Review

The genetic history of Europeans

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The evolutionary history of modern humans is characterized by numerous migrations driven by environmental change, population pressures, and cultural innovations. In Europe, the events most widely considered to have had a major impact on patterns of genetic diversity are the initial colonization of the continent by anatomically modern humans (AMH), the last glacial maximum, and the Neolithic transition. For some decades it was assumed that the geographical structuring of genetic diversity within Europe was mainly the result of gene flow during and soon after the Neolithic transition, but recent advances in next-generation sequencing (NGS) technologies, computer simulation modeling, and ancient DNA (aDNA) analyses are challenging this simplistic view. Here we review the current knowledge on the evolutionary history of humans in Europe based on archaeological and genetic data.

Major turning points in the evolutionary history of Europeans

Human evolutionary history includes all the complex demographic, natural selection, and stochastic processes that have shaped our species. Despite the limitations of genetic and archaeological data to inform on all the details of human evolution, they constitute an irreplaceable source of information to appraise the key episodes that are likely to have had major impacts on patterns of genetic, morphological, and cultural variation. When considering AMH in Europe, three such critical periods are apparent: (i) the expansion of AMH out of Africa and their colonization of Europe approximately 45 000 years ago (45 ka), (ii) the last glacial maximum (LGM) and the formation of uninhabitable areas in Europe between 27 and 16 ka, and (iii) the arrival of Neolithic culture in southeast Europe and its spread throughout the rest of the continent between 9 and 5 ka. Here we review genetic evidence describing these major demographic episodes in the context of archaeological and chronological data.

Appearance of AMH in Europe and the LGM

It is generally accepted that all humans today are descended from AMH that evolved around 160 to 200 ka in Africa ([1] and references therein). However, recent NGS-based analyses (see Glossary) of two prehistoric genomes, one composite from three Neanderthals [2], and one from an as-yet morphologically uncharacterized hominin from Siberia [3,4], are shifting the prevailing view of a strict recent African origin for AMH ancestry because both studies indicated that AMH may have interbred with archaic humans. Nevertheless, the majority of genomic diversity in living humans outside Africa is still believed to have originated in AMH who left Africa between 50 and 70 ka. Until recently, the earliest date for the first appearance of AMH in Europe had been set to around 42 to 43 ka solely based on their proposed association with Aurignacian artifacts (Table 1) [5,6]. New direct radiocarbon dates of fossils support this view and indicate that AMH appeared in Europe by 44.2–41.5 calibrated (cal.) ka BP at Kent's Cavern in southern England [6] and by 45–43 cal. ka BP in Grotta del Cavallo, Italy [7], whereas Neanderthals did not survive in most of Europe and the Caucasus after 39 cal. ka BP [8,9].

After the disappearance of the Neanderthals and particularly during the LGM, the northern parts of Europe were covered by ice sheets, leaving humans to survive in poorly resourced environments [10,11]. Parts of northern Europe were either completely abandoned [12] or sparsely populated [13]. The archaeological record of this period catalogs a complex series of interrelated material cultures that vary in their geographic ranges and temporal durations (Table 1). Spatial patterns of material culture change have been interpreted as indicating colonization of regions up to 52°N latitude during the Gravettian, followed by partial or complete retreat of most northern populations by 24 ka, and recolonization of these regions by 20–16 ka, with some continuity of occupation in more southern latitudes [14]. However, the extent to which material cultures correspond to distinct human populations, and to which their distribution changes through time correspond to demographic processes, remains unclear. Although it is likely that some component of modern European ancestry can be traced back to one or more refugial populations around the time of the

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Glossary

Calibrated before present: carbon isotope ratio measurements are reported in uncalibrated (uncal.) radiocarbon years 'before present' (BP), where 'present' is set at 1950. This determination can be calibrated (cal.) to calendar years using a calibration curve which primarily charts variations over time in atmospheric carbon isotope ratio.

Coalescent theory: a retrospective model of population ancestry that provides a probabilistic description of the genealogy of a gene going back to a single ancestral copy, given a particular demographic history. It provides a means of simulating genetic data under different historical scenarios in a very computationally efficient manner, and it benefits from considering only the sample and not the whole population.

Cultural diffusion: in the context of the Neolithic transition, a model in which farming spread by transmitting a set of technologies and skills, including agricultural crops and livestock, but without any substantial movement of populations.

Demic diffusion model: in the context of the Neolithic transition, a diffusion process of farming populations without substantial admixture with local hunter-gatherer populations, implying that the latter did not contribute substantially to the ancestry of modern Europeans.

Founder effect: a loss of genetic diversity which occurs when a new population is established by a small number of individuals (founders) from a larger source population. Owing to random sampling, the new population will carry only a subset of the total genetic variation of the parental population.

F_{ST} distance: a statistical index representing the proportion of overall genetic variation that is explained by differences between subpopulations. It can be used as a measure of allele frequency difference between population samples; F_{ST} values range from 0 to 1, with lower values indicating greater genetic similarity between two samples.

LGM refugia: during the LGM large areas of Europe, including most mountain regions, were covered by glaciers. The peninsulas of southern Europe, in other words Iberia, Italy, and Balkan, remained ice-free and were core areas for the survival of temperate animal and plant species, and potential source regions for their postglacial recolonization of the continent.

Mesolithic: the archaeological cultures of late hunter-gatherer-fishing populations that existed mainly in northern Europe approximately between ~11.5 and 5.5 ka BP, before and during the emergence of agriculture.

Neolithic transition: the prehistoric transition from mobile hunting-gathering to a sedentary, food-producing lifestyle with a subsistence base that mostly relied on domesticated crops and livestock, storage, and the use of pottery.

Next-generation sequencing (NGS): a set of DNA sequencing methods that are fundamentally different from classical Sanger sequencing and that allow extremely large numbers of DNA sequencing reads (>1 billion reads for some technologies) to be obtained in parallel in a single machine run. The first of these technologies to be introduced was the Roche-454 system [67], followed by a range of other technologies [68].

Principal component (PC) map: a synthetic map plotting a principal component of genetic variation (usually summarizing many loci) in a geographic area. One map can be produced per principal component and the first map (PC1) summarizes the largest amount of genetic variation.

Population continuity: a situation whereby a population at one timepoint is descended entirely from a population at an earlier timepoint (usually in the same region). Although complete population continuity is unlikely, except in the case of an isolated island population, it forms a natural null-hypothesis in genetic studies of population history and is frequently invoked in archaeology.

Spatially explicit modeling: the simulation of population histories, usually including the inheritance processes that shape patterns of genetic variation, that explicitly takes into account the geographic location of individuals and populations, and often also the effects of environmental and topological features. For convenience, and to take advantage of well-developed population genetic theory, this is usually done in a demic framework whereby geographic space is split up into a grid, although continuous space modeling is also possible.

Upper Paleolithic: the prehistoric period, which in Europe began during the Late Pleistocene at about 50 ka BP, and lasted until the onset of the Holocene, ~11.5 ka BP. It is associated with an increase in symbolic and technological complexity, and, in Europe, is associated with the first appearance of anatomically modern humans (AMH).

LGM, the number, location, and distribution of these refugia remain uncertain ([15] and references therein). To understand the contribution of genetic studies to this problem it is first necessary to consider the different ways genetic data can be used to draw inferences about the past.

Approaches to interpreting genetic variation

Many studies of modern human genetic variation, particularly of mitochondrial DNA (mtDNA) and Y-chromosome data, have applied phylogeographic analysis (e.g., [16,17]), an approach whereby branches or clades on a phylogenetic tree, together with their estimated ages, are assumed to correlate with demographic episodes of interest. There are some demographic scenarios where this inference approach is justified, such as when populations are small and are related purely through a process of bifurcation with no subsequent admixture (i.e., the population history is as tree-like as a gene tree); however, such scenarios are probably absent from the human past, and phylogeographic inference of human variation brings with it considerable risks of misinterpretation [18]. Nonetheless, phylogeographic inference remains popular (e.g., [17]) despite widespread criticism (e.g., [18–20]) and the development of other more robust approaches. The latter involve either interpretation of population genetic statistics that summarize patterns of variation and that often have convenient properties – such as acting as proxies for real evolutionary processes (under 'ideal' conditions) or explicit modeling of population genetic history (Box 1).

Paleolithic versus Neolithic contribution

As mentioned above, the recolonization of northern Europe by refugial populations may have had profound effects on the genetic diversity of Europeans. However, it is difficult to distinguish these effects from those of the Neolithic transition, which happened only a few thousand years later. The Neolithic transition has been widely studied, and was traditionally considered to be the major demographic process in Europe. Over 30 years ago a seminal study [21] used principal component analysis (PCA) to create synthetic maps of Europe summarizing spatial allele frequency variation in 39 classical markers (e.g., blood groups). The first principal component map (PC1) displayed a Southeast–Northwest cline, which was attributed to the 'wave of advance' or 'demic diffusion' of farmers from the Near East into Europe, with gradual changes in allele frequencies believed to be due to the absorption of local hunter-gatherer populations into farming communities. This interpretation assumed that, during the diffusion process, local admixture between indigenous hunter-gatherers and the advancing wave of farmers was minimal. Consequently, the current European gene pool was interpreted as consisting mainly of genetic variation originating in Near Eastern/Anatolian (NE/A) Neolithic populations, with only a small contribution from pre-Neolithic Europeans. This approach was later extended using data on allele frequencies for over 130 classical markers [22], which yielded similar results.

However, the extent to which composite allele frequency clines indicate the direction of past population movements has been questioned [23,24]. It has been shown that, in some circumstances, PC1 may lie perpendicular to the direction of population expansion, whereas other components may reflect mathematical artifacts [25–27]. In addition, although the observed genetic clines may indeed result from the spread of Neolithic peoples [28,29], they may also have arisen from serial founder effects during the expansion of late Pleistocene refugial populations or the first AMH into Europe at around 45–40 ka [30,31], (see Box 1). Thus, PCA analyses can provide useful summaries of some of the data and can sharpen intuitions when

Box 1. Modeling human genetic history in space and time

Making inferences about human evolutionary history using patterns of genetic and other sources of variation is challenging principally for four reasons: (i) our true evolutionary history is sufficiently complex that no genetic or archaeological dataset will have enough information to fully recover it; (ii) different evolutionary histories can give rise to the same patterns in genetic or other data (equifinality); (iii) because of the underlying stochastic nature of inheritance (drift) and mutation, identical evolutionary histories run multiple times will give rise to different patterns in genetic data (evolutionary variance); and (iv) some evolutionary processes can generate counter-intuitive patterns in genetic data (emergence; e.g., allele surfing [26]). Computer simulation constitutes a powerful approach for examining hypotheses of human evolution, and can be used to integrate information from various fields such as genetics, archaeology, and paleo-environmental studies [41]. Most importantly, simulation approaches – especially those that are spatially explicit – allow researchers to maximize (i) and accommodate (ii), (iii), and (iv).

The utility of simulation approaches was shown in a seminal study published in 1986 [72] that consisted of the simulated colonization of Europe by early Neolithic farmers in a large wave of migration starting from the Near East. During this demographic and spatial expansion, gene flow was allowed between local hunter-gatherers and migrating Neolithic farmers. The resulting pattern of allele frequencies was compared to the one observed in contemporary Europeans. This study demonstrated that allele frequency clines, from the southeast of Europe toward the northwest (SE–NW), such as those observed in contemporary Europeans, could be generated by demic diffusion during the Neolithic. This approach was further pursued and extended, confirming that SE–NW genetic clines over Europe are compatible with Neolithic demic diffusion, even with a very low level or absence of genetic contribution by local hunter-gatherers [28,30], and that those clines could have been generated not only by the Neolithic transition but also by the arrival of AMH in Europe, or by both, implying that the simple observation of SE–NW genetic clines over Europe is not indicative, by itself, of the amount of genetic replacement during the Neolithic [31]. Indeed, clines could be generated not only by admixture between two different populations but also by a series of founder effects – either during the arrival of early modern humans or during the advance of Neolithic farmers.

Spatially-explicit computer simulations are thus extremely useful to understand how geographic patterns of genetic variability could have been shaped (e.g., [41,73]). Europe has been the main focus of this approach, firstly because abundant genetic data exist from this area, and secondly because its archaeology is particularly well-documented, which has resulted in a range of explicit and testable alternative scenarios of settlement [74]. Although models are necessarily relatively simple compared to reality, the simulation approach aims at understanding underlying processes and offers a theoretical framework to which empirical data can be compared and integrated. One of its main advantages is that potential improvements are almost unlimited because one may add new features to the model as new information becomes available either in the genetic or in the archaeological domains.

developing hypotheses of population history, but they should be interpreted with caution because they (i) do not provide direct tests for those hypotheses, (ii) are not necessarily direct proxies for the demographic processes that shaped them [25,26], (iii) only represent a limited proportion of the overall genetic variation present (e.g., the first two principal components of genetic variation in European genome-wide data [32] only explain 0.45% of the total variation), and (iv) can be biased by data quantity and quality. PCA and other clustering approaches can provide attractive and illuminating data summaries, and reveal interesting and sometimes surprising relationships among populations, but they are no substitute for explicit testing of competing demographic and evolutionary hypotheses [33].

Therefore, because the age of the observed clines cannot be assessed directly, and in the absence of clear-cut genetic markers of Paleolithic/Mesolithic or Neolithic origin, genetic studies have delivered diverse and often conflicting inferences on the contribution of NE/A farmers to the modern European gene pool. Estimates for this contribution have varied from 20% to 70% ([34] and references therein). In addition to the shortcomings of intraspecies phylogeographic inferences noted above, the incongruence between studies may be the outcome of a range of factors. First, different genetic loci each have their own genealogical histories which, because of the stochastic nature of inheritance, will to a greater or lesser extent be decoupled from each other as well as from the population history itself. It is therefore necessary either to account for this decoupling statistically or to combine information from multiple loci to build a composite picture of population relationships. Second, the archaeological record indicates that the Neolithic transition in Europe was not a single

Table 1. Chronology, subsistence, and geographic distribution of the main archaeological cultures of western Eurasia

Culture	Subsistence	Period[a]	Geography
Middle Paleolithic	Hunter-gatherers	300–30 uncal. ka BP	Western Eurasia
Upper Paleolithic (UP):	Hunter-gatherers	50–11.5 uncal. ka BP	Western Eurasia
Proto-Aurignacian/Initial UP	Hunter-gatherers	50–40 uncal. ka BP	Western Eurasia
Aurignacian	Hunter-gatherers	45–30 cal. ka BP	Western Eurasia
Gravettian	Hunter-gatherers	32–23 cal. ka BP	Western Eurasia
Solutrean	Hunter-gatherers	25–19 cal. ka BP	SW France, Iberia
Magdalenian	Hunter-gatherers	19–13.5 cal. ka BP	Western and Central Europe
Epigravettian	Hunter-gatherers	20–10 cal. ka BP	Europe
Mesolithic	Hunter-gatherers	11.5–5.5 cal. ka BP	Western Eurasia
Epipaleolithic	Hunter-gatherers, cultivators	20–11.5 cal. ka BP	Near East, Anatolia
Pre-Pottery Neolithic	Farmers	11.5–8.3 cal. ka BP	Near East, Anatolia
Pottery Neolithic	Farmers	8.3–7.8 cal. ka BP	Near East, Anatolia
Early Neolithic	Farmers	8.5–6 cal. ka BP	SE/Central Europe
Middle/Late Neolithic	Farmers	6.5–4.5 cal. ka BP	SE/Central Europe

[a] BP, before present; cal., calibrated; ka, kiloannum (1000 years); uncal., uncalibrated.

continuous dispersal process from the NE/A into Europe, but instead involved a series of punctuated maritime and land movements along both coastal routes and major river valleys ([35] for various contributions). The extent of interbreeding with local hunter-gatherers is therefore likely to show considerable regional variation. Third, studies differ in the type of molecular data considered [classical allele frequencies, DNA sequences, single nucleotide polymorphisms (SNPs), short tandem repeats (STRs)] and the kind of inference approaches used. Consequently, these results are often difficult to compare [31]. Fourth, different inferences from the widely used sex-specific inheritance systems (mtDNA or the Y chromosome) might reflect real differences in the contributions of male and female Neolithic farmers to the European gene pool (e.g., [36]) rather than revealing overall population processes. Indeed, mtDNA data appear inconsistent with a large Neolithic replacement [31], whereas this is not the case for Y-chromosome data ([36–38]; but see [39]). The differing estimates could possibly indicate an expansion of incoming male farmers, with a more limited contribution of female-line ancestry, although this hypothesis requires further testing. Admixture-based estimates of European Neolithic ancestry using autosomal data are close to 50% overall, or slightly higher, with a gradual Southeast–Northwest decrease [40].

Model-based approaches, which involve the simulation of genetic and/or other data under different evolutionary scenarios (e.g., [30,31,41,42]; Box 1), hold the potential to overcome these difficulties and assess the validity of different hypotheses. In a model-free or implicit model mode of enquiry it is often difficult to assess which of a set of plausible explanations best fits the observed data. For example, allele frequency clines may result from stochastic processes such as mutation and genetic drift, as well as from demographic processes such as isolation-by-distance (but see [30]), admixture of two genetically differentiated populations, range expansion, or variable selective pressures (see [23]). Likewise, the assumption that a relatively low contribution of incoming Neolithic farmers to the ancestry of modern Europeans is indicative of a cultural rather than demic diffusion model (e.g., [40]) is not necessarily valid because demic diffusion could spread genetic variants of hunter-gatherers that accumulate on a wave of advance, even if the assimilation of hunter-gatherers on the wave front is low (e.g., [31,43]).

Paleogenetics

As described above, modern-day genetic diversity provides only indirect evidence about the evolutionary history of past populations. About two decades ago, paleogenetics, the analysis of aDNA (Box 2), started adding to our understanding of evolutionary processes by providing direct data on the genetic diversity of past populations. We will therefore focus on how inferences based on paleogenetic data have contributed to our understanding of prehistoric population dynamics in Europe.

To date, the majority of aDNA studies have been restricted to short fragments, predominantly from the hypervariable region-1 (HVR-1) of the mtDNA genome, and this is of somewhat limited utility. However, recent technical progress has resulted in the first studies investigating genome-wide data, starting with the genome of a 4500 year old paleo-Eskimo at 20-fold coverage [44], followed by a low-coverage composite genome for Neanderthals [2], and another low-coverage genome from a morphologically uncharacterized hominin fossil from Denisova cave [3]. More recently, a sevenfold coverage genome of the Tyrolean iceman [45], and partial genomic data (27–97 Mb) from four Scandinavian individuals dating to the Neolithic [46], have been published. The proceeding from classical PCR protocols to novel NGS technology will no doubt further improve our understanding of human migrations and population processes.

Box 2. aDNA and next-generation sequencing (NGS)

The key advantage of aDNA data is the ability to time-stamp genetic diversity, instead of inferring past patterns from modern data. However, aDNA analyses also suffer from limitations such as variable (depending on environmental factors) and temporally limited DNA survival, problems with sequence authenticity, and of the representativeness of samples that yield reliable results [75,76]. Some of these problems have largely been solved by the application of NGS technologies [77]. Most importantly, NGS does not rely on targeted PCR amplification of the aDNA molecules using primers. Therefore, NGS is able to obtain useful sequence information from shorter DNA fragments than can PCR. Because the number of endogenous aDNA molecules in historical, archaeological, and paleontological samples increases exponentially with decreasing fragment length (e.g., [2]), NGS permits access to a much larger fraction of endogenous aDNA than does PCR. In addition, contaminating modern DNA tends to be longer, and consequently the ratio of endogenous to contaminating DNA shifts in favor of the former when using NGS compared to PCR [78,79], an effect that applies to both shotgun sequencing (e.g., [2,3,44]) and hybridization capture approaches [79–82]. Another key advantage of NGS is that it allows the use of degradation patterns to discriminate between modern DNA contaminations and ancient degraded DNA [70,83]. However, it should be noted that 'older' contaminations that have been present on and in the bones already for some time, for example since excavation in the field, may resemble aDNA and thus be difficult to discriminate [78].

Although the end of classical PCR methods is near, the use of NGS is no guarantee for reliable data (e.g., [84]). Therefore, certain precautions should be taken when working with ancient human DNA, such as the use of dedicated facilities separate from the modern and post-PCR DNA laboratories, appropriate controls and – most importantly – a critical attitude toward any data obtained [85]. However, the risks of obtaining erroneous results with NGS are smaller than for PCR, and the amount of data that can be obtained for the same amount of money and effort is much greater. Finally, it should be noted that NGS so far has only been applied to a few well-preserved samples. The extent to which it can be applied to highly degraded samples is currently unknown.

Hunter-gatherers from Upper Paleolithic to the Neolithic

Modern DNA data have shown that genetic similarities in present day Europeans are characterized by a strong correlation with geography (e.g., [32,47]) but, as mentioned above, the extent to which this pattern can be attributed to the initial colonization of Europe by AMH, the recolonization of Europe after the retreat of the ice sheets, the Neolithic transition, or general isolation-by-distance effects is unclear. Ancient mtDNA sequences recovered from three Upper Paleolithic and 14 Mesolithic and Neolithic hunter-gatherers all belong to the mtDNA haplogroup U [48], currently found at frequencies between 1 and 7% in most modern European populations, but at up to 20% in Baltic populations and around 40% in Saami. Interestingly, almost all pre-Neolithic hunter-gatherers from Central and Northeastern Europe sequenced to date, and the majority of European post-Neolithic hunter-gatherers, carry U-type mtDNA [48,49] (Figure 1a,c). There are three exceptions: two Italian individuals with N* and pre-HV types [50], and one from Sweden [46]; the latter dating to the late Neolithic and possibly being the result of an admixture event with incoming farmers. In all other hunter-gatherer samples, the now common mtDNA lineages H, T, K, and J are absent, suggesting that these mtDNA lineages were introduced during the Neolithic period.

A change in the genetic makeup of Europeans during the Neolithic transition is also suggested by the recent genomic study of three Neolithic hunter-gatherers and a Neolithic farmer from Scandinavia [46]. The Neolithic farmer appears to be more closely related to modern southern Europeans, whereas the hunter-gatherers were more closely related to modern Northern Europeans. Furthermore, comparison of allele-sharing between the ancient hunter-gatherer and various modern Europeans, versus that of ancient farmers and various modern Europeans, showed that the two are negatively correlated. This is consistent with the interpretation that these ancient samples represent two of the major sources of diversity in Europeans today. However, no modern individual was found to have a profile similar to that of the Neolithic hunter-gatherers, suggesting that their patterns of diversity no longer exist. These individuals come from a period rather late in the Neolithic, and may be the outcome of long-lasting interactions between various farmer and hunter-gatherer groups in Northern Europe. Although based on a limited sample, this study is consistent with others using aDNA in indicating a genetic distinction between late hunter-gatherers and early farmers, and it demonstrates some of the potential of genome-wide NGS data. To date, all paleogenetic studies of hunter-gatherers and early farmers are consistent with a scenario whereby

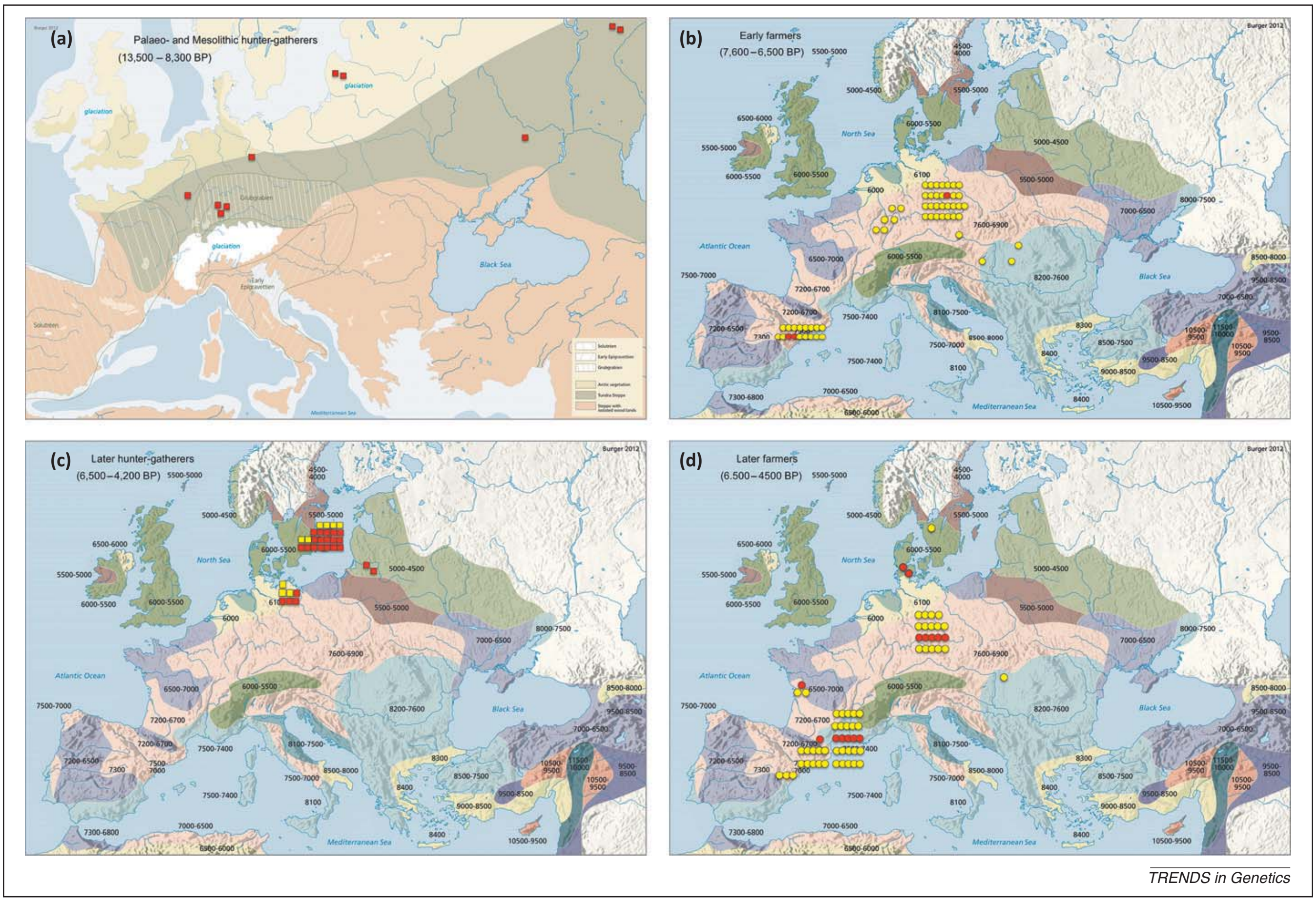

Figure 1. Maps showing Europe in times slices and depicting the locations from which ancient mitochondrial DNA (mtDNA) sequences were retrieved. Squares represent hunter-gatherer individuals and circles represent farming individuals. Lineages belonging to the U-clade are shown in red. Other lineages are shown in yellow. **(a)** Paleolithic and Mesolithic hunter-gatherers 13 500–8300 BP (plotted on a map of Europe during the last glacial maximum ca 22 000 BP). All Pleistocene hunter-gatherers analyzed to date carry mitochondrial lineages that belong to one of the U-clades: U2, U4, or U5. **(b)** Early farmers 7600–6500 BP. The map illustrates the approximate arrival times and duration of the earliest Neolithic cultures (in years BP). Very few of the early farmers belong to one of the U-clade mtDNA haplotypes, indicating discontinuity between Paleolithic/Mesolithic hunter-gatherers and early farmers [48,64]. **(c)** Later hunter-gatherers 6500–4500 BP. Whereas early hunter-gatherers carry exclusively mitochondrial U-lineages, later hunter-gatherers show additional lineages that are also present in early farming groups (b), pointing to a possible admixture between the groups or a change in lifestyle of former farmers back to hunting-gathering in Northern Europe. **(d)** Later farmers 6500–4500 BP. Compared to the period of the first appearance of farmers, late farmers have a significantly higher frequency of U-lineages. This can be explained by increasing rates of admixture between farmer and hunter-gatherer groups during this period and by the adoption of a farming lifestyle by hunter-gatherers. The maps are adapted from [69] and show datapoints from [46,48,51,54–56,61,62,64,70,71]. Abbreviation: BP, before present.

farmers immigrated into Europe from the South and Southeast, and a substantial fraction of the Mesolithic nuclear gene pool survived in modern populations of Northern Europe.

Farmers of the Neolithic

An increasing number of studies have reported mtDNA and also some Y-chromosome data from Neolithic individuals. In 2005, a study reported mtDNA sequences from 24 human specimens of the Early Neolithic Linearbandkeramik (LBK) culture. These mtDNA sequences were compared to 484 modern Europeans from the same geographic region. The authors found that 25% of the Neolithic farmers carried mtDNA haplogroup N1a, which is found at only 0.2% in modern Europeans (also [51]). A later study [48] compared 20 late hunter-gatherer sequences from Central and Northern Europe (ranging in time from 15500 to 2300 cal. BP) to 25 LBK farmers, and found a high F_{ST} value between these samples. Because temporal differentiation arises through genetic drift, particularly in small populations, serial coalescent simulations under a range of population size histories were used to test the hypothesis of population discontinuity. Population continuity was rejected, and the authors concluded that LBK farmers were not primarily the descendants of post-LGM hunter-gatherers in central Europe, but instead moved into the area around 7.5 ka (Figure 1b), probably from the northern Balkans [52]. Interestingly, paleogenetic studies on cattle indicate an origin for intensified dairying culture in the same region [53], as do simulation studies on the origins of lactase persistence–dairying gene–culture coevolution [41]. Population continuity between a late hunter-gatherer Pitted Ware Culture population and modern Scandinavians, including Saami, was also rejected using coalescent simulation [49], although a study of mtDNA haplotypes from 92 Mesolithic to Medieval Age specimens from Denmark suggested that the overall distribution of mtDNA haplogroups is similar to that of extant Scandinavians [54].

There are currently only a few studies, including those described above, which have investigated both Mesolithic and Neolithic individuals. However, many population-level studies have investigated Neolithic or Bronze Age DNA samples from different regions of Europe. The emerging pattern is not homogenous but is instead one of regional variation in the inferred mode of the spread of farming. For example, one study [55] reported Neolithic mtDNA sequences from the Hungarian plain that included the N1a sequence previously found in a Central European LBK sample [56], as well as sequences that are found in modern Central and Eastern Asians, suggesting the possibility of appreciable gene flow between these regions. However, a formal population genetic comparison with other data sets is still pending, and issues have been raised concerning the true age of some of these samples [57]. Another group [58] studied mtDNA sequences from 11 Middle Neolithic specimens from Granollers (Catalonia, northeast Spain) dated to 5.5 ka. Correspondence analysis indicated similarity between these ancient and modern Iberian samples and was interpreted by the authors as showing population continuity in the region from the Middle Neolithic onwards. Similarly, a restriction fragment length polymorphism (RFLP) study of 120 skeletons from four Neolithic and Bronze Age Basque sites reported some degree of similarity with modern Basques, based on the presence or absence of specific haplogroups in the serial samples [59]. A comparison of 49 mitochondrial DNA sequences from Upper Paleolithic, Mesolithic, Neolithic, and Bronze Age sites in Northern Spain with other European mitochondrial data from prehistoric periods, using F_{ST} distances and associated multidimensional scaling plots [60], indicated no statistically significant differences among hunter-gatherer samples from Iberia, Central Europe, and Scandinavia, but did show differences between hunter-gatherers and later Neolithic, Bronze Age, and modern samples. However, the Neolithic samples from Spain were distinct from the LBK sample from both Central Europe and modern European samples, suggesting heterogeneity among European Neolithic populations. Differences between seven Neolithic mtDNA sequences and six Y-chromosome haplotypes from the Avellaner Cave in northern Spain [61] and Central European Neolithic aDNA samples were interpreted as supporting a distinct Mediterranean expansion route for the spread of the Neolithic in Europe. The same authors also reported 29 mtDNA sequences from the Late Neolithic cave site at Treilles in Southern France, which were similar to those found among modern day Europeans [62]. Finally, a study compared mtDNA variation in 23 Bronze Age Sardinians with that of 254 modern individuals from two regions of the island, Ogliastra – a probable genetic isolate – and Gallura [42]. Using coalescent simulation and approximate Bayesian computation, the authors examined a range of hypotheses and found that continuity between the Bronze Age population and that from the Ogliastra region, but not from the Gallura region, best fitted the data, indicating a complex scenario in which two geographically proximal populations have distinct demographic histories.

In contrast to mtDNA, ancient Y-chromosome data has until recently been less informative, but a single Y-chromosome haplotype (G2a) in 20 of 22 male individuals from the Late Neolithic cave site at Treilles [62] led to the hypothesis that a small male founding population arrived in Southern France, probably by a maritime route from the eastern Mediterranean, in the early Neolithic. The same haplotype was also found in five of six individuals from the Avellaner Cave [61] and in one out of three Central European LBK individuals [63]. If authentic, the presence of the Y-chromosome haplogroup G2a in 26 of 31 Neolithic individuals from Germany, France, and Spain is both surprising and intriguing, but this requires further examination.

The only high coverage genome sequence of a prehistoric European individual is that of the Tyrolean Iceman, Ötzi, a 5300 year-old individual from South Tyrol, which was recently reported at 7-fold coverage [45]. Comparison with 1300 contemporary Europeans indicated closest genetic affinities with southern Europeans, particularly inhabitants of the Tyrrhenian Islands. Intriguingly, this is also the region where the Y-chromosome haplotype of the Iceman is found at highest frequency, and this haplotype belongs to the same G2a haplogroup described above. Although the Iceman is a single individual, this result suggests that the spatial structure of European genetic variation was not fixed in the Neolithic but continued to be reshaped by subsequent demographic processes. However, it should be noted that, in both recent reports of Neolithic genome-wide data [45,46], one of the main inference approaches relied on interpretation of the first few principal components, and this can be misleading, as discussed above.

Taken together, the aDNA studies indicate that Central Europe experienced an introgression of early farmers around 7500 years ago, although their place(s) of origin remain unknown ([48], but see [63] for speculation on this point). Coalescent simulations based on mtDNA also show that modern day Central Europeans are neither solely descendants of late hunter-gatherers nor of early Neolithic farmers from that region [48]. A lack of direct continuity between early Iberian farmers and modern Spaniards [64], as well as between late hunter-gatherers from Sweden and modern Scandinavian populations [49], further indicates that population turnover may have been common during the Holocene, a conclusion also supported by genome-wide data from Scandinavian Neolithic hunter-gatherers and farmers [46] and by the modern affinities of the Tyrolean Iceman genome. Strong similarity has been seen among all Neolithic and pre-Neolithic hunter-gatherers examined to date, both from mtDNA analysis and some limited genome-wide data [46,48,49]. Some degree of continuity between Middle Neolithic northeast Iberian populations and modern Spaniards [58], as well as between Late Neolithic and modern southern French populations [62], has been inferred based solely on the interpretation of mtDNA haplogroup distributions. Because almost no hunter-gatherer sequences have been analyzed from southern Europe, the genetic impact of early farming migrations into this region remains to be determined.

The inferred patterns of discontinuity between Neolithic and modern populations in Europe raise questions about which demographic processes reshaped European genetic variation after the Neolithic transition. It is reasonable to assume at this stage that varying admixture proportions between incoming farmers and local hunter-gatherers had an important impact upon the modern European gene pool [65] (Figure 1c,d). However, estimating the extent and nature of these admixture processes in different regions of Europe, and at different times, will require further genomic studies using fossil human DNA as well as the analysis of other sources of data such as craniometric analysis of Mesolithic and Neolithic humans, genetic studies of early domestic crops and livestock, and radiocarbon dates [66] (Box 3).

Box 3. The transition to agriculture in Europe

Human migration patterns can be studied by a large number of anthropological, archaeological, and genetic methods. Here we review some of these in the context of their contribution to the study of the emergence of farming and its spread in Europe.

Craniometric information can serve as a useful proxy for biological relatedness [86,87]. Model-bound craniometric analyses indicate that the affinities between early Neolithic populations in central Europe and the Near East are best explained by a demic diffusion model, but that in northeastern Europe local hunter-gatherers adopted cultural elements from contemporaneous farming communities without evidence of a major genetic contribution from Central and southeast Europe [88–90].

Archaeozoological studies of the origins and spread of the earliest four domestic livestock species – pig, goat, sheep, and cattle – have provided new perspectives on agricultural dispersals as well as on the manner and nature of early domestication processes. They indicate that all four species were first domesticated in the region of southeastern Anatolia, Syria, Iraq, and western Iran around 10 500 years ago, and were probably first introduced into Europe by dispersing farmers from Anatolia [91,92]. Paleogenetic studies of livestock species are in agreement with this view [53,93,94], but in the case of pig they point to post-Neolithic introgression from European wild boar [95].

The important role of dairying during the domestication process of ungulates is underlined by results of residual analyses of fats from late Neolithic ceramic vessels from western Anatolia [96] and in Europe [97], and from genetic studies of the prevalence of lactase persistence (LP) [41,62,98–102].

In addition, archaeological data on settlement patterns and sizes [103], skeletal demographic data [104], and the distribution of summed radiocarbon dates, have been analyzed to detect diachronic fluctuations in population size [66] and inform on the Neolithization process. Carbon, nitrogen, oxygen, sulfur, and strontium isotope ratios in bones, teeth, and dental calculus, of both humans and animals, have been used to provide information about changes in diet and migration patterns in both hunter-gatherers and farmers (e.g., [105–108]).

Concluding remarks

aDNA data in combination with descriptive analyses and explicit modeling approaches have opened a new avenue to investigate the evolutionary history of AMH in Europe and assess the extent to which major environmental and cultural changes (such as the LGM or the Neolithic transition) have impacted upon the structure of modern genetic diversity. Improvements in aDNA techniques have provided glimpses into the patterns of genetic variation in past populations, but, despite a growing number of prehistoric individuals examined, the field still suffers from a paucity of data from the information-rich autosomal genome. Single-sex inheritance systems (mtDNA and the Y chromosome) contain useful information on population histories, but stochasticity in the genealogical process means that such data need to be analyzed with caution – preferably in an explicit modeling framework that accounts for such 'noise' – before meaningful inferences can be drawn. Fortunately, coalescent theory provides a useful framework for this, although modeling of genome-wide data is less well developed at present. Many researchers still use descriptive or phylogeographic approaches that are best thought of as hypothesis-generation methods but which lack a formal framework for testing these hypotheses. This holds true both for single- and multi-locus studies. However, the advent of NGS technology (Box 2), together with the undeniable conceptual progress made in population genetics and computer simulations (Box 1), have started to overcome both shortcomings.

Future research should also reveal the effects of post-Neolithic demographic processes, including migration events, which preliminary data suggest had a major impact upon the distribution of genetic variation. These include events associated with Bronze Age civilizations, Iron Age cultures, and later migrations, including those triggered by the rise and fall of Empires. Challenges remain in being able to sequence aDNA routinely from serial samples in the range of megabases, and in the development of software that allows spatially-explicit simulation of genome-scale data, but advances in these areas are now a weekly occurrence and the stage is set for a rapid increase in our knowledge on the evolutionary history of AMH in Europe.

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