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ARTICLE

European Neolithization and Ancient DNA: An Assessment

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Neolithic processes underlying the distribution of genetic diversity among European populations have been the subject of intense debate since the first genetic data became available. However, patterns observed in the current European gene pool are the outcome of Paleolithic and Neolithic processes, overlaid with four millennia of further developments. This observation encouraged paleogeneticists to contribute to the debate by directly comparing genetic variation from the ancient inhabitants of Europe to their contemporary counterparts. Pre-Neolithic and Neolithic paleogenetic data are becoming increasingly available for north and northwest European populations. Despite the numerous problems inherent in the paleogenetic approach, the accumulation of ancient DNA datasets offers new perspectives from which to interpret the interactions between hunter-gatherer and farming communities. In light of information emerging from diverse disciplines, including recent paleogenetic studies, the most plausible model explaining the movement of Neolithic pioneer groups in central Europe is that of leapfrog migration.

The transition from a food-collecting economy to a food-producing economy (Neolithic transition) started in the Near East about 10,000 years ago and was associated with a dramatic change in the European archeological record. The Neolithic transition is thought to have highly transformed European populations' economy, demography, and genetics. The processes implied during neolithization are intrinsically linked to the question of the ancestry of modern Europeans and consequently are a subject of intense debate in prehistoric archeology and population genetics.

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The spread of agriculture from central Europe to the Atlantic involved four major diffusion events. The earliest evidence of the Mesolithic to Neolithic transition is associated with two distinct diffusion waves. The first of these spread along the Mediterranean coast and is associated with Impressed Ware and Cardial culture; the second wave spread along the Danubian route and is associated with the Linearbandkeramik culture (LBK). Finally, the Funnel Beaker Culture (TRB) of southern Scandinavia and Neolithic Britain and Ireland completed the Neolithic expansion in north and northwest Europe. The character and chronology of the transition in various parts of Europe, and for the various diffusion waves, remain controversial.

Despite the continual improvement of archeological data across Europe, whether the Neolithic transition involved an extensive movement of people (demic diffusion) from the areas where farming first appeared or the spread of agricultural technologies mainly through cultural contacts involving little migration (cultural diffusion) is still widely debated. A model of neolithization as a result of migration has been omnipresent in archeological discourse since the works of G. Childe.[1] The rapid spread of LBK settlements in central Europe was notably viewed as a classic example of prehistoric migration. However, more recent studies attach much greater significance to indigenous adoption of agricultural technology and contacts between invading farmers and local foragers.[2,3] Existing archeological evidence suggests that the spread of agriculture across Europe was complex, with a succession of migration phases and local admixture.[4–7] As a consequence, some authors believe that, whether it occurred through contact or migration, the introduction of farming alone cannot explain the establishment of Neolithic societies.

Clearly, different regions must have experienced different combinations of cultural exchanges and migratory movements. Various scenarios, ranging from demic diffusion to leapfrog colonization, frontier mobility, contact, and exchange have consequently been proposed to account for the complexity of the Neolithic diffusion.[8,9] The debate remains open since a recent synthesis on the spread of agriculture in Neolithic western Europe has revealed that it was everywhere both chronologically and economically much more abrupt than had hitherto been envisaged. Consequently, the four major spreads of agriculture noted earlier all appear to be compatible with the immigration of at least a considerable proportion of farmers.[10]

The relationship between Neolithic processes and the distribution of genetic diversity among modern European populations has been the subject of intense debate since the first genetic data became available. Over the past forty years a fascinating dialogue has consequently developed between archeology and genetics.

The pioneering collaboration between the geneticist Cavalli-Sforza and archeologist A. Ammerman provided the first scientific model explaining the origins and spread of farming in western Eurasia.[11] The first isochrone maps, plotting similarly dated sites on a map of Europe as a series of isochrones or contour lines, showed a broad southeast-northwest gradient. This finding suggested a roughly constant rate of neolithization across Europe along the gradient axis. Ammerman and Cavalli-Sforza then proposed the mixed demic diffusion hypothesis for Neolithic diffusion, suggesting that productive farming communities with reliable subsistence bases gradually expanded as their populations increased. The regular spread rate was considered to be demonstrative of a wave of advance that combined two features, logistic population growth and random local migratory diffusion or range expansion.

The demic diffusion model was supported by the broad allele-frequency gradients observed for "classical" genetic markers, including non-DNA markers such as allele frequencies for blood groups, the tissue antigen HLA system, and some enzymes.[11,12] Principal-component analyses were used to summarize the variation in gene frequencies across Europe. The results were presented as contour maps (component by component) to show the changes in gene frequencies with geography.[12] The map of the first PC, accounting for about 27% of the total variation in gene frequencies across Europe and the Near East, revealed a gradient from the southeast to the northwest. The map's resemblance to the radiocarbon map for the Neolithic expansion was immediately obvious and considered as strong evidence of the demographic impact of Neolithic farmers dispersing from the Near East. Despite the fact that only about 27% of the gene variation was linked to the gradient observed, the idea of a major dispersion of Neolithic farmers throughout Europe, together with the idea that most modern Europeans shared Neolithic ancestry, solidified in the scientific community's minds. The clines subsequently observed during studies of autosomal DNA and Y-chromosomal markers matched those described at the protein level.[13,14] Using the same analytical methodologies, all nuclear markers indicated a possible Near Eastern origin for the ancestral European population.

A major problem of genetic clines is that they cannot be dated. Zvelebil[15,16] notably pointed out that multiple human dispersals into Europe may have taken place via Anatolia, because this is one of the main possible points of entry into the continent, and may have generated the same genetic gradient. Clines tell us nothing about when they were generated; one identified by PC analysis might be the result of an accumulation of many dispersals.[16] In 2005, the first simulations proposing to elucidate European neolithization also questioned the interpretation of the clines. Currat and Excoffier[17] showed that allele frequency clines over Europe were equally probable under a pure demic diffusion model or a pure acculturation model. The results also implied that allele frequency clines could be equally well explained by the first expansion of humans into Europe. Moreover, the authors demonstrated that ascertainment bias, consisting of selecting single nucleotide polymorphisms (SNPs) with high frequencies, could cause overestimation of the clinal patterns and hence of the Neolithic contribution to the European gene pool. It was concluded that proponents of a demic diffusion model could have overestimated the genetic impact of the Neolithic diffusion and the uniformity of its spread.

The model of European genetic ancestry shifted away from the Neolithic diffusion model toward an emphasis on autochthonous Paleolithic origins, through the phylogeographic analysis of maternally inherited mitochondrial DNA lineages (mtDNA) and paternally inherited Y-chromosome lineages. In the 1980s, thanks to the development of molecular biology technologies, it became possible to analyze not only gene products, but the DNA sequences of the genes themselves. The direct acquisition of DNA sequences allowed reconstruction of the genealogical history of the locus under study and led to the development of the phylogeographic approach.[18]

Phylogeography can be viewed as the mapping of gene genealogies in space and time.[19] It combines three elements: the phylogenetic tree of the locus concerned, the illustration of geographic distribution of lineages on the tree, and the time depth of these lineages. The time scale of phylogenies is provided by converting the diversity of lineages to age estimates by means of a molecular clock.

Heated debate followed the publication of mitochondrial data suggesting that only a small fraction of modern Europeans were descended from Near Eastern farmers.[18] Indeed, phylogeographic analysis of mtDNA variants revealed that about 20% of mitochondrial haplogroups coalesced in Neolithic times, whereas the majority of mtDNA lineages appeared to descend from founders of Middle or Late Upper Paleolithic origin. It appeared that, following the maternal line of descent, only a minority of European ancestors were Near Eastern farmers and that more than three quarters of the surviving lineages in modern Europeans were the result of the acculturation of indigenous foraging peoples. These findings were considered to support the integrationist model described by Zvelebil,[8] which involves both pioneer colonization of farming groups and acculturation and/or genetic exchange with the surrounding foraging communities. Proponents of a demic diffusion model responded that the ages of particular genealogical lineages observed in a geographical region do not necessarily correspond to the arrival of the population in that region.[20] However, applying founder analysis to mtDNA lineages[21] and using the phylogeographic approach on Y-chromosome haplogroups[22] confirmed that Neolithic admixture represented about 20%-25% of the current European gene pool and thus primarily supported the integrationist model. At this point, both mitochondrial and Y-chromosome analyses appeared to be moving toward a consensus, consistent with the archeological evidence, indicating that a quarter or less of the European gene pool was contributed by Neolithic Near Eastern lineages.

In spite of the apparent consensus between mtDNA and Y-chromosome data, recent studies show that the extent of modern European populations' Neolithic ancestry remains controversial. For example, a large survey of autosomal microsatellite loci recently allowed estimation of the admixture rates in European populations.[23] On average, the Near Eastern contribution was calculated to be between 46% and 66%. In addition, admixture estimates showed a strong, significant negative correlation with distance from the Near East. The latest study on extant European Y chromosome variation, analyzing the geographical distribution of Y haplogroup R1b1b2 microsatellite diversity,[24] concluded that this haplogroup's distribution was best explained by diffusion from a single source in the Near East via Anatolia during the Neolithic. The authors concluded that most European Y chromosomes originated during the Neolithic expansion. They proposed that the contrast of this pattern with that shown by maternally inherited mtDNA suggests a unique role for males during the transition.

The compilation of these genetic studies gives pause for thought: Despite the extent and variability of data accumulated on current European populations, genetic studies still lead to conflict regarding estimates of Neolithic input to the present population and processes implied in Neolithic diffusion. Recognizing that conflicting estimates are expected because different investigators use different loci, approaches, and assumptions, this assessment explains why the debate between archeologists and geneticists has often been marked by a degree of mutual confusion. Moreover, the different temporal and spatial scales on which the two disciplines operate and the different questions they address only reinforce this confusion.[25]

If one point of consensus can be proposed at the moment, it is that there has been a major shift in opinion concerning the ancestry of Europeans. This change has been driven largely by the combined forces of archeology and genetics: Neolithic farmers from the Near East participated in European ancestry, but the majority of European genetic lineages have their roots in the European Paleolithic.[26]

Genetic analyses of the extant European gene pool contribute to the study of the Neolithic transition. However, the patterns observed in the current European gene pool are the outcome of Paleolithic and Neolithic processes overlaid with four to ten millennia of subsequent demographic processes, which may have blurred the picture. This observation encouraged paleogeneticists to participate in the Neolithic transition debate, proposing direct genetic comparisons of ancient inhabitants of Europe to their contemporary counterparts. Indeed, ancient DNA (aDNA) obtained from pre-Neolithic and Neolithic human remains appears to offer much more specific information concerning individuals and groups of individuals who can be culturally situated and archeologically dated. Paleogenetic evidence should, therefore, play a primary role in gaining a deeper understanding of the Neolithic transition and in reconciling archeological and genetic perspectives.

NEOLITHIC AND PALEOGENETICS

The development of techniques enabling the analysis of DNA from ancient remains allows the genetic ancestry of ancient inhabitants of Europe to be directly compared to their contemporary counterparts. Although paleogenetics is confronted with numerous inherent problems, it provides a unique opportunity to determine to what extent later European populations truly do retain the genetic legacy of earlier groups. Dozens of papers proposing ancient sequences from pre-Neolithic and Neolithic groups are now available and mainly concern the mtDNA genome, which is more easily obtained from ancient remains (Fig. 1). Specifically concerning the Neolithic transition, distinguishing between demic and cultural diffusion mechanisms has been regarded as a question of genetic continuity or discontinuity (Box 1).

The majority of paleogenetic studies available today allow discussions concerning the Neolithic wave that spread along the Danubian route and was associated with the LBK and its derivatives. The first published studies proposed paleogenetic data for Neolithic groups from Germany, Hungary, Austria,[27] the Czech Republic,[28] and western France.[29] Haak and coworkers[27] focused on human remains originating from the LBK and Alföldi Vonaldiszes Keramia (AVK) cultures. Both are associated with the beginning of farming in the temperate regions of Europe around 5500 BC (Fig. 2). Originating in Hungary and Slovakia, LBK spread rapidly as far as the Paris Basin and the Ukraine.[2] A total of 24 out of 57 Neolithic skeletons from 16 sites of the LBK/AVK cultures in Germany, Austria, and Hungary (5500-5000 cal BC; sites 9-18 in Figs. 1 and 2) yielded successful amplifications and reliable HVR-I sequences (hypervariable region I of the mitochondrial genome). Interpretations focused primarily on the characterization of a high frequency of the mitochondrial N1a lineage in the LBK area. Today, this lineage is exceedingly rare, but widespread, in Europe. The genetic discontinuity described between Neolithic farmers and extant Europeans was considered to be evidence that the first agriculturists had limited success in leaving a genetic mark on the female lineages of modern Europeans. Setting aside the possibility of significant post-Neolithic migrations, the

Figure 1. Location of European Mesolithic and Neolithic communities for which ancient DNA sequences are available. 1, Hohler Fels[31]; 2, Villabruna[51]; 3, Spiginas[31]; 4, Donkalnis[31]; 5, Chekalino[31]; 6, Lebyazhinka[31]; 7, Bad Durrenberg[31]; 8, Hohlenstein[31]; 9, Derenburg[27,33]; 10, Eisleben[27]; 11, Halberstadt[27]; 12, Flomborn[27]; 13, Schwetzingen[27]; 14, Seehausen[27]; 15, Unterwiederstedt[27]; 16, Vaihingen[27]; 17, Ecsegfalva[27]; 18, Asparn Scheltz[27]; 19, Vedrovice[28]; 20, Gurgy; 21, Kretuonas[31]; 22, Péré[29]; 23, Mezzocorona[51]; 24, Borgo Nuovo[51]; 25, Eulau[47]; 26, Fraselgarden[34]; 27, Cami de Can Grau[36]; 28, Les Treilles[37]; 29, Otzi[52]; 30, Orstorf[31]; 31, Fridtorp[34]; 32, Ajvide[34]; 33, Ire[34]; 34, Drestwo[31]; 35, Dudka.[31]

authors proposed a Paleolithic ancestry for modern Europeans, claiming that small pioneer groups carried farming into new areas of Europe. Surrounding hunter-gatherers, who outnumbered the original farmers, then adopted the new techniques (leapfrog colonization).

Although the N1a lineage was not recovered from the six individuals analyzed from Vedrovice[28] (Czech Republic, 5300 cal BC; site 19 in Figs. 1 and 2), a specific N1a haplotype first described in an AVK human remain from Hungary[27] (site 17 in Figs. 1 and 2) was later discovered in a French megalithic burial chamber from the Middle Neolithic (site 22 in Figs. 1 and 2).[29] This raised questions concerning the possibility of lineage diffusion through farmer pioneer groups as far as western France.[29] A more recent study proposed a detailed reconstruction of N1a haplogroup phylogeography.[30] The authors of this study proposed that the N1a lineages characterized in LBK and AVK contexts[27] originated from different sources, such as eastern Europe, central Europe, and/or the Near East. The results were considered to accord with a leapfrog colonization process in central Europe with admixture between indigenous "Mesolithic" N1a and arriving "Neolithic" N1a individuals.

More recently, important paleogenetic results have been published for late European hunter-gatherers from central and northern Europe. These results allowed the theory of an exogenous origin of the first Neolithic farmers to be tested directly. Bramanti and coworkers[31] obtained 22 mtDNA sequences from late European hunter-gatherer skeletons (sites 1, 2-8, 30, 34, and 35 in Figs. 1 and 2). The sample spanned a long time, with remains from the Late Upper Paleolithic and Mesolithic (13400 cal BC to 2300 cal BC), as well as a very large geographic region from Germany and Poland to Lithuania and Russia. A surprisingly high frequency of mtDNA lineage U, 82%, was discovered. Most of the hunter-gatherers were shown to share mtDNA types that are relatively rare in central Europeans today. Discontinuity between late hunter-gatherers, early farmers, and extant European populations from central Europe was considered persuasive evidence that the first farmers were not the descendants of local hunter-gatherers, but immigrated into central Europe at the beginning of the Neolithic transition. Results indicated that the migrating farmers, at least initially, did not mix significantly with the resident female hunter-gatherers. Post-Neolithic events would have remodeled the genetic pool, which explains the discontinuity with extant Europeans.

The representativeness of the hunter-gatherer sample studied by Bramanti and colleagues can, however, be questioned. This sample encompassed a large geographic area and temporal span, thus forming a highly artificial population. The chronological heterogeneity of the sample implies that it incorporates pre-Neolithic individuals from central and northeast Europe, as well as hunter-gatherers postdating the first farmers' arrival in the region (50% of samples, Fig. 2), who may already have incorporated variants from incoming Neolithic groups. In that respect, late hunter-gatherers, such as those of the Ostorf site in Germany (around 3000 cal BC, site 30 in Fig. 2)[31] may already have incorporated mtDNA lineages brought by the first farmers, who arrived around 5500 BC. Adding even more confusion to the analyses, the hypothesis that Ostorf people were former farmers who became fishermen around 3300-3200 cal BC has yet to be resolved.[32] The geographical heterogeneity of the sample may make the neolithization processes appear simple, when in reality they may have been quite variable at the regional scale.[3]

The latest paleogenetic study focusing on central European neolithization[33] provided new mtDNA data for 21 individuals from the Derenburg graveyard of the LBK culture (Germany; 5500-4900 cal BC; site 9 in Figs. 1 and 2). This first LBK group genetically characterized at the "population" level was combined with previously analyzed LBK groups.[27] Comprehensive phylogeographic and population genetic analyses were done to locate the LBK farmers' origins within the broader Eurasian region and to trace potential dispersal routes into Europe. Genetic affinities between LBK groups and modern-day Near Eastern and Anatolian populations were uncovered, supporting the hypothesis of major genetic input from this area during the advent of farming in Europe. A possible geographic route for the dispersal of Neolithic lineages into central Europe was also proposed along the natural corridor of the Danube and Dniester rivers. However, mtDNA pools for regions from central Europe were also shown to be closely affiliated to that of the LBK population. This accords well with archeologists' proposal of an LBK origin in modern-day Hungary.[2] Further, the LBK population, as compared to modern populations, presented unique genetic features, confirming that major demographic events continued to take place in Europe after the Early Neolithic. The large-scale model supported by the

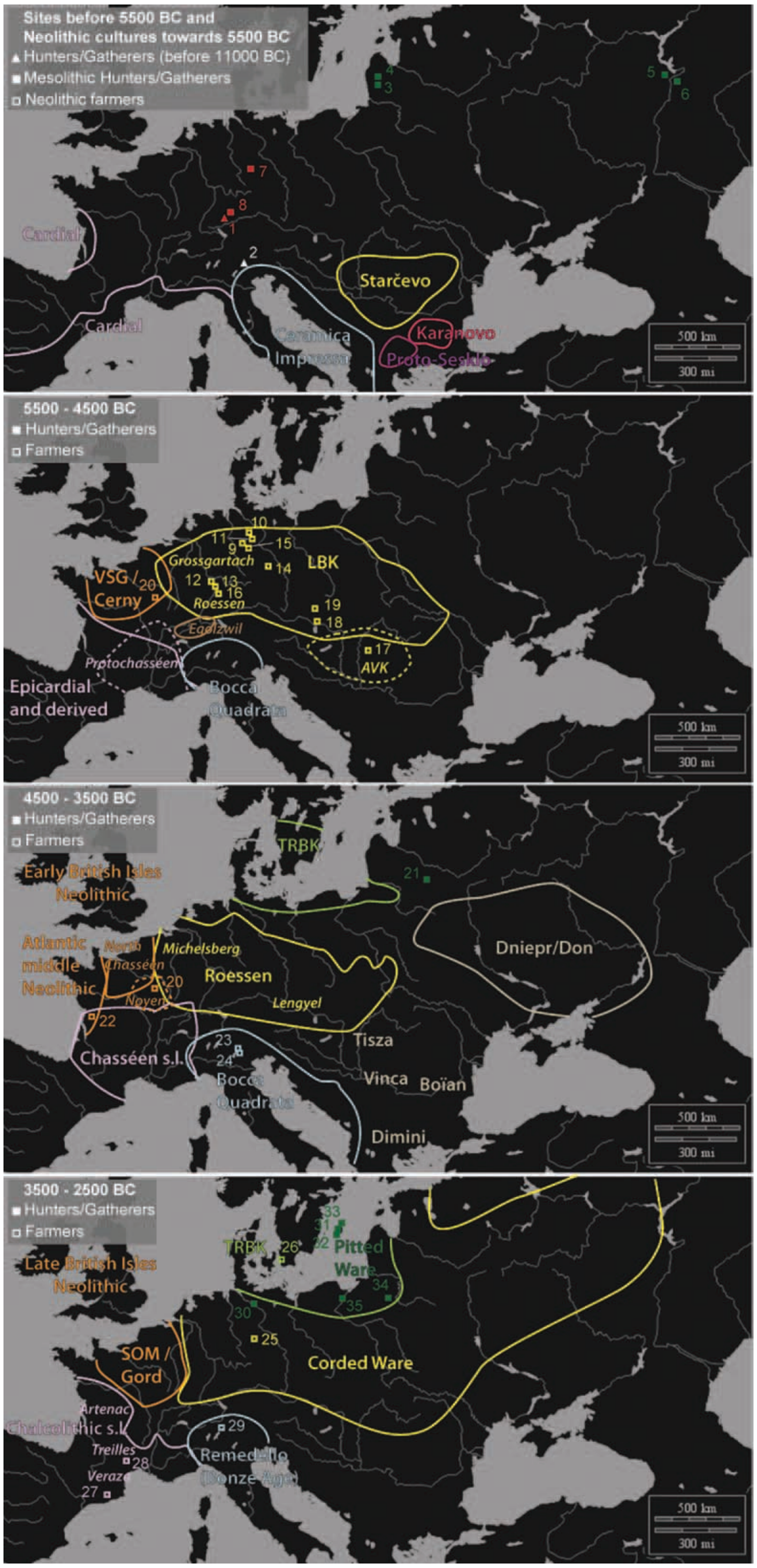

Figure 2. Geographical and chronological localization of European Mesolithic and Neolithic communities for which ancient DNA sequences are available and main cultures associated, after Guilaine 1998,[4] Zvelebil 2001,[8] and Gronenborn 2003[3] (numbers refer to populations listed in Figure 1). VSG, Villeneuve-Saint-Germain; LBK, LinearBandKeramik; AVK, Alföldi Vonaldíszes Kerámia; TRBK, TrichterRandBecherKultur or Funnel Beaker Culture; SOM, Seine-Oise-Marne. (Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.)

authors was that of a demic diffusion model with genetic input from the Near East into central Europe.[33] This large-scale demic diffusion model was combined with a leapfrog colonization model at the regional scale, which would explain the relative speed of the LBK expansion in central Europe, the clear genetic Near Eastern connections seen in pioneer settlements, and a sizeable but less important input of Mesolithic populations to modern European genetic variation. Quantification of the Near East contribution still appears problematic, however. Notably, we can question the accuracy of selecting only "informative" haplotypes that have limited geographic distribution and are unequally spread among present-day populations to unravel LBK groups' genetic affinities with modern Eurasian populations. Moreover, Haak and coworkers[33] propose inferring the origin of LBK mtDNA lineages according to modern European populations' gene pools. Since a clear genetic discontinuity between past and modern Europeans is regularly confirmed, this proposition may be questionable.

Paleogenetic studies have also been conducted in Scandinavia (sites 26 and 31 to 33 in Figs. 1 and 2).[34] This region is unique in that it maintained one of the last major hunter-gatherer complexes in Neolithic Europe, the Pitted Ware Culture (PWC). In Scandinavia, the neolithization process is linked to the appearance of the Funnel Beaker Cultural complex (FBC or TRB) around 3200 BC. However, Early Neolithic immigrant farmers appear to have been replaced by Middle Neolithic PWC hunter-gatherers from the northeast Baltic around 3000 BC. Analyzing 19 skeletons from the PWC (3300-2500 cal BC), Malmström and co-investigators[34] found a high incidence of mitochondrial haplogroups U4 and H1b (the two cannot be clearly distinguished at the HVR-I level), U5, and U5a, all of which are rare among contemporary Scandinavians. The results indicated that people of the PWC were not the direct ancestors of modern Scandinavians and supported the theory that PWC groups were replaced by a new wave of farmers. However, a recent study on ancient populations from Denmark[35] proved the presence of Hg U4 and U5 in two human remains from the Neolithic site of Damsbo (2200 cal BC) and one from the Bronze Age site of Bredtoftegard (1300-1500 cal BC). Melchior and coworkers[35] proposed that predominantly Hg U lineages persisted among Neolithic/Bronze Age population samples in southern Scandinavia, in direct contradiction to Malmström and colleagues'[34] conclusions suggesting Mesolithic communities' replacement by a renewed wave of farmers.

Only two paleogenetic studies are available that relate to the neolithization associated with Impressed Ware and Cardial culture of the Mediterranean coast.[36,37] However, the sites concerned are chronologically distant from the first Neolithic establishments known in these regions. These sites (27 and 28 in Figs. 1 and 2) date to the end of the Neolithic, even the Chalcolithic, and their usefulness in shedding light on neolithization processes can be questioned. The profound social transformations associated with the arrival of the metal must notably be taken into account, particularly as regards the networks of exchanges of raw materials, objects, and, most importantly, people.

Whereas genetic discontinuity between the present and Neolithic periods characterizes central Europe, the opposite diffusion pattern, genetic continuity, was encountered in Catalonia.[36] HVR-I sequences were obtained from 11 individuals originating from the site of Cami de Can Grau[36] (3500-3000 cal BC; site 27 in Figs. 1 and 2). These sequences showed no significant difference in genetic composition as compared to the extant populations from the Iberian Peninsula. The investigators concluded that the genetic impact of Neolithic groups that migrated to Iberia was considerable enough to be maintained up to present day. This result appeared to agree with archeological evidence that the Impressed Ware complex represented a demic intrusion.[38] The authors of this study concluded that whereas the dispersal of agriculture in central Europe could have followed a cultural diffusion model, results in the Mediterranean suggest a demic diffusion model. However, the Neolithic group studied in Catalonia (3500-3000 cal BC) largely postdates arrival of the Cardial Neolithic in the region at about 5500 BC.[39] Without paleogenetic data from more ancient periods and communities, such as hunter-gatherers from the region, we cannot exclude the possibility that the regional gene pool has been stable since the Mesolithic or Paleolithic, bringing the demic diffusion model into question.

The latest report of DNA sequences from southern European Neolithic groups concerned the collective burial at Treilles Cave in Aveyron, France (site 28 in Figs. 1 and 2), dating to the end of the Neolithic period (3000 BC).[37] The exceptional DNA conservation in the 53 analyzed samples allowed the study of short tandem repeats (STRs) and SNPs located in the nuclear (Y-chromosome and autosomes) and mitochondrial genomes. Close parental relationships within the necropolis, imbalance in sex ratio (22 males and 2 females), and greater genetic diversity for mtDNA sequences than Y-chromosome ones could be determined. Results suggest that the community that used this burial site was patrilocal or engaged in a particular funeral rite. The maternal lineages appeared to have more diversified origins in space and time than did Y-chromosomes. However, both markers were interpreted as indicating a greater Mediterranean than central European influence in the peopling of southern Europe during the Neolithic transition. The same criticism as that of Sampietro's study can be leveled at this one: The Neolithic group studied largely postdates the Neolithic arrival in the region (around 5500 BC) and thus cannot illuminate the processes implied in Early Neolithic colonization. The situation is perhaps even more complex in France, for this is one of the few European regions where the two great currents, Mediterranean and Danubian, came into both direct and indirect contact with each other.

Overall, paleogenetic studies available for southwest Neolithic groups highlight the importance of taking into account the archeological dates of the human remains under study, especially as they relate to the neolithization chronology of the region concerned. While it is conceivable to draw conclusions concerning the arrival of the first farming groups in a certain region from paleogenetic analyses of Early Neolithic human remains, it appears impossible to do so with farming groups who postdate the original neolithization processes by several centuries or millennia. In the case of the latter, it is impossible to decide if the groups concerned correspond to "pure" Neolithic groups descendant from the original farming communities established in the region, to the admixture of farmer and hunter-gatherer communities, or to hunter-gatherer communities who adopted a Neolithic way of life. It is also impossible to determine if the groups considered derived only from the initial potential Neolithic migration in the region or from subsequent migrations, possibly from different sources.

A major conclusion can be drawn in light of the information now emerging from paleogenetic studies. It appears that genetic discontinuity between Neolithic European populations and maternal contemporary European populations is the rule, at least for central and north Europe, where data are more numerous. It is now essential to understand and characterize the processes underlying genetic discontinuity among the pre-Neolithic, Neolithic, and post-Neolithic periods.

LIMITS OF PALEOGENETIC DATA

Despite the great potential of using the paleogenetic approach to achieve a better understanding of neolithization processes, the inherent problems of the discipline include DNA authenticity and sample representativeness, as well as the potential implications of these difficulties in formulating hypotheses to explain European neolithization.

First, studies using ancient DNA are plagued by a unique set of methodological problems, including template degradation and contamination with modern exogenous DNA.[40,41] Due to technical difficulties induced by the minute and degraded nature of ancient DNA and the consequent risk of contamination, the authentication of amplified sequences has become a major interest in the field. This is especially true when dealing with human remains.[42] Results are usually validated through the determination of potential contamination sources, the use of decontamination techniques, and the application of "criteria of authenticity."

Since it appears that there is no way to guarantee complete removal of contaminant DNA, information on the handling history of human remains and the application of criteria of authenticity remain fundamental.[43] It is worth noting that paleogenetic studies on late European hunter-gatherers and Neolithic farmers did not always meet those criteria. Indeed, human remains under study often came from old excavations and information on their handling history was often lacking. In such cases, the repeatability of results was presented as strong evidence of sequence authenticity. However, if the samples were highly contaminated before the analysis, the replication criterion provides no way to detect contamination, since independent replications can yield the contaminant in all replicas.[44] Moreover, information on sequence repeatability was sometimes missing. Cloning of the amplification products to detect PCR artifacts associated with postmortem template modification and/or contamination sometimes appears to have been omitted.

We believe, nevertheless, that the sequences proposed by the previous studies are mostly authentic; the best evidence of this is the genetic discontinuity measured between the past human groups analyzed and extant potentially contaminating populations, and the fact that different sequences were found for different human remains. Even so, we strongly urge that all information supporting sequence authenticity, including that relating to handling history or careful contamination controls during initial sample collection, cloning of all PCR products, and systematic replications for all samples, be systematically provided so the scientific community can better evaluate and accept proposed results. It is worth noting that non-PCR based approaches (next generation sequencing technologies), which have been recently developed and applied to anatomically modern human remains (for example, by Krause and colleagues[45]), appear particularly promising in respect to contamination problems. This is because endogenous DNA can be relatively easily differentiated from exogenous contaminant DNA through differentiation of degradation patterns. These technologies should emerge as a major approach to future paleogenetic analyses of pre-Neolithic and Neolithic remains.

The second problem that is inherent in the paleogenetic approach is the representativeness of the samples under study. For the moment, ancient DNA samples are very limited; a total of 40 mtDNA sequences from hunter-gatherers from 15 Paleolithic/Mesolithic sites and 109 farmers from 20 Neolithic sites have been analyzed (Fig. 1). It is obvious that such samples will not permit complete resolution of the debate surrounding neolithization processes. However, this sample begins to shed some light on the demographic history of central Europeans. Continuous improvement of the ancient DNA database will, without doubt, provide critical insight into this topic.

In addition to the paucity of ancient samples available, the geographical and temporal representativeness of samples can also be questioned. The best illustration is given by the hunter-gatherer sample studied by Bramanti and associates,[31] as discussed earlier. It is also essential to take into account the absolute chronology of the human remains under study, especially as they relate to the neolithization chronology of the region concerned, as discussed previously with regard to paleogenetic studies conducted in southwest Europe.

The genetic picture presented by aDNA studies currently is primarily based on mtDNA results, the reason being that mtDNA, because of its higher frequency in living cells, is conserved in ancient samples better than is the nuclear genome. Studies of maternally inherited mtDNA allow inferences to be drawn only for females. It is conceivable that analyses of the Y chromosome or nuclear markers would present different patterns. Ammerman, Pinhasi, and Bánffy[46] argued that marriage patterns could explain extinction of the N1a lineage after the Neolithic[27] if, for example, early male Neolithic farmers mated with Mesolithic women. However, such scenarios cannot currently be tested since nuclear markers are rarely obtained from ancient DNA. The rare cases in which nuclear DNA has been obtained for Neolithic groups are reported in two studies by Haak and coworkers[33,47] and Lacan and colleagues.[37] Three male remains from the Derenburg graveyard were successfully typed for Y chromosome SNPs, permitting determination of their Y haplogroup affiliation but providing little phylogeographic information.[33] In contrast, characterization of Y-chromosome lineages in male remains from the Treilles collective burial permitted researchers to discuss genetic affinities with extant Mediterranean populations.[37] The exceptional achievement of sequencing autosomal, mitochondrial, and Y-chromosome markers from the few Late Neolithic human remains found at the Eulau site[47] (Germany, 2600-2500 cal BC) allowed researchers to provide evidence of the oldest nuclear family ever recovered in one burial. Combined with isotope data, the evidence indicated that this Late Neolithic society was exogamous and patrilocal. A patrilocal pattern was also proposed for the group buried at Treilles.[37] Whereas studies suggesting patrilocal societies concerned very Late Neolithic communities, this point could demonstrate the importance of exercising caution when drawing conclusions based solely on maternally inherited mtDNA.

The last problem inherent in a paleogenetic approach is working on samples originating from funerary contexts. It is widely accepted in archeological contexts that samples recovered from funerary structures do not necessarily represent the population as a whole. For example, it has been demonstrated that only a small portion of the LBK population could have been interred in regular burials and that about 80% of the dead were treated in a way that left hardly any archeological trace.[48] Burials, therefore, may represent a specific group of exceptionally treated people. It is essential to discuss such recruitment bias when interpreting paleogenetic data.[29] Moreover, cemeteries may have functioned over long times and could therefore include individuals corresponding to distinct biological groups or populations. For example, the cemetery at Flomborn[27] was used for as long as 150 years[49]; the graveyard at Derenburg was used over three centuries.[33] Long activity periods are also proposed for other LBK funerary sites.[48,50] Considering the chronological distribution of the burials may further complicate inferences of LBK identities. Once again, such bias must be taken into account to facilitate wider discussions of regional neolithization processes.

HUNTER-GATHERER AND FARMING COMMUNITIES' GENETIC AFFINITIES

Since drachronic comparisons can introduce a certain number of biases into interpretation (Box 1), a classic phylogeographical analysis of ancient populations may shed new light on hunter-gatherer and farming communities, genetic affinities. This approach is, of course, qualitative, since aDNA datasets are still rare for pre-Neolithic and Neolithic periods. To illustrate genetic affinities among European Neolithic communities, all HRV-I sequences available for hunter-gatherer (40 sequences)[31,34] and Neolithic human remains (109 sequences, 5500-2500 cal BC)[27–29,31,33,36,37,47,51,52] were compiled without any selection. These included three sequences from the Gurgy necropole, France, 4800-4550 cal BC (unpublished data) (Fig. 2). The sequences were aligned using the MEGA4 program.[53] A median-joining network connecting pre-Neolithic and Neolithic HRV-I mitochondrial sequences was constructed for nps 16055-16400 by using NETWORK 4.6.

The resulting median joining network (Fig. 3) is not wholly satisfactory because it was constructed using an aDNA dataset too poor to be representative of the periods concerned and because it takes into account only HVR-I sequences (nps 16055-16400); the latter is responsible for creating artificial links between different haplogroups. Nevertheless, this network does highlight different types of interesting genetic affinities, especially for the north-central European region for which data are more substantial.

1. Haplotypes are shared among hunter-gatherer communities from north-central Europe, even those which are distant in time and space. This could reflect some genetic continuity between ancient and late hunter-gatherer communities, as well as potential gene flow among distant hunter-gatherer groups. It could also imply intensification in communication among groups in most regions of continental temperate Europe beginning in the Late Mesolithic.[2]
2. Farmers from LBK communities share haplotypes with late hunter-gatherers from Germany and Sweden who postdate the Neolithic arrival. This observation could either highlight gene flow between culturally distinct communities between 5500 and 3000 cal BC or reflect a degree of hunter-gatherer acculturation, with genetic continuity between the ancient hunter-gatherer gene pool and LBK farmers.
3. Haplotypes shared among LBK communities from Germany underline their genetic proximity. This could reflect some genetic homogeneity in this cultural group, deriving from a shared origin and/or consequent gene flow inside this cultural group.
4. Early Neolithic sequences from north or west France are grouped with or are identical to Neolithic sequences from central Europe (Hungary and Germany). This could highlight the diffusion of haplotypes from central to western Europe, linked to the Neolithic diffusion.

5. The same diffusion of haplotypes can be proposed from central Europe to Sweden (Figs. 2 and 3), but is associated, for the moment, with fewer haplotypes.

6. It is interesting to note that many mtDNA haplotypes from the Late Neolithic Treilles site (south France) are shared with sequences from Early Neolithic groups from central Europe. This could be related to the fact that France is one of the few European regions where the two great currents, Mediterranean and Danubian, responsible for the diffusion of the Neolithic in Europe, came into direct or indirect contact with each other. However, the important chronological gap between groups (about 2,000 years) does not allow us to propose any reliable conclusion concerning these genetic affinities.

Phylogeographical arguments (2-5) based on paleogenetic data appear to support a model of leapfrog Neolithic migration in north-central Europe; that is, the migration of pioneer Neolithic groups in central Europe as far as western and northern Europe. This migration would have been followed by admixture with indigenous hunter-gatherers combined with hunter-gatherer groups' acculturation, eventually leading to hunter-gatherers outnumbering the original Neolithic migrants.

ARGUMENTS FOR LEAPFROG COLONIZATION ALONG DANUBIAN EXPANSION

All genetic data accumulated for present-day European populations show that Neolithic colonization from the Near East did indeed take place in Europe and that the descendants of colonists are present in extant central European populations. Nevertheless, the precise contribution of Near East genes to the European gene pool remains problematic, since different proportions of Near Eastern lineages in the European gene pool are regularly proposed. Paleogenetic arguments have recently contributed to this question through phylogeographic analysis of the N1a haplogroup,[30] as well as a recent analysis of the origins of LBK.[33] Both approaches suggested that lineages characterized in LBK and AVK contexts originated from different sources, including the Near East and eastern and central Europe. This is in total accordance with archeological data.

The LBK culture is reported to have originated in present-day western Hungary and southwestern Slovakia.[2] Archeological data and 14C dates[7] confirmed that Neolithic groups crossed the Danube valley between 6000 and 5800 BC (white pottery phase of the Starčevo culture). This precocious neolithization established farming in the eastern part of the Carpathian Basin as early as 6000-5500 BC (northeastern Hungary, Körös-Starčevo). Expansion of the Körös-Starčevo to the northern part of the Carpathian Basin would then have been constrained by an ecological barrier in the upper Tisza Basin and northern Transdanubia. Adaptation to the new ecological conditions would have taken at least 300-400 years and would have corresponded to the transition between Körös and Linear Pottery. LBK culture must have then emerged from the long interaction among Starčevo farmers, Körös communities, and Mesolithic hunters in Transdanubia during the earlier 6th millennium BC.[2] The rapid population expansion in the LBK area must therefore have involved lineages brought by the agriculturalist newcomers from Anatolia, mixed with lineages from assimilated Balkan foraging populations.[2,33,54]

If one assumes that currently available Mesolithic data apply to the pre-Neolithic Danubian, which remains to be proved, we can consider that paleogenetic data are beginning to provide evidence that early LBK farmers expanding in central Europe were not the descendants of local hunter-gatherers, but rather immigrants who came into central Europe at the onset of the Neolithic.[31] These results would indicate that the LBK expansion in central Europe was accompanied by a substantial influx of people from outside the region. Moreover, a certain genetic homogeneity can be observed among LBK communities (Fig. 2), which highlights their common origin, as well as potential gene flow. This suggests the maintenance of intense connections and exchanges among LBK communities throughout their expansion into central Europe. Since Childe,[1] the spread of LBK settlements in central Europe has indeed been viewed as a classical example of prehistoric migration. The abrupt and uniform start of the Neolithic in central Europe, the remarkable speed of the LBK expansion (500 years), and the general uniformity of the culture across a territory of nearly a million square kilometres might indicate that neolithization was fuelled to a considerable degree by a migration of people.[23] Archeological evidence of leapfrog colonization could explain the rapid spread of farming communities through the fertile lowland basins and river valleys in the Balkans and central Europe.[5] Migrant LBK groups preferentially settled in areas of highly fertile soil, creating "small islands of farmers in the immense sea of foragers."[55] This very particular locational preference demonstrated by LBK settlements in central and western Europe appears to indicate swift expansion into favorable landscapes rather than slow, population-driven movement.[56]

MtDNA analyses support substantial human migration in central and north or western Europe. MtDNA results indeed suggest that the Neolithic most heavily affected regions in southeast, central, northwest, and northeast Europe, and that this was potentially linked to the LBK expansion.[13] However, the Neolithic lineages proposed as markers for the LBK culture and post-LBK dispersals, based on extant European populations analyses (notably lineage J1a, T1)[18,21] were, until now, virtually absent from the Neolithic gene pool as characterized. Available data for LBK groups instead indicate elevated or high frequencies of haplogroups T, N1a, K, and W (Fig. 3).[33] Such haplogroups appear to be unique to LBK populations, differentiating them from both Europe and the Near East, and indicating that further significant genetic changes

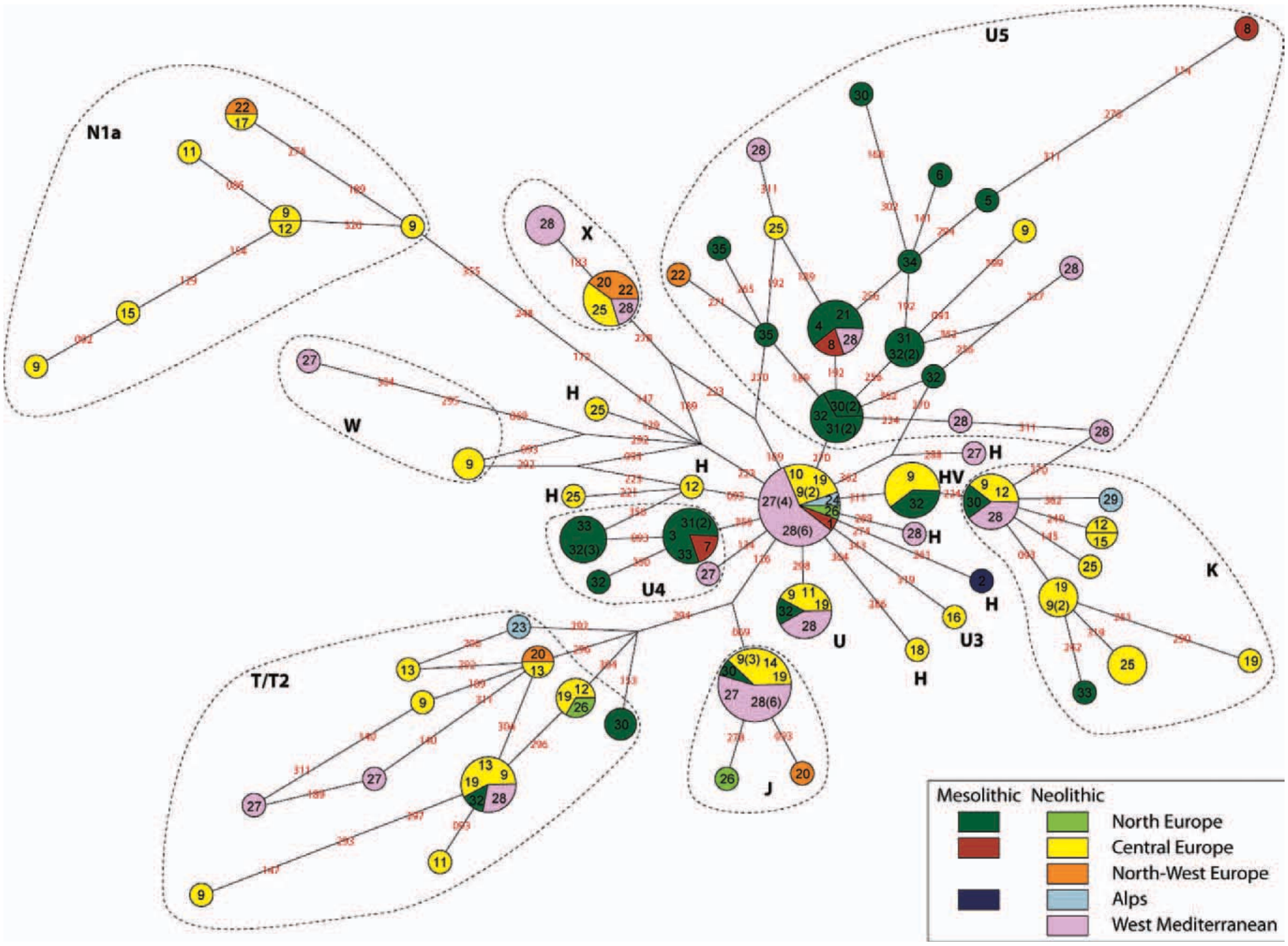

Figure 3. Median-joining network of HVR-I sequences (nps 16055-16400) available today from late European hunter-gatherers and Neolithic farmers (numbers refer to populations listed in Figure 1). (Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.)

took place in Europe after the Early Neolithic. These findings particularly reinforce the considerable value of aDNA for testing hypotheses derived from analyses of extant populations. Other paleogenetic arguments highlight a specific human migration linked to the LBK expansion. The coevolution of European lactase persistence and dairying recently has been linked to the LBK origin and expansion in central Europe.[57] The diffusion of specific lineages of domestic pigs in central Europe clearly mirrored the Danubian route.[58] Since human groups usually keep piglets born from domestic sows, this pattern was considered to reflect human movement. Craniometric data also support a migration of Neolithic groups toward north-central Europe.[59]

Results from Bramanti and coworkers[31] indicate that farming communities did not, at least initially, mix significantly with the resident female hunter-gatherers in central Europe. The first farming communities are indeed perceived as Neolithic enclaves in a Mesolithic environment. The original LBK communities were shown to have been settled in areas only marginally exploited by hunter-gatherers,[60] suggesting mutual exclusion in spatial terms that might be consistent with a model of contemporaneity and co-existence between culturally different communities. The general model supported by paleogenetics and archeology is mosaic colonization with scattered farming settlements linked together by exchange networks, co-existing initially with indigenous hunter-gatherer communities. This same pattern has been proposed for western Europe.[61]

Potential subsequent gene flow between LBK and hunter-gatherer communities may, however, be proposed, citing the sharing of some specific haplotypes between groups (Figs. 2 and 3). Furthermore, the genetic discontinuity characterized between the first farmers and extant European populations would reflect progressive dilution of the farmers' lineages in the "Mesolithic" gene pool. Indigenous hunter-gatherers would have adopted the new culture and/or mixed with farming communities, and may have outnumbered the original farmers. This scenario is clearly echoed by genetic analyses of extant European populations that still contain a high proportion of lineages originating from pre-Neolithic periods, which were interpreted as the result of acculturation of indigenous foraging peoples during the Neolithic transition.[21] In this case, genetic differentiation between farmers and hunter-gatherers (potentially unravelled by aDNA analyses) might have eroded over time as the effects of subsequent gene flow blurred the picture. These paleogenetic arguments mirror the development of a more culturally homogeneous landscape that began to develop with the exchange of ceramics, stone tools, livestock, furs, and presumably also individuals between farmer and hunter-gatherer communities. Gronenborn[2] has indeed interpreted the intensification of exchanges among groups as an increase in economic exchange between farmers and terminal Mesolithic communities, during which the latter were gradually assimilated.

Strontium isotope analyses have indicated that between 30% and 50% of analyzed LBK burials were of nonlocal origin.[62] These individuals could represent hunter-gatherers who married into farming communities[62] or residents of LBK settlements in the highlands who relocated upon marriage.[63] In any case, strontium isotope analyses of LBK skeletons from the Rhine Valley suggest that many people made residential changes during this period. Whether it involved foragers or farmers, the observed pattern of female migration is consistent with genetic evidence of patrilocality in European prehistory. These observations are characterized in the context of a period of prolonged stasis, some centuries after farmers initially settled, during which the interaction took place between hunter-gatherer and farming communities.

Phylogeographic analyses of Neolithic groups highlight genetic affinities among LBK communities and both French and Swedish farming groups of the Middle Neolithic (Fig. 3). This observation supports the hypothesis that substantial mtDNA lineages were dispersed by farmers into western and northern Europe through later population movements. Richards[54] remarked that the presence of Near Eastern lineages in northwest Europe appeared to be inconsistent with Zvelebil's model[8], which suggests that a long-term frontier was established on the North European Plain, and that the transition to farming in north, northwest, and southwest Europe took place largely by acculturation. Extant paleogenetic data support the hypothesis that farming groups indeed migrated to west and north Europe, even if their lineages did apparently become diluted later in the Mesolithic gene pool.

Sites presenting evidence of LBK culture spread into a vast area of central Europe, stretching from southwest Ukraine and Moldova in the east to the Paris Basin in the west. Shortly before 5000 cal BC, the LBK advanced toward the Paris Basin, where it formed the Rubané Récent du Bassin Parisien.[64] Further west in France, settlements related to the LBK tradition are attributed to the Bliquy-Villeneuve-Saint Germain (BVSG) group.[65,66] Scarre[61] has suggested that the BVSG sites of Normandy and the Loire might represent small, dispersed pioneer groups who moved into areas of the landscape that complemented those occupied by hunters-gatherers. Similarities in settlement type and material culture support the argument that these BVSG sites indeed represented a movement of populations in a westward direction.

Between 4400 and 4000 cal BC, farming was introduced from central Europe into northern Poland and Germany by enclave forming, isolated settlements of the LBK and derivative traditions, such as the Stichbandkeramik (SBK), Lengyel. The first extensive farming communities in northern Poland, Germany, Denmark, southern Norway, and Sweden belonged to the Funnel Beaker (TRB) culture and date to 3200 BC in southern Scandinavia. Archeological evidence demonstrates that the gradual establishment of farming communities in regions of this area unfolded hand in hand with the development of contacts, trade, and exchanges with communities that retained hunting, fishing, and gathering practices.[67] In terms of economic orientation and subsistence practices, this area corresponded to a mosaic of hunting-gathering and farming communities in the Stone Age farming period.[67]

Parallel processes have been identified in western France and the North European Plain that correspond to the initial settlement of farming community enclaves, followed by intensification of the exchange of materials and, presumably, individuals between farmer and hunter-gatherer communities. These interactions culminated in the formation of hybrid material culture complexes such as the Cerny Culture in France and the earliest TRB in north Europe.[61] It is possible that both hunter-gatherer and farming groups contributed to the subsequent formation of new cultures. Paleogenetic and archeological evidence thus support a model in which genetically isolated farming "islands" in western and northern Europe, derived from the LBK culture, may have diffused through time as a result of gene mixing with local populations.

In conclusion, in central Europe, a combination of colonization and contact is emerging as the most likely mechanism for the Neolithic transition,[8] in accordance with archeological, genetic, and paleogenetic evidence. In central Europe, colonization by farmers appears to have occurred mainly through leapfrog colonization, with farming groups targeting patches of fertile soil for enclave-like settlements. These local migrations by farmers of mixed genetic origin were later accompanied by a local adoption of farming by indigenous hunter-gatherer communities through contacts around the initial settlement. The acculturation of hunter-gatherer communities may have led to the diffusion of farmers' genetic islands in time through gene mixing with local populations. At the regional scale, this process may have caused important gene replacement in favor of lineages brought by indigenous hunter-gatherers. In consequence, the agricultural transition in central Europe appears to have been accomplished by local hunter-gatherer communities, with varying degrees of gene flow from Neolithic farmers.

Box 1. Paleogenetic Data and Genetic Continuity or Discontinuity

The question of neolithization processes has been regarded by paleogeneticists as a question of genetic continuity or discontinuity between Neolithic farmers and extant European populations. However, such a relationship between genetic continuity or discontinuity and neolithization processes can be discussed only in light of a complete diachronic dataset. In the case of central Europe, Haak and co-investigators[27] first characterized genetic discontinuity between LBK farmers and extant Europeans and considered that the Neolithic transition was mainly a result of cultural diffusion. However, when paleogenetic data became available for some late hunter-gatherers from the region and the genetic discontinuity between late hunter-gatherers, first farmers, and extant Europeans was characterized,[31] they revealed a more complex picture than an undisturbed genetic link between contemporary Europeans and their Paleolithic forbears. The genetic continuity identified between Neolithic farmers and extant populations from Catalonia[36] was considered to be in agreement with a demic Neolithic intrusion in the Iberian Peninsula. However, without paleogenetic data from more ancient periods and communities (that is, hunter-gatherers from the region), we cannot exclude the possibility that the regional gene pool has been stable since the Mesolithic or Paleolithic. This would imply not that Neolithic culture was effectively brought by intrusive Neolithic groups,[39] but that the Neolithic transition in the region was mainly conducted by indigenous communities. In this case, intrusive Neolithic groups would have had a minor impact on regional gene pools. Finally, in the case of Scandinavia, the genetic discontinuity between PWC hunter-gatherer communities and extant Swedish populations was interpreted as the result of a renewed wave of farmers that replaced hunter-gatherer communities.[34] A more recent study finally demonstrated the persistence of Hg U lineages, associated with Mesolithic communities,[31,34] in Neolithic and Bronze Age populations from Denmark.[35] Only three sequences have so far been obtained for the crucial Neolithic/Bronze Age periods in Denmark; clearly, further studies are required.[35] In any case, these analyses illustrate the need for complete diachronic datasets to discuss regional genetic continuity or discontinuity.

Although specific gene pool transformations can be identified only through the comparison of continuous diachronic aDNA datasets, ideally from the same region, aDNA samples are, for the moment, rare, and data from crucial periods are sometimes lacking. Moreover, diachronic data can concern different regions, complicating interpretations in terms of genetic continuity. The late hunter-gatherers analyzed by Bramanti and colleagues[31] are from geographically diverse locales, including sites in Germany, Poland, Lithuania, and Russia. Since they are compared to LBK farmers from Hungary, Austria, and Germany, we can wonder if the samples are appropriate to test genetic continuity in north-central Europe. In this case, the geographical and temporal structures of the communities could remain inextricable during genetic analyses. In consequence, we can ask ourselves if there is any acceptable argument that allows us to assume that currently available Mesolithic data apply to the pre-Neolithic Danubian.

Most aDNA studies demonstrate genetic discontinuity between past and present populations, and then indicate that contemporary European ancestry is not a living fossil of the Paleolithic maternal deme. This assessment is regularly confirmed throughout Europe, regardless of the period in question.[68-73] The developing characterization of the European Neolithic gene pool strengthens this theory of mitochondrial discontinuity between Neolithic farmers and current Europeans. The model proposed[27] to explain such discontinuity in central Europe is the diffusion of farmers' lineages by small pioneer groups migrating into new areas of the continent, followed by the dilution of their genes in the hunter-gatherer gene pool. However, the interpretation of such a pattern can vary, as different evolutionary processes may have altered the patterns of maternal lineage frequency and their distribution in populations since the Neolithic period. The evolutionary processes most often invoked to explain genetic discontinuity are migratory or demographic events. If genetic drift has been refuted through simulations,[27] numerous and repeated regional migrations during the Neolithic and post-Neolithic periods could have had substantial influence on the European genetic record. Founder effects and bottlenecks must also have had a dramatic impact, resulting in striking genetic discontinuity between past and present populations. Moreover, it still remains unclear whether or not the mtDNA genome has undergone selective pressure. The possibility that mtDNA, due to its control of cellular metabolism, played a central role in the selection process of human populations is still widely debated.[74] If mitochondrial function and variability indeed play a role in adaptive selection, natural selection may have changed the European mitochondrial gene pool over time, causing certain mitochondrial lineages to disappear while others have come to dominate the genetic landscape.

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. We do not know if the maternal lineages identified in different archeological sites provide a snapshot of the whole Neolithic population or of only a socially differentiated group.[29] The progressive disappearance of elite lineages through time has been recently described in Hungary,[75] where rapid and comprehensive dilution of the haplogroups associated with the Magyar conquerors was demonstrated. Such parameters should be kept in mind when proposing explanations for evolution of the European gene pool.

PERSPECTIVES

The extant European gene pool is the result of various demographic and evolutionary mechanisms, which have led to a striking genetic discontinuity between present and past populations. As a consequence, paleogenetic analyses of human remains appear particularly promising as a method to shed further light on ancient evolutionary processes such as the Neolithic transition in Europe. The accumulation of more aDNA data on pre-Neolithic human samples remains crucial to a better understanding of the processes implied in European neolithization. In this context, we expect that next-generation sequencing technologies, which allow for more reliable identification of authentic endogenous DNA, as well as the simultaneous and more economic analysis of numerous samples, will play a crucial role in the future.

Accumulating aDNA data for late hunter-gatherers and farmers in central Europe seems to conform to a broad leapfrog migration pattern during the Neolithic transition in this region. The convergence of arguments from archeology, anthropology, genetics, and paleogenetics gives weight to the idea that pioneer groups of LBK culture and their derivatives migrated from central Europe to as far as western and northern Europe. Nevertheless, it is necessary to confirm this model through genetic analyses of more numerous pre-Neolithic and Neolithic samples from central, northern, and western Europe. More diachronically and synchronically representative samples are essential to clarify the processes that led to the Neolithic transition in these regions. In addition, analyzing more localized paleogenetic samples remains crucial to efficient explanation of specific regional processes.

The leapfrog dispersal of farmer communities in hunter-gatherer territories could also correspond to the processes encountered in recent agricultural dispersals. It would be interesting to compare genetic results obtained for the European Neolithic period with those for more recent agricultural dispersals involving potentially similar mechanisms to evaluate the reliability of genetic arguments.

Different processes may have led to the Neolithic transition in north and south Europe. It is probable that the Paleolithic population was smaller in the Mediterranean area, a less productive area for hunting and gathering, than it was in Atlantic and central Europe. In consequence, the intrusion of Neolithic farming groups in these regions could have had a greater impact on the extant gene pool. However, paleogenetic datasets are even rarer in south Europe. Only additional data from this area will allow the proposal of new interpretations regarding the Cardial diffusion.

The genetic history of Europe has undergone significant transformation over time, resulting in genetic discontinuity between modern-day Europeans and their ancient maternal forbearers. The genetic diversity observed today in European populations cannot be explained by admixture between hunter-gatherers and early farmers alone. If Neolithic farmers appear, in light of paleogenetic data, to have left only limited genetic traces among contemporary Europeans, the picture revealed today does not correspond to substantial genetic continuity between modern groups and Paleolithic hunter-gatherers. Instead, the aDNA evidence suggests a more complex picture than an undisturbed genetic link between contemporary Europeans and their Paleolithic forbearers. 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.

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