Ancient DNA: A Window to the Past of Europe

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Key Words

Ancient DNA · Mitochondrial DNA · European populations · Neolithic transition · Genetic continuity

Abstract

Objectives: The history of European populations is characterised by numerous migrations or demographic events that are likely to have had major impacts on the European gene pool patterns. This paper will focus on how ancient DNA (aDNA) data contribute to our understanding of past population dynamics in Europe. **Methods:** Technological challenges of the palaeogenetic approach will be discussed. With these limitations in mind, it will be shown that the acquisition of aDNA now permits a glimpse of how human genetic diversity has changed, spatially and temporally, in Europe, from the Palaeolithic through to the present day. **Results:** Although early modern human DNA sequences come only from rare exceptionally well-preserved specimens, genetic samples of a reasonable size are becoming available for the Mesolithic and the Neolithic periods, permitting a discussion of regional variation in the inferred mode of the spread of farming. Palaeogenetic data collected for ancient and more recent periods regularly demonstrate genetic discontinuity between past and present populations. **Conclu-**

sions: The results indicate that only large diachronic aDNA datasets from throughout Europe will permit researchers to reliably identify all demographic and evolutionary events that shaped the modern European gene pool.

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Introduction

The history of European populations is characterised by numerous migrations and demographic events that likely have had major impacts on the patterns of the European gene pool. Three notable events have been suggested as having profoundly affected the genetic diversity of Europeans:

- First, the initial colonisation of the continent by anatomically modern humans approximately 45,000 years ago [1]. The question of interbreeding of anatomically modern humans with archaic humans, a topic that is highly debated since the publication of the complete Neanderthal genome [2], will not be addressed in this paper.
- Second, the Last Glacial Maximum, which was accompanied by important changes in the distribution of the human populations, i.e. the retreat of most

northern populations into different southern refugees, followed by the recolonisation of Northern Europe after the retreat of the ice sheets by 20,000–16,000 years ago.

- Third, the spread of the Neolithic culture in Europe between 9,000 and 5,000 years ago. The transition from a food-collecting to a food-producing economy, the Neolithic transition, is associated with dramatic changes in the European archaeological records. This Neolithic transition has been widely studied and is traditionally considered to be *the* major demographic process in Europe [3, 4].

In the last 30 years, the gene pool of current European populations has been widely analysed to better understand the demographic history of these populations. As a result, specific components of the European gene pool have been directly linked to the different migratory events described above [see 5 for a review]. However, because of the complexity of population dynamics in Europe, the extent to which the present-day genetic pattern can be attributed to the key episodes previously presented is still unclear. For example, genetic studies have delivered numerous, but often conflicting, inferences on the impact of Neolithisation on the modern European gene pool [see 6 for a review]. Indeed, even if geneticists now agree that the Neolithic farmers participated in the construction of the European gene pool, the importance of this Neolithic contribution remains unclear, and it can vary from 20 to 70% according to the genetic markers analysed or the analytical methods used [see 7–10, for example]. This assessment can be easily explained by the fact that modern-day genetic diversity provides only indirect evidence about the evolutionary history of past populations. The patterns observed in the current European gene pool are the outcome of Palaeolithic and Neolithic processes, overlaid with 4,000 years of further developments that have blurred the picture. This observation encouraged palaeogeneticists to contribute to the debate by directly comparing genes from the ancient inhabitants of Europe to their contemporary counterparts. The goal of this paper is to show how the acquisition of ancient DNA (aDNA) data at different dates and places across the continent now permits us to catch a glimpse of how human genetic diversity has changed both temporally and spatially in Europe. Offering valuable insights into the evolution of the European gene pool at key moments in history, palaeogenetic evidence plays a primary role in providing a deeper understanding of the European populations' evolution as well as raising new questions concerning populations' dynamics.

aDNA and Methodological Limits

Before presenting several key studies dealing with European populations' palaeogenetics, no one can evade the question of the limitations that are intrinsic to the field of aDNA. Indeed, studies using aDNA are plagued by a unique set of methodological problems, such as variable and temporally limited DNA survival, problems with sequence authenticity, and the representativeness of samples that yield reliable results [11, 12]. The first limit concerns the ability to obtain authentic aDNA from archaeological specimens. The preservation of DNA molecules and then the success rate of studies vary importantly from one specimen to another and can be a function of various factors, including environmental parameters, burial circumstances, and storage conditions before and after excavation. In concert with the limited number of well-documented archaeological specimens available for analyses, this point automatically leads to a low representativeness of samples that can yield reliable results. There are still only a limited number of aDNA samples; for example, a total of 100 Neolithic farmers' and 14 Mesolithic hunter-gatherers' mitochondrial sequences have been obtained to date, and it is obvious that such samples will not permit the complete resolution of the debate surrounding Neolithisation processes [6]. However, even this small sample size begins to shed some light on these processes in some specific regions, as we will see, and the continuous improvement of the aDNA database will doubtlessly provide critical insight into this topic. In addition to the paucity of ancient samples, the geographical and temporal representativeness of samples can also be questioned. Finally, we must always keep in mind that aDNA studies deal with human remains originating from funerary structures that are intrinsically biased. This point will be specifically addressed at the end of the paper.

Due to the technical difficulties induced by the nature of minute and degraded aDNA and the consequent risk of contamination, the authentication of amplified sequences represents a major interest in the field [13]. This is especially true when dealing with human remains. The results are usually validated through the determination of potential contamination sources, the use of decontamination techniques, and through the application of 'criteria of authenticity' [14]. All information supporting sequence authenticity must then be systematically provided so that the scientific community can better evaluate and accept the proposed results [13].

We must also keep in mind that the genetic picture presented by the aDNA studies is currently based primarily

on mitochondrial DNA (mtDNA) results. The mtDNA in ancient samples is better conserved than the nuclear genome because of its higher frequency in living cells. Studies of maternally inherited mtDNA allow inferences to be drawn for only one sex, and it is conceivable that analyses of the Y chromosome or nuclear markers would present different patterns. The majority of aDNA studies presented in this paper have been restricted to short fragments amplified from hypervariable region 1 (HVR-1) of the mitochondrial genome. However, the advent of second-generation sequencing has recently revolutionised the study of aDNA, enabling researchers to move from the study of a few short fragments of DNA into the so-called palaeogenomic era. The recent development of second-generation sequencing (or next-generation sequencing – NGS) technologies has greatly benefited the field of aDNA [see 15 for a review]. Notably, unlike classical PCR approaches that rely on targeted PCR amplification of aDNA molecules using primers, the NGS approach allows for massive sequencing of ancient DNA molecules without prior targeted amplification. As a consequence, the new approach permits access to shorter fragments and to a much larger fraction of endogenous aDNA than PCR does. Moreover, new techniques provide degradation arguments (including short fragment read length, nucleotide misincorporations, and an increased fraction of purines close to sequence reads termini) permitting to clearly distinguish between ancient endogenous degraded reads and modern intact contaminants [16]. Finally, the more recent development of true single molecule sequencing [17] will likely bring us into a new era of ancient genomics.

The achievement of the first ancient human nuclear genome, from an extinct Palaeo-Eskimo from Greenland [18], demonstrated the great power of past genomics in addressing past demographic history of now-extinct cultures as well as in dating migration events with high confidence. This study demonstrated that it was possible to sequence the genome of an ancient human to a level that allowed for SNPs and population analyses to be run. The next technical challenge was to extend such a sequencing approach to material outside the permafrost regions. Moreover, past genomics have also demonstrated that interpretations of migrations and populations' relationships relying on mtDNA only could be misleading [19], and that as such, palaeogenomics should be of great relevance to our current understanding of European records. During recent years, NGS has been applied to a few well-preserved samples, and this approach has provided novel important autosomal data from ancient European remains [20, 21].

Despite the numerous inherent problems that confront palaeogeneticists, this field provides a unique opportunity to determine to what extent later European populations retain the genetic legacy of earlier groups. We will review the main contributions of the field to the understanding of past European population dynamics in the next section.

aDNA and the European Palaeolithic Gene Pool

The determination of early modern human DNA sequences has been confined, until now, to exceptionally well-preserved specimens. The majority of aDNA sequences obtained to date are from sites in Kostenki in Russia and Dolni Vestonice in the Czech Republic and the cave of Paglicci in Italy. The NGS approach has provided the complete mitochondrial genome from a 30,000-year-old modern early human from the Kostenki site in Russia [22]. The characterisation of the U2 mitochondrial haplogroup confirmed that haplogroup U has a deep coalescence and may thus be older than other mitochondrial haplogroups in Europe. Three Gravettian individuals were analysed from the Paglicci cave of Southern Italy, a site that has been dated to 23,000 BP [23, 24]. In this case, the analysis of amplified fragments of the HVR-1 of the mitochondrial genome did not permit a complete resolution of the individuals' haplogroups. Complete mitochondrial genomes were reported for three Gravettian remains from Dolni Vestonice (dated at 21,000 BP) and confirmed the overwhelming presence of haplogroup U in Palaeolithic European specimens [25]. Finally, mitochondrial data have been obtained from several specimens from the Magdalenian period in Spain and in Germany, and these have demonstrated the occurrence of haplogroups H, U, and derivatives during this period [25–27].

As a consequence of the scarcity of human remains from that period and the inherent methodological difficulties of aDNA conservation, the Palaeolithic European genetic pool remains poorly understood. Although the data demonstrate the presence of H and U sub-haplogroups in the Palaeolithic European mitochondrial gene pool, they do not allow any estimates concerning group diversity or differentiation. It is actually impossible to test whether some genetic differentiation between human groups echoes the cultural diversity highlighted for the periods concerned. Likewise, no inferences can be drawn concerning demographic events and population dynamics during the Upper Palaeolithic period. The NGS

approach will doubtless provide crucial information about this period in the future.

Finally, it is interesting to note that some specific mitochondrial haplogroups that have been proposed to coalesce during the Palaeolithic period after the analysis of extant Europeans [haplogroups HV, HV1, J, T, and I, for example; see 5, 8] have not been detected yet among human remains from that period.

aDNA and the European Mesolithic Gene Pool

The picture concerning the Mesolithic period is actually becoming clearer. Despite the fact that the aDNA dataset for the Mesolithic hunter-gatherers predating the Neolithic expansion presently includes only 14 human remains (from Lithuania, Poland, Germany, and Spain), all point out striking mitochondrial haplogroup homogeneity. Indeed, a surprisingly high frequency of mtDNA lineage U (approximately 80%, represented by sub-haplogroups U4 and U5) was discovered, and most of the ancient hunter-gatherers were shown to share mtDNA types that are relatively rare in Europeans today [20, 21, 24, 26]. This haplogroup composition, which is very particular and clearly different from that found in extant European populations, lends support to the fact that modern populations cannot be the direct descendants of the pre-Neolithic groups. Moreover, the characterisation of a remarkable genetic uniformity and little phylogeographic structure for Mesolithic European communities suggests a common origin for all pre-Neolithic individuals. The picture drawn is in agreement with a population of highly mobile groups that may have exchanged goods and individuals continent-wide.

Very recently, the NGS approach has permitted the acquisition of autosomal DNA data from two exceptionally well-preserved 7,000-year-old Mesolithic individuals from a site in Northwestern Spain [La Braña; see 21]. The data show that both Braña individuals fall outside of the range of modern European genetic variation, including Iberians, but they may show genetic affinities with extant populations from Northern Europe. In addition to confirming a clear regional genetic discontinuity between Mesolithic groups and extant populations from the same region, these results may confirm a potential gene flow between distant hunter-gatherers' communities.

Interestingly, the most complete palaeogenetic results that have been published for late European hunter-gatherers concern Central and Northern Europe [26], a region where substantial data for Neolithic farmers have also been obtained from.

aDNA and the European Neolithic Transition

Neolithic 'revolutions' represent the origin of huge changes in lifestyles, social structures, ideologies, techniques, and land use [28]. The Neolithic transition has been widely studied and is traditionally considered to be *the* major demographic process having shaped the European gene pool. Archaeological evidence and radiocarbon records suggest that the spread of agriculture over Europe has been complex, with a succession of migration phases and local admixture [3, 4]. The processes underlying the agricultural expansion have long been a topic of considerable debate. What originally was perceived as a simple dichotomy between demic versus cultural diffusion is now generally recognised as a more complex set of processes involving cultural and population dispersion as well as variable patterns of population admixture [29].

The genetic study of the individuals who lived during the Neolithic transition adds precious information to our understanding of the local Neolithisation processes. As a consequence, our paper will first focus on early, or pioneer, farmer groups. The study of mtDNA from early Neolithic human remains has mainly focused on two different regions: the Central European plain connected with the LBK (Linearbandkeramik) expansion route, and the Northern regions of Iberia linked with the Cardial Pottery culture. The first general assessment that can be proposed is that farmers appear to have introduced a new set of mitochondrial lineages into Western Europe. Indeed, the Mesolithic-Neolithic transition is characterised by substantial gene pool changes, with a significant reduction in the abundance of the U haplogroups found in Mesolithic hunter-gatherers together with the sudden appearance of a dozen new haplogroups (H, HV, I, J, J1, K, N1, T, T2, W, and X; fig. 1a). The genetic discontinuity observed between the pioneer farmer groups and the predecessor Mesolithic communities clearly favours the hypothesis that a substantial migration accompanied Neolithisation.

At present, the majority of aDNA studies concern early Neolithic communities from Northern and Central Europe. Relevant analyses have been conducted on human remains originating from Linear Pottery culture sites [or LBK; see 30, 31]. This culture is associated with the onset of farming in the temperate regions of Europe around 5,500 BC. A total of 45 Neolithic skeletons from sites in Germany, Austria, and Hungary have yielded reliable HVR-1 sequences. The initial interpretations have focused primarily on the characterisation of a high frequen-

Fig. 1. Mitochondrial haplogroup composition of hunter-gatherer and farmer groups' gene pools: **a** before 4,500 BC and **b** after 4,500 BC.

cy of the mitochondrial N1a lineage in the LBK area. Because this lineage is exceedingly rare but widespread in Europe, the results lend support to the view that the first agriculturists had limited success in leaving a genetic mark on the female lineages of modern Europeans. A specific N1a haplotype described in an early Hungarian farmer was later characterised in a French megalithic burial chamber from the middle Neolithic period, raising questions concerning the possibility of lineage diffusion through farmer pioneer groups as far as Western France [32]. Genetic affinities among LBK groups and modern-day Near-Eastern and Anatolian populations were also uncovered, supporting the hypothesis that there was major genetic input from this area during the advent of farming in Europe. However, extant regions from Central Europe were also shown to have mtDNA pools that were closely affiliated with the LBK population, lending support to the view that lineages characterised in LBK contexts originated from different sources, including the Near East and Eastern and Central Europe. Finally, the LBK population presented unique genetic features when compared to modern populations, confirming that major demographic events continued to take place in Europe after the early Neolithic period. A large-scale model pro-

posed for the LBK expansion is one of demic diffusion with genetic input from the Near East into Central Europe. This large-scale demic diffusion model could be combined with a leapfrog colonisation model at the regional scale, which would explain (i) the relative speed of the LBK expansion in Central Europe, (ii) the clear genetic Near-Eastern connections seen in pioneer settlements, and (iii) a sizeable (but less important) input of Mesolithic populations to modern European genetic variation.

Important palaeogenetic results published several years later, describing late European hunter-gatherers from Central and Northern Europe, allowed the theory of an exogenous origin of the first Neolithic farmers to be tested directly. Bramanti et al. [26] obtained mtDNA sequences from 22 late European hunter-gatherer skeletons. The discontinuity between late hunter-gatherers, early farmers, and extant European populations from Central Europe provided persuasive evidence that the first farmers were not the descendants of local hunter-gatherers but immigrants who had come into Central Europe at the onset of the Neolithic transition. The results indicated that the migrating farmers, at least initially, did not mix significantly with the resident female hunter-gatherers.

Despite the fact that ancient Southwestern/Mediterranean biological data are typically underrepresented (mainly due to bone preservation issues), relevant palaeogenetic data have been recently reported for early Neolithic groups from Northeast and Northwest Iberia (for a total of 54 individuals). Samples of aDNA from early Neolithic settlers in Northeast Iberia have been obtained from sites dating back to the Cardial culture [33]. The Neolithic groups that have been analysed were characterised by a high frequency of haplogroups Hg N* and X1, and they showed a clear genetic discontinuity from modern populations. Bayesian coalescence-based simulations demonstrated that the results were compatible with a pioneer colonisation of Northeast Iberia at the early Neolithic period, characterised by the arrival of small genetically distinctive groups showing genetic connections with the Near East. Clear distinct gene pools were characterised for early Neolithic sites in the Cantabrian fringe of Iberia [including the absence of haplogroups N* and X1; see 27], suggesting maritime colonisation by small and genetically differentiated Neolithic groups [34]. Finally, aDNA data obtained from several hunter-gatherers from the Northwestern region of Iberia confirmed a clear genetic discontinuity between hunter-gatherers and farming groups.

In light of the information now emerging from palaeogenetic studies, it appears that genetic discontinuity between late hunter-gatherers, early Neolithic groups, and maternal contemporary counterparts is the rule. Phylogeographical arguments based on palaeogenetic data appear to support a model of leapfrog Neolithic migration in North-Central Europe and Northern Iberia [6].

Interestingly, all regions appear to have seen their early Neolithic gene pool modified by later evolutionary processes. Indeed, most of the studies performed to date on samples from the middle and late Neolithic periods show the disappearance of the haplogroups characteristic of pioneer farmers, suggesting that some genetic modifications could have taken place during the entire Neolithic period [35–38]. This point has been very recently confirmed by the analysis of a time transect spanning the >3,500 years of the Central European Neolithic period [from early to late Neolithic; 39]. Altogether, the findings demonstrate that the frequencies and the distribution of mitochondrial lineages brought by the first Neolithic farmers were highly modified during the Neolithic period (as well as later periods, as we will further describe).

To conclude, the increasing number of palaeogenetic data available for the pre-Neolithic and Neolithic periods provide crucial clues, including the following:

- There is a genetic break between late hunter-gatherers and early Neolithic communities, which points out substantial migration accompanying European Neolithisation.
- Genetic affinities between the early Neolithic populations and Near-Eastern modern-day populations were detected, suggesting a genetic input from the Near East during the Neolithic period. Nevertheless, this Near-Eastern input appears to have been subsequently remodelled through varying levels of interaction with local hunter-gatherers during the Neolithic expansion [31].
- The emerging pattern suggests heterogeneity among European Neolithic populations, with a clear regional variation in the lineages brought by farmer groups and varying relative biological contributions of indigenous Mesolithic hunter-gatherers across the continent.
- Palaeogenetic data for the middle or late Neolithic period suggest that migratory/demographic events would have later remodelled the groups' genetic pool throughout the Neolithic period, which could partially explain the observed discontinuity with extant Europeans (of course along with 4,000 years of further developments).

aDNA and the Post-Neolithic Evolution of the European Gene Pool

It appears that the genetic structure of European populations was not fixed in the Neolithic period; rather, it continued to be reshaped by subsequent demographic processes. Interesting palaeogenetic data have been obtained for more recent periods in a variety of regions (fig. 2). The majority of the studies note regional genetic discontinuity between ancient European and modern-day populations. A compilation of 41 papers reporting palaeogenetic data for ancient European populations can be found in table 1 [20–27, 30–33, 35–38, 40–64]. It is worth noting that, among the studies discussing (dis)continuity between ancient and extant populations from the same region, 83.3% concluded genetic discontinuity, whereas 25% of the papers reported genetic continuity (two studies reported continuity with some extant groups and discontinuity with other groups). However, only a fraction of the studies used adequate statistical tests (37.5%) or coalescence-based simulation (45.8%), and these approaches are absolutely crucial to provide a persuasive quantitative argument of genetic (dis)continuity. Despite this shortcoming, as 92.3% of the studies that used such statistical/simulation approaches noted genetic discontinuity between extant Europeans and their ancient forbearers, we can conclude that a large majority of palaeogenetic studies highlight a genetic break between ancient and extant European populations.

A Genetic Break with the Past

On the whole, we have observed that the large majority of aDNA studies concluded that there is genetic discontinuity between ancient and extant Europeans. Various demographic and evolutionary mechanisms may have led to this genetic break with the past, including the strong likelihood of genetic contributions from migratory peoples that occurred during and after the Neolithic period. This gene flow may have been so significant that genetic signals from the earlier inhabitants of Europe were all but obliterated. Founder effects, genetic drift and bottlenecks must also have had dramatic impacts. In addition, it remains unclear whether the mtDNA genome has undergone selective pressure [65]. If mitochondrial function and variability play a role in adaptive selection, natural selection may have changed the face of Europe over time, causing certain mitochondrial lineages to disappear, while others have come to dominate the genetic

Fig. 2. A map of ancient European populations genetically analysed (for more information, see also table 1).

landscape. All of these events, either alone or in combination, have resulted in a general striking genetic discontinuity between past and present populations.

Finally, we believe that social parameters may also have played a significant role in lineage evolution, especially since the appearance of potentially hierarchic communities in the Neolithic period. As has already been mentioned, the ancient samples generally originate from funerary structures and may therefore not be representative of ancient populations as a whole. Indeed, putative selective mortality, cultural factors related to burial, environmental factors related to post-depositional processes, and methodological factors related to the excavation and analysis of skeletal samples could all potentially bias samples. We would like to particularly emphasise the impact of specific funeral practices on the representativeness of ancient samples. Funeral practices could include the selection of buried individuals according to their sex, their age, or their kinship. In the latter case, caution should be taken when making genetic diversity inferences from ancient funerary groups because an uncharacterised kinship can significantly bias allele frequencies. In addition, the study of late Neolithic sites has suggested exogamy and patrilocality [35–37], two practices that must have led to strong divergence between mitochondrial and Y diversities in ancient samples.

Funeral practices may also be driven by social criteria. We often do not know if the maternal lineages identified at different archaeological sites provide a snapshot of the whole population or only of a socially differentiated group. The question of social stratification has been posed for Etruscan groups, and palaeogenetic results may support the hypothesis of elite class extinction during an assimilation process [63]. The progressive disappearance of elite lineages through time has also been described in Hungary, where the rapid and comprehensive dilution of the haplogroups associated with the Magyar conquerors has been demonstrated [60]. The same question can even be posed for more remote periods, such as the Neolithic. For example, some authors have noted that only a small portion of the LBK population could have been interred in regular burials and that approximately 80% of the dead were treated in a way that hardly left any archaeological trace [66]. Burials, therefore, may represent a specific group of exceptionally treated people.

The question appears similarly important for middle Neolithic monumental funerary structures. For example, the extraordinary construction at Prissé (a 100-m-long mound constructed between 4,500 and 4,000 cal BC in West-Central France), compared with the very small number of individuals deposited in the chamber (8), supports the idea of selection and highlights discussions

Table 1. Compilation of papers reporting palaeogenetic data for ancient European populations

Popu- lation ¹	Ref.	Country	Region	Archaeological site(s)	Period	Sequences obtained, n	With extant populations continuity	discontinuity	Stati- stical tests	Coales- cence- based models
1	20	Sweden	Gotland	Ajvide	2,800–2,000 BC	3		YES	yes	yes
2	21	Spain	Leon	La Braña-Arintero, Leon	5,000 BC	2		YES	yes	yes
3	22	Russia		Kostenki	30,000 BP	1	n.d.	n.d.		
4	23, 24	Italy		Paglicci cave	25,000–20,000 BP	3	n.d.	n.d.		
5	25	Russia, Czech Republic, Germany, Italy, Luxembourg		Kostenki, Dolni Vestonice, Oberkassel, Continenza, Paglicci, Loschbour, Iceman	39,500–4,550 BP	11	n.d.	n.d.		
6	26	Lithuania, Poland, Russia, Germany		Hohler Fels, Chekalino, Lebyazhinka, Falkenstein Höhle, Bad Dürrenberg, Hohlenstein-Stadel, Spiginas, Donkalis, Kretuonas, Dudka, Drestwo, Ostorf	13,400–3,000 BC	22		YES	yes	yes
7	27	Spain	Navarra	Paternabidea, La Pasieta, La Chora, Erralla, Aizpea, Los Cascajos, Fuente Hoz, Urtiaga	12,300 BP–1,430 BC	49		YES	no	no
8	30	Austria, Germany, Hungary		Asparn Schletz, Eilsleben, Flomborn, Schwetzingen, Seehausen, Flomborn, Unterwiesedter, Halberstadt, Vaihingen an der Enz, Derenburg Meerensstieg II, Ecsegfalva I	5,500–5,000 BC	24		YES	yes	yes
9	31	Germany		Derenburg Meerensstieg II	5,200–4,900 BC	21		YES	yes	yes
10	32	France	West	Prissé-la-Charrière	4,200 BC	3	n.d.	n.d.		
11	33	Spain	Catalonia	Can Sadurni, Chaves, Sant Pau del Camp	5,500–3,700 BC	13		YES	yes	yes
12	35	Germany		Eulau	2,600 BC	9	n.d.	n.d.		
13	36	France	Aveyron	Les Treilles	3,000 BC	29	n.d.	n.d.		
14	37	Spain	Catalonia	Avellaner cave	5,000 BC	14	n.d.	n.d.		
15	38	Spain	Catalonia	Cami de Can Grau Granollers	3,500–3,000 BC	11	YES	YES	no	no
16	40	Spain	Basque Country	Aldaieta	3,300 BC–700 AD	34		YES	no	no
17	41, 42	Czech Republic	Moravia	Vedrovice	5,300 BC	6	n.d.	n.d.		
18	43, 44	Italy	Sardinia	Teresa di Gallura, Fluminimaggiore, Perdasdefogu, Alghero	1,300–700 BC	23	YES with specific extant populations		yes	yes
19	45	Spain	Andalusia	Priego de Cordoba	1,100–1,300 AD	61		YES	no	no
20	46	Portugal		not specified	5,500–4,750 BC	35		YES	no	no
21	47	Russia	Northwest	Yuzhnyy Oleni Ostrov, Popovo, Bol'shoy Oleni Ostrov	7,500–3,500 BP	34		YES	yes	yes
22	48	Italy	Trentino- South Tyrol	Villabruna, Mezzocorona, Borgo Nuovo	12,300–4,000 BC	4	YES		no	no
23	49	Spain	Castellon	Costa Lloguera, Puig de la Nau	Chalcolithic–Iberian	9		YES	no	no
24	50	Spain	Castellon	Malpaso Cave, Assud de Villareal, Costa Lloguera, Maçanet, Castellet de Bernabé, Los Villares, Puig de la Nau, Torrello Bonerot, Tossal de Sant Miquel, Mas d'Arago	Chalcolithic–Bronze Age–Iberian	20	n.d.	n.d.		

Table 1 (continued)

Popu- lation ¹	Ref.	Country	Region	Archaeological site(s)	Period	Sequences obtained, n	With extant populations		Stati- stical tests	Coales- cence- based models
							continuity	discontinuity		
25	51	Italy	Tuscana	Casenovole, Adria, Volterra, Castelfranco di Sotto, Castelluccio di Pienza, Magliano, Marsiliana, Tarquinia, Capua, Firenze, Pisa, Livorno, Massa Carrara, Siena, Grosseto	700 BC–1,400 AD	57	YES with specific extant populations	YES with specific extant populations		yes
26	52	Italy	Tuscana	Firenze, Pisa, Livorno, Massa Carrara, Siena, Grosseto	900–1,400 AD	27	YES	YES	no	yes
27	53	Spain	Northwest	San Juan Ante Portam Latinam, Pico Ramos, Pico Ramos, Longar, Urratxa	3,300–1,400 BC	121		YES	no	no
28	54	Germany		Kromsdorff, Calden, Odagsen, Panker	3,400–2,800 BC	8	n.d.	n.d.		
29	55	Sweden	Gotland, Gokhem	Ajvide, Fridtorp, Ire, Frålsögården	3,500–2,000 BC	22		YES	yes	yes
30	56	Denmark		Bøgebakken, Tybrind Vig, Strø Bjerge, Damsbo, Kyndeløse, Hulbjerg, Hestehavebakken, Egtvedpiggen, Borum Eshøj, Bredtøftgård, Vårpelev, Himlingøje, Skovgaarde, Simonsborg, Bøgebjerggård, Galgedil, Kongemarken, Riisby	500 BP–1,450 AD	56		YES	yes	no
31	57	Denmark		Kongemarken	1,000–1,250 AD	9	n.d.	n.d.		
32	58	Spain	Catalonia	Mas Castellar, Illa d'en Reixac, Puig de Sant Andreu	700–100 BC	13	YES		no	no
33	59	Spain	Catalonia	Montanissell Cave	3,200 BP	8	n.d.	n.d.		
34	60	Hungary		Sarretudvar-Hizofold, Harta-Freifelt, Oroshaza-Gorbics tanya, Besenyotelek-Szorhat, Aldebro-Mocsaros, Eger-Szepasszonyvolgy, Fadd-Jegeshegy, Izsak-Balazspuszta, Szabadkigyos-Palliget, Mozs-Szarazdomb, Ormenyktut, Szegvar-Oromdulo, Lebeny-Kaszas, Zalavar-Kapolna	900–1,100 AD	27		YES	no	no
35	61, 62	England, UK		Leicester, Norton, Dover, Market Lavington, Norwich	300–1,020 AD	48		YES	no	no
36	63, 64	Italy	Tuscana	Adria, Volterra, Castelfranco di Sotto, Castelluccio di Pienza, Magliano, Marsiliana, Tarquinia, Capua	700–300 BC	30		YES		yes

¹ The numbers are the same as in figure 2.

N.d. = Not determined/done; yes = response for the use of statistical tests or coalescence-based models; YES = response for continuity/discontinuity with extant populations.

about the potential status of the deposited individuals [32]. It appears essential to discuss such social recruitment bias when interpreting palaeogenetic data.

Finally, cemeteries may have functioned over long periods of time and could therefore group together individuals corresponding to distinct biological groups or populations. A precise analysis of the human remains' dating must automatically accompany genetic analysis to verify group homogeneity at the site level. A recent study has proposed palaeogenetic data from human remains belonging to the Starčevo-Körös-Criş cultures [67]. Anthropologists and archaeologists specialising in this region later noted serious errors in the dating of the skeletons investigated, which significantly influenced the interpretation of the genetic data [68]. The conclusion of these specialists was that 'all efforts to produce authentic aDNA data are worthless if the archaeological background to a genetic project is missing or insufficiently explored' [68].

This conclusion can be applied to the aDNA field as a whole. It appears crucial that the genetic structure in ancient samples and the archaeological identity of individuals under study be systematically investigated within each archaeological site, and these data should be taken into account to propose reliable evolutionary scenarios for ancient European populations. This task necessitates a real interdisciplinary approach including archaeology and aDNA research. Only the increasing volume of palaeogenetic data, originating from various archaeological contexts, will permit to overcome the limitations linked to small sample size (more prone to the bias previously exposed).

Conclusions

Palaeogenetic data highlight the significant transformations that the genetic history of Europe has undergone and the resulting genetic discontinuity between modern-day Europeans and their ancient forbearers. Migratory and demographic processes have mainly been inferred from patterns in modern mtDNA under the implicit assumption of genetic continuity among people dwelling in the same region during different time periods. Our paper notes that genealogical continuity between ancient and modern populations in the same area does not seem to be a safe general assumption but rather a hypothesis that, when possible, should be tested using aDNA analysis. On the whole, the aDNA approach has revealed additional levels of complexity in the population history of Europe-

ans that were undiscovered from the study of only modern populations. Even if aDNA datasets are actually not sufficient to precisely reconstruct each prehistoric event that shaped the European gene pool, they do offer new perspectives on several aspects of the peopling of the continent.

All of these arguments justify broadening the sampling of aDNA in both time and space. The multiplication of aDNA studies should help to elucidate local demographic processes and to refine our knowledge of the migration patterns across the continent. Only many large diachronic aDNA datasets from throughout Europe will permit researchers to reliably identify all demographic and evolutionary events that shaped the modern European gene pool. Notably, future research should reveal the effects of post-Neolithic demographic processes because available data suggest that they had a major impact on the distribution of genetic variation. Such an increase in available aDNA data is also essential for the field to adopt a less qualitative approach, permitting more computational modelling, which should lead to a more precise identification of scenarios that can explain modern genetic data.

Finally, the palaeogenomic approach has clearly proved to be of high relevance in reconstructing human migrations and populations' relationships, potentially providing arguments contradicting with previous scenarios relying on mtDNA only. If the palaeogenomic approach has been applied so far to a few well-preserved European human samples, its next development will without any doubt be of great relevance to our understanding of the European records.

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