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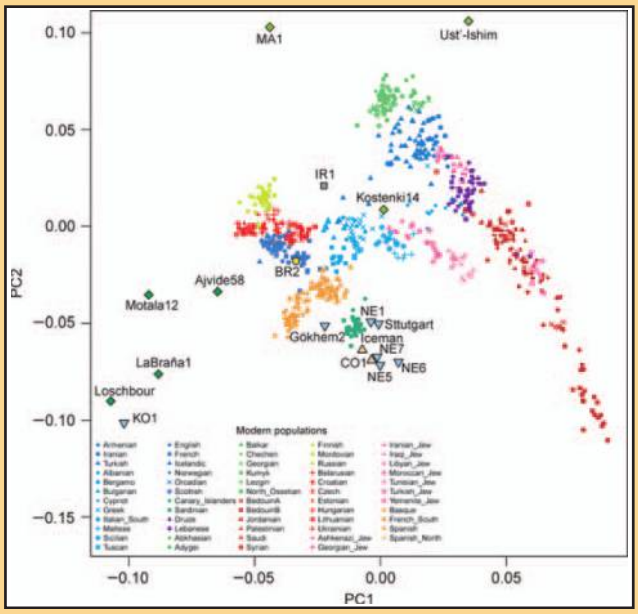
Modern humans' paleogenomics and the new evidences on the European prehistory

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Abstract The modern human settlement of Europe is a complex process with overimposed migrations and regionalisation episodes, as well as adaptive processes that shaped the genome of modern populations. New developments in massive sequencing techniques allow now the generation of an unprecedented amount of genomic data, including the genotyping of a large number of single nucleotide polymorphisms (SNPs) and the generation of complete ancient genomes. These paleogenomic data will have much more resolution for studying the past than genomic data from present-day individuals because migration, admixture and population replacement can confound the interpretation of current genetic diversity. However, their interpretation will require a real multidisciplinary effort, involving population geneticists, archaeologists and anthropologists. Here, we explain how paleogenomics is providing new insights into a wide range of topics such as demography, undetected migrations, mating strategies or adaptive processes of prehistoric European populations.



Statement of significance The study of the prehistoric modern humans' settlement of Europe has been until recently restricted to fields such as archaeology and physical anthropology. However, with the advent of the new sequencing technologies in the last few years, it is increasingly possible to retrieve massive genomic data from these past populations, and to directly test previous hypothesis on human population movements and affinities. We provide here a guideline of the main areas of study of the past that will likely be impacted by paleogenomic studies in the next years.

Keywords Ancient DNA, Modern human beings, Demography, Adaptation, Migration, Europe

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Data availability The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are contained within the paper.

Introduction

Anatomically modern human beings (AMH) were the only inhabitants of Europe after the disappearance of the Neandertals, around 40000years ago (Higham *et al.* 2014) [a more recent date, around 28000years ago, had been previously proposed (Finlayson *et al.* 2006) although it is now debated). New direct radiocarbon dates of fossils indicate that AMH appeared in Europe by 44200–41500years BP at Kent's Cavern (England) (Higham *et al.* 2011) and by 45000–43000years BP in Grotta del Cavallo (Italy) (Benazzi *et al.* 2011). Archaeologists have identified different material cultures that varied in temporal scale and geographical areas: Aurignacian, Gravettian, Solutrean and Magdalenian. This period of AMH occupation of Europe was marked by the Last Glacial Maximum (LGM) that took place between 26500 and

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19000 years ago (Peter et al. 2009). During its cold peak, large parts of the continent were covered with ice sheets, at the same time pushing human beings into southern refugia such as the Iberian, the Italian and the Balkan peninsulas that were relatively limited in resources.

The Mesolithic is the first period of the Holocene and comes at the end of the LGM. Its chronology varies depending on the continental region, but it dates from about 11500–5500 years BP. As the ice sheet disappeared, hunter-gatherer groups began to inhabit forest land and open tundra that had earlier been covered by ice. Hunting of species such as aurochs, red deers and wild boars became a specialised task for the Mesolithic hunters, which seem to be composed by small and highly mobile groups (Whittle and Cummings 2007). This was accompanied by some technological innovations, most notably the microlithisation of flint tools.

The scenario radically changes with the arrival of the next cultural period, the Neolithic, defined by a subsistence strategy based on domestic animals and crops. The first Neolithic groups entered Europe through Anatolia around 8,500 years BP, coming originally from the Near East, where the first archaeological sites can be traced back to 11,500 years ago (Renfrew and Bahn 1991). These pioneers established in Greece and the Balkans, and later spread west and northwest, following two different routes: the Mediterranean coast to the west (associated with the Cardial ware culture) and the Danube river to the centre and north of Europe [associated with the Linearbandkeramik (LBK) or Linear Pottery culture] (Pinhasi *et al.* 2012). It is likely, although more difficult to prove, that maritime movements along the Mediterranean could have sped the process (Fernández *et al.* 2014; Paschou *et al.* 2014). Overall, the expansion was probably complex and varied in intensity from one area to another, depending primarily on ecological conditions favourable to agriculture and also on local human population densities. Nonetheless, by 5,000 years BP, the Neolithisation of continental Europe was complete, and the hunting and gathering way of life had forever disappeared in this region of the world. Subsequently, other economic changes such as the introduction of the wheel, wool and milk production, and the increased use of animal power for farming tasks took place.

The Neolithic represents a real demographic revolution in European history, to the point that all modern European societies derive from that cultural, social and ecological turning point (Antia *et al.* 2003; Wolfe, Dunavan and Diamond 2007). Human groups became sedentary and established large settlements that allowed an increase in population size and density; this generated the emergence of peasants – the oldest substratum of contemporary society (Diamond 1997). Material culture saw also the development of pottery and polished tools that later diversified in several traditions.

There seems to be a period of stability from the Early to the Middle Neolithic, as judged from the archaeological record. However, the Late Neolithic and the Chalcolithic (or Copper Age) sees the emergence of technical developments related to copper metallurgy and also the appearance of different types of pottery, including the Corded Ware and the Bell Beaker (BB) ceramics. The expansion of these ceramic traditions, which could have involved either cultural diffusions or human migrations, has also been the subject of academic debates for many years. In any case, the Copper, the Bronze and the subsequent Iron Age represent a social turning point, marked by the formation of social elites and the emergence of complex societies in prehistoric Europe (Czebreszuk 2004).

Until recently, the origin of the human settlement of Europe has mainly been studied from archaeological data. However, ancient DNA, the recovery of genetic material from preserved human skeletal remains, resulted in a new source of independent evidence for other fields such as archaeology, anthropology and forensics. Interestingly, some of the conflicting hypotheses on the European prehistory can be resolved from the analysis of ancient genomes.

For about 20 years, the only possible technical approach to ancient DNA was the polymerase chain reaction (PCR) and the cloning of PCR products. Due to the methodology employed, researchers working on the genetics of past human populations were limited to study single genetic loci, mainly uniparental markers such as maternally inherited mitochondrial DNA (mtDNA) (Brandt *et al.* 2013) and, to a lesser extent, the paternally inherited Y-chromosome (Lacan *et al.* 2011a, 2011b). Despite having some interpretative advantages such as lack of recombination, high mutation rates and well-established phylogenies, uniparental markers (mtDNA and Y-chromosome) are greatly affected by random demographic processes (Mourier *et al.* 2012), and they constitute very coarse markers for unravelling complex population dynamics at a small geographic scale.

The emergence of palaeogenomics (the study and analysis of ancient genomes), triggered by the development of next-generation sequencing (NGS) technologies after 2005, Mardis (2008) and Metzker (2010) have provided a new powerful source of information that can be used to test previous hypotheses regarding human evolution and diversity.

With the advent of these NGS technologies, the years 2012–2014 have seen for the first time the publication of complete prehistoric European genomes (Table 1; Fig. 1). The first one was the genome of the exceptionally well-preserved Tyrolean Ice man, Ötzi, dated to about 5300 calibrated years BP (cal BP) (Keller *et al.* 2012). The second one was the retrieval of a Mesolithic hunter-gatherer from La Braña-Arintero site in Spain dated 7800 cal BP (Olalde *et al.* 2014). More recently, additional Mesolithic and Neolithic genomes from Scandinavia, Luxembourg, Germany and Hungary have been published (Gamba *et al.* 2014;

Table 1 List of ancient Eurasian specimens with a published complete genome sequence (cutoff of >1 × coverage)

Sample name	Period	Age (cal. BP)	Mean coverage	Sex	mtDNA Haplogroup	Y Haplogroup	References
Ust'-Ishim	Pleistocene hunter-gatherer	46 880–43 210	42	Male	R	K(xLT)	Fu *et al.* (2014)
Kostenki14	Pleistocene hunter-gatherer	38 700–36 200	2·42	Male	U2	C1	Seguin-Orlando *et al.* (2014)
MA-1	Pleistocene hunter-gatherer	24 423–23 891	1·2	Male	U	R	Raghavan *et al.* (2014)
Motala12	Holocene hunter-gatherer	8311–7466	2·4	Male	U2e1	I2a1b	Lazaridis *et al.* (2014)
Loschbour	Holocene hunter-gatherer	8170–7940	21·6	Male	U5b1a	I2a1b	Lazaridis *et al.* (2014)
LaBrana1	Holocene hunter-gatherer	7940–7690	3·4	Male	U5b2c1	C1a2	Olalde *et al.* (2014)
Ajvide58	Holocene hunter-gatherer	4900–4600	2·22	Male	U4d	I2a1	Skoglund *et al.* (2014)
KO1	Neolithic farmer	7730–7600	1·24	Male	R3	I2a	Gamba *et al.* (2014)
NE1	Neolithic farmer	7260–7020	22·12	Female	U5b2c	–	Gamba *et al.* (2014)
NE5	Neolithic farmer	7160–6940	1·04	Male	J1c1	C1a2	Gamba *et al.* (2014)
NE6	Neolithic farmer	7250–6900	1·18	Male	K1a3a3	C1a2	Gamba *et al.* (2014)
Stuttgart	Neolithic farmer	7050–6750	19·1	Female	T2c1d1	–	Lazaridis *et al.* (2014)
NE7	Neolithic farmer	6440–6310	1·14	Male	N1a1a1a	I2a	Gamba *et al.* (2014)
Gokhem2	Neolithic farmer	5050–4750	1·33	Female	H1c	–	Skoglund *et al.* (2014)
Iceman	Copper Age	5309–5055	7·6	Male	K1f	G2a2a1b1	Keller *et al.* (2012)
CO1	Copper Age	4850–4650	1·13	Female	H	–	Gamba *et al.* (2014)
BR2	Bronze Age	3220–3060	21·25	Male	K1a1a	J2a1	Gamba *et al.* (2014)
IR1	Iron Age	2930–2780	1·31	Male	G2a1	N	Gamba *et al.* (2014)

mtDNA: mitochondrial DNA.

Lazaridis *et al.* 2014; Skoglund *et al.* 2014), some of them to high coverage (this refers to the average number of DNA sequences covering each position of the genome; the higher the coverage, the better the quality of the genome sequence and the higher the chance to determine accurate genotypes). Moreover, the first European Upper Palaeolithic genome – that from the Russian site of Kostenki – has also been published (Seguin-Orlando *et al.* 2014), opening the study of European Pleistocene populations with complete paleogenomes.

Importantly, the interaction between archaeologists, palaeontologists and researchers from the emerging field of palaeogenomics has traditionally been plagued by a lack of collaborative efforts. Nevertheless, the possibility of working with partial or complete ancient European genomes is a unique opportunity for reconstructing the past following a real multidisciplinary approach.

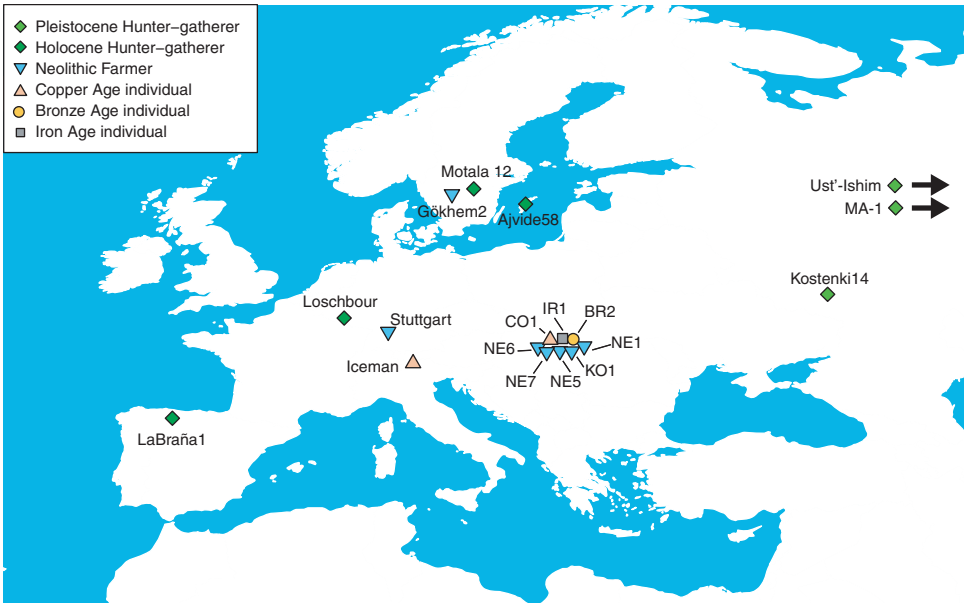


Figure 1 Geographic location of ancient Eurasian individuals whose complete genome (at least 1 × coverage) has been sequenced

Human contamination in ancient samples

Contamination of human samples with exogenous, modern DNA, sometimes deriving from ancient specimen handling during excavation and prior to the arrival to the laboratory (Sampietro *et al.* 2006), has been traditionally considered one of the major problems in ancient DNA research. Some precautions on how to handle samples for genetic analyses (e.g. using gloves and avoiding extensive cleaning and manipulations) have been established (Fortea *et al.* 2008). Unfortunately, it is impossible to know *a priori* if a specimen is contaminated and the extent of their endogenous DNA preservation. However, the NGS technologies have revealed a nucleotide misincorporation pattern at the ends of the ancient DNA sequences that can be used as a proxy for authenticity (Briggs *et al.* 2007). Additionally, the retrieval of the mtDNA genome at very high coverages allows now a precise estimate of the sample contamination (Krause *et al.* 2010). At genomic level, heterogeneities in the sexual chromosomes can also be used to estimate the level of intrusive DNA (Green *et al.* 2010; Rasmussen *et al.* 2011).

With the potential of NGS techniques to retrieve complete genomes and monitor contamination, even specimens such as Kostenki-14 (Seguin-Orlando *et al.* 2014), excavated decades ago, are now susceptible of being genetically analysed.

Demographic inferences and social practices

Genetic diversity is correlated with population size; the larger the population, the higher the diversity. In fact, demographic history has shaped our genomes and left traces on them that can still be recognised. From the study of prehistoric genomes, we can make varied inferences about group size, reproductive rules and social practices of these ancient populations.

With the limited number of available genomes, we already have evidences that Mesolithic foragers had a reduced genetic diversity, lower than modern Europeans and Neolithic farmers at the nuclear genome level (Skoglund *et al.* 2014). This can also be observed by looking at uniparental markers: so far, the 11 Mesolithic individuals analysed with NGS technologies show U2, U4 or U5 mtDNA haplotypes (Fu *et al.* 2013; Lazaridis *et al.* 2014; Sánchez-Quinto *et al.* 2012; Skoglund *et al.* 2014), a reduced diversity that contrasts with that of upcoming Neolithic farmers, where H, HV, V, K, J, T2, X, W and N1a (together with U haplotypes) have been observed (Brandt *et al.* 2013; Gamba *et al.* 2012, 2014; Haak *et al.* 2005, 2010; Hervella *et al.* 2012; Lazaridis *et al.* 2014; Sampietro *et al.* 2007; Skoglund *et al.* 2012) (Table 1). Less data are available for the Y-chromosome, not only due to the high coverage required to make an accurate inference of the haplotype but also because some of the samples analysed are obviously females. Even so, the genetic uniformity of the paternal line is more dramatic than that seen at the mtDNA. Ajvide58, Motala 12 and Loschbour carry an I2a1 haplotype (this is probably also the case of Motala 2, 3 and 9 that have low coverage genomes; Lazaridis *et al.* 2014) while La Braña 1 carries an extremely rare C1 haplotype [reported as C6 by Olalde *et al.* (2014)]. Interestingly, one early and one late Neolithic individuals from Hungary also carry a I2a lineage, while a 37 000-year-old individual from Kostenki-14 and two late Neolithic samples from Hungary share a C1 lineage with La Braña 1, suggesting paternal genetic continuity from the Upper Palaeolithic to the Mesolithic (Seguin-Orlando *et al.* 2014) and from foragers into farmers (Gamba *et al.* 2014; Table 1). These observations are further confirmed with the clustering of one sample (KO1) from the early Neolithic Körös culture site of Tiszaszőlős-Domaháza (dated 7730–7600 cal BP and coming from a short-lived settlement) among former Mesolithic foragers (Gamba *et al.* 2014); in addition, its predicted phenotype shows a blue-eyed male with dark skin, similar to La Braña 1, Motala 12 and Loschbour. Importantly, KO1 represents the first genetic evidence of a hunter-gatherer being incorporated into a farming community, adding further complexity to the social aspects of the Mesolithic–Neolithic transition.

In contrast to the high mtDNA diversity among early farmers, its paternal counterpart, the Y-chromosome, appears to be restricted to few lineages, notably the G2a lineage (also present in later individuals such as Ötzi) (Haak *et al.* 2010; Keller *et al.* 2012; Lacan *et al.* 2011a, 2011b; Table 1). These genetic observations based on the uniparental markers among farmers are compatible with a mating strategy based on patrilocal residence (i.e. men stay in the communities they were born while women move to their husband's communities). This could make sense in the first farming communities that needed to have a male-based control of resources and territorial defence.

Endogamy and consanguinity

Runs of homozygosity (ROH) are long chromosomic sections within an individual genome where both the paternal and the maternal copies are identical by descent. Depending on their length and frequency, can either be attributed to recent consanguinity (long, >1.6 Mb, ROH tracks) or endogamy due to long-term reduced population size (short, <1.6 Mb, ROH tracks). Precisely, the previously mentioned KO1 specimen from Hungary displays about 150 short ROH segments, a much higher number than subsequent Neolithic samples that have values ranging between 50 and 100 (Gamba *et al.* 2014). This observation indicates that this specific individual was coming from a small and endogamic population. It is remarkable, however, that no such long ROH segments have been found in the available Mesolithic genomes such as La Braña 1, Motala 12 and Loschbour (Lazaridis *et al.* 2014;

Olalde *et al.* 2014). It seems likely that despite their low population size, the foragers had social rules that were able to effectively avoid inbreeding in their hunting groups.

Recent selective sweeps in europeans

Methods for detecting positive selection at the genetic level are based on the principle that a selected allele changes in frequency more quickly than a neutral locus on the same genomic background. Using different detection methods applied to genetic variation data in modern populations, we have now a complete list of alleles that have purportedly been recently selected in Europeans, thus offering a unique window into the adaptive forces that shaped our evolutionary history (Grossman *et al.* 2013). Even though none of these methods can accurately date the selective event, thanks to the ancient genomes already available, we are now able to track the trajectories of these alleles over time and space, allowing direct access to the evolutionary history of adaptations in European populations.

Recent findings have already shown that some predominant traits in Europeans such as lactase persistence or LP (e.g. the continuing ability to digest milk in the adulthood due to a mutation in a regulatory element of the LCT gene) have been driven to current high frequencies much more recently than previously thought (Gerbault *et al.* 2011). The selective advantage of LP is the possibility of having a constant nutritional intake from dairy – the only source of lactose – especially in regions such as northern Europe that are less favourable to farming. The conspicuous absence of the persistence allele in many Neolithic individuals from different places in Europe (in a recent study of five millennium transect from the early Neolithic to the Iron Age in Hungary (Gamba *et al.* 2014), the first LP allele appears in a sample that is only 3000 years old) suggests that the rise of the LP mutation could be related to pastoralism practices spreading during the Late Neolithic or even later. A recent model was based on the assumption that LP first underwent selection about 5500 years ago, possibly associated with the LBK culture in central Europe (Itan *et al.* 2009). However, the growing evidence from Neolithic genomes seems to point to an even more recent origin. If confirmed, it can constitute one of the strongest cases of positive selection ever described in human beings, driving the LP allele to almost fixation in many European populations in no more than a few thousands of years (Gerbault *et al.* 2011).

The LP selective sweep (selective sweep is defined as the increase in frequency of an allele favoured by selection as well as other alleles linked to the selected variant) had previously been predicted to be a post-Neolithic event. However, a bigger surprise was to discover that acquisition of light skin pigmentation in Europeans might also be post-Neolithic (Olalde *et al.* 2014; Wilde *et al.* 2014). Newly available ancient genomes suggest that selection of derived alleles in the two crucial skin pigmentation genes, SLC24A5 and SLC45A2, is decoupled in time by 2000 years; a selective sweep first occurred for SLC24A5 (the gene with the strongest effect in light skin colour (Lamason *et al.* 2005; Norton *et al.* 2007)) and later for SLC45A2 (Gamba *et al.* 2014). This explains why many farmers, including some late Neolithics, still retain the ancestral allele at SLC45A2 (Bronze Age and Iron Age individuals with the ancestral allele could correspond to posterior migrations from the east where the 'light' allele was not yet prevalent). In addition, Motala 12, a Mesolithic hunter-gatherer from Sweden, is heterozygous for SLC24A5 (Lazaridis *et al.* 2014), which might suggest that previous Mesolithic populations were not totally homogeneous for the phenotype, and that some light skin alleles were already present in Europe (but maybe not in Western Europe) before the arrival of the Neolithic. In the future, new ancient genomes from different periods and adjacent geographical areas such as the Near East or Western Asia will help to fully understand the timing of crucial selective processes.

Although genomic data are still scarce, the temporal screening of selective events associated with some European-specific alleles with medical interest will also be relevant to model past selective events. For instance, alleles related to pathogen resistance or susceptibility to disease, like the CCR5-delta 32 mutation (resistant to HIV infection) that is thought to have recently risen to relatively high frequencies in Europeans (5–18%) (Novembre, Galvani and Slatkin 2005) could be screened in the ancient genomes. Moreover, we could be able to detect past epidemics that may have impacted the genomic composition of prehistoric (and also historic) European populations. These events could eventually be related to demographic declines observed in some archaeological horizons.

Uncovering migrations and population turnovers

The recent political and social context has an influence on the archaeologists' attitudes regarding the interpretation of the past. Since the 1960s, most of the theoretical developments in British archaeology – as well as in other European countries – have favoured local developments as the explanation for cultural changes and thus have been quoted as 'anti-migrationists' (Härke Heinrich 1998). Indeed, supporting large-scale population movements is seen as academically anachronistic in many European institutions. However, recent advances in archaeogenetics are placing migration and population turnover again as a central mechanism in the shaping of current European genetic diversity. In addition, a dynamic metapopulation system, as invoked in the analysis of Kostenki-14 genome (Seguin-Orlando *et al.* 2014), could also account for some of the population patterns observed.

A large variety of past migratory movements, some of them undetected by archaeology, are now

susceptible of being scientifically explored in space and time, and this is taking place irrespective of the views of scholar's archaeological traditions. For instance, a genomic study based in modern data (Patterson *et al.* 2012) detected a possible influx of central Europeans into the Iberian Peninsula circa 4000 years ago that seemingly remains uncharacterised from an archaeological point of view. Ancient DNA data from this period could help to resolve this genomic turnover and also to correlate it with cultural evidence. More recently, a large-scale analysis of modern European genomes together with several ancient ones have revealed three different ancestral population components in the gene pool of Europeans (Lazaridis *et al.* 2014): West European hunter-gatherers, early European farmers of Near Eastern origin, and a mysterious ancient north Eurasian population [related to the Mal'ta boy (Raghavan *et al.* 2014)] that apparently entered the continent from the East in the Late Neolithic. The arrival of this north Eurasian component could be associated with the clear genomic shifts detected in Bronze and Iron Age individuals from Hungary (Gamba *et al.* 2014). The three ancestral population models have recently been challenged by the publication of the Kostenki-14 genome (Seguin-Orlando *et al.* 2014) that points to the presence of the Near East component much earlier than the suggested (Lazaridis *et al.* 2014) Neolithic period. Ultimately, the precise number and nature of those ancestral components could be elucidated with the additional sequencing of more specimens.

These new ancient DNA studies provide evidence that many cultural horizons are associated with the arrival of foreign people, but the reverse can also be true; potential migration movements described from the spreading of some material culture could in fact be revealed to be just the consequence of cultural diffusion.

In order to study patterns of genetic affinities between ancient individuals and extant populations, different statistical methods are routinely applied in paleogenomic works. Principal Component Analysis (PCA) (Patterson, Price and Reich 2006) is a straightforward way to visualise genetic variation by reducing the observed dimensions into a number of uncorrelated principal components that account for most of the variance in the data. In a PCA (Fig. 2, Table 2) including modern West Eurasian populations from the Human Origins array (Lazaridis *et al.* 2014; Patterson *et al.* 2012), together with ancient individuals sequenced to at least 1× coverage (on average, one DNA sequence spanning each nucleotide of the genome), several features can be observed: (1) Mesolithic hunter-gatherers are beyond the extant European genetic diversity, (2) all Early farmers cluster

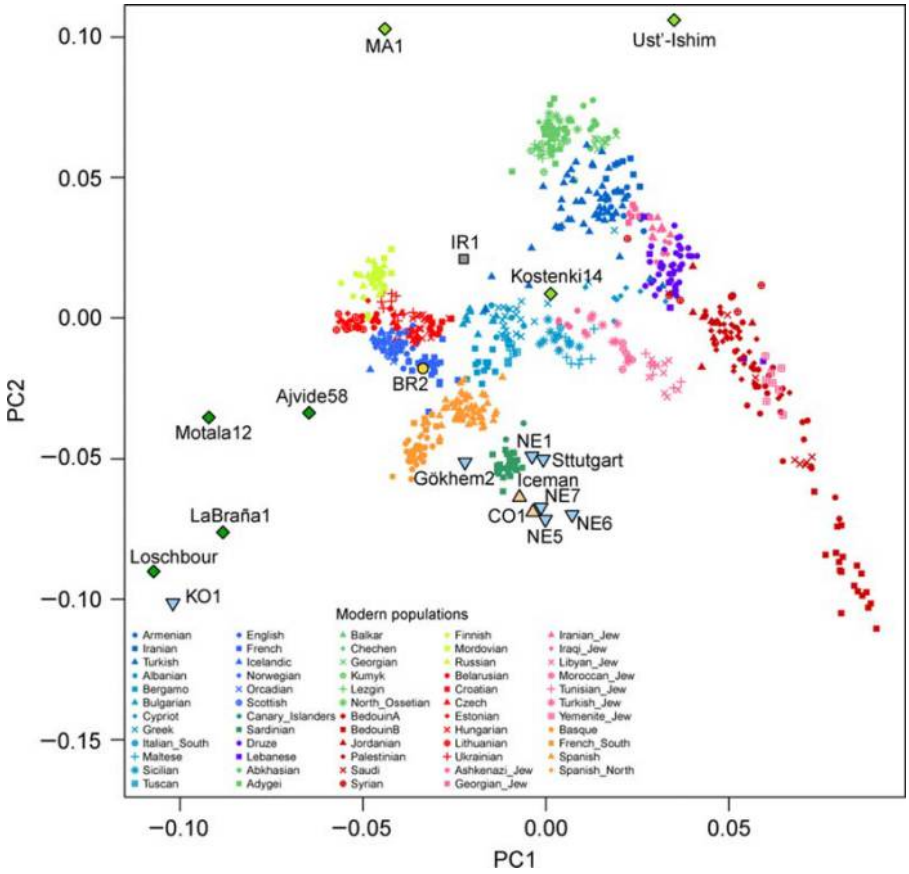


Figure 2 Principal Component Analysis (PCA) of present-day West Eurasian populations and ancient individuals. Colour key for ancient individuals as in Fig. 1. We performed individual PCAs on each ancient sample together with the modern dataset [randomly sampling one allele at each single nucleotide polymorphism (SNP) position]. Then, individual PCAs were combined using Procrustes transformation as previously described (Skoglund *et al.* 2012; Wang *et al.* 2010)

Table 2 Number of SNPs used for each individual PCA

Sample	Number of SNPs
Ust'-Ishim	483 822
Kostenki-14	457 297
MA-1	347 805
Motala12	423 877
Loschbour	409 521
LaBrana1	447 805
Ajvide58	267 505
KO1	337 552
NE1	404 808
NE5	286 039
NE6	325 561
Stuttgart	406 810
NE7	315 664
Gokhem2	210 539
Iceman	422 128
CO1	303 493
BR2	431 504
IR1	331 392

SNPs: single nucleotide polymorphisms; PCA: Principal Component Analysis.

together with southern Europeans, and specifically with Sardinians, (3) Scandinavian, Western European and Upper Palaeolithic hunters seem to form different clusters, suggesting some kind of ancient phylogeographic structure, and (4) current European diversity is shaped during quite recent (from the Copper Age onwards) migrations associated with cultural developments that combine previous population components. Again, these analyses provide independent genetic evidence that can be corroborated on the archaeological record.

The future of palaeogenomics

We are currently entering in the golden era of palaeogenomics, which will provide researchers with an unprecedented amount of information for the reconstruction of human past. In the next few years, we will see many, perhaps hundreds, of the ancient human genomes retrieved, and tens of thousands of modern human genomes routinely sequenced. All archaeological hypotheses involving population movements and affinities of cultural horizons will be explored from this emerging body of genomic data, including highly debated archaeological problems such as the timing of the spreading of the Indo-European languages or the real nature of the BBs expansion. However, for such enterprise to be successfully achieved, crucial archaeological information, such as precise radiocarbon dates, funerary contexts, regional developments and general cultural patterns will be needed. We believe this is a unique opportunity for building real multidisciplinary groups involving the different scientific fields dealing with the study of the past.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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