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Through 40.000 years of human presence in Southern Europe: the Italian case study

Serena Aneli[1*], Matteo Caldon[1], Tina Saupe[2], Francesco Montinaro[2,3], Luca Pagani[1,2]

1 Department of Biology, University of Padova, Padova, Italy
2 Estonian Biocentre, Institute of Genomics, University of Tartu, Tartu, Estonia
3 Department of Biology, Aldo Moro University of Bari, Bari, Italy

*corresponding author. Email: serena.aneli@unipd.it

Abstract

The Italian Peninsula, a natural pier across the Mediterranean Sea, witnessed intricate population events since the very beginning of human occupation in Europe. In the last few years, an increasing number of modern and ancient genomes from the area has been published by the international research community. This genomic perspective started unveiling the relevance of Italy to understand the post-Last Glacial Maximum (LGM) re-peopling of Europe, the earlier phase of the Neolithic westward migrations, and its linking role between Eastern and Western Mediterranean areas after the Iron Age. However many open questions are still waiting for more data to be addressed in full.

With this review, we summarize the current knowledge emerging from the available ancient Italian individuals and, by re-analysing them all at once, we try to shed light on the avenues future research in the area should cover. In particular, open questions concern i) the fate of pre-Villabruna Europeans and to what extent their genomic components were absorbed by the post-LGM hunter-gatherers; ii) the role of Sicily and Sardinia before LGM; iii) to what degree the documented genetic structure within the Early Neolithic settlers can be described as two separate migrations; iv) what are the population events behind the marked presence of an Iranian Neolithic-like component in Bronze Age and Iron Age Italian and Southern European samples.

Keywords: ancient genomes; peopling of Italy; human migrations

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Introduction

An increasingly detailed portrait of ancient Europe has been emerging in the last few years thanks to the joint analyses of archaeological findings, and the pattern of modern and ancient genetic variations elucidating past cultural transitions. The coarse edges of the European mosaic have been traced by three major contributions arriving in different periods and from different homelands: the first hunter-gatherers on the European ground, the Neolithic farmers from Anatolia, and the Bronze Age herders from the Steppe [1,2]. However, even though we have certainly found the tesserae composing the main figure, we still lack some fine details and we are far from putting together the blue sky pieces.

In this context, due to its position at the centre of the Mediterranean Basin, Italy is an ideal country to unearth the genetic footprints of at least the past demographic events in Southern Europe, thus offering the opportunity to add new pieces of the puzzle until clearer pictures emerge from our past. Indeed, its geographical features and the mild climate have been attracting people both near and far during the main stages of the peopling of the continent [3].

Since Upper Palaeolithic, around 45,000 years ago, modern humans have inhabited there [4,5], and during the LGM (18,000-20,000 years ago) Italy and the other Southern Mediterranean areas were used as refugia from the North. The Italian Peninsula also played a major role in spreading the farming lifestyle, through at least two diffusion routes: one started from Apulia, where the most ancient findings associated with farming were discovered and following the Eastern coast reached the North, while the other started from East Sicily and travelled up along the Tyrrhenian coast [6].

During the Neolithic and for thousands of years later, the Mediterranean Sea itself contributed to shortening the distances and making Italy one of the gateways to the European continent: first acting for millennia as a barrier separating the African and the European continents, and then turned into a bridge as the first Bronze Age seafarers started to travel in open water [7]. Another hallmark of the Italian landscape contributed significantly to the cultural ferment of such times: the Alps. Indeed, they were so rich in copper that some Copper Age cultures arose in Italy, at Remedello and Rinaldone in the North and Gaudo in the South [8]. The diffusion of these metalworkers is evident in Ötzi, the South-Tyrolean Iceman. He carried a copper axe of the Remedello type, which was produced in Northern Italy using the ores of Tuscany [9,10]. During the Bronze Age, at least two ancestral sources moved through Italy, the Steppe-related component and an Iranian farmer-related component, whose amount steadily increased during the Iron Age period [11,12]. Moreover, the distribution of these components is still visible in the genomes of modern Italians [13].

Later on, the rise of the Roman civilization and its tight network of trade, political, religious, and cultural connections over one of the largest empires in the ancient world contributed to shuffle and mingle different genetic components across the Mediterranean, Continental Europe and Northern Africa [7]. With all this back and forth, the population inhabiting the country have been accumulating the genetic traces of these ancient wanderings, thus exhibiting the largest genetic diversity in Europe [13].

In the last few years, the scientific community has collected a huge amount of information from human remains discovered worldwide and through a wide range of time. Such data represent an extraordinary asset to solve the outstanding questions concerning the demographic events experienced by ancient populations.

In this review, we collected and analysed all the genetic data of ancient Eurasian individuals which have been generated so far (Figure 1, Supplementary Figure 1, Supplementary Figure 2 and Supplementary Table 1), and taking Italy as a case study we recapitulate the main steps of the peopling of Europe through the lens of Archaeology, Anthropology, and Genetics, highlighting outstanding questions that may hopefully be addressed in the coming years.

Chronological sections

From the first peopling of Europe to the Villabruna HG

The first humans inhabiting Italy were archaic humans: remains of putative *Homo erectus* [14], *Homo heidelbergensis*/Ceprano and Neanderthal were found throughout Italy and date back from as early as 1 million years ago to ~40 thousand years ago (kya) [15–17]. The *Homo sapiens* occupation of the Italian Peninsula started quite early and intriguingly overlaps the Neanderthal one. Notably, the Grotta del Cavallo [4] provides one of the earliest evidence of Sapiens in Europe and it is associated with a cultural package, the Uluzzian technology, which may show influences from both human groups. Ancient genomic evidence [18] showed that the Italian genetic substrate was connected with the one of continental Europe, with archaeological sites like Paglicci (33kya) and Ostuni (28kya) showing genetic affinities with the so-called “Vestonice cluster”. This group of individuals spanned all the way from the Russian Steppe to Central Europe until the beginning of the last Ice Age (25-20kya) and falls outside of the human diversity described by modern human samples (Supplementary Figure 3). These Italian sites are all associated with Gravettian material culture (33-21kya), which is argued to have provided a substrate for the in-situ development of Early Epigravettian, the landmark of the fossil record dating to the Last Glacial Maximum (LGM) and potentially pointing to the role of Central and Southern Italy as glacial refugia.

During the LGM, at around 20-19kya, Italy by and large disconnected from the rest of continental Europe by the Alpine Icecap [19], but was in continuity with the Balkan Peninsula through land bridges spanning the Adriatic Sea. These connections provided crucial for the establishment of trade and cultural exchanges with groups from the Balkans and the Black Sea region [19], which ultimately resulted in genetic exchanges. The most notable evidence of such a gene flow is represented by the so-called Villabruna replacement, which is represented by the arrival of genetic components with a higher affinity to contemporary Near Eastern groups and which formed the basis for the post-Ice Age European genetic landscape [18]. The earliest evidence of such a massive replacement is represented by the Tagliente2 sample from Riparo Tagliente (Verona, Northern Italy) dated to 17kya (16,980-16,510 cal BP) and for which a whole-genome shotgun sequence is available [20]. Although based on mtDNA evidence alone, earlier traces of Villabruna-like genome may be seen in some Paglicci samples dated to 19kya, at the very end of LGM [21]. Villabruna and Tagliente2 (in yellow, Figure 2A and Figure 2B) fall at the centre of the other post-Villabruna HG samples, from which they differentiate towards Italy and the rest of Europe (Figure 2B), showing the pivotal role of these early Italian samples in explaining the subsequent Western Hunter Gatherers (WHG) genetic diversity. The emerging scenario hence seems to place Italy as the first step of the re-colonization of post-Ice Age Europe by settlers coming from the East potentially as early as 19 or 20kya. This population movement introduced in Southern Europe, through demic diffusion, the Epigravettian cultural package [20] and may have initiated other cultural transition given the presence of a similar genetic component found in the genome of the individual ElMiron, who lived ~18.5kya in Spain and that was associated to Magdalenian culture [18]. A similar, parallel movement from the Balkan/Black Sea region towards the North, with genetic contributions from Central/East Eurasia, may explain the origin of Eastern Hunter Gatherers (EHG), who indeed seem to align along an orthogonal axis from the one described by the WHG (Figure 2B), although this is beyond the scope of the current review. While LGM can be seen as the major cause for the disappearance of pre-Villabruna people in Central and Northern Europe, it is still poorly understood what may have caused the disappearance of the genetic components related to the Vestonice cluster in later individuals in Italy. One scenario may involve the Epigravettian package brought by the Villabruna people as the key for their ultimate success, although more Southern Italian samples from 20-15kya may show a less dramatic scenario, perhaps providing a clue to interpret the so-called WHG component in light of pre and post Villabruna admixtures. Another outstanding question is the role of the major Italian islands (Sicily and Sardinia) during the pre and post-Villabruna periods. We know from Grotta d'Oriente (Sicily) that WHG arrived there at least as early as 14kya [22]. However, the presence of Vestonice-like individuals in Sicily and of Vestonice or Villabruna-like individuals in Sardinia is an as yet unexplored chapter.

Neolithic and Copper Age

The term "Neolithic Revolution" refers to the five millennia-long process (approximately between 12,000 and 7,000 ya) involving dramatic changes in the behavior, culture, and ecology of the human populations, culminating in the domestication of plants and animals, and the almost ubiquitous adoption of pottery. The Western Eurasian origin of these changes has been geographically placed in the Fertile Crescent, an area including the Southern part of Anatolia, modern-day Western Iran, Iraq, Syria, Lebanon, Israel, Palestine, Jordan, and Egypt. After their emergence, techniques and artefacts associated with Neolithic culture spread very fast, reaching Iberia already by ~7,450 ya [23]. The how and when of its expansion in Europe has been a very debated subject till the last decades, in which a spectrum of purely demic/purely cultural models was advocated by many scholars, including geneticists [24]. Although many genetic studies, including the pioneering ones started by Cavalli Sforza and colleagues, tentatively attempted to address the Neolithic Revolution dynamics [24,25], the ultimate verdict was reached only recently, when the analysis of genetic material extracted from human remains confirmed a mostly demic diffusion, with a complex genetic interaction with local hunter gatherers [2,26–28]. It is globally accepted that this demic diffusion followed both a Mediterranean route (following the Mediterranian coasts, including Italy) and a continental route (Balkan route reaching Central and Western Europe through the Danubian valley), with the terminal fringes of this migration admixing again. Furthermore, quantitative and systematic analysis of the archaeological records found support for an expansion characterised by "boom and bust" [29], in which expansions were followed by stasis. For example archaeological records, such as the Impressed/Cardial Ware (Southern Europe along the Mediterranenan shores) and Linear ceramic cultures (LBK, Central Europe) named after the peculiar practice of pottery ornamentations, suggest the existence of at least two different routes of colonisation of Europe. Although the global outlines of the European Neolithization are now known, many specific and local aspects are missing. As an example, despite their marked difference in Archaeology, it is not clear how these groups from the two waves were differentiated.

In addition, clear genetic signals of this double expansion have not been found so far [1]. This could possibly depend on the small sample size of the available ancient populations, combined with the relatively low quality of genotype and sequence data, not having enough power to recover the subtle differences between the two Neolithic founder populations. However, with the increase of ancient samples examined, subtle differences between Central-West and Central-East Europe started to emerge, with samples from Western and Eastern Europe forming two distinct clusters in Principal Component Analyses (PCA, Figure 2C) [11,28,30,31]. *F*4 statistics and related methods (qpAdm) showed that this separation is possibly due to different amounts of ancestry related to WHG. It may be possible that the populations spreading through Central and Mediterranean routes mixed differently with residing HG populations, with the former witnessing a higher number of admixture events, in line with some archaeological evidence suggesting a sparser HG population in (Southern) Italy. On the other hand, the genetic drift and founder effect acting independently in the two groups might have given origin to differences in allele frequency and haplotype pattern, which had not been uncovered so far. Notably, when focusing on Italy, most Italian Neolithic samples are closely related to the Central European cluster (Figure 2C,D), with the only exception of Peninsular Chalcolithic and Neolithic Sardinian individuals (Figure 2D, pink and orange circles) which are more related to the Mediterranean cluster. A recent analysis based on a newly developed Y-chromosome enrichment capture approach uncovered two different H2 sub lineages in Late and Post-Neolithic samples which are differentially present in populations from the Mediterranean and Central Europe, possibly reflecting the two diffusion routes followed by farmer populations [32].

Nevertheless, many aspects of the Neolithic diffusion West of the Adriatic have not yet been characterised. The lack of Neolithic samples from Southern Italy, the first Italian area to be interested by the Neolithic wave, makes it impossible to understand the most probable genetic sources. Another open question concerns whether multiple streams reached the Eastern shores of the Adriatic, as cultural differences in Sicilian and Adriatic Italy tentatively suggest [6].

Bronze Age

European populations were just swept up by the Neolithic wave that another time of great demographic and cultural turmoil was approaching. The Eurasian Bronze Age (between around 5,000–3,000 ya) was a period of major cultural changes, indeed, with the introduction of new metalworking techniques and breakthrough innovations, such as the wheel, the chariots and the ox-drawn plough, the rising of the first cities and the intensification of commercial networks whose routes for selling metal goods connected people from far and wide [33]. However, whether these changes were gradually diffused by the movement of people or the spreading of ideas remained disputed for a while.

The initial answers to this debate came from the Pontic-Caspian steppes, where the Yamnaya, a nomadic culture based on sheep and cattle herding, emerged at least 5,000 ya. The *kurgan*, a round tumulus or barrow built upon a grave, was one of their distinctive elements, representing also an important step in the direction of modern civilization because, for the first time, single rather than collective graves were introduced [34]. Gradually, elements of their culture began to be found in Europe and Central Asia, up to the foothills of the Altai Mountains. From the genetic point of view, the Yamnaya population was a mixture of different ancestries: EHG and CHG-related ancestry as well as traces of the first farmers of Northern Iran, the latter possibly deriving from the Maikop culture. This combination of ancestries started appearing in Europe with the Corded Ware culture as early as 4,750 ya [1,33]. Actually, about three-quarters of their genome derives from Yamnaya-related populations, while the remaining part has been inherited from the local farmers. Along with part of their DNA, the Corded Ware shared with the Yamnaya many other traits, such as the large burial mounds, the intensive use of horse and herding, a male-centred culture and the finely executed copper axes [1]. Another European society shared some cultural as well as genetic legacy from the Yamnaya - the Bell Beakers - but, in contrast to the Corded Ware [35,36], they exhibited a high genetic heterogeneity: while Iberian Bell Beakers were genetically indistinguishable from the people who lived there earlier [37], Bell Beakers from Central Europe showed a considerable amount of their ancestry deriving from the steppe populations. With this Yamnaya ancestry, the third ancestral component of the European genetic make-up and the one contributing the most to it had been found [2]. At that point, the European genetic puzzle was apparently completed with the Corded Ware and Bell Beaker migrations as the major spreaders of the high level of steppe-related ancestry we see in modern-day Europeans, at least in Northern and Central Europe [13].

Southeastern Europe was the first European region to receive the steppe-related ancestry, with sporadic individuals showing this component in Bulgaria as early as 6,700-6,500 ya [38], to the point that during the Bronze Age almost everyone there harboured around 30% of steppe-related ancestry. Also Sardinia and Sicily received this ancestry through the expansion of the Bell Beaker complex after ~4,459 ya [12,39]. More specifically, the timing of the Steppe-related ancestry arrival in Italy was a central topic of a recent study, examining human remains from Northeastern and Central Italy dated to the Chalcolithic and Bronze Age [31]. As a confirmation of other Central European studies[37], they found a gradual increase in such ancestry over time, with the first appearance in Early Bronze Age Italians (Italian Bell Beaker ~4,000 ya [37], Italian Remedello ~3,900 ya [33], and Grottina dei Covoloni del Broion dated ~3,800 [31]. Moreover, Saupe and colleagues retraced the arrival of the Steppe-related ancestry in Northeastern Italy as early as 3,900 ya and in Central Italy as early as 3,550 ya.

The Bronze Age Aegean civilizations, mainly represented by Minoans and Myceneans, also harboured up to a quarter of their ancestry ultimately connected with ancient populations from Caucasus and Iran. Nonetheless, only the Mycenaean individuals also showed the EHG-related component [40]. For this reason, at least in the Minoans, the three-way split ancestral contribution is not completely fulfilled and raises other issues about who brought the Caucasus/Iran-related component into Europe.

The widespread commercial networks, facilitated by the introduction of chariots and the technological advances in seafaring, allowed long-distance mobility never seen before. In this context, the geographical position of Italy at the centre of the Mediterranean Sea could help reconcile the cryptic source of the Caucasus-related ancestry, and several aDNA studies based on Italian archaeological sites came out in the last few years also trying to address this issue [7,11,12]. The work from Antonio et al. examined the genetic transitions experienced by Central Italians which, during the Iron Age, would have given origin to the founders of the Roman civilization [7]. While they detected the suggestive presence of a small amount of the Caucasus/Iran-related component as early as the Neolithic period, they observed an increase of that component during the Bronze and Iron Age (between ~4,800 and 2,850 ya), possibly due to increased trade-driven mobility.

The same long-distance mobility with the resultant genetic transitions has been highlighted by the other recent studies focusing on the islands of the Western Mediterranean Sea: the Balearic Islands, Sardinia, and Sicily [11,12]. Both Sardinia and Sicily received the cultural influxes of the Bell Beaker complex after ~4,450 ya [41,42], but similarly to the Iberian situation, that happened without bringing along the steppe-related ancestry: indeed, Beaker-associated individuals in both islands do not harbour steppe ancestry [12,37]. The steppe ancestry started appearing in Sicily around 4,150 ya, during the Early Bronze Age, as demonstrated by qpAdm modelling on autosomal genetic variation and Y-chromosome analyses, which highlighted two notable outliers carrying up to 20% and 40% (Sicily_EBA8561 e Sicily_EBA11443). Starting from the Middle Bronze Age (3,750-3,450 ya), Sicilian samples show a considerable shift in the PCA towards Minoans and Mycenaeans (Figure 3A, Minoans and Mycenaeans are grouped in the Balkan area in green) and can be modelled with the Iranian-related component in a percentage around 15%. This data opens up the fascinating possibility that this mysterious ancestry must have reached Southern Italy before the occupation of the southern coastal areas of Italy (Magna Graecia). Conversely, ancient Sardinians showed a higher degree of genetic continuity from the Neolithic to the Bronze Age, with almost all individuals showing similar proportions of Anatolian Neolithic and WHG ancestries. This peculiar situation strongly differs from many other European regions, where the Bronze Age brought huge demographic and cultural turmoil and suggests genetic isolation from mainland populations [11]. Two fascinating exceptions regard two outliers showing the former a huge proportion of Northern African ancestry (around 77%) and the latter carrying Eastern Mediterranean or steppe-related components [12]. During the Middle Bronze Age, about 3,550 ya, archaeological evidence showed the appearance of the Nuragic culture, named after the Sardinian most characteristic stone towers called *nuraghi*. Despite the absence of the Iranian-related ancestry until the Iron Age, this period was characterized by the intensification of trades and cultural exchanges bridging together the lands facing the Mediterranean, as testified, for instance, by the arrival of copper goods from Cyprus in the Late Bronze Age Sardinia [43] and the presence on the island of Mycenaean, Levantine and Cypriot traders. Nevertheless, evidence of gene flows altering the genetic continuity did not appear until after the Nuragic period [11].

Iron Age

The Iron Age, the latest protohistoric period before the beginning of Antiquity History and Historiography, in Italy and Western Europe started between the 2nd and the 1st millennium BCE (Before Common Era) and ended with the Roman conquest. This period was characterised by the development of new metallurgic techniques and the consequent mass appearance of iron artifacts in archaeological sites. It was also a period of demographic growth, social, and political stratification as well as the intensification of trade networks. Multiple Iron Age populations characterized by different languages and cultures lived in Italy but, genetically speaking, they started to approximate the modern Italians [7].

The most particular case is represented by the Sardinians: a strong Anatolian Neolithic footprint is visible in the Iron Age samples due to the persistent isolation in previous times and despite the documented contacts with Mycenaeans and Phoenicians [11]. Phoenician presence has been dated to as early as the late 9th century BCE [44]. Indeed, Eastern Mediterranean genetic influxes have been found among the post-Nuragic individuals in Phoenician/Punic sites, which could be modelled as direct immigrants or strict relatives of the newcomers. Moreover, also the diffusion of Steppe and Iranian-related ancestries, as well as a North African component that likely arrived during the Punic (Chartaginean) domination, has been highlighted in Iron Age samples [11].

As Sardinia, also Sicily was an important Punic trading post, especially on the western coast. Despite the paucity of samples from this area, the presence of Northern African ancestry on the island could be tentatively reconducted to the Iron Age (or at least Antiquity), because, although absent in previous time layers, it's present in modern Sicilians [11,12]. In addition to the Punics, the other main shapers of the Iron Age Sicilian genetic make-up were the Greeks, who established colonies on the island (and South Italy) starting from the 8th century BCE [39]. According to recent findings, the Greek colonization was a more gradual and peaceful process than that described by ancient historians, characterized by greater interaction and close cohabitation between newcomers and local people [45]. This is probably the reason why modern southern Italians display a varying amount of Greek genetic influence [39], which could be described as a "Mediterranean continuum". The Greek-speaking communities in South Italy (Grecani in Calabria and Griko in Apulia) are clearly placed in this *continuum* and, although there may have been a more recent migration during the Byzantine times (Early Middle Ages), those Greek-speaking communities are believed to have lived in Southern Italy probably since the Magna Graecia times.

If on the one hand Phoenicians and Greeks Mediterranean-wide trade network left genetic traces in Sardinians and Southern Italians, on the other hand, it surely had a cultural impact on Etruscans, a population who lived in the Central Italian area named Etruria (between Lazio, Umbria and Tuscany). The Etruscan civilization and, specifically its earliest phase called Villanovan culture, dates back to the 9th century BCE and ended with the slow and gradual Roman assimilation from the 6th century BCE. However, where the Etruscans came from is still uncertain. Both ancient and modern historians have different opinions on the matter and, while some researchers believe in an autochthonous process of formation from the previous Villanovan society, others claim an Anatolian origin [46,47]. For instance, a multi-step origin explaining the modern Tuscans genetic composition has been proposed: a proto-Etruscans population, born in a Southeastern region of the Middle East, would have migrated to the Caucasus, then to Lydia and finally arrived in Central Italy at the beginning of Iron Age [48], in a way resembling the steps of the mythological migrations of Dionysus (Zagreus). However, it is clear that more genetics studies are needed to shed light on this disputed matter.

During the later Iron Age, Etruscans were replaced in their political influence on Central Italy by Rome's rule, a new town that arose in Lazio on the banks of the Tiber river. Central Italy and Rome inhabitants of the 1st millennium BCE exhibited high genetic variability, in particular, they showed a relevant Steppe-related ancestry, an increase in the Iranian-Neolithic component, respect to previous times, and the appearance of the first Northern African signatures [7]. The appearance of such different contributions is the main consequence of the great mobility of people, which increased even more in the later stages of Rome's history during the Republic (509-27 BCE) and the following Empire periods (27 BCE-CE 476). During the Iron Age and the first stages of Antiquity, long-distance mobility was promoted by the cosmopolitan nature of the Roman Empire and people from far and wide arrived in Rome, creating a melting pot of languages, cultures, and genes. In particular, many people came from the East (Greece, Syria, Egypt), the richest and most densely inhabited region of the Empire, thus resulting in a genetic shift towards the Eastern Mediterranean areas in individuals from this period (Figure 3C and Supplementary Figure 4A). Figure 3C,D clearly point toward the high genetic heterogeneity within Roman and Central Italian individuals, which is a direct consequence of the key role of Rome as the geographical, cultural, and political crossroad of

Eastern and Western Mediterranean. These figures also show that the genetic makeup of Italy during the Iron Age was close to the pattern of modern-day populations.

Antiquity and the making of modern Italians

From the 3rd century CE, the Roman Empire went into a deep crisis due to a mixing of causes like strong political instability and military anarchy, population decrease (wars and epidemics), economic stagnation, and invasions from non-Roman populations. The Empire was also divided into two: the Eastern Roman Empire with the capital Byzantium (today Istanbul, Turkey) and the Western Roman Empire with capital Rome. The latter, poorer in resources and weaker, fell definitively in 476 CE with the deposition of the last Roman emperor by the barbarian commander Odoacer [49].

The split of the Empire also had repercussions on the Italian genetic composition. Antonio et al. observed a shift from the Eastern Mediterranean to continental Europe in Central Italian samples (Supplementary Figure 4), which was probably resulted from the reduction of the East-West mobility, the demographic drop, and the arrival of people from Central and Eastern Europe. Besides the Romans, the Longobards were another relevant population for the Italian cultural landscape during the Late Antiquity. Samples from the Longobard cemetery of Collegno show genetic affinities to Bronze Age people from Central and Eastern Europe [50]. However, the genomic background of Northern Italian people preceding the Longobards arrival is still unknown. Thus, it is unclear if they contributed in diffusing the Central European genetic component and what was their real genetic contribution to the Italian inhabitants. Altogether, with the end of the Antiquity and the beginning of the Middle Ages (5th century CE), the Italian genomic scenario reached most of the modern time composition with a high level of individual variability.

The stratified contribution of each time layer to contemporary allele frequencies is evident in Figure 4, where we magnify the time-dependent allele frequency changes for five variants which have been reported to be genome-wide significant signals of selection among the three main ancestries composing the modern European genetic make-up - hunter-gatherer, early farmer and steppe people [51]. The variants in Figure 4 are associated with lactose metabolism, immune system, skin pigmentation, and eye color [52–60]. However, the fact that little has changed after Iron Age and Antiquity does not mean that movements of people across Europe and Italy would have come to an end after that time, rather the incoming populations would have not been so highly diverse as they were before [61].

Homogenizing effects introduced by the pervasive Neolithic and Bronze Age demic diffusions, paired with a steady post-Neolithic population growth that made local populations less and less prone to major genetic turnovers, established a strong genetic continuum all across West Eurasia, making geographic distance the best predictor of similarity and dissimilarity between populations [62]. Therefore, contemporary Italians can be seen as the inhabitants of a pier stretched across the Mediterranean Sea, whose genome has been shaped by millennia of landings that made it resonate with the ones of the neighboring populations ashore.

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Materials and Methods

Datasets

We collected all available ancient human DNA data from the literature, covering a period from Upper Palaeolithic to historical times and mostly coming from Western Eurasia.

Specifically, we downloaded the 1240K+HO dataset from Dr David Reich laboratory, which comprises present-day and ancient DNA data (version V42.4; https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data [2,7,18,23,27,28,33,35–38,40,50,51,63–104]). In addition, we added: (i) Bronze Age Southern Levant DNA data [105]; (ii) ancient DNA data from the Balearic Islands, Sicily, and Sardinia [12]; (iii) ancient samples from Sardinia spanning the Middle Neolithic to present days [11]; (iv) the Italian Chalcolithic and Bronze Age samples [31]; (v) the genotypes of an Italian hunter-gatherer from the Late Epigravettian site of Riparo Tagliente dated around 17,000 years ago [20].

Finally, we also recovered additional modern-day Italian samples [13].

We converted the dataset to the PLINK format using *convertf* (for the dataset in EIGENSTRAT format) and the software PLINK 1.9[106] for the vcf file of [11].

We extracted only those modern and ancient samples from the 1240k+HO coming from locations at latitudes higher than 22 (22.23) and longitudes between -15 (Canary Islands) and 60 (roughly the political border between Iran and Afghanistan). We kept the Mbuti individuals from Congo (HGDP project). We removed items with the string "*Ignore*" in their "Group.Label" column of the *.anno* file. Finally, we removed those whose "Assessment" column did not contain the string "*PASS*". If there were duplicates among ancient individuals, we selected the sample with the highest number of SNPs hit on autosomal targets.

To further refine the backbone of the modern-day European genetic variability, without mixing different genotyping techniques, we selected the modern samples flagged as "*PASS (genotyping)*" ("Assessment" column) and we removed those coming from Uzbekistan, Kazakhstan, Algeria, Morocco, Tunisia, Libya, as well as some populations from Russia. We excluded SNPs on sexual chromosomes, rare variants (minor allele frequency higher than 0.0001) and those with more than 5% missing data and in the modern samples using PLINK.

We extracted the bulk of variants built on modern samples from each dataset and we merged them with PLINK. Finally, we excluded the ancient samples with more than 5,000 missing SNPs and we converted the dataset to the EIGENSTRAT format using *convertf.*

Our final dataset contained 2,146 ancient and 1,091 modern samples (Supplementary Table 1 and Supplementary Table 2).

We assigned each sample to a geographic macro-area among "Northern Europe", "Western Europe", "Eastern Europe", "Central Europe", "Continental Italy", "Peninsular Italy", "Sicily", "Sardinia", "Balkans", "Middle East" and "Northern Africa" and to a period among "Hunter-gatherers", "Neolithic", "Bronze Age", "Iron Age", and "Antiquity" based on the available cultural information (Supplementary Figure 1A and Supplementary Figure 2). We additionally distinguished, among the hunter-gatherers, the Pre- and Post-Villabruna samples using 18,000 years ago as a threshold.

Derived Allele Frequencies variability of selected variants

We computed the derived allele frequencies for the twelve genetic variants, which have been found under selection among hunter-gatherers, early farmers and steppe ancestry [51]. Given that some of these SNPs were missing in the Human Origin arrays, we used the 1240K dataset (V42.4) which contains the same ancient samples of the 1240K_HO and less modern samples (Supplementary Table 3 contains the list of modern samples of the 1240K dataset).

Principal Components Analysis

We performed principal component analysis using the *smartpca* function implemented in EIGENSOFT software 8.0.0 [107]. Specifically, we projected the ancient samples' genotypes onto the principal components inferred from the modern individuals' genetic variability. We used the options "*lsqproject*" and "*shrinkmode*".

f_3-statistics

To explore the genetic affinities among ancient samples, we performed a series of Outgroup f_3 [108,109] in the form (X, Y; outgroup: Mbuti) using the qp3Pop function (version 650) implemented in ADMIXTOOLS [108]. Since the Mbuti population is an outlier with respect to the samples analysed in this review, the resultant f_3 measures the shared drift between X and Y (i.e., the common branch length from the outgroup), in a way that the higher is the f_3, the closer is the relatedness between the two populations.

We grouped the non-Italian samples into higher cultural labels, which are shown in the column "Higher.Label" of Supplementary Table 1 and we performed the Outgroup f_3, after removing samples flagged as outliers or with family relationships with other individuals (Supplementary Table 4).

Finally, we computed the distance matrices from the Outgroup f_3 results by subtracting the f_3 values from 1 and we performed a classical (metric) Multi Dimensional Scaling (MDS) from such distance matrices using the function *cmdscale* in the stats package of R (version 4.0.4). When necessary, we iteratively removed the outliers and recomputed the MDS.

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Competing interests

The authors declare that they have no competing interests.

Data Availability

All data used in this work are publicly available.

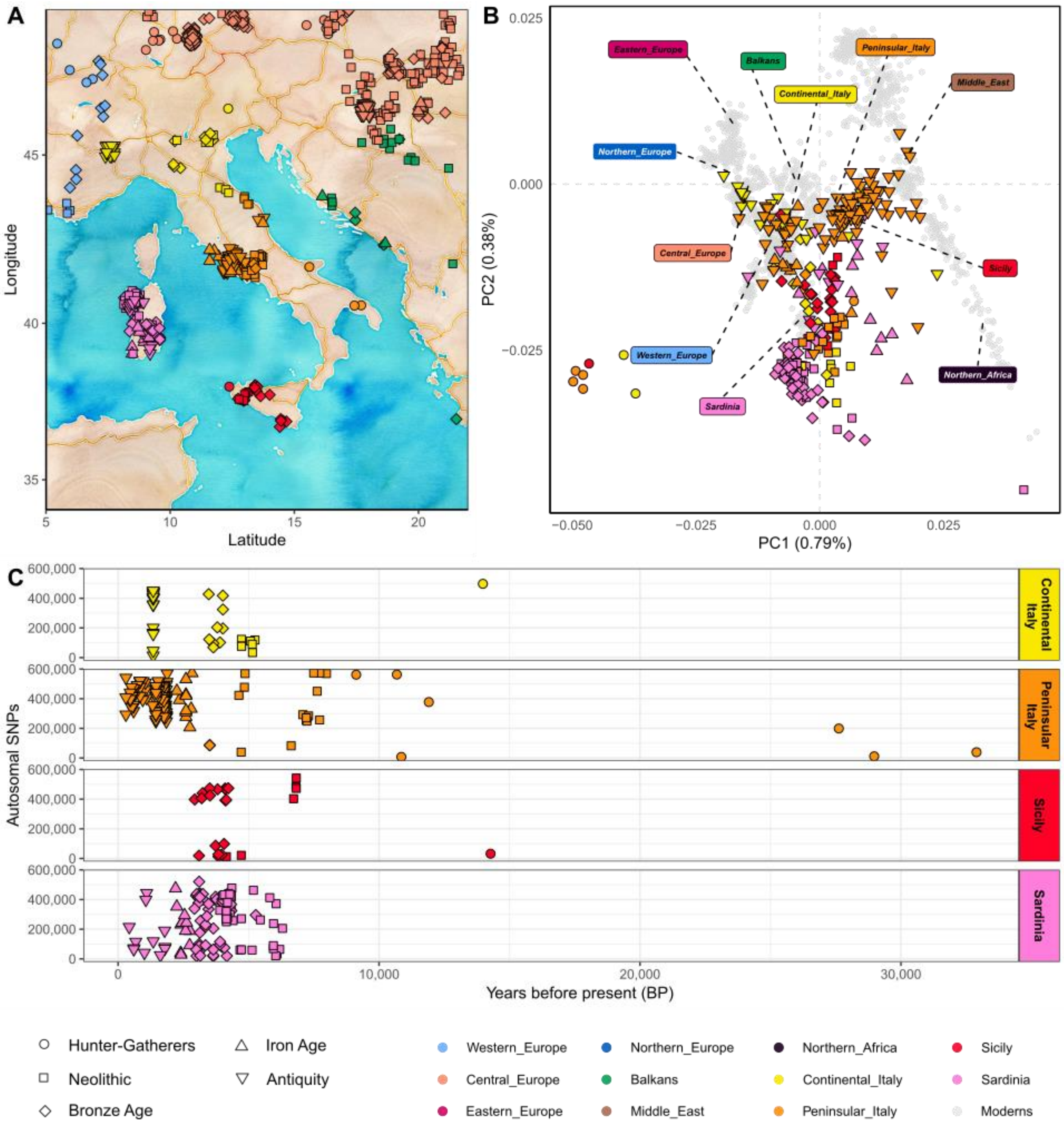

Figure 1. ***Ancient Italian individuals discussed in this review.*** *A) Geographical location of comparative and Italian ancient individuals The Italian ancient individuals are divided into four main categories: Continental Italy (yellow), Peninsular Italy (orange), Sicily (red), and Sardinia (pink); B) Principal component analysis (PCA) projecting the ancient individuals onto the genetic variation of present-day individuals (in grey). C) Chronological distribution (x-axis) and the number of autosomal SNPs of the Italian ancient individuals (y-axis, proxied by the number of available SNPs from the standard 1240K_HO (Human Origin array) SNP set). See also* Supplementary Table 1 *and* Supplementary Figure 2*.*

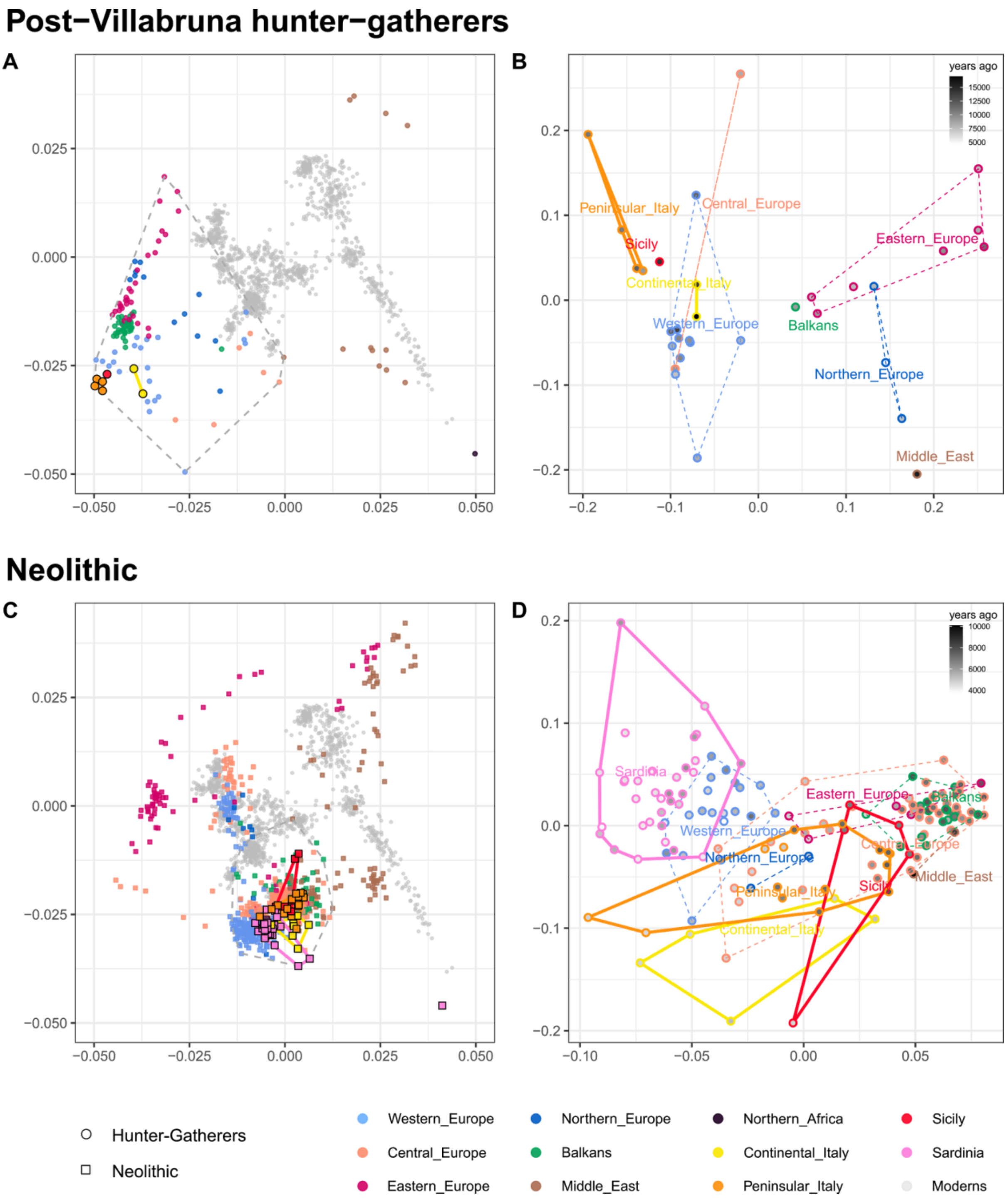

Figure 2. ***PCA and outgroup-f3 multidimensional scaling (MDS) for samples dated back to Paleo-Mesolithic (hunter-gatherers) and Neolithic periods (A-B and C-D, respectively).*** *Samples were divided into four groups according to their chronological and cultural affiliation and projected onto the genetic variation of modern West Eurasians (left column, panels A, C) along with a number of modern reference samples from neighbouring regions. Samples within the dashed grey polygons were also used to construct a pairwise matrix of outgroup f3 distances in the form of f3(Mbuti; X, Y) on which an MDS was performed, hence providing a complementary visualization to the PCA. The chronological dating, based on mean date BP (before present), was used to color-code the samples (right column, panels B, D).*

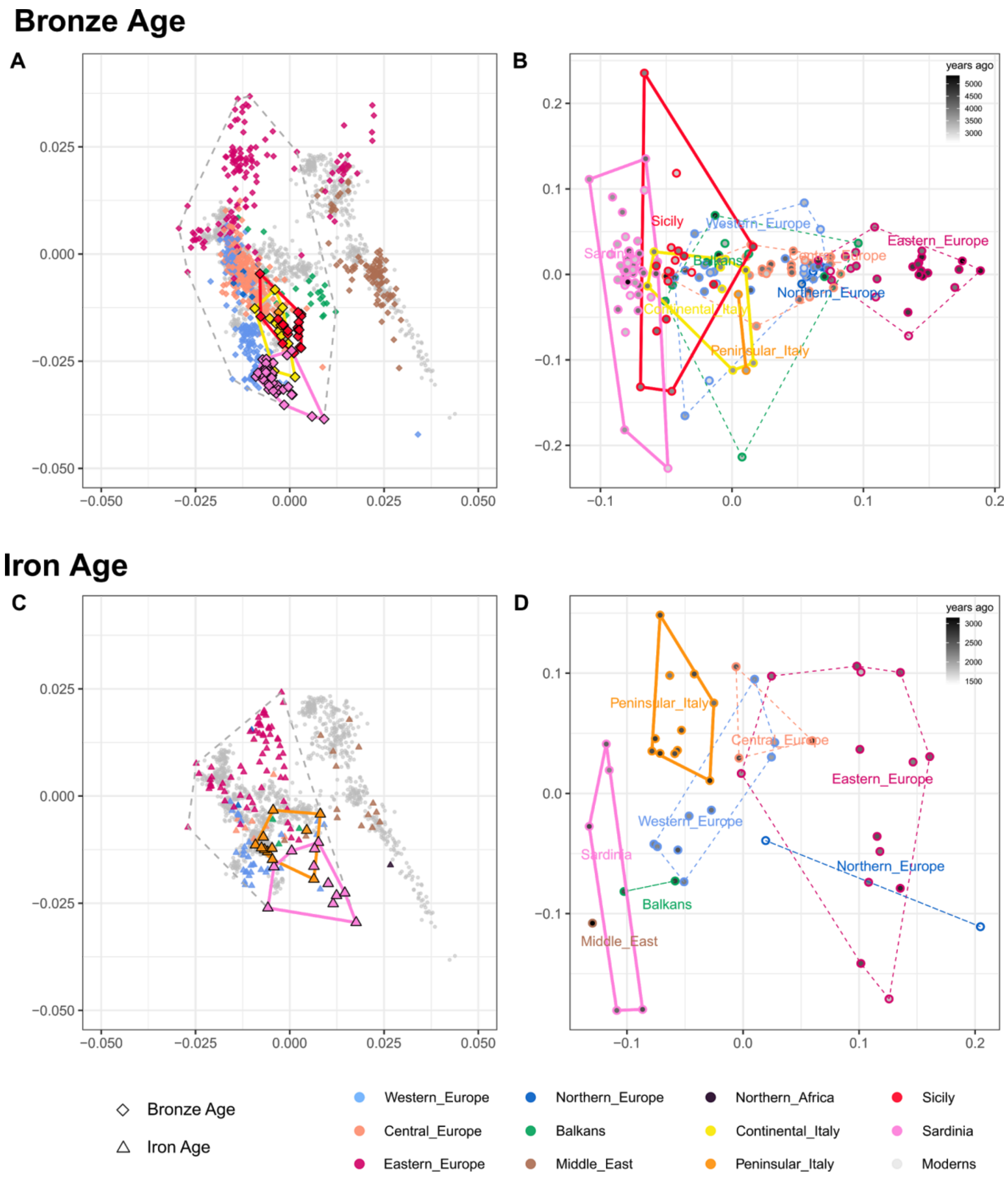

Figure 3. ***PCA and outgroup-f3 multidimensional scaling (MDS) for Bronze and Iron Age samples (A-B and C-D, respectively).*** *Samples were divided into four groups according to their chronological and cultural affiliation and projected onto the genetic variation of modern West Eurasians (left column, panels A, C) along with a number of modern reference samples from neighbouring regions. Samples within the dashed grey polygons were also used to construct a pairwise matrix of outgroup f3 distances in the form of f3(Mbuti; X, Y) on which an MDS was performed, hence providing a complementary visualization to the PCA. The chronological dating, based on mean date BP (before present), was used to color-code the samples (right column, panels B, D)*

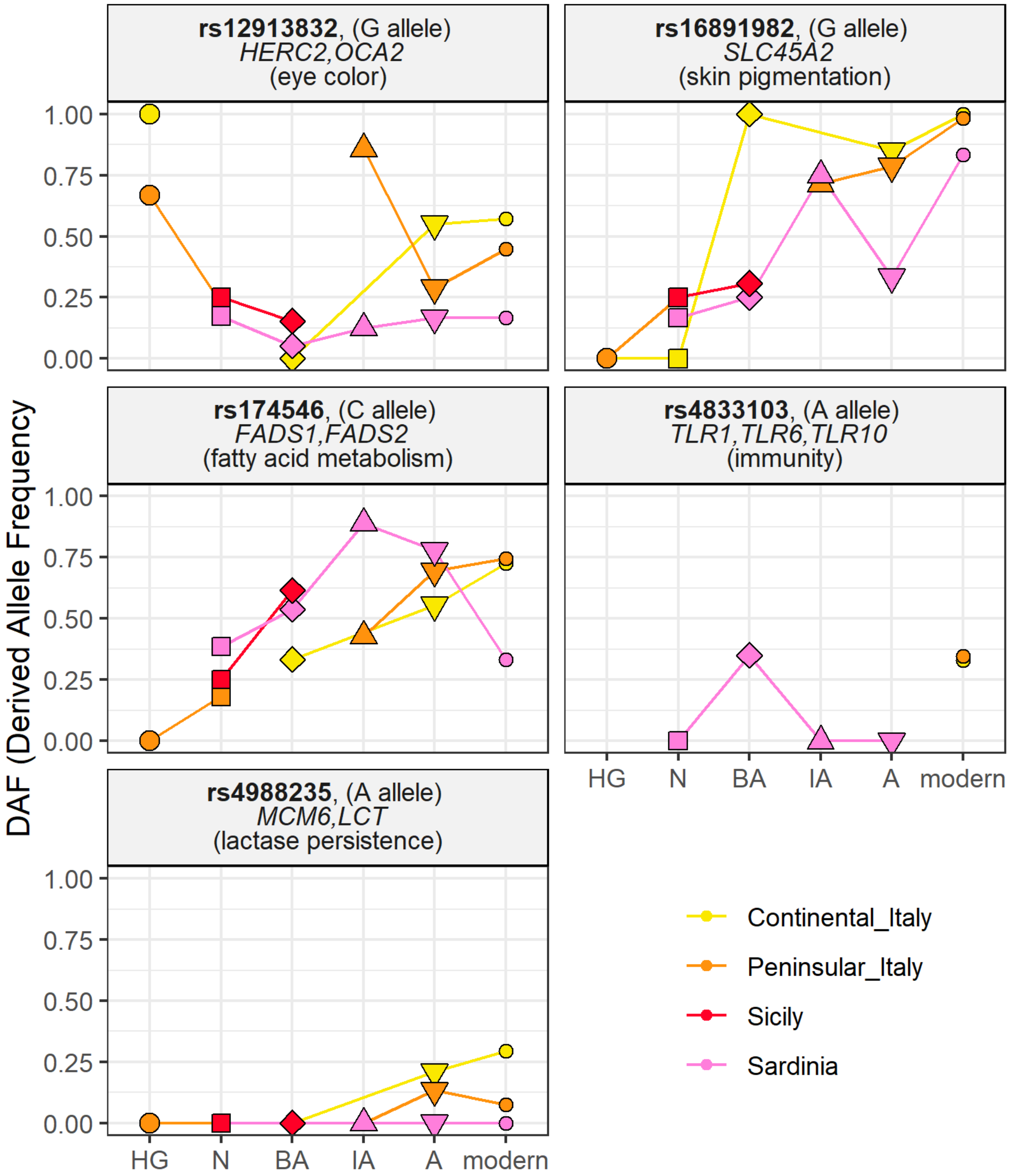

Figure 4. ***Derived allele frequencies across the four Italian macro-areas for five genetic variants reported to be the strongest significant signals of selection in Mathieson et al. 2015.*** *The name of the variant (in bold), the allele for which the genetic frequencies are computed (the derived one), the name of the gene, and the associated function are shown above each plot.*

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