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Report

Genomic Affinities of Two 7,000-Year-Old Iberian Hunter-Gatherers

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Summary

The genetic background of the European Mesolithic and the extent of population replacement during the Neolithic [1–10] is poorly understood, both due to the scarcity of human remains from that period [11–18] and the inherent methodological difficulties of ancient DNA research. However, advances in sequencing technologies are both increasing data yields and providing supporting evidence for data authenticity, such as nucleotide misincorporation patterns [19–22]. We use these methods to characterize both the mitochondrial DNA genome and generate shotgun genomic data from two exceptionally well-preserved 7,000-year-old Mesolithic individuals from La Braña-Arintero site in León (Northwestern Spain) [23]. The mitochondria of both individuals are assigned to U5b2c1, a haplotype common among the small number of other previously studied Mesolithic individuals from Northern and Central Europe. This suggests a remarkable genetic uniformity and little phylogeographic structure over a large geographic area of the pre-Neolithic populations. Using Approximate Bayesian Computation, a model of genetic continuity from Mesolithic to Neolithic populations is poorly supported. Furthermore, analyses of 1.34% and 0.53% of their nuclear genomes, containing about 50,000 and 20,000 ancestry informative SNPs, respectively, show that these two Mesolithic individuals are not related to current populations from either the Iberian Peninsula or Southern Europe.

Results and Discussion

La Braña 1 and 2 mtDNA HVR1

PCR amplified, cloned, and sequenced mitochondrial (mtDNA) HVR1 sequences, generated in two independent laboratories, indicate that La Braña specimens (Figure 1) belong to the U5b haplotype (16192T-16270T) (see Table S1 available online). Although the observation of the same haplotype in both individuals could be explained through matrilineal family relationship, the emerging picture of genetic uniformity within European populations during the Mesolithic suggests this might not be the case.

These two novel sequences were aligned against all previously reported mtDNA HVR-1 sequences from European Paleolithic, Mesolithic, and Neolithic individuals, accounting for a total number of 166 sequences (each 253 bp in length). Serial coalescent simulations showed low support for a population model of genetic continuity from Mesolithic to Neolithic populations, suggesting that mtDNA variation better fitted population models where European Paleolithic/Mesolithic populations were replaced during the Neolithic transition (see Supplemental Experimental Procedures).

La Braña 1 Complete mtDNA Genome

We subsequently captured and sequenced a mtDNA library from La Braña 1 on an Illumina Hi-Seq 2000 platform at the Center for GeoGenetics in Copenhagen, Denmark (see Supplemental Experimental Procedures). The number of raw reads generated was 44,581,347, of which 19,993,417 uniquely mapped to the human mtDNA reference genome (rCRS). Sequences starting and ending in the same nucleotide were collapsed because they could derive from the same template molecules. The clonality of the sample was relatively high, and after the collapse, only 5,488 reads were kept. Nevertheless, it was possible to retrieve the complete mtDNA with a final 28× coverage and 16,450 sites covered at least once (Figure 2). The La Braña 1 mtDNA haplotype was an U5b2c1 (Tables S2 and S3), according to the standard PhyloTree classification [24] and the HaploGrep online tool for haplogroup attribution [25].

MtDNA Contamination Estimates

To estimate the potential modern DNA contamination in the generated results, we followed several approaches. First, we estimated the phylogenetic assignment of the nucleotide positions differing from the mtDNA human reference (rCRS) in the light of the known mtDNA tree (Table S2) [25]. We then searched for heterogeneities (that could either be contaminants, heteroplasmic sites, or damage) in those positions (Table S2), finding the U5b consensus sequence in 92% of the reads. Thus, the upper limit for mtDNA contamination is 8% (2%–13%, 95% C.I.).

Second, we searched for the identical 16192T-16270T HVR1 mtDNA haplotype (between positions 16022 and 16400) using an in-house database (compiled by F. Calafell) and found that it is residually present at a 0.4% frequency in modern populations from the Iberian Peninsula, as estimated from 2,749 published mtDNA sequences. At a pan-European level, the same haplotype is found in only 40 out of 22,807 (0.18%) published mtDNA individual sequences.

Third, we analyzed the ratio of nucleotide residues at the 5′ and 3′ ends of the reads. It has been demonstrated that ancient DNA templates exhibit 5′ and 3′ overhangs, resulting

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Figure 1. The Two Mesolithic Skeletons as They Were Discovered

The images show the skeletons as they were accidentally discovered in 2006. Above, La Braña 1; below, La Braña 2.

in inflated cytosine deamination rates and changes from cytosine to thymine residues at the 5′ ends and from guanines to adenines at the 3′ ends [22, 26–28]. This particular nucleotide misincorporation pattern has been observed in a number of ancient samples that have been subject to deep sequencing, suggesting that it is a specific trait of ancient DNA sequences [19]. We have analyzed the base composition at the sequence ends, finding the described signal of cytosine deamination (Figure S1), thus suggesting that La Braña 1 consensus sequence is in fact endogenous. In conclusion, most of the sequences retrieved show a nucleotide misincorporation pattern typical of ancient DNA sequences, derive essentially from a single individual, and show a phylogenetically coherent haplotype that is rare in modern Iberian populations.

La Braña 1 and 2 Shotgun Genomic Data

For La Braña 1, 42,396,337 raw sequence reads were obtained, of which 6,113,535 mapped to the human reference genome (Hg18). After collapsing them to remove clonal reads and paralogs, 728,880 uniquely mapped reads remained (Table S4). The number of reads recovered from La Braña 2 was much lower, with 15,670,532 original reads, of which 364,578 could be uniquely mapped (Table S4). This represents a shotgun efficiency of 1.7% and 2.3% for La Braña 1 and 2, respectively, higher than the efficiency figures found in other samples from the Iberian Peninsula [27] but significantly lower for instance than those obtained for Vindija Neanderthals [29]. The rather high clonality can be explained by an initial low copy number of DNA template in the ancient extracts.

The generated data covered 41,320,020 nucleotide positions for La Braña 1 and 16,876,146 for La Braña 2; thus, about 1.34% and 0.53% of the La Braña 1 and 2 genomes were retrieved, respectively. The read average length was 74.7 and 59.5 nucleotides, respectively (Table S4), shorter than the 85.7 nucleotides observed in the mtDNA reads (Table S3) but similar to the length previously reported for DNA extracted from Neanderthal remains [30]. The ratio of X chromosome versus Y chromosome sequences was close to 9:1 (n = ~18,000 versus n = ~2,000 reads, and n = ~8,000 versus n = ~1,000 for La Braña 1 and 2, respectively), consistent with the length ratio between both sex chromosomes. This would confirm the previous anthropological identification of La Braña specimens as males.

A worldwide genomic principal component analysis (PCA) with data from the 1000 Genomes Project [31] places La Braña 1 and 2 near, but not within the variation of current European populations (Figure S2). However, when compared exclusively to European populations, La Braña 1 and 2 fall closer to Northern European populations such as CEU and Great Britons than Southern European groups such as Iberians or Tuscans (Figure 3). With 1KGPomni chip [31] data, the PCA generates a similar pattern (Figure S3), although the general geographic structure is less clear because of the limited number of SNPs (see Supplemental Experimental Procedures).

Genomic Contamination Estimates

To obtain a direct estimate of contamination in the nuclear data, we screened for heterogeneity in the nucleotides among X chromosome overlapping reads (discarding C to T and G to A changes attributable to DNA damage [22]). In the 204,207 nucleotide positions covered by at least two reads in La Braña 1, 341 (0.166%) showed conflicting nucleotides; in the 11,012 positions covered by three or more reads, the figure was 0.4%. In La Braña 2, there was heterogeneity in 143 of the 67,623 nucleotides covered by three or more reads, yielding a similar figure of 0.2% of potential contamination. Less numerous Y chromosome heterogeneities in two or more reads (91 out of 21,203 nucleotides and 31 out of 8,567 for La Braña 1 and 2, respectively) yielded similar values (0.4% and 0.36%). These contamination figures are overestimates, because sequencing errors are likely included in the existing reads.

Mesolithic Genetic Affinities

Previous studies of ancient mtDNA have shown that U5 haplotypes were common among Mesolithic Europeans, especially in Central and Eastern parts of Europe. For instance, a high incidence of U5 haplotypes (about 65%) has been detected in hunter-gatherer individuals from various sites across central and Eastern Europe [14]. U5b haplotypes have also been reportedly recovered from Mesolithic skeletons found in Aizpea in Navarra, Spain (dated to about 6,600 years before present) [32] and Reuland-Loschbour in Luxembourg (dated to about 6,000 years BC) [33], as well as from the skeleton known as "Cheddar Man" that was found in Gough's Cave, England [34]. Although no detailed methodological information has been reported for these two latter studies, the fact that the haplotype found in the mtDNA HVR1 is 16192T-16270T (without the U5a-defining nucleotide 16256T), seems

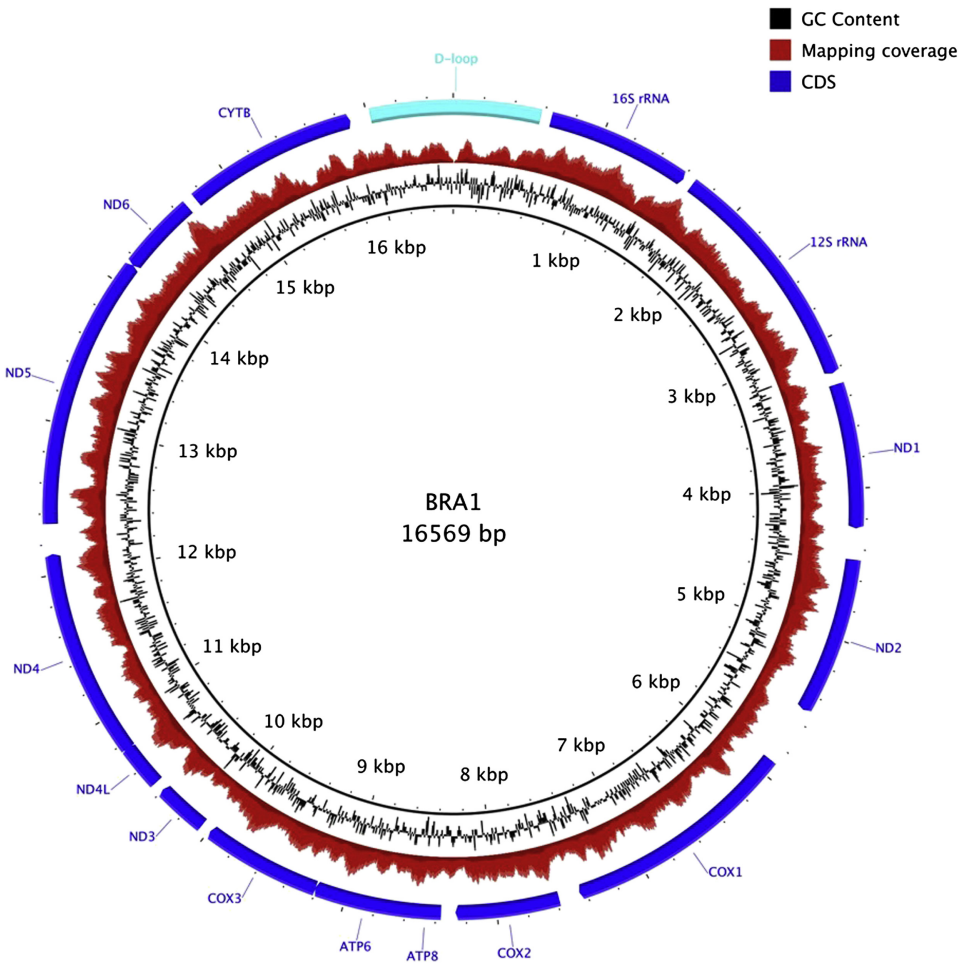

Figure 2. The La Braña 1 Complete Mitochondrial Genome

Mapping coverage of unique DNA reads (in red) and the mtDNA GC content. Coverage is correlated with CG content as shown in [19] and [30].

to indicate that the haplotype is in fact U5b, not U5a as reported in both cases [33, 34].

U5b haplotypes are thus present in 9 out of 16 Mesolithic sites with genetic information available (56.3%), comprising 12 of the 27 individuals so far analyzed (44.4%). This surprisingly widespread presence of U5b includes present day Lithuania (Donkalnis and Kretuonas sites), Poland (Dudka site), Germany (Hohlenstein-Stadel and Falkensteiner Höhle sites), likely Luxembourg (Reuland-Loschbour site) and England (Gough's Cave), and Spain (Figure 4).

It is generally accepted that the most ancient European mitochondrial haplogroup, U5, arose in Europe [6]. The coalescence time estimate from molecular data for the U5 is ~25–30 thousand years (ky) and for its subhaplogroups U5a and U5b ~16–20 and ~20–24 ky, respectively [35]. The time estimate for U5b1c is 12.8 ky [35]. U5 haplotypes are also found in Neolithic and present day populations, although their frequency is moderated as compared to the Mesolithic, ranging from about 1% in some places along the Mediterranean up to 5%–8% in continental Europe [14]. The exceptions to this trend are the Saami populations, in northern Scandinavia, where haplogroup U5 (and mainly subhaplogroup U5b) ranges from 26.5% to 56.8%, depending on the population [36].

The genetic uniformity of the European Mesolithic hunter-gatherers that apparently carried U5 haplotypes in very high frequencies is surprising, considering the time span and also the vast geographic area involved, enlarged now with these two new haplotypes from the Iberian Peninsula. This suggests minimal geographic structure across Europe during the Mesolithic. The fact that all Mesolithic mtDNA haplotypes so far described derive from the U haplogroup suggests a common origin for the Mesolithic foragers, probably deriving from a small founding population. Being hunter-gatherers and thus highly mobile groups probably prevented the generation of any geographical structure, at least in continental Europe.

La Braña 2 specimen was found with typical Mesolithic personal ornaments, consisting in 24 perforated atrophic red deer canines that were used embroidered on a cloth. The widespread presence of these perforated red deer canines in other Mesolithic sites, but especially in those from Central and Northern Europe [37, 38], suggests that La Braña individuals had tight cultural affinities with those distant regions (Figure 4). It is not known, however, if this genetic uniformity observed during the Mesolithic is a trait shared with the Upper Paleolithic modern human populations or if it is a specific feature from this period. In any case, the posterior

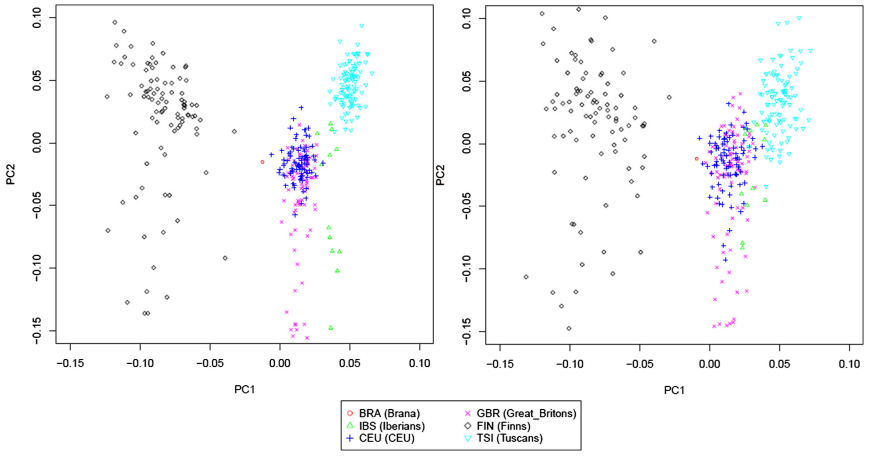

Figure 3. PCA Analyses of the Two Mesolithic Individuals

Left, La Braña 1; right, La Braña 2. The analyses were generated using 47,742 SNPs for La Braña 1 and 32,339 SNPs for La Braña 2, and five current European populations (Finns, Iberians, Great Britons, Tuscans, and CEU) [31].

European population affinities during the Neolithic seems to be much more complex and heterogeneous, involving probably spatial and temporal demographic movements likely related to the strategies of food production [17]. It is noteworthy that this latter scenario also received the highest support using a serial coalescent framework and Approximate Bayesian Computation (Figure S4; Supplemental Experimental Procedures).

In the genomic analysis, it is interesting to see that the La Braña individuals do not cluster with modern populations from Southern Europe, including those from the Iberian Peninsula. The first PC separates a north-south distribution, whereas the second follows a general east-west pattern in modern Europeans. The position of La Braña individuals in the 1000 Genomes Project data and the 1KGPomnichip PCAs suggests that the uniform Mesolithic substrate could be related to modern Northern European populations but may represent a gene pool that is no longer present in contemporary Southern European populations. In the latter PCA, where the origin of each Iberian sample is known, it is possible to see that the Mesolithic specimens are not related to modern Basques, contrary to what has been previously suggested in some recent studies [39].

Ancient genomics from Neolithic individuals from Scandinavia [18] supports that the spread of agriculture into Europe involved the expansion of populations from the Middle East that eventually assimilated the contemporaneous hunter-gatherers. Modern European populations seem to derive essentially from those Neolithic migrants [18]. Until now, however, the genetic affinities of the Mesolithic populations to the modern Europeans were largely unknown. Our partial La Braña 1 and 2 genomic data show that modern Iberian populations are not descendants of the local hunter-gatherers inhabiting the same region prior to the arrival of farmers and thus support a genetic shift in that region between the Mesolithic and modern populations.

Accession Numbers

The GenBank accession number for the mtDNA sequence reported in this paper is JX186998.

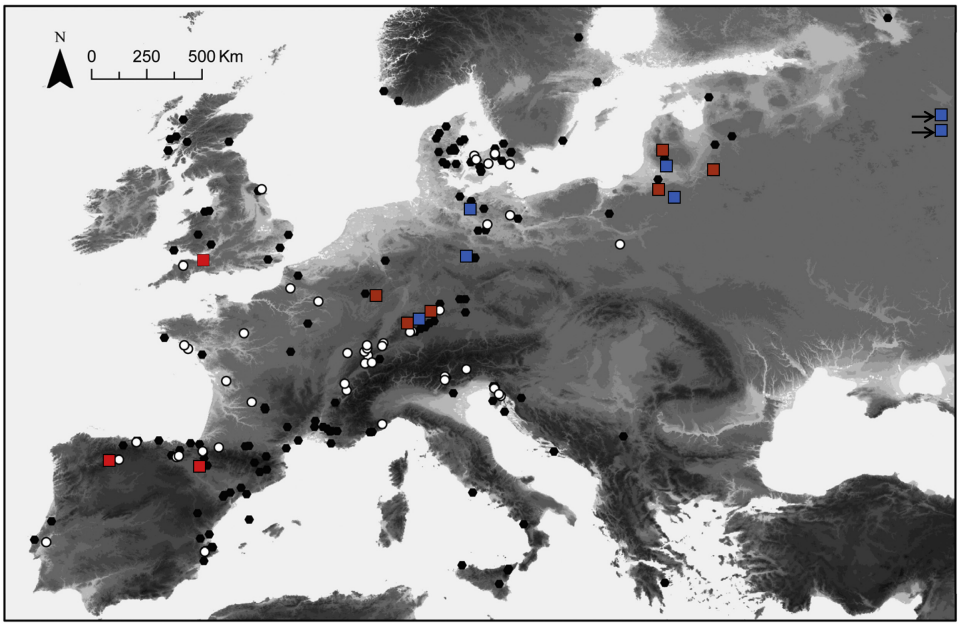

Figure 4. Evidence of Genetic and Cultural Uniformity during the European Mesolithic Period

Black circles, Mesolithic sites with ornaments; white circles, Mesolithic sites with perforated red deer canines (including La Braña site); squares, Mesolithic sites with genetic data; blue squares, U4 and U5 mtDNA lineages; red squares: U5b haplotypes (including La Braña site).

Supplemental Information

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.06.005.

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