unadmixed northern European genetic ancestry (Piedmont\_N.Longobard), then we obtain a ~20% contribution to the C.Italy\_Early.Medieval cluster (Fig. 5B). This finding is consistent with a genetic input of northern European ancestry in central Italy during the Longobard period. However, the influence of other Germanic tribes in Italy like the Ostrogoths could also have enhanced the observed genomic shift.

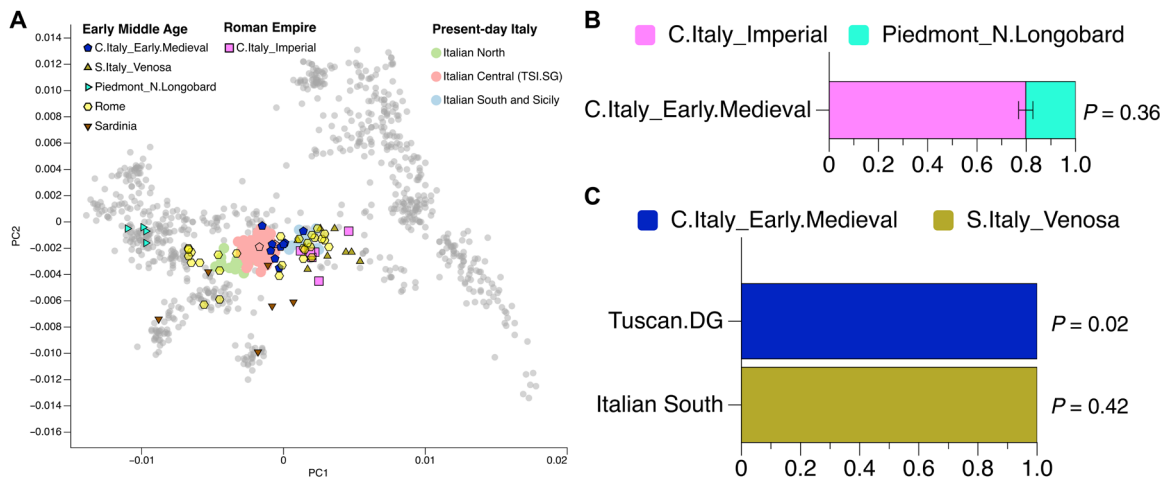
Since modern-day central Italians largely overlap in PCA space with C.Italy\_Early.Medieval individuals (Fig. 5A), we tested the consistency of the former group deriving from the latter. To enhance resolution, qpAdm was implemented with present-day worldwide populations in the reference set. No present-day Italian populations are consistent with deriving from the C.Italy\_Early.Medieval cluster ( $P$  values below 0.05), although high-coverage genomes from Tuscany (Tuscan.DG) (32) yielded no grounds for strong rejection of genetic continuity ( $P = 0.02$ ) (Fig. 5C and tables S2E and S4G). This suggests that the genetic makeup of present-day central Italian populations was largely formed at least by 1000 CE. To investigate whether an analogous picture is observed in contemporaneous individuals from southern Italy, data from the Early Medieval

archeological site of Venosa in Basilicata were similarly analyzed. With the exception of VEN002, all Venosa individuals (S.Italy\_Venosa) broadly overlap modern-day southern Italian populations in PC space and can be jointly modeled in qpAdm as deriving from the same stream of ancestry ( $P = 0.42$ ) (Fig. 5, A and C). In PCA space, most Medieval and Early Modern individuals from Rome fall in an intermediate position between Early Medieval groups from Tuscany and Basilicata (Fig. 5A). This distribution is thus consistent with the current north-south genetic cline that mirrors geography (33, 34) (fig. S4), with Italy bridging the genetic gap between Europe and the eastern Mediterranean.

To investigate the potential influence of sex biases in these genomic transformations, we computed the frequency of uniparental markers through time. The mtDNA diversity does not seem to change substantially before and after year 1 CE (fig. S5A). By contrast, the newly reported central Italian individuals from 800 to 1 BCE show ~75% frequency of the Y-chromosome haplogroup R1b, mostly represented by the R1b-P312 polymorphism and its derived R1b-L2, that diffused across Europe alongside steppe-related ancestry in association with the Bell Beaker complex (16). This suggests that this R1b Y-chromosome lineage spread into the Italian peninsula with steppe-related movements during the Bronze Age. In the first millennium CE, its frequency is reduced to ~40% with higher occurrence of Near Eastern-associated Y-chromosome lineages such as J (fig. S5B). While we cannot rule out substantial female mobility, the marked shift in Y-chromosome haplogroup frequency indicates that male mobility played an important role in the observed genetic turnovers from the Imperial period onward.

## DISCUSSION

Genomic analyses of 82 ancient individuals spanning ~2000 years of Italian history from Tuscany, Lazio, and Basilicata have revealed major episodes of genetic transformation. Across the first interval of our central Italian temporal transect (800 to 1 BCE), most individuals form a homogenous genetic cluster (C.Italy\_Etruscan), indicating that the sporadic presence of individuals with ancestry tracing back to other regions did not leave a substantial local genetic legacy. In



**Fig. 5. Genetic placement and modeling of Early Middle Age and present-day individuals.** (A) PCA of Italian individuals from the Imperial to the Early Medieval periods (symbols with outline) and present-day Italians (symbols without outline). Gray dots represent present-day west Eurasian individuals (see Fig. 2A). (B) Proportion of northern ancestry from Longobard-related individuals in C.Italy\_Early.Medieval as estimated with qpAdm (table S4E). (C) Proportion of C.Italy\_Early.Medieval and S.Italy\_Venosa ancestry in modern-day central Italians (Tuscan.DG) and southern Italians, respectively, as estimated with qpAdm (table S4G).

particular, and contrary to previous suggestions [see, e.g., (9, 10)], the Etruscan-related gene pool does not seem to have originated from recent population movements from the Near East. Etruscans carry a local genetic profile shared with other neighboring populations such as the Latins (table S2F) from Rome and its environs despite the cultural and linguistic differences between the two neighboring groups (17). A large proportion of the C.Italy\_Etruscan genetic profile can be attributed to steppe-related ancestry, confirming a trend observed in most other European regions, that this genetic component also reached central Italy during the Bronze Age (18) but earlier than it reached Sardinia (27, 35). Since it is estimated that the split between Proto-Italic and descendant languages (e.g., Latin, Oscan, and Umbrian) took place during the second millennium BCE (36), their presence in Italy cannot directly be correlated with the at least one millennium-older movement of Yamnaya-related groups out of the Steppe. Instead, with the assumption that incoming steppe-related groups were responsible for the initial arrival of Indo-European languages in Europe, more evolved forms of Indo-European, including Italic, may have spread across Italy at a later stage. The only three Beaker complex-related individuals sequenced so far from northern Italy dated to around 2000 BCE reveal a nonubiquitous presence of steppe-related ancestry, suggesting that the admixture process was ongoing (16). The historically documented persistence of the non-Indo-European Etruscan language in Etruria indicates that this speech community was maintained despite a large-scale admixture, a situation similar to the Basque region in Iberia where a non-Indo-European language endures today (24). This linguistic persistence, combined with a genetic turnover, challenges simplistic assumptions that genes equal languages and suggests a more complex scenario that may have involved the assimilation of (early) Italic speakers by the Etruscan speech community, possibly during a prolonged period of admixture over the second millennium BCE. This scenario is made plausible by the recent finding of steppe-related ancestry in central Italy as early as 1650 BCE followed by an increase of this component through time (18). Although Etruscan is considered a relict language, which survived in central Italy until the Imperial Period, it was not isolated. Instead, Etruscan seems to be linked to both Rhaetic, a language documented in the eastern Alps in a population that ancient historians claim to have migrated from the Po valley, and to Lemnian, a language putatively spoken on ancient Lemnos in the Aegean Sea. This leaves the question open as to whether these enigmatic “Tyrsenian” languages may somehow relate to sea-borne expansions from the eastern Mediterranean (37). However, the lack of Iranian-related ancestry in C.Italy\_Etruscan might also suggest that the close linguistic affinity across the Mediterranean Sea could represent population movements departing from the Italian peninsula.

The earliest individual in our dataset with a nonlocal genetic signature is radiocarbon dated to the seventh century BCE (CAM002) and exhibits a central European genetic profile. During the Early Iron Age, the Hallstatt culture associated with Celtic-related groups occupied the region north of the Alps. Although there is archeological evidence for exchange of goods and techniques between the Etruscan civilization and northern cultural groups from the eighth century BCE, extensive direct contacts are reported only later, during the La Tène cultural phase, when Celtic-related groups spread into northern Italy bordering Etruscan territories (38–40). In the presented dataset, we find another individual (VET005) radiocarbon dated to the third century BCE with a genetic profile that overlaps with individual CAM002, despite their ~400-year discrepancy in radiocarbon age.

This suggests continuity in the source of the central European genetic ancestry sporadically found in Etruria from the Hallstatt to the La Tène cultural periods.

In the last four centuries BCE, we identify a higher proportion than in the previous four centuries of individuals carrying nonlocal genetic ancestries, which show the greatest affinities to the Near East and northern Africa. This could be explained by increased interconnections between Etruria and other regions, not only for those societies associated with harbors but also for those in the hinterland. An emblematic case of such cross-continental connectivity is observed at the archeological site of San Germano in Vetulonia (VET), where even within the same tomb, there is a clear genetic transition from a local genetic profile in the eighth to sixth century BCE to central European- and north African-related ancestries in the fourth to third century BCE. During the latter period, a similar northern African genetic signal is observed in two other individuals from a distantly located site [Tarquinia (TAQ)]. While more data from this temporal horizon are needed to determine whether these findings represent a general phenomenon, it is possible that the arrival of this ancestry was influenced by the expansion of the Carthaginian Empire across the Mediterranean (41, 42).

The vast majority of individuals from the first millennium BCE, however, show large levels of genetic continuity for more than 800 years from the post-Villanovan period to the end of the Roman Republic. While in Etruria we do not detect increased affinity toward central European ancestry, we are unable to exclude the possibility that admixture took place across neighboring regions between populations with similar genetic profiles, such as with Latin-associated groups (table S2F). However, the marked genetic stability in Etruria over almost a millennium accords with the historical record, which describes its assimilation into the Roman Republic as a political rather than a demographic process, further evidenced by the maintenance of Etruscan culture and its language in the region for centuries (3).

By sharp contrast, all analyzed individuals from the Roman Imperial and Late Antique periods (1 to 500 CE) show a marked shift in ancestry toward populations of the eastern Mediterranean. While the strength of this shift might be influenced by the changing frequency of different burial practices—such as cremation and inhumation—among groups through time (43, 44), it clearly depicts the role of the Roman Empire in the large-scale displacement of people in a time of enhanced upward or downward socioeconomic and geographic mobility. In central Italy, including around Rome itself (17), the incoming ancestry detected so far mainly originated from the Near East rather than other areas of the Empire. The genetic replacement of ~50% of the preceding Etruscan-related gene pool was likely influenced by the movement of slaves and possibly soldiers, along with a larger pattern of human mobility from the eastern Mediterranean toward Italy (45–49). In the Roman Empire, citizenship was progressively extended to more classes of free people until the Edict of Caracalla in 212 made it universal among them, and expanding citizenship likely facilitated intermixture between local and other populations. Our new data from Etruria show that the influx of Near Eastern ancestry spread far beyond the greater capital region itself and suggest that this broader pattern of population movement may have affected larger portions of the Italian peninsula.

Continuing our genomic transect into the Early Middle Ages (500 to 1000 CE), we observe an additional genetic transition in some of the former Etruria territories through the spread of northern European-related ancestries. Admixture models are consistent with

this genetic component being introduced from previously published individuals associated with the Longobard culture, although other cultural groups may have contributed as well. Thus, settlers expanding across large parts of the Italian peninsula after the collapse of the Western Roman Empire and the establishment of the Longobard Kingdom might have left a traceable impact on the genetic landscape of central Italy. Last, our analyses identify broad population continuity between the Early Medieval times and today in the regions of Tuscany, Lazio, and Basilicata, suggesting that the main gene pool of present-day people from central and southern Italy was largely formed at least 1000 years ago.

In conclusion, our study sheds light on five main aspects of Italian population history. First, individuals associated with the Etruscan culture carried a high proportion of steppe-related ancestry, despite speaking a non-Indo-European language. If the Etruscan language was indeed a relict language that predated Bronze Age expansions, then it would represent one of the rare examples of language continuity despite extensive genetic discontinuity (24, 50). The steppe-related ancestry in Etruscans may have been mediated by Bronze Age Italic speakers, possibly through a prolonged admixture process resulting in a partial language shift. Second, after the Bronze Age admixture, the Etruscan-related gene pool remained generally homogeneous for almost 800 years, notwithstanding the sporadic presence of individuals of likely Near Eastern, northern African, and central European origins. Third, eastern Mediterranean ancestries replaced a large portion of the Etruscan-related genetic profile during the Roman Imperial period. Fourth, a substantial genetic input from northern European ancestries was introduced during the Early Middle Ages, possibly through the spread of Germanic tribes into the Italian peninsula. Fifth, the genetic makeup of present-day populations from central and southern Italy was mostly in place by the end of the first millennium CE.

Although a broader geographic analysis of aDNA across Italy would be needed to substantiate the above conclusions, the observation of extremely similar ancestry shifts in Tuscany and northern Lazio to those reported for the city of Rome and its surroundings implies that historical events during the first millennium CE determined large-scale genetic transformations over an extended portion of the Italian peninsula. An analogous turnover is observed in Iberia between Iron Age and modern-day populations (24). This suggests that the Roman Empire might have left a long-lasting demographic contribution to the genetic profile of southern Europeans, bridging the gap between European and Near Eastern populations on the genetic map of western Eurasia. Additional archeogenetic datasets from other regions of the Empire will be pivotal to better pinpoint the genetic origins of incoming groups and to discern regionally specific patterns of admixture.

## MATERIALS AND METHODS

### Archeological sampling

The archeological material analyzed in this project derives from different collection campaigns. All specimens were sampled with the approval of the appropriate institutions for the handling of archeological samples and/or in collaboration with local scientists and curators listed among the authors of this study. The set of samples from Chiusi (Siena) derives from the Museum of Anthropology and Ethnology of Florence University, curated by coauthor M.Z. The set of samples from Tarquinia (Viterbo) derives from the Anatomy

Institute of Leipzig University, curated by coauthor H.F. The set of samples from Venosa (Potenza) derives from the Museum of Civilizations in Rome, Italian Ministry of Cultural Heritage (Mi-BACT), curated at the time of sampling by coauthor L.B. and later by coauthor A.S. The set of samples from Casenovole (Grosseto) derives from Soprintendenza per i Beni archeologici della Toscana (prot. 0002442 16/02/2015, CI. 34.16.04/301). The set of samples from Poggio Renzo (Siena) derives from Soprintendenza per i Beni archeologici della Toscana (prot. 000954 22/06/2016, C.I. 34.16.04/280). The set of samples from Campiglia dei Foci (Siena), Magliano in Toscana (Grosseto), Marsiliana d'Albegna (Le Pianacce, Grosseto), and Volterra (Pisa) derives from the Superintendence of Archaeology, Fine Arts and Landscape for Firenze, Pistoia, and Prato, curated at the time of sampling by coauthor E.P. and later by coauthor A.R. The sample from Marsiliana d'Albegna (Poggio di Macchiabuia, Grosseto) derives from the Department of History and Cultural Heritage of Siena University, curated by coauthor A.Z. The sample from Chiostraccio cave derives from the Prehistory and Anthropology collection of Siena University, curated by coauthor S.R. The set of samples from Vetulonia (Grosseto) and Poggio Pelliccia (Grosseto) derives from the Department of History, Archeology, Geography, Art and Entertainment of Florence University, curated by L.C. Authorization for the identification and acquisition of data from prehistoric material in the provinces of Siena and Grosseto is granted to the Department of Physical Sciences, Earth, and Environment by Soprintendenza per i Beni archeologici della Toscana (prot. 10413 20/06/2011, CI. 34.31.01/53-43). For a more detailed description of the analyzed sites and samples, see Supplementary Text and table S1A.

### aDNA sample processing and quality control

The skeletal samples analyzed in this study were processed in dedicated clean room facilities at the Max Planck Institute for the Science of Human History in Jena, Germany (MPI-SHH); the University of Tübingen, Germany; and the University of Florence, Italy. Prior to sampling, specimens were microCT scanned and/or photographically documented as part of the processing workflow record. Sampling of the petrous parts of the temporal bones was performed by cutting the petrous bone using an electric saw and drilling the densest area surrounding the cochlea, as described in the work of Pinhasi *et al.* (51). Teeth were sampled by drilling from the tip of the root toward the crown or by sawing along the junction between root and crown and directly drilling inside the pulp chamber. DNA was extracted from around 50 to 100 mg of bone or tooth powder following an established protocol (52). Negative and cave bear positive controls were included in each extraction batch. Samples were incubated in 1 ml of extraction buffer [0.45 M EDTA (pH 8.0) and proteinase K (0.25 mg/ml)] at 37°C for at least 16 hours, in some cases followed by an additional hour at 56°C (53). The suspension was centrifuged, and the supernatant was first transferred into a binding buffer containing guanidine hydrochloride and then into silica columns (High Pure Viral Nucleic Acid Large Volume Kit, Roche) as described in the work of Korlević *et al.* (54). After two washing steps using a wash buffer (High Pure Viral Nucleic Acid Kit, Roche), DNA was eluted in EBT buffer [10 mM tris-Cl (pH 8.5) and 0.05% Tween 20] in two rounds for a final volume of 100  $\mu$ l. A double-stranded and dual-indexed Illumina DNA library was prepared from 20 to 25  $\mu$ l of each extract, following previously published protocols (55, 56). For most samples (table S1A), deaminated cytosines resulting from DNA damage were kept or partially



removed using a uracil-DNA-glycosylase treatment (UDG-half) that retains the characteristic aDNA damage pattern in the terminal two nucleotides at the 5' and 3' ends of the DNA fragments (57), while for one sample (UDC\_P), those were entirely removed (UDG-full) (58). Negative and positive controls were taken alongside each experiment. One aliquot of each library was quantified using IS7/IS8 primers (55) and the DyNAmo SYBP Green quantitative polymerase chain reaction (qPCR) kit (Thermo Fisher Scientific) on the Light-Cycler 480 (Roche). Subsequently, libraries were double indexed with unique combinations of indexes [8 base pairs (bp) in length each] through a 10-cycle PCR reaction using PfuTurbo DNA Polymerase (Agilent Technologies). After amplification, the indexed products were purified over MinElute columns (Qiagen), eluted in 50  $\mu$ l of EBT buffer, and quantified with IS5/IS6 primers (55), following the method described above. Indexed libraries were then amplified to reach a total of  $10^{13}$  DNA copies per reaction using Herculase II Fusion DNA Polymerase (Agilent Technologies) and the IS5/IS6 primer set. After another purification round, each amplified library was quantified on a TapeStation system using D1000 kit (TapeStation Nucleic Acid System, Agilent 4200). A 10 nM equimolar pool of all libraries was then prepared for shotgun sequencing. The pooled double-indexed libraries were sequenced on an Illumina HiSeq 4000 instrument for a depth of  $\sim 5$  million read cycles, using a single-end configuration ( $1 \times 75 + 8 + 8$  cycles). Libraries prepared from the blanks were also pooled and paired-end ( $2 \times 50 + 8 + 8$  cycles) shotgun sequenced on an Illumina NextSeq500 platform. Reads were analyzed with EAGER v1.92.59 (59) to assess human endogenous DNA quantity and quality. Samples that showed a percentage of endogenous DNA  $> 0.1\%$  and the presence of the typical CtoT and GtoA substitutions toward the 5' and 3' fragment ends, respectively, were selected to be enriched for  $\sim 390,000$  or  $\sim 1.24$  million genome-wide informative SNPs, following an established in-solution capture enrichment assay (390K or 1240K capture, respectively) (60). One individual (UDC\_P) was directly shotgun sequenced. Before capture, the selected libraries were further amplified with IS5/IS6 primers to reach a DNA concentration of 200 to 400 ng/ $\mu$ l and subsequently quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Moreover, whole-mtDNA capture (61) was carried out for samples that showed low coverage of the human mitochondrial genome after nuclear capture enrichment. Captured DNA products resulting from the different enrichment strategies were either single-end or paired-end sequenced on an Illumina HiSeq 4000 platform.

### Radiocarbon dating

For the 50 individuals selected for radiocarbon dating (Fig. 1B and table S1A), a sample usually below 1 g of petrous bone or tooth was sent to the CEZ Archaeometry gGmbH, Mannheim, Germany. Collagen was extracted from the specimens, purified by ultrafiltration (fraction,  $> 30$  kDa), freeze-dried, and combusted to  $\text{CO}_2$  in an elemental analyzer.  $\text{CO}_2$  was converted catalytically to graphite. The dating was performed using the MICADAS-AMS of the Klaus-Tschira-Archäometrie-Zentrum. The resulting  $^{14}\text{C}$  ages were normalized to  $\delta^{13}\text{C} = -25\%$  (62) and calibrated with the INTCAL13 dataset (63) using the SwissCal 1.0 software (64).

### Quantification and statistical analyses

#### Postsequencing data processing

The sequenced libraries were demultiplexed, allowing for a maximum of one mismatch in each index, and preprocessed through the

EAGER pipeline (v1.92.59) (59). We clipped adapter sequences and discarded single-end reads shorter than 30 bp using AdapterRemoval (v2.2.0) (65). With this tool, we also performed the merging of paired-end sequencing data by keeping only read pairs with an overlapping region of at least 10 bp. Sequences were subsequently mapped against the human reference genome *hg19* using BWA (v0.7.12) (66) with seeding option disabled, stringency parameter set to 0.01, and a mapping quality filter of 30. Clonal molecules were then removed with DeDup (v0.12.2) (59), which considers both start and end coordinates of the reads to identify and discard identical sequences.

#### Authentication of aDNA

Before genotype calling, we estimated the level of contamination in the mitochondrial and nuclear genome to authenticate the retrieved DNA using multiple methods described below. Extraction and library preparation blanks were also analyzed to control for potential background contamination during the laboratory steps. (i) DNA damage and read lengths were inspected and quantified using mapDamage2.0 (67). We checked for the presence of aDNA typical features as a short average length of the DNA sequences ( $\sim 40$  to 70 bp) and an increased proportion of miscoding lesions at the terminal positions of the molecules. (ii) The genetic sex was inferred for each individual by calculating the normalized X-ratio (coverage on targeted X-chromosome SNPs/coverage on targeted autosomal SNPs) and Y-ratio (coverage on targeted Y-chromosome SNPs/coverage on targeted autosomal SNPs). To remove substitutions potentially deriving from aDNA damage, we trimmed the first two and last two positions of each read (one position for the UDG-full library) and ran sex determination on the newly generated BAM files. The expected results for uncontaminated specimens are an X-ratio  $\sim 1$  and Y-ratio  $\sim 0$  for females and both X- and Y-ratio  $\sim 0.5$  for males. Individuals that fall in an intermediate position between male and female are assigned to undetermined sex and indicate the presence of DNA contamination. ANGSD (v0.910, method 1) (68) was next run on male individuals to estimate nuclear contamination by measuring the rate of heterozygosity observed on polymorphic sites of the X-chromosome after accounting for sequencing errors in the flanking regions, which are likely to be monomorphic. All male samples with a level of X-chromosome contamination below 6% and at least 140 X-chromosome SNPs covered twice were considered suitable for further analyses. (iii) Both males and females were tested for human mitochondrial contamination using schmutzi (parameters: -notusepredC -uselength) (69), which iteratively determines the endogenous mtDNA sequence while also estimating human mitochondrial contamination given a comparative database of modern-day potential Eurasian contaminant mitogenomes. For individuals showing relatively low proportion of mtDNA molecules compared to the nuclear DNA (mt/nuclear DNA ratio  $< 200$ ), mtDNA contamination estimates (below 5%) were considered a reliable indicator for nuclear contamination (70). Four of the 86 individuals reported in this study were excluded from population genetic analyses because of high contamination levels (ETR015 and CSN012) or unavailability of enough SNPs covered to perform reliable individual-based analyses such as PCA (ETR004 and VET011), i.e., less than 31,000 SNPs covered on the 1240K panel and around half of that on the Affymetrix Human Origins panel ( $\sim 600\text{K}$  SNPs) (table S1A) (71).

#### Genotyping and merging with datasets

To reduce the impact of deamination-induced misincorporations in downstream analyses, genotyping was carried out on the BAM

files generated after trimming 2 bp at the ends of each read for non-UDG and UDG-half libraries and 1 bp for the UDG-full library. A pseudo-haploid genotype was reconstructed for each individual with pileupCaller by performing a random allele call between high-quality bases (Phred-scaled base quality score  $\geq 30$ ) aligning to each 390K or 1240K SNP positions covered at least once (<https://github.com/stschiff/sequenceTools>). Subsequently, genotyped data were merged with the Human Origins panel, the 1240K dataset from the Simons Genome Diversity Project (32), and ancient individuals reported in v37 of the dataset available at <https://reich.hms.harvard.edu>, plus the addition of relevant ancient populations (17, 24, 72–75).

### Kinship relatedness

To avoid bias caused by grouping closely related individuals into a population, we computed for each unique pair of individuals the average pairwise mismatch rate (PMR) across all autosomal SNPs covered at least once for both of the two individuals being compared (76). PMRs were consequently used to calculate the coefficient of relatedness as  $\alpha = ((\text{PMR}_{\text{unrelated}} - \text{PMR}_{\text{pair}}) / \text{PMR}_{\text{identical}})$ . Pairs with  $\alpha > 0.2$  were considered first- or second-degree related, and for each pair, only the individual with the higher number of SNPs was kept for population genetic analyses. Suggested genetic kinships were confirmed through mtDNA and Y-chromosome haplogroups assessment. Two pairs of samples from Vetulonia, i.e., VET006-VET009 and VET003-VET004, were found to belong to a single individual; thus, their data were merged (table S1B). Moreover, we found four family clusters with up to four individuals at Venosa, two family clusters of three individuals at Tarquinia, and kinship among four adult individuals at Casenovole (fig. S2).

## Population genetic analyses

### Principal components analysis

We used the software smartpca from the EIGENSOFT package (v16000) (77) to compute a West Eurasian PCA using 50 present-day populations genotyped for the Human Origins dataset. We projected ancient individuals with more than 15,500 overlapping SNPs using the option lsproject: YES onto PC1 and PC2. Moreover, since the number of individuals is much lower than the number of SNPs, the estimated principal scores tend to be biased, exhibiting a regression toward 0 (28). To correct for this shrinkage effect when predicting PC scores, we selected in smartpca the parameter “shrinkmode: YES” (figs. 2A, 3A, 4A, and 5A).

To explore at a finer scale how the genetic makeup of the ancient individuals under study relates to the genomic variability of present-day Italian populations, we merged our data with a previously published genome-wide dataset of Italian individuals (78). The dataset consists of 737 individuals genotyped on the Illumina (San Diego, CA, USA) CoreExomeChip v.1.1 array. The individuals were selected to be representative of 20 Italian provinces (i.e., the four grandparents born within the same province) uniformly distributed along the entire peninsula and the two major islands (Sardinia and Sicily; fig. S4). PCA was calculated on the modern Italian individuals including 168,649 SNPs overlapping between the CoreExomeChip and the 1240K capture panel. The ancient individuals were then projected to this PCA space with the same methodology detailed before.

### ADMIXTURE

We run the clustering algorithm ADMIXTURE (79) in unsupervised mode on the Human Origins dataset after the removal of individuals

with more than 99% missing data, genetically related individuals, and non-European ancient individuals. Furthermore, we removed all pre-Nuragic ancient individuals from Sardinia (27, 35) to prevent ancient Sardinians maximizing a component at low  $K$  values. We included a subset of 20 randomly selected present-day Tuscans from the 1000-genome project (TSI.SG) for a total of 4118 individuals. After linkage disequilibrium (LD)-based SNP pruning (--indep-pairwise 200 25 0.2), we performed five independent runs for  $K$  between 3 and 16. In Fig. 2 (B and C), we report  $K = 11$ , which had the second lowest cross validation error after  $K = 13$  for the run with the lowest likelihood where Iranian Neolithic and Steppe-related ancestries were distinguished in two distinct components.

### F-statistics

$F_3$ - $f_4$  statistics were calculated using the ADMIXTOOLS suite (<https://github.com/DReichLab>) on the 1240K dataset.  $F_4$ -statistics were initially used to define genetic groups as  $f_4(\text{Onge.DG}, X, \text{Cluster}, \text{Individual})$  where  $X$  are the cornerstones of West Eurasian genetic variation [WHG, Anatolia\_N, Morocco\_EN, Morocco\_Iberomaurusian, Russia\_Yamnaya\_Samara, Iran\_Ganj\_Dareh\_N, and EHG (three Karelia\_HG, one Samara\_HG, and one Popovo)], and *Cluster* are individuals from the same temporal intervals closely related in PCA space (C.Italy\_Etruscan, C.Italy\_Imperial, S.Italy\_Venosa, C.Italy\_Early.Medieval, and C.Italy\_Etruscan.Afr). *Individual* MAS001, VEN002, ETR014, and VET006.9 provided at least one comparison with  $Z_{\text{score}} > |3|$  and were analyzed separately like UDC\_P, the only individual whose genome-wide data are produced through shotgun sequencing rather than through capture (table S2A).

The presence of admixture between C.Italy\_Etruscan and 29 present-day African, European, and Near Eastern populations or 256 ancient West Eurasian and African populations was initially measured with the statistic  $f_3(\text{C.Italy_Etruscan}, \text{present-day and ancient populations}; \text{Test})$  where *Test* was C.Italy\_Etruscan.Ceu, C.Italy\_Etruscan.Afr, C.Italy\_Imperial, and C.Italy\_Early.Medieval (table S4, A and B). Similarly, we determined whether C.Italy\_Etruscan and C.Italy\_Imperial are consistent with forming a clade with *Test* in the  $f_4(\text{Onge.DG}, \text{present-day and ancient populations}; \text{C.Italy_Etruscan/C.Italy_Imperial}, \text{Test})$  (table S2, B to D). For the youngest clusters in our dataset (C.Italy\_Early.Medieval and S.Italy\_Venosa), this test was performed in comparison to present-day populations with  $f_4(\text{Onge.DG}, \text{present-day populations}; \text{C.Italy_Early.Medieval/S.Italy_Venosa}, Y)$  where  $Y$  are present-day populations from Italy and Greece (Bergamo.DG, Tuscan.DG, Greek.DG, and TSI.SG) (table S2E).

Comparisons with individuals from ancient Rome were performed with  $f_4$ -statistics after removing PCA outliers from each group described in the work of Antonio *et al.* (17) (table S2F). To inspect whether Neolithic and Copper Age individuals from Rome are closer to Iron Age and Republic period individuals from Rome or from Tuscany/northern Lazio, we performed  $f_4(\text{Onge.DG}, \text{Rome_Neolithic/Rome_CopperAge}; \text{C.Italy_Etruscan\_UDC\_P}, \text{Rome\_Iron\_Republic})$ . Moreover, we evaluated whether Neolithic, Copper Age, and Iron Age/Republic period individuals from Rome have more affinity to the C.Italy\_Etruscan cluster split into pre- and post-Roman Republic (preRR and postRR, respectively) with  $f_4(\text{Onge.DG}, \text{Rome_Neolithic/Rome_CopperAge/Rome_Iron\_Republic}; \text{C.Italy_Etruscan\_preRR}, \text{C.Italy_Etruscan\_postRR})$ . In addition, we tested whether the C.Italy\_Etruscan cluster is closer to Iron Age individuals from Rome associated to the Etruscan or Latin culture with  $f_4(\text{Onge.DG}, \text{C.Italy_Etruscan}; \text{Rome\_IronAge\_Latin}, \text{Rome\_IronAge\_Etruscan})$ .

Last, the highest affinity between Rome\_Iron\_Republic and C.Italy\_Etruscan rather than other central Italy genetic clusters identified here was confirmed with  $f_4$ (Onge.DG, Rome\_Iron\_Republic; C.Italy\_Etruscan, *Cluster*) (table S2F).

#### qpAdm modeling

The software qpAdm v810 was used on the 1240K dataset to test two populations being consistent ( $P$  value above 0.05) to be equally related to a set of reference groups or to model one population resulting from the admixture of two source populations (one-way and two-way admixture, respectively). Initially, all newly produced genetic clusters from Iron Age and Republic period Central Italy (C.Italy\_Etruscan, C.Italy\_Etruscan.Afr, C.Italy\_Etruscan.Ceu, C.Italy\_Etruscan\_MAS001, and C.Italy\_Etruscan.Afr\_VET006.9) were tested as deriving entirely from contemporaneous or older West Eurasian populations (145 ancient groups from Bronze Age and Iron Age) in comparison to a set of 15 reference groups (Ethiopia\_4500BP.SG, Ust\_Ishim\_HG.DG, Russia\_Kostenki14, Belgium\_GoyetQ116\_1, Czech\_Vestonice16, Russia\_MA1\_HG.SG, Italy\_Villabruna, EHG, Iran\_Ganj\_Dareh\_N, Jordan\_PPNB, Israel\_Raqefet\_M\_Natufian, Morocco\_Iberomaussian, Anatolia\_N, Germany\_LBK\_EN, and Russia\_Yamnaya\_Samara) (table S4A). Afterward, the same groups were modeled as a two-way admixture between previously published Neolithic to Bronze Age individuals from Italy and either Russia\_Yamnaya\_Samara or Germany\_Bell\_Beaker (table S4B). This test was repeated with C.Italy\_Etruscan added to the reference population set (table S4C). In addition, we tested the possibility that the two individuals grouped in C.Italy\_Etruscan.Ceu (VET005 and CAM002) are recently admixed between C.Italy\_Etruscan and Germany\_Bell\_Beaker using the tool DATES (25), which studies ancestry covariance patterns of two sources to infer how long they admixed before the date of a studied individual. Furthermore, contemporaneous and immediately younger clusters than C.Italy\_Etruscan (i.e., C.Italy\_Etruscan.Afr, C.Italy\_Etruscan.Ceu, C.Italy\_Etruscan\_MAS001, C.Italy\_Etruscan.Afr\_VET006.9, and C.Italy\_Imperial) were tested as mixtures between C.Italy\_Etruscan and the 158 ancient groups from Bronze Age and Iron Age West Eurasia and North Africa, using the same set of reference populations as outgroup plus Italy\_Remedello\_BA.SG (table S4D). Similarly, C.Italy\_Early.Medieval and C.Italy\_Early.Medieval\_ETR014 were modeled as a two-way admixture between the preceding C.Italy\_Imperial cluster and 59 ancient West Eurasian groups mainly dated after the Bronze Age (table S4E). Last, we attempted to model present-day Italian populations (Bergamo.DG, Tuscan.DG, and TSI.SG) as resulting from a two-way admixture between C.Italy\_Early.Medieval and 46 post-Bronze Age ancient West Eurasian groups (table S4F).

To increase resolution in discerning admixture patterns or continuity in the last time slice of our temporal transect, from Early Medieval to current populations from the same region, we performed qpAdm analyses on the Human Origins dataset using 17 present-day populations in the reference set (Ami, Basque, BedouinB, Biaka, Chukchi, Eskimo\_Naukan, Han, Iranian, Ju\_hoan\_North, Karitiana, Mbuti, Papuan, Russian, Sardinian, She, Ulchi, and Yoruba). We tested whether any present-day Italian population in this dataset (Sicilian, Italian\_South, Italian\_North, TSI.SG, Bergamo.DG, and Tuscan.DG) is consistent deriving directly (one-way) from one of the newly defined genetic clusters (table S4G).

We lastly explored the possibility of modeling all newly defined genetic groups from central Italy as a mixture of maximum of five distal genetic components (table S4H) represented by Anatolia\_N,

WHG (LaBran1.SG, Loschbour.DG, and KO1.SG), Russia\_Yamnaya\_Samara, Iran\_Ganj\_Dareh\_N, and Morocco\_EN compared to a set of 10 reference populations [Ethiopia\_4500BP.SG, Ust\_Ishim\_HG.DG, Czech\_Vestonice16, Russia\_MA1\_HG.SG, Italy\_Villabruna, EHG, Caucasus hunter-gatherers (CHG; Georgia\_Kotias.SG and Georgia\_Satsurblia.SG), Jordan\_PPNB, Israel\_Raqefet\_M\_Natufian, and Morocco\_Iberomaussian]. We started to model each group represented by more than one individual in this study as a five-way admixture then identified the nested models that minimize the number of sources, and lastly, we rerun the program including only those sources. For C.Italy\_Etruscan and C.Italy\_Etruscan.Ceu, the three-way admixture models between Anatolia\_N, WHG, and Russia\_Yamnaya\_Samara were repeated, this time including Iran\_Ganj\_Dareh\_N to the reference group to test for the presence of the latter component in the newly produced Iron Age genomes from central Italy (table S4H).

#### Haplogroup assignment of uniparental markers

mtDNA analyses were first performed on 1240K captured data. Samples that returned insufficient data to reliably determine the mtDNA haplogroup were selected for mtDNA enrichment. The 1240K and mtDNA captured sequences were preprocessed with EAGER (v1.92.59) as described above and aligned to the mitochondrial reference genome (*rCRS*) using CircularMapper, a tool that takes into account the circularity of the mtDNA (59). We reconstructed the endogenous mitochondrial consensus sequences with the log2fasta program as part of the schmutzi package (69) and applied a quality filter of more or equal to q10, q20, and q30. The resulting consensus sequences were then analyzed with HaploFind (80) to determine the corresponding mtDNA haplogroups. Reads with mapping quality of  $\geq 30$  and base quality of  $\geq 30$  overlapping Y-chromosome SNPs found in the ISOGG database [v.15.64 ([www.isogg.org/tree](http://www.isogg.org/tree))] were genotyped and manually assigned to Y-chromosome haplogroups (table S1A and fig. S5) (81).

#### SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <https://science.org/doi/10.1126/sciadv.abi7673>

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