

News From the West: Ancient DNA From a French Megalithic Burial Chamber

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ABSTRACT Recent paleogenetic studies have confirmed that the spread of the Neolithic across Europe was neither genetically nor geographically uniform. To extend existing knowledge of the mitochondrial European Neolithic gene pool, we examined six samples of human skeletal material from a French megalithic long mound (c.4200 cal BC). We retrieved HVR-I sequences from three individuals and demonstrated that in the Neolithic period the mtDNA haplogroup N1a, previously only known in central Europe, was as widely distributed as western France. Alternative scenarios are discussed in seeking to explain this result, including Mesolithic

ancestry, Neolithic demic diffusion, and long-distance matrimonial exchanges. In light of the limited Neolithic ancient DNA (aDNA) data currently available, we observe that all three scenarios appear equally consistent with paleogenetic and archaeological data. In consequence, we advocate caution in interpreting aDNA in the context of the Neolithic transition in Europe. Nevertheless, our results strengthen conclusions demonstrating genetic discontinuity between modern and ancient Europeans whether through migration, demographic or selection processes, or social practices. *Am J Phys Anthropol* 000:000–000, 2010. © 2010 Wiley-Liss, Inc.

The transition from a food-collecting economy to a food-producing economy, or the “Neolithic transition,” is associated with dramatic change in the European archaeological record. For western Europe, the available archaeological evidence from the Early Neolithic suggests two distinct Neolithic cultural traditions (Guilaine, 2003; Scarre, 2005; Lüning, 2008): the first of these spread along the Mediterranean coast (associated with Impressed Ware and Cardial culture) and the second one stretched along the Danube valley and then across the plains of central and northern Europe (associated with the Linearbandkeramik culture or LBK). It is often assumed that the dissemination of the Neolithic way of life took a more complex turn when arriving in the western part of Europe. The meeting of the two main currents of the west-European Neolithic expansion coupled with interactions with surviving Mesolithic societies may have created a mosaic pattern. Western France is one of the regions where these two diffusion waves met and mixed around 5,000 cal BC (Laporte, 2005; Scarre, 2007). In this area, the archaeological record indicates clear interaction between late hunter-gatherers and the earliest Neolithic. Contact between Neolithic and Mesolithic groups does not, however, obscure the variability in material cultures among the pre-existing Mesolithic hunters-gatherers.

Despite the ongoing accumulation of archaeological and genetic data across Europe, it remains unclear whether the spread of the Neolithic was caused by move-

ment of people (demic diffusion model) or by cultural diffusion involving little human migration (acculturation). Archaeological evidence suggests that the spread of agriculture over Europe has been a complex and discontinuous process with a succession of migration phases and local admixture (Guilaine, 2000, 2003; Guilaine and Manen, 2007; Bocquet-Appel, 2009). One region where the various “steps” of the Neolithic progression are in

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evidence is the area running east-west along the middle latitudes of Europe, up to the Atlantic coast in France (Rasse, 2008). Whether the “steps” seen here can be linked to major climatic events or to global changes in the northern hemisphere (such as the North Atlantic Oscillation) is open to question. A consequence would however have been that different regions experienced different blends of cultural exchange and migratory movement. A highly simplified and polarized dichotomy between migration and acculturation appears implausible. Various scenarios have accordingly been proposed to account for the complexity of the Neolithic diffusion process (Zvelebil, 2001; Miracle and Robb, 2007).

Genetic studies carried out on modern Europeans have given conflicting results as to the level of Neolithic contribution to the present day gene pool. Broad allele frequency gradients across Europe were first observed for protein and nuclear markers (Menozzi, 1978; Ammerman and Cavalli-Sforza, 1984; Chikhi et al., 2002). These clines were interpreted as strong evidence for the mixed demic diffusion hypothesis. This theory posits a demic expansion concurrent with intermarriage with the local hunter-gatherers. It was later shown, however, that allele frequency clines could be equally well explained by a pure demic diffusion or a pure acculturation model (Currat and Excoffier, 2005). These clines could hence be associated with the initial expansion of humans into Europe as easily as with Neolithic diffusion. Moreover, Currat and Excoffier (2005) demonstrated that ascertainment bias (the selection of single nucleotide polymorphisms with high frequencies) could cause overestimation of the clinal patterns. It was concluded that Ammerman and Cavalli-sforza (1984) could have overestimated the impact of the Neolithic diffusion as well as the uniformity of its spread. The model of European genetic ancestry later shifted away from the Neolithic diffusion model toward an emphasis on autochthonous Palaeolithic origins through the analysis of maternally inherited mitochondrial DNA (mtDNA). Heated debate followed the publication of mitochondrial data that suggested a weak Neolithic contribution to the European gene pool (about 20%; Richards et al., 2000). The debate is far from closed, however, since the most recent genetic data show a considerable Neolithic contribution to the European gene pool in studies of the nuclear genome. A large survey of autosomal microsatellite loci confirmed that the Near Eastern, and presumably Neolithic, contribution was a major element of the European gene pool (between 46% and 66%; Belle et al., 2006). The latest study analyzed the geographical distribution of microsatellite diversity of the Y haplogroup R1b1b2 and concluded that it was best explained by spread from a single source in the Near East via Anatolia during the Neolithic (Balareshue et al., 2010).

Paleogenetic studies have recently entered the debate, permitting direct comparison of the ancient inhabitants of Europe with their contemporary counterparts. The first study of Haak et al. (2005) focused on human remains associated with the LBK and AVK (Alföldi Vonaldiszes Keramica) cultures in central Europe. Both cultures mark the onset of farming in temperate regions of Europe (7,500 BP). Originating in Hungary and Slovakia, the LBK spread rapidly as far as the Paris Basin and the Ukraine (Gronenborn, 2007). Analyses identified high frequencies of the N1a mitochondrial haplogroup, which is currently rare in Europe (Haak et al., 2005). Evidence of genetic discontinuity between

the first European farmers and current European populations led the authors to conclude that the early Neolithic agriculturalists had limited impact on modern European female lineages. Setting aside the possibility of significant post-Neolithic migrations, they proposed a Paleolithic ancestry for modern Europeans. Nonetheless, two recent publications (Bramanti et al., 2009; Malmström et al., 2009) reported sequences from late European hunter-gatherers that demonstrated genetic differentiation between ancient hunter-gatherers, early Neolithic farmers, and modern European groups. These results have been interpreted either as evidence of demic diffusion at the onset of the Neolithic and/or as evidence for post-Neolithic replacement. It is possible however, that these aDNA studies compared hunter-gatherer populations with Neolithic populations distant from them in space and time. In this case, the Neolithic populations would not be expected to be directly descended from the hunter-gatherer populations. In addition, many hunter-gatherer sequences came from groups far younger (4th to 5th millennium BC; Bramanti et al., 2009; Malmström et al., 2009) than the Neolithic groups with which they were compared (6th millennium BC, Haak et al., 2005). In light of this information, it is impossible to exclude the possibility that more recent hunter-gatherer groups may have included descendants from earlier Neolithic populations.

Paleogenetic data were also obtained for Neolithic communities from the Iberian Peninsula [in Basque populations (Izagirre et al., 1999) and Catalonia (Sampietro et al., 2007)] and the Eastern Italian Alps (Di Benedetto et al., 2000), where the gene pool have been shown to be stable since the Neolithic. In the Catalonia region, the genetic continuity was interpreted to result from a major Neolithic contribution to the gene pool of the Iberian Peninsula, favoring the argument of Neolithic demic diffusion in the Mediterranean area (Sampietro et al., 2007). Thus, ancient DNA (aDNA) studies indicate that the spread of the Neolithic was neither genetically nor geographically a uniform process in Europe. Interpretations based on paleogenetics are, however, obviously limited by the small aDNA dataset available and by the fact that previous studies have focused on local Neolithic groups distant from one another in space and time and located mainly in central and northern Europe. Nevertheless, the accumulation of genetic snapshots from past European populations represents a promising way to better understand the impact of the Neolithic transition on the European gene pool.

To extend the geographical range of European aDNA data, we sampled the remains of six Neolithic individuals from west-central France. The samples came from Péré tumulus C at Prissé-la-Charrière (Deux-Sèvres) (project directors Laporte, Joussaume, Scarre; Scarre et al., 2003) (see Fig. 1). This 100-m long mound (see Fig. 2) was constructed between 4,500 and 4,000 cal BC. Such long mounds are at the center of the debate on the emergence of monumental funerary structures in western Europe, some centuries after the establishment of farming communities. The final form of the Prissé-la-Charrière long mound was the result of a complex architectural sequence. The mound contains three separate burial chambers, each associated with a distinct stage within the overall sequence of construction. The human remains analyzed in this study were deposited on the pavement within chamber III, a passage grave

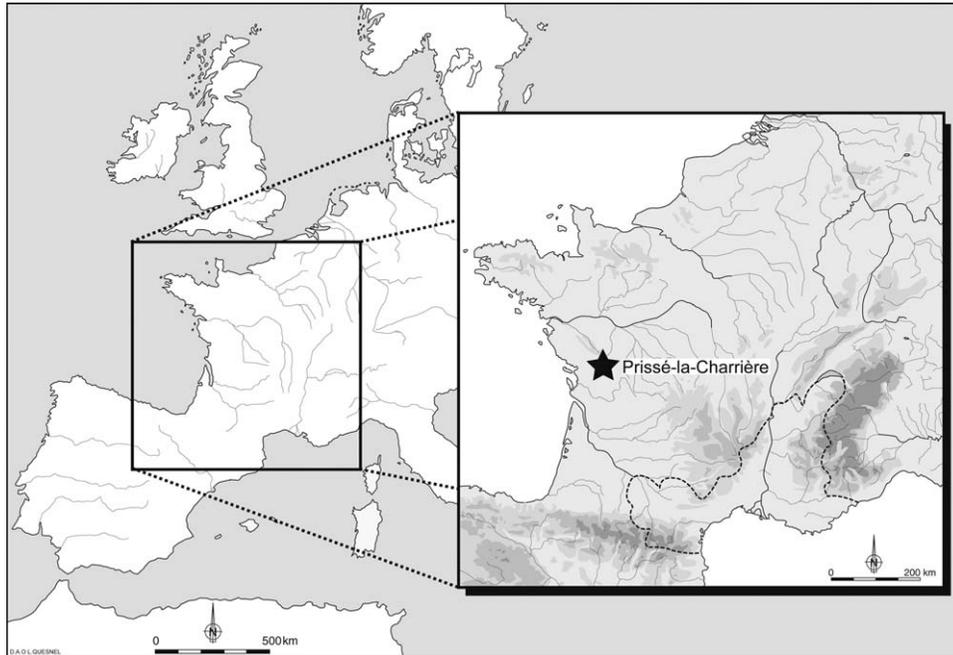


Fig. 1. Location of Prissé-La-Charrière.



Fig. 2. The Prissé-La-Charrière long mound during excavation. Photo L. Laporte. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

surrounded by a circular dry stone cairn. The discovery is exceptional in having survived totally intact, free from human or animal perturbations, since the Neolithic (see Fig. 3). Remains of at least eight individuals, corresponding to four adults and four children, had been deposited in the chamber. Sampling was conducted on six well-preserved remains with all due precautions against contamination (Table 1). DNA was extracted from teeth and partial HVR-I sequences of the mitochon-

drial genome were amplified. Our results reveal that the N1a mitochondrial lineage, found at high frequencies in the central European LBK/AVK groups (Haak et al., 2005), was present as far west as western France and was therefore more widely distributed in European Neolithic populations than has been previously demonstrated. We discuss alternative scenarios to explain our results, each of which is equally consistent with the regional archaeological data.

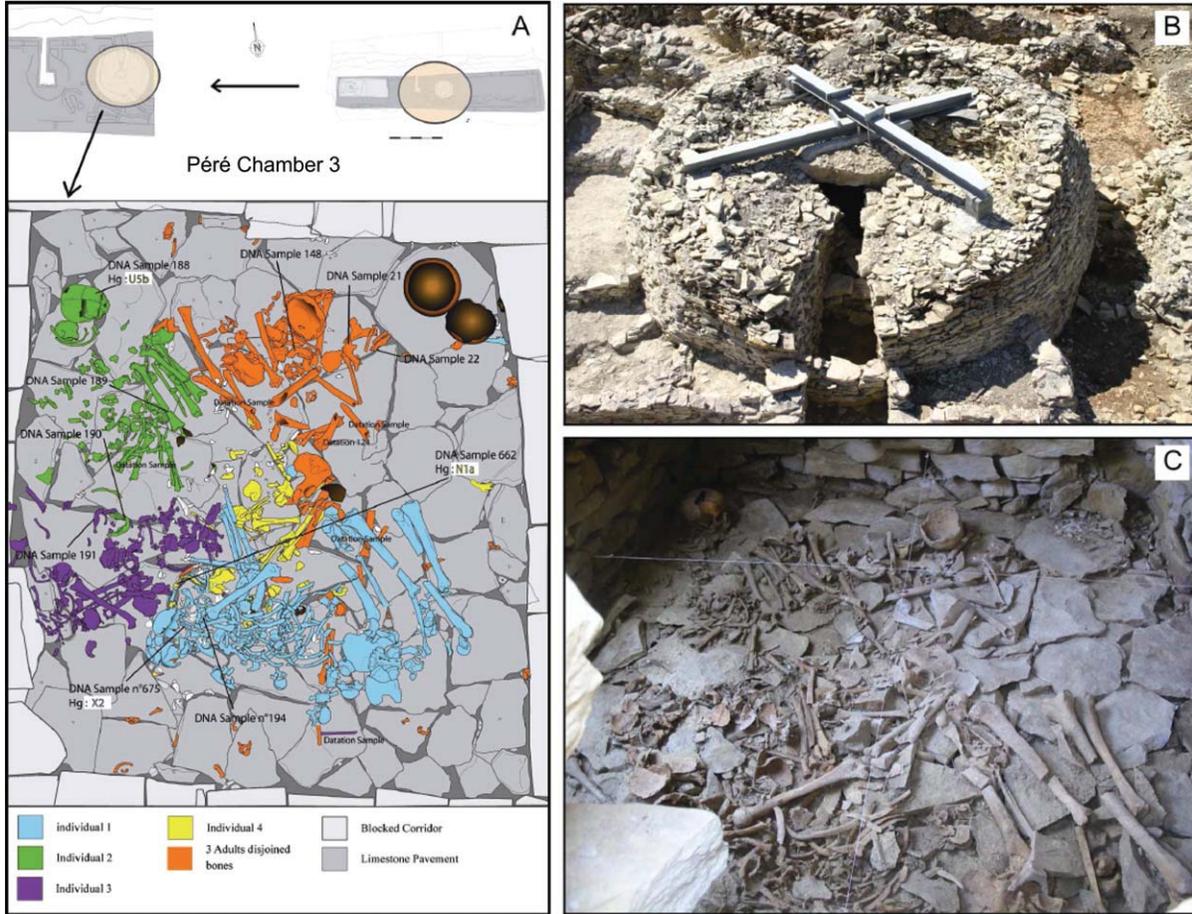


Fig. 3. A-B/Chamber III of the Prissé-la-Charrière mound with entrance passage, initially enclosed within a circular dry-stone cairn. Photo L. Laporte. A-C/Location of individuals and samples collected for paleogenetic analysis. Drawing H. Duday and L. Soler. Photo and interpretation, L. Soler.

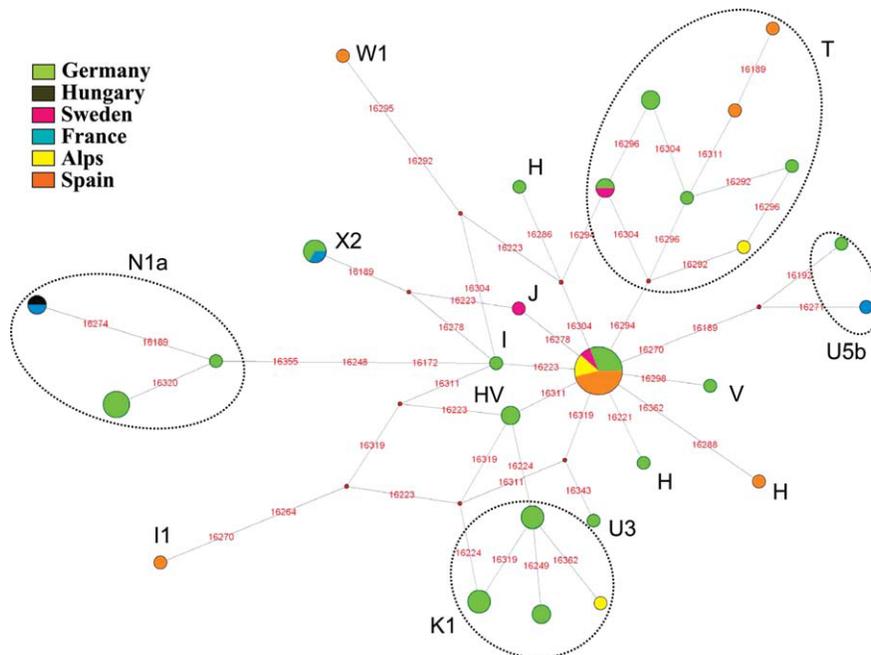


Fig. 4. Median-Joining network of European Neolithic mitochondrial sequences. Data encompass mtDNA HVR-I segment (np 16165–16390).

TABLE 1. Sample information (age determination according to Ubelaker, 1984; sex determination according to Bruzek et al., 1996; n.d. nondetermined)

Individual	Sample codes	Position	Color on the plan	Sex	Age at death	Radiocarbon age	Number of clones analyzed	mtDNA haplogroup
Prissé 1	195 and 675	Bones in connection along southern wall	Blue	Male	Young adult	4340–4171 cal.BC (OxA-15063)	81	X2
Prissé 2	188 and 189 and 190	Bones in connection in North-east corner	Green	Unknown	12 years ± 30 month	4336–4076 cal.BC (OxA-15960)	52	U5b
Prissé 3	191	Bones in connection enter of chamber	Purple	Unknown	6 years ± 24 month	4340–4175 cal.BC (OxA-15062)	–	n.d.
Prissé 4	662	Disjoined bones	Yellow	Unknown	6 years ± 24 month	–	51	N1a
Prissé 5	21–22	Disjoined bones	Orange	Unknown	Adult	–	–	n.d.
Prissé 6	148	Disjoined bones	Orange	Unknown	Adult	–	–	n.d.

MATERIALS AND METHODS

Samples

The skeletal material under study comes from recent excavations directed by Laporte, Joussaume, and Scarre in tumulus C at Prissé-la-Charrière (Deux-Sèvres, Joussaume et al., 1998; Laporte et al., 2002, 2006; Scarre et al., 2003). The absence of disturbance since the Neolithic provided a unique opportunity to better understand the burial practices. Spatial analysis of the skeletal material provided arguments (persistence of specific articulations after initial decomposition, consistency of the distribution of disarticulated elements) in favor of bodies deposited sitting along the walls that subsequently collapsed into the empty chamber space. Detailed analysis of bone distribution and associations allowed the identification of eight individuals. We analyzed six of the eight individuals present in the chamber; the others were omitted from the analyses because of their unsuitable state of conservation (Table 1). For each individual, a complete mandible or complete skull was collected in situ during the excavations, with all precautions against contamination (including wearing of mask and gloves). Samples were directly deposited in hermetic sterile bags and conserved at -20°C as soon as possible. Long bones from individuals Prissé 1 to Prissé 3 were dated (Table 1) as well as three bones from the yellow disarticulated bones group (that could not be attributed to an individual): 4,345–4,240 cal BC (OxA-14953), 4,339–4,173 cal BC (OxA-15059), and 4,333–4,070 cal BC (OxA-15061).

All the following analyses were performed at the ancient DNA facilities of the Laboratory of Past Human Populations, UMR PACEA (Université Bordeaux 1, Bordeaux, France) in a laboratory dedicated to analyses of ancient DNA. All pre-PCR procedures were conducted under sterile conditions in an aDNA room (characterized by a high-pressure system, filtered incoming air, UV light irradiation, bleach cleaning of every surface, and laminar flow hood) and the laboratory equipment and reagents used were DNA-free. Workers systematically wore clean-room overalls, shoe protection, face masks, and gloves. DNA isolation, PCR reagent preparation, and mixing of PCR reagents and DNA extracts were conducted in three separate dedicated rooms of the aDNA laboratory. These laboratories are spatially distant from the main laboratory, where PCR amplifications and subsequent analyses take place (at opposite ends of the same building), to avoid contamination by amplified DNA.

Ancient DNA extraction

Before isolation, teeth were separated from mandibles or jaws in a sterile laminar flow hood (with positive air pressure, UV irradiation, and bleach cleaning). As the samples were not supposed to be contaminated by any source of external DNA (in light of the sampling precautions), they were not treated before the isolation procedure.

DNA was isolated from the teeth of six individuals (Table 1), corresponding to three adults and three children. DNA was extracted using a phenol/chloroform protocol (Hughes et al., 2006). Each tooth was first reduced to powder. After overnight decalcification and protein digestion at 55°C with agitation (EDTA 0.5 M, pH = 8.5, proteinase K 1–2 mg/mL, *N*-lauryl Sarcosyl 0.5%), the supernatants were extracted using phenol-chloroform-

TABLE 2. HVR-I polymorphisms observed in ancient individuals from Prissé and potential sources of contamination (R1 to R13 = researchers who have been in contact with samples)

	16069	16093	16126	16172	16176	16189	16194	16209	16223	16234	16248	16270	16271	16274	16278	16290	16298	16311	16355	16357	16362	Haplogroup	
Anderson	C	T	T	T	C	T	A	T	C	C	C	C	C	G	C	C	T	T	C	T	T	H	
R1	T	C	U5
R2	And
R3	H
R4	.	C	G	C	T	H
R5	.	C	J1c
R6	T	.	C	C	H
R7	.	.	.	T	H
R8	A	H
R9	H
R10	H
R11	C	HV0
R12	T	H
R13	H
PERE 1	nd	nd	nd	.	.	C	.	T	X2
PERE 2	nd	nd	nd	.	.	C	T	U5b
PERE 4	nd	nd	nd	.	.	C	.	T	.	.	T	.	.	A	N1a

isoamyl alcohol (25:24:1) organic extraction. Subsequently, the aqueous phase was concentrated in 100 μ L of sterile distilled water with Centricon-30 columns (Amicon[®]) according to the manufacturer's instructions. An extraction blank was systematically coextracted with the ancient Neolithic samples during each extraction session. No more than two ancient samples were coextracted at the same time. At least two independent DNA isolations were undertaken from each sample.

Amplification, cloning, and sequencing

PCR amplifications were performed on the HVR-I region of the mitochondrial DNA control region. Partial sequences of 225 bp of the HVR-I (nps 16,165 to 16,390) were obtained using a set of three overlapping primer pairs: Ms1 (Jehaes et al., 2001), L16210 (nps 16,190–16,210) and H16322 (nps 16,302–16,322), and Ms3 (Jehaes et al., 2001). PCR amplifications were performed in a 25 μ L reaction volume containing 6.5 mM MgCl₂, 0.4 mM dNTP, 0.66 mg/mL BSA, 1 μ M each primer, 2.5 μ L GeneAmp 10 \times PCR Buffer (Perkin-Elmer), 0.25 μ L DNA extract, and 1.25U AmpliTaq Gold[™]. PCR was run for 55 cycles at 94°C for 45 s, 56°C for 45 s, and 72°C for 45 s. At least two independent DNA isolations were undertaken from each sample, and at least two separate amplifications of each mitochondrial fragment were performed on each DNA extract.

To detect possible contamination by external DNA, extraction and amplification blanks were used as negative controls. Despite strict precautions against contamination during excavation and laboratory work, all persons who had been in contact with the Prissé samples were also typed to monitor potential contaminations. All 12 Europeans involved in the excavation, sampling, or genetic analyses were genetically typed and their HVR-I sequences were compared with those obtained from ancient remains (Table 2).

As the products of ancient DNA amplification generally contain a large number of artifacts generated by DNA degradation and *Taq* polymerase errors, the PCR products were systematically cloned using the Topo TA cloning kit (Invitrogen[®]). HVR-I ambiguities were resolved by analysis of multiple clones and the authentic sequences were always deduced from the "consensus" among several clones of several amplification products and several extracts.

Phylogenetic analysis

Mitochondrial sequences obtained from the Prissé remains have been deposited in the GenBank database (accession number FJ839887 to FJ839889). To illustrate genetic affinities among European Neolithic communities, Prissé sequences were compared with 50 mtDNA sequences previously described for Neolithic human remains: 32 sequences from Germany [23 sequences from LBK culture, 6th millennium cal BC, (Haak et al., 2005; Bramanti et al., 2009), and 9 sequences from Corded Ware culture, 3rd millennium cal BC, (Haak et al., 2008), respectively], one sequence from Hungary [AVK culture, 7th–6th millennium cal BC (Haak et al., 2005)], three sequences from the Alps [Mezzocorona and Borgo Nuovo, 5th millennium cal BC (Di Benedetto et al., 2000), Ötzi, 4th millennium cal BC (Ermini et al., 2008)], 11 sequences from Spain [Granollers in Catalonia, 4th millennium cal BC (Sampietro et al., 2007)],

and three sequences from Sweden [Fralsegarden, 3rd millennium cal BC (Malmström et al., 2009)].

The 53 resulting sequences were aligned using the MEGA3.1 program (Kumar et al., 2004). A median-joining network connecting the Neolithic mitochondrial sequences was constructed for nps 16,165–16,390 by using NETWORK 4.516 (www.fluxus-engineering.com).

RESULTS

The paleogenetic analysis revealed heterogeneous DNA conservation among the remains from Prissé. Reproducible sequences could be easily obtained from three out of the six samples, whereas the remaining samples contained too few template molecules to be successfully amplified or replicated. The relatively satisfactory preservation of DNA in the Prissé long mound may indicate that limestone geology, such as the one encountered at this site, can ensure good preservation of both skeletal remains and DNA.

The haplotype composition observed for the three Neolithic remains and for all the researchers who directly participated in this study (from people working in the field to those working in the laboratory) is summarized in Table 2. We reproducibly retrieved partial HVR-I sequences (nps 16,165 to 16,390) from three human remains (Prissé 1, 2, and 4, Table 1), one adult and two children deposited during different stages of use of the burial chamber. Corresponding sequences could be unambiguously assigned to haplogroups X2, U5b, and N1a (Table 2 and Supporting Online Information).

MtDNA haplogroup X2, characterized by polymorphisms 16189C, 16223T, and 16278T, could be unambiguously replicated from individual Prissé 1. The X2 sequence described from Prissé corresponds to the central node of the sub-haplogroup that is found at low frequencies throughout Europe and in current French populations (Rousselet and Mangin, 1998; Dubut et al., 2004; Richard et al., 2007). This haplotype has also been described in German Neolithic remains from the Corded Ware Culture [3rd millennium cal B.C. (Haak et al., 2008)]. We cannot exclude the presence of X2 in Basque Neolithic remains (Izagirre et al., 1999), although corresponding RFLP analyses do not allow the X and T haplogroups to be differentiated (individuals defined as T/X by authors).

The characterization of polymorphisms 16189C, 16270T, and 16271C in the remains of individual Prissé 2 allowed for the identification of mtDNA haplogroup U5b. This specific U5b haplotype, characterized by the specific mutation in np 16271, has never before been described in France (Rousselet and Mangin, 1998; Dubut et al., 2004; Richard et al., 2007), or in the databases of modern populations (Richards et al., 2000; GenBank database; mtDNA manager database, Lee et al., 2008; Genographic dataset, Behar et al., 2007). Subhaplogroup U5b has also been encountered in German Neolithic remains from the Corded Ware Culture (Haak et al., 2008) and in the hunter-gatherers studied by Bramanti et al. (2009), although in both instances, the branches concerned were distinct from the U5b in the Prissé sample. It is, however, worth noting that haplogroup U5 has been encountered in surprising frequency in the hunter-gatherers studied by Bramanti et al. (2009) and could correspond to a Mesolithic heritage.

The combination of polymorphisms 16172C, 16189C, 16223T, 16248T, 16274A, and 16355T in individual Prissé 4 is characteristic of the mtDNA haplotype N1a.

This mitochondrial lineage has already been encountered in high frequency in LBK/AVK farmers (Haak et al., 2005). More specifically, the haplotype identified in Prissé 4 has been reported only once before, in AVK remains from Ecsegfalva (Hungary, 6000 BC; Haak et al., 2005). This result demonstrates the presence of the N1a lineage in Neolithic populations from central Europe to western France. As noted by Haak et al. (2005), the frequency of N1a is very low in modern European populations (0.14%) including France (0.27%; Rousselet and Mangin, 1998; Dubut et al., 2004; Richard et al., 2007).

Many studies have demonstrated the risks of contamination with external DNA when dealing with ancient material, all the more likely with ancient human DNA (Gilbert et al., 2005; Malmström et al., 2005). All analyses performed in our study followed the basic authenticity criteria proposed by paleogeneticists (Richards et al., 1995; Gilbert et al., 2005) including: (i) the use of a dedicated laboratory, (ii) ensuring the reproducibility of the results, and (iii) the cloning and sequencing of the amplification products to detect PCR artifacts associated with postmortem template modification and/or contaminations. Nevertheless, human contamination can never be ruled out entirely and only the combination of independent evidence can allow the probability of authenticity to be assessed. As a consequence, we provide all lines of evidence that make us confident about the authenticity of the ancient sequences retrieved in our study:

- i. Strict precautions were followed during all procedures (including precautions during excavation) and proved to be effective, because all researchers who directly participated in this study (from people working in the field to those working in the laboratory) were genotyped and their sequences were never observed during analyses. However, European sequences were randomly found in clones (28% of the sequences obtained). These specific sequences are regularly observed in the laboratory, whatever the project tackled (including samples from Polynesia or South America), in clones from samples or negative controls. They are not reproducible for a specific sample and are different from researchers' sequences. These facts lead us to suspect the contamination of PCR reagents (Leonard et al., 2007). It was relatively easy, however, to discard those contaminating sequences from our analyses because they were largely in the minority when compared with endogenous sequences.
- ii. For each skeletal sample, we conducted two independent DNA extractions, followed by at least two independent PCR amplifications for each extract and mitochondrial fragment, and we systematically cloned the PCR products and obtained the same sequence.
- iii. The pattern of mutations among clones was found to be consistent with that previously described for ancient DNA (Hofreiter et al., 2001).
- iv. Different mtDNA fragments gave identification of the same lineage.
- v. The detection of the specific N1a, U5b, and X2 haplotypes in Neolithic remains, all of them rare in modern-day European populations, is unlikely to correspond to contamination events.

The sequences obtained from the Prissé remains were compiled with previously published Neolithic mitochondrial sequences. The resulting Median Joining Network is

shown in Figure 4. We are aware that this network is not wholly satisfactory because it was constructed using a partial HVR-I sequence (nps 16,165–16,390), the small size of which could be responsible for creating artificial links between different haplogroups. Moreover, the Neolithic groups included are distant in space and time (from Spain to Hungary and from the 6th to 3rd millennium cal BC) and that too can lead to artificial associations. This network does however highlight two types of genetic affinity that appear especially interesting. First, the numerous sequences shared among LBK groups from Germany underline their genetic proximity. Second, it appears that the Neolithic sequences from Prissé are grouped with (or are identical to) Neolithic sequences from central Europe (i.e., from Hungary and Germany). It is possible, however, that this relationship results from the over-representation of Neolithic sequences from central and northern Europe in the current aDNA dataset.

DISCUSSION

The paleogenetic results obtained for the Prissé Neolithic group provide useful information on two distinct issues: Neolithic funerary practices, and the Neolithic transition in western France. The fact that the chamber has not been entered since the Neolithic gives special importance and significance to the paleogenetic data concerning funerary practices. The presence of different mtDNA haplogroups indicates mtDNA variability among the individuals deposited in this burial chamber. The extraordinary construction at Prissé compared with the very small number of individuals deposited in the chambers supports the idea of selection and highlights discussions about their potential status. For the first time, mitochondrial data have allowed us to demonstrate that individuals deposited in a megalithic burial chamber were not maternally related. Of course, other types of kinship (including polygamy) cannot be excluded, but this result has significance for the interpretation of the social composition and selection of the individuals buried in this Middle Neolithic passage grave.

Our results also contribute the first paleogenetic arguments to discussions of the Neolithic transition in the region. The construction of monuments in northwest Europe, starting during the second quarter of the 5th millennium cal BC, represents a pattern of behavior for Neolithic communities that set them apart from their Mesolithic predecessors. The earliest Neolithic impact detected through material cultures in western France is related to the north-west Mediterranean complex. The earliest relevant sites are dated to the second half of the 6th millennium cal BC (Laporte, 2005). The extension toward the Atlantic of the Early Neolithic of central European origin forms another current of Neolithic expansion. Shortly before 5,000 cal BC, the LBK advanced toward the Paris Basin where it formed the Rubané Récent du Bassin Parisien (RRBP) (Jeunesse, 2001). In Brittany, settlements related to the LBK tradition are attributed to the BVSG group (Blicquy-Ville-neuve-Saint-Germain; Cassen, 1998; Scarre, 2007). Different traditions of monumentality, the construction of funerary chambers built above the ground using large stones, tend to converge and mix during the third quarter of the 5th millennium cal BC (Laporte, in press). The Prissé sequence is a good example of this accretional monumentalism, a process, which continues throughout

the second half of the 5th millennium (Scarre et al., 2003).

In the regional context, the presence of the N1a mitochondrial lineage in the Prissé burial chamber (also frequent in LBK/AVK samples) is particularly striking. Alternative explanatory scenarios or working hypotheses can be proposed although we are aware that only the future accumulation of paleogenetic data from diverse Neolithic groups originating in different regions of Europe and different periods will allow us to determine which of these scenarios is most plausible.

The widespread distribution of the N1a lineage in Early and Middle Neolithic northwestern Europe may indicate genetic continuity from Mesolithic populations. This scenario would support a Mesolithic contribution to the earliest Neolithic of Atlantic Europe. This would imply that the N1a lineage was already common in indigenous north European populations and that the spread of the Neolithic was principally the result of cultural diffusion. Although so far the N1a lineage has not been encountered among late European hunter-gatherers in central and north Europe (Bramanti et al., 2009; Malmström et al., 2009), it is worth noting that less than half of the hunter-gatherers' paleogenetic data come indeed from the pre-Neolithic period (predating LBK expansion). Finally, no paleogenetic data currently exist for the Mesolithic period in Western Europe. This prevents any conclusion being drawn about N1a occurrence during the Mesolithic period in those regions.

The sharing of N1a between LBK/AVK farmers and the Neolithic individual from Prissé-la-Charrière could result from the diffusion of this specific lineage through the expansion of farming groups. This hypothesis would partly link the Neolithic transition in continental Europe to the emergence of the Atlantic megalithic tradition. It does not account for the variety of architectural forms adopted on the Atlantic seaboard of Europe, however, nor explain the genesis of the megalithic monuments as a whole (Joussaume et al., 2006), especially in Western France (Laporte et al., 2004). A link between the plan of LBK long houses and that of similarly trapezoidal Neolithic tombs in Northern Europe was first proposed by Childe (1949). Recent discoveries have considerably reduced the geographical and chronological distances between these elongated funerary monuments and the last LBK-derived long houses (Laporte et al., 2004). At Prissé, however, it is unclear whether the deposition of bodies in Chamber III is strictly related to the final long mound construction, because this passage grave was first surrounded by a circular structure. In any case, this scenario would imply the westward migration of small pioneer groups of LBK descendants (LBK-derived cultures) as far as western France. Simulations have shown that a variant present at the front of an expansion could occasionally travel with the wave of advance and be carried over long distances (Klopfstein et al., 2006). However, such a surfing phenomenon would imply a continuous and progressive colonization from central Europe. Such a diffusion model conflicts with archaeological evidence suggesting a succession of migrations coupled with local admixture which must have transformed the gene pool of the migrants. Genetic exchanges between hunter-gatherers and farming groups seem initially, however, to have been limited, at least on the female side (Bramanti et al., 2009). Moreover, stable isotope analyses at LBK cemeteries indicate a pioneer stage characterized by high proportion of allochthonous

(farmers) individuals, which decreases and concerns mainly young women in later stages (Bentley et al., 2003). These results suggest exogamy and patrilocality, two practices that have so far been documented only for Late Neolithic communities (Haak et al., 2008). The surfing phenomenon might not therefore be in such conflict with the archaeological evidence since the admixture may have not occurred during the initial stages of diffusion and may not necessarily have transformed the maternal gene pool of all migrants. Finally, the genetic affinities between LBK groups highlighted by phylogenetic analyses (see Fig. 4), combined with the pooling of the Prissé sequences with LBK/AVK sequences from Germany and Hungary, would support this second scenario (even though such genetic affinities must be confirmed through the analysis of more geographically representative aDNA datasets).

The last scenario would propose a genetic link between communities from the Carpathian Basin or central Europe and western France, at a period later than the initial Neolithic transition, which may have involved long-distance matrimonial exchanges. This hypothesis might then echo the established long-distance exchange of prestige goods in France, and more widely during the Middle Neolithic, although none of these prestige goods specifically indicate direct relationships between Atlantic Europe and Carpathian region.

We consider that the sample of European Neolithic mitochondrial sequences obtained so far does not indicate which of these scenarios is to be preferred. Consequently, we believe that Neolithic aDNA data should be interpreted with great caution, because the data are limited in quantity and relate to groups that are distant in space and time (each of which must have undergone a distinct transition to the Neolithic). Our current belief is that the only way to reliably uncover the processes that led to the expansion of the Neolithic in Europe is by the accumulation of paleogenetic data for both the pre-Neolithic and Neolithic periods in different European regions. The developing characterization of the European Neolithic gene pool, however, appears to strengthen mitochondrial discontinuity between Neolithic farmers and current Europeans.

Our study confirms the widespread distribution of N1a in Neolithic populations in Northwest Europe, and its subsequent decrease in the European gene pool. The disappearance of a lineage can be caused by demography, migration, or selective processes. In the case of N1a, a spread through small Neolithic pioneer groups, followed by dilution in the majority Palaeolithic gene pool, may have led to its current low frequencies. Such later dilution could explain the genetic discontinuity measured between Early Neolithic groups and current populations on the one hand (the former still presenting measurable Neolithic input; Haak et al., 2005; this study), and on the other, the continuity observed between later Neolithic communities and modern Europeans (the Neolithic component has already been diluted; Izagirre et al., 1999; Sampietro et al., 2007). Social parameters may also have played a significant role in lineage evolution. We do not know if the maternal lineages identified among the Prissé individuals provide a snapshot of the whole Neolithic population or only of a socially differentiated group. The decrease of N1a could, for example, be explained by the progressive disappearance of elite lineages through time. Such a phenomenon has recently been described in Hungary, where Tömöry et al. (2007) demonstrated the rapid and comprehensive dilution of the hap-

logroups associated with the Magyar conquerors. Finally, large-scale movements of people in Europe are recorded in both the archaeological record and in historical accounts. Numerous and repeated regional migrations since the Neolithic may have had the same impact on the European gene pool as the Mesolithic-Neolithic transition. Indeed, numerous paleogenetic studies of ancient European populations have demonstrated that the genetic history of Europe is characterized by significant transformations through time, resulting in frequent genetic discontinuity between modern-day Europeans and their ancient maternal forebears (see Levy-Coffman, 2005 for review). As a consequence, post-Neolithic migrations could easily explain the extinction of mitochondrial lineages.

The Neolithic human remains from Prissé are exceptional on several counts. They represent the first Neolithic mitochondrial sequences from France and they are associated with the emergence of megalithic monuments in Western Europe. Our results shed new light on Neolithic population genetics, showing that the N1a lineage was widespread throughout central and Western Europe. The distribution of this mitochondrial lineage suggests alternative hypotheses for the evolution of the Neolithic population that appear equally consistent with the archaeological data. Such results could notably indicate a link between LBK diffusion and one individual at least from Prissé, among others of different maternal lineages in chamber III. They do not necessarily demonstrate a major LBK contribution to the genesis of the Neolithic megalithic tradition in Western Europe as a whole. We believe that further paleogenetic analyses of European Mesolithic and Neolithic groups, from different regions (notably within France) and different periods, will give critical insight into the adoption and spread of the Neolithic. Our data agree with previous paleogenetic studies and suggest that demographic events during the Neolithic and post-Neolithic period, along with various evolutionary processes, have resulted in a striking genetic discontinuity between past and present European populations. As a result, contemporary Europeans cannot simply be viewed as the direct descendants of either Neolithic farmers or the indigenous Paleolithic inhabitants of Europe. Great caution is necessary when making historical inferences from modern population data alone.

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