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The Basque Paradigm: Genetic Evidence of a Maternal Continuity in the Franco-Cantabrian Region since Pre-Neolithic Times

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Different lines of evidence point to the resettlement of much of western and central Europe by populations from the Franco-Cantabrian region during the Late Glacial and Postglacial periods. In this context, the study of the genetic diversity of contemporary Basques, a population located at the epicenter of the Franco-Cantabrian region, is particularly useful because they speak a non-Indo-European language that is considered to be a linguistic isolate. In contrast with genome-wide analysis and Y chromosome data, where the problem of poor time estimates remains, a new timescale has been established for the human mtDNA and makes this genome the most informative marker for studying European prehistory. Here, we aim to increase knowledge of the origins of the Basque people and, more generally, of the role of the Franco-Cantabrian refuge in the postglacial repopulation of Europe. We thus characterize the maternal ancestry of 908 Basque and non-Basque individuals from the Basque Country and immediate adjacent regions and, by sequencing 420 complete mtDNA genomes, we focused on haplogroup H. We identified six mtDNA haplogroups, H1j1, H1t1, H2a5a1, H1av1, H3c2a, and H1e1a1, which are autochthonous to the Franco-Cantabrian region and, more specifically, to Basque-speaking populations. We detected signals of the expansion of these haplogroups at ~4,000 years before present (YBP) and estimated their separation from the pan-European gene pool at ~8,000 YBP, antedating the Indo-European arrival to the region. Our results clearly support the hypothesis of a partial genetic continuity of contemporary Basques with the preceding Paleolithic/Mesolithic settlers of their homeland.

The settlement history of Europe has been punctuated by several major episodes over the past 50,000 years. These include the first arrival of modern humans from Africa during the Upper Paleolithic, the Late Glacial re-peopling of Europe from southern refugia, the Postglacial recolonization of deserted areas after the end of the Younger Dryas, the farming-related population expansion of Near Easterners into Europe during the Neolithic, and the small-scale migrations along continent-wide economic exchange networks beginning from the Copper Age onward.^{1–3} Furthermore, a number of Urheimat hypotheses suggest the proto-Indo-European culture completed its expansion to Europe between the fifth and first millennia BCE, leaving the Basque language the only remnant of the antedating culture in Western Europe.⁴ The way in which these events have left their genetic footprints on the current European gene pool has been the focus of intense genetic research over the last decades. At the genome-wide scale, European populations appear rather homogeneous, and there are small existing differences that broadly correlate with geography.^{5,6} The most noticeable pattern uncovered was a clear distinction between northern and southern Europeans.^{5,7–9} Despite the contribution of these genome-wide data sets in evaluating the degree of popula-

tion structure and admixture, they are of more limited use than uniparentally inherited genomes for the detection of subtle population migrations, the timing of such episodes, and gender-biased events. Indeed, the study of Y chromosome and mitochondrial DNA (mtDNA) variation has extensively improved our understanding of the different spatial and temporal sources that contributed to the genetic structure of modern Europeans.³ For example, recent Y chromosome data suggest that most present-day European Y chromosomes were contributed by the Near Eastern Neolithic package,¹⁰ although alternative, more complex scenarios have been proposed.^{11,12} Conversely, founder analyses of mtDNAs suggest that less than 15% of European haplogroups can be traced back to the Neolithic expansion.³

The Late Glacial and Postglacial reoccupation of Europe from refugial areas has played a major role in shaping the gene pool of modern European populations and indicates the critical role of climate change in European demographic history. Several, independent lines of evidence point to the resettlement of much of western and central Europe after the Ice Age from the Franco-Cantabrian region, an area that includes the southern half of France and the northern strip of Spain looking at the Bay of

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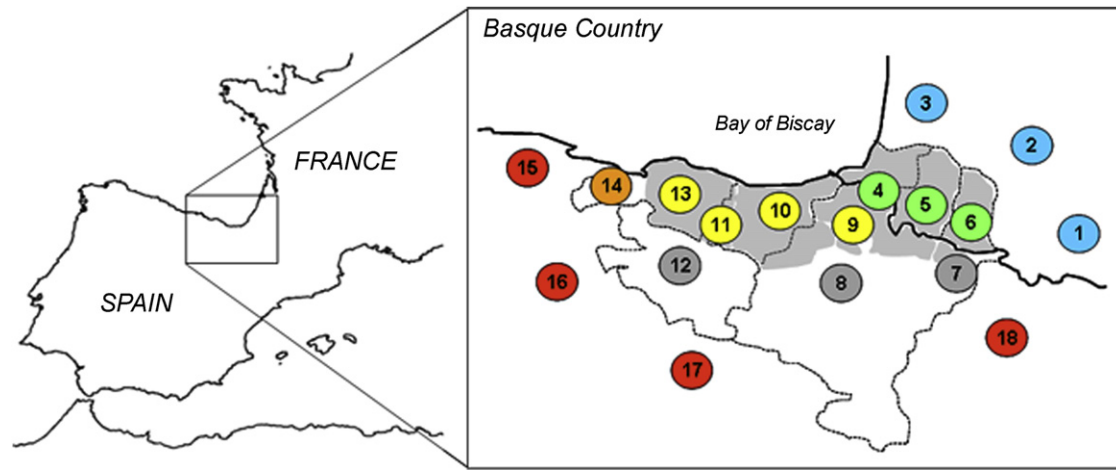


Figure 1. Geographic Location of the Studied Populations

The sampling strategy adopted was centered on the Basque-speaking provinces and included the immediately adjacent non-Basque-speaking regions. Grey shaded areas correspond to regions where the Basque language is currently spoken. Individuals were sampled in 18 different regions that, on the basis of geographic location, linguistic affiliation and surnames, were grouped into six major zone categories. These include Zone A (in blue), French-speaking regions that historically spoke Gascon (Bigorre, Bearn, Chalosse); Zone B (in green), Basque-speaking regions located in France (Lapuri/Baztan, Lapuri Nafarroa, Zuberoa); Zone C (in gray), regions where Basque was spoken up to the last century (Roncal and Salazar valleys, central western Nafarroa, Araba); Zone D (in yellow), Basque-speaking regions located in Spain (northwestern Nafarroa, Gipuzkoa, southwestern Gipuzkoa, Bizkaia); Zone E (in orange), a Spanish-speaking region in the Basque country (western Bizkaia); and Zone F (in red), four Spanish-speaking regions (Cantabria, northern Burgos, La Rioja, northern Aragon). Details on sample size per region are provided in [Table S1](#).

Biscay. On the basis of archaeological data, the Franco-Cantabrian refuge seems to have been the most densely populated region of Europe throughout all the Upper Paleolithic;¹ it has a strong signal of both range and size expansions at ~15,000 years before present (YBP) as the Magdalenian industry spread from the southwest into the rest of Europe.² Genome-wide studies have revealed that the clinal distributions of some parameters are compatible with a prehistoric population expansion from southern to northern Europe,^{5,9} but no indication of the time-depth of the underlying events has been provided. Here again, phylogeographic studies based on mtDNA variation have, by and large, supported the Franco-Cantabrian refuge scenario that all mtDNA haplogroups expanded from this region and present Late Glacial or Postglacial expansion times, that is H5 at ~13,000 YBP and V, H1, and H3 at ~11,000 YBP.^{3,13–16} In this context, the study of the Basques, unquestionably the most emblematic population inhabiting the Franco-Cantabrian region, can be particularly informative.

Above all, Basques are characterized by their unique language, Euskara, a non-Indo-European isolate that is not considered a member of any extant language family. The present-day Basque population is located on both sides of the western Pyrenees, and Basque is currently spoken by around 25% of the population. The corresponding area is divided into seven provinces, four in Spain and three in France ([Figure 1](#)). Toponymic data suggest a larger territory was previously occupied by ancient Basques, stretching from the Garonne River in the north to the Ebro River in the south. The linguistic isolation of the Bas-

ques, together with their outlier position with respect to a large set of classical genetic markers,^{17–19} has strengthened the view of Basques as being a genetic isolate with the greatest degree of genetic continuity with the early European, Paleolithic hunter-gatherers. However, this popular vision has been challenged by a number of recent, differing studies.

At the genome-wide scale, Basques are close to other Europeans,^{20–22} and the genetic component dominating European populations is observed at the highest proportions among Basques.^{20,23} This quantitative distinctiveness of Basques has been nevertheless contested by another study.²⁴ From a Y chromosome perspective, Basques are differentiated from other populations, with the exception of the neighboring Gascons,^{25,26} but the Mesolithic status of Basques has been challenged by a more recent study.¹⁰ From an mtDNA perspective, conflicting patterns have also emerged. All major mtDNA haplogroups observed among contemporary Basques are shared with the general European maternal pool.^{27–33} Although haplogroup H has been shown to be the most dominant among Basques,^{13,14} very different frequencies of other minor haplogroups have been reported. Indeed, several rare mtDNA variants within H2a5, U8a, J1c1, and J2a have been suggested to be autochthonous to some Basque groups but are absent in others.^{31,34,35} Likewise, distances between Basque populations have been found to be larger than between Basques and other Iberian groups, pointing to strong local isolation and limited gene flow between Basques and surrounding populations.³² This plethora of conflicting results, probably attesting to the complex

demographic matrilineal history of Basques, have led to differing opinions regarding the putative genetic continuity between present-day Basques and Paleolithic Europeans^{27–29,31,34,35} and, more generally, with respect to the role played by the Franco-Cantabrian refuge in the postglacial recolonization of Europe.^{13,32}

We hypothesized that part of the uncertainty regarding the origin of the Basques might be attributed to the unsatisfying level of mtDNA resolution achieved so far. Indeed, most studies of the Basque population have been based solely on the sequencing of the hypervariable segment (HVS)-I or on complete mtDNA sequences of targeted, low-frequency haplogroups in few individuals.^{34,35} Furthermore, because the time-depth of the major European haplogroups is in the range of 10,000–50,000 YBP,³ the study of their variation is less relevant to the case of the Basques and the spread of Near Eastern farmers and Indo-European languages, which is considered to have occurred 3,000–7,000 YBP.⁴ It is possible, though, that a distinguishing feature will exist at the level of complete mtDNA sequences of specific haplogroups. Here, we aimed to increase knowledge of the origins of the Basque people, the putative genetic continuity between present-day Basques, and pre-Indo-European Neolithic peoples and, more generally, the role of the Franco-Cantabrian refuge in the Postglacial repopulation of Europe. To this end, we characterized the maternal ancestry of Basque- and non-Basque-speaking populations from the Franco-Cantabrian region and, by sequencing complete mtDNA genomes, we focused on haplogroup H—the most dominant haplogroup in Europeans and in Basques in particular (~45%).^{13,14,34,36} In contrast to genome-wide analysis and Y chromosome data where the problem of poor time estimates remains, a new timescale has been recently established for the mtDNA,³⁷ making this genome at present the most informative marker for studying European prehistory.

We sampled a large collection of 908 individuals from 18 different geographic locations (Figure 1 and Table S1, available online), from whom we collected detailed ethnological and geolinguistic data. Our sampling strategy was centered on the seven Basque-speaking provinces in France and Spain, here referred to as the “Basque Country,” and included the immediately adjacent non-Basque-speaking regions. Inclusion criteria comprised an autochthonous origin confirmed by surnames and birthplaces of each individual, their parents and grandparents. Furthermore, we favored the inclusion of participants of more than 60 years old to increase the probability of a deep local ancestry. On the basis of geographic location, linguistic affiliation, and surnames, individuals were attributed to six major zone categories: French-speaking regions that historically spoke Gascon (Zone A), Basque-speaking regions located in France (Zone B), regions where Basque was spoken up to the last century (Zone C), Basque-speaking regions located in Spain (Zone D), a Spanish-speaking region in the Basque Country (Zone E), and Spanish-speaking regions (Zone F)

(Figure 1 and Table S1). It is worthy of mention that in the Spanish-speaking regions E and F, Basque languages have most likely been spoken up to medieval times (Zuazo³⁸ and references therein). For all individuals, written informed consent was obtained, and Ethics Committees at Institut Pasteur (n° RBM 2005.012) and Université Michel de Montaigne Bordeaux 3 approved all procedures.

To characterize the maternal ancestry of all individuals, we first typed a panel of 22 coding region SNPs that are diagnostic of the major haplogroups of the mtDNA phylogeny and sequenced the entire control region, including both HVS-I and HVS-II.³⁹ Variable positions throughout the control region were determined from positions 16,024–16,569 and 1–573. The 22 coding region SNPs were analyzed on all samples by SNaPshot typing with the multiplex GenoCoRe22.⁴⁰ We used this data set to assign each of the mtDNA genomes to its respective nodal haplogroup. The majority of samples, 420 of the 908 (46.3%), belonged to haplogroup H (Table 1 and Table S2), as expected in populations of western European descent.^{13,14,34,36} All but three of the remaining samples belonged to haplogroups that characterize the western Eurasian mtDNA landscape, including U5, J1, J2, V, and T (Table S2). One individual from the Basque-speaking region of Guipuzcoa and one from the Spanish-speaking region of Burgos belonged to the sub-Saharan African haplogroups L2 and L3h, supporting previous observations of low frequencies of sub-Saharan African haplogroups in northern Iberia.^{29,30,32} In addition, one individual originating from the French region of Bigorre belonged to haplogroup C, which mostly characterizes East Asian populations.^{41,42}

To gain insight into the haplogroup that accounts for most of the maternal variation of populations from the Basque Country and immediately adjacent regions, we sequenced the complete mtDNA genomes of all individuals (i.e., 420 samples) belonging to haplogroup H. We then integrated these data into the global haplogroup H phylogeny, inferred from a large data set of complete mtDNA genomes from individuals of pan-European origin, and focused on the phylogenies of the specific haplogroups characterizing our sample from the Franco-Cantabrian region (Figures S1–S6). These phylogenies were reconstructed by evaluating all previously published and novel haplogroup H complete mtDNA sequences in order to identify the most parsimonious solution and aided by the mtPhyl software. The 420 samples could be assigned to 129 different haplogroup H sub-haplogroups, with 59 appearing as singletons (Tables S3 and S4). Remarkably, although three different haplotypes were required to support the labeling of a new haplogroup, only nine (2.1%) samples could not be related to either a previously or a herein described haplogroup and were therefore labeled as H. Numerous haplotypes matched previously reported complete mtDNA sequences sampled throughout the European continent. However, our analyses revealed

Table 1. Haplogroup H Variation in the Basque Country and Adjacent Regions

	Geographic Zones						Total
	A	B	C	D	E	F	
Sample size	164	193	175	231	21	124	908
H samples	61	82	78	125	14	60	420
H frequency	37.2	42.5	44.6	54.1	66.7	48.4	46.3
Autochthonous H Haplogroups^a							
H1av1	0.0	1.0	0.6	2.6	0.0	0.8	1.1
H1av1a	0.0	1.0	0.0	2.2	0.0	0.0	0.8
H1e1a1	3.1	3.1	0.6	0.0	0.0	0.0	1.3
H1j1	0.0	0.5	4.0	2.6	9.5	0.8	1.9
H1j1a	0.6	0.5	0.6	0.9	0.0	1.6	0.8
H1j1a1	0.0	0.0	0.0	1.3	0.0	0.0	0.3
H1j1a2	1.2	1.6	0.0	0.0	0.0	0.0	0.6
H1j1b	0.0	2.6	0.6	3.9	0.0	0.0	1.7
H1j1c	0.0	0.0	0.0	2.2	0.0	0.0	0.6
H1t1	0.0	0.0	1.1	0.4	0.0	0.0	0.3
H1t1a	1.8	3.6	2.9	3.0	0.0	1.6	2.6
H1t1a1	0.0	0.0	0.0	3.0	0.0	0.0	0.8
H2a5a1	2.4	0.5	0.0	2.2	0.0	0.0	1.1
H2a5a1a	0.6	1.0	0.6	3.5	0.0	0.0	1.3
H3c2a	0.6	1.6	2.3	1.3	0.0	0.0	1.2
H3c2a1	0.0	1.6	0.0	0.0	0.0	0.0	0.3
Total frequency of autochthonous haplogroups	10.4	18.7	13.1	29.0	9.5	4.8	16.6
Proportion of autochthonous H variation	27.9	43.9	29.5	53.6	14.3	10.0	36.0

^aFrequency of autochthonous haplogroups in the different geographic zones of the Basque Country and adjacent regions.

six dominant haplogroups that display an internal structure within the Basque Country and bordering regions and explain 17% of all mtDNA variation and 36% of haplogroup H variation of our data set (Table 1). These haplogroups, in descending order of frequency, included H1j1, H1t1, H2a5a1, H1av1, H3c2a and H1e1a1 (Figure 2). Only H1j1, H1t1 and H2a5a1 have been previously labeled,^{32,34,43,44} and the remaining three are baptized herein for the first time. Of these haplogroups, only H2a5a1 has been previously suggested to be autochthonous to the Basque Country.^{32,34,43,44} Most importantly, these six haplogroups were found to be virtually absent in the comparative data set of more than 7,000 complete mtDNA genomes (D.M.B., unpublished data) from populations of predominantly western European origin.

The most dominant H haplogroup of the Franco-Cantabrian region is H1j1, which accounts for 12.4% of

haplogroup H variation of our data set (Figure S1). This haplogroup has only been observed in four previously reported individuals of Basque origin (D.M.B., unpublished data).⁴³ Likewise, haplogroup H1t1, which explains 8.1% of haplogroup H variation, displays its highest frequencies in the Basque-speaking populations from France and Spain, and has been previously observed only among Basques (Figure S2).³² Haplogroup H2a5a1 accounted for 5.2% of haplogroup H variation. Here, we have further resolved the phylogeny of H2a5a1, now termed H2a5a1a, and all previously reported individuals carrying this haplogroup^{34,44} belong indeed to the herein refined H2a5a1a (Figure S3). Haplogroup H1av1, which accounts for 4% of H haplogroup variation, has been found so far only in our data set and its distribution is restricted to Basque-speaking populations and immediately adjacent Spanish-speaking populations (Figure S4). Finally, haplogroups H3c2a (Figure S5) and H1e1a1 (Figure S6), which explain 3.3% and 2.9% of haplogroup H variation, respectively, have each been observed only once in a composite sample from Spain.¹³ Overall, the exclusive geographic distribution of H1j1, H1t1, H2a5a1, H1av1, H3c2a, and H1e1a1 among Basque-speaking populations and immediately adjacent populations and their absence from a large data set of populations of western European-descent strongly suggest that these haplogroups are indeed autochthonous to the region.

Furthermore, significant matrilineal structure within this geographic region was observed in our database. The frequency of haplogroup H per se was not significantly different between the six geographic zones (Table 1), indicating that the mere comparison of low-resolved haplogroups is not informative enough to detect fine population structure. However, the proportions of the autochthonous haplogroups varied dramatically, and significantly, between Basque- and non-Basque-speaking regions (χ^2 test, $p < 0.01$). These six haplogroups accounted cumulatively for 44%–54% of the total haplogroup H variation among Basque-speaking populations from France and Spain (zones B and D), whereas only for 10%–14% among Spanish-speaking regions (zones E and F) (Table 1). Intermediate frequencies of ~28% were observed in French-speaking regions and in Spanish regions that historically spoke Basque (zones A and C). The somewhat closer affinity between French speakers (i.e., Gascons) and Basque speakers than between Spanish speakers and Basque speakers is concordant with previous observations based on the Y chromosome.^{25,26}

We next estimated the time-depth of these autochthonous haplogroups within the H phylogeny, by means of the ρ statistic (mean sequence divergence from the inferred ancestral haplotype) by using a mutation rate estimate for the complete mtDNA sequence and a correction for purifying selection.³⁷ All autochthonous H haplogroups coalesce at recent times ranging from 3,187 to 5,057 YBP with an average of 3,836 YBP (Table 2). These time estimates attest to a rather homogeneous signal of expansion

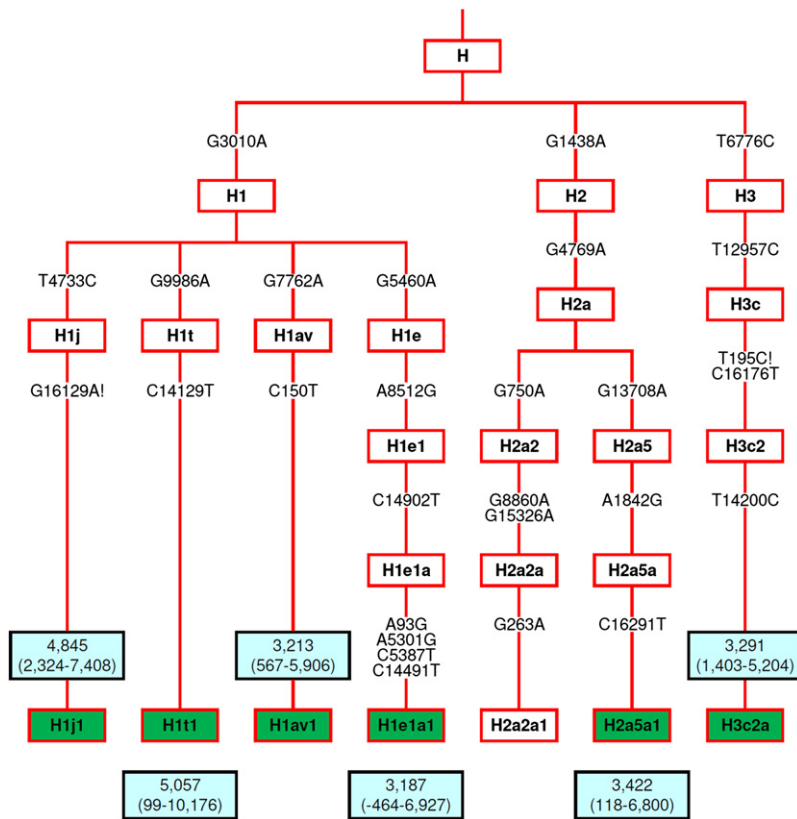


Figure 2. Phylogenetic Tree of the Six Autochthonous Haplogroups

The phylogeny represents the roots of H1j1, H1t1, H2a5a1, H1av1, H3c2a, and H1e1a1 and their coalescence age estimates based on complete mtDNA sequences. Mutations shown on the branches indicate the actual descendant nucleotide state relative to the root of haplogroup H. Detailed phylogenies of each of the six autochthonous haplogroups are presented in Figures S1–S6.

pean prehistory. First, the haplogroup H dissection indicates that populations from the Basque Country and adjacent regions, rather than the Basque population per se, are characterized by numerous low-frequency autochthonous haplogroups, each explaining ~2%–6% of the region's contemporary maternal ancestry, along with other H haplogroups that present a pan-European distribution and are observed at even lower frequencies. However, the autochthonous haplogroups are clearly more frequent within contemporary Basque speakers. Second, the identification of these autochthonous haplogroups attests

to some period of genetic isolation of populations inhabiting the region from the rest of Europe. Interestingly, such a partial genetic isolation of the Basques well parallels their linguistic affiliation, as they speak a language that is considered a linguistic isolate. Third, our findings suggest that the contemporary haplogroup H variation observed among Basque populations can be traced back to at least two different temporal sources. One ancient deme, preceding the arrival of Indo-European peoples and languages to the region, is represented today by a number of surviving autochthonous haplogroups, H1j1, H1t1, H2a5a1, H1av1, H3c2a, and H1e1a1, which diverged from the pan-European mtDNA pool ~8,000 years ago and experienced successive expansions at ~4,000 YBP. A more recent genetic contribution to the region is attested to by the seemingly punctuated introgression of pan-European H haplogroups, which are shared with other European populations, through the historical events affecting this region in the last millennia.

Collectively, our analyses of a large sample of complete mtDNA genomes demonstrate the need for such high-resolution sequence-based studies and large comparative databases to uncloak maternal ancestry patterns and subtle levels of population stratification. These patterns were previously masked in studies based on the typing of pre-ascertained panels of coding region SNPs and/or HVS-I/II sequence variation. Specifically, the study of Basque populations, located at the epicenter of the region that was a major source for the repopulation of Europe after the Ice Age, further improves our understanding of the maternal ancestry of the modern inhabitants of the Franco-Cantabrian region and, more generally, of Euro-

pean prehistory. First, the haplogroup H dissection indicates that populations from the Basque Country and adjacent regions, rather than the Basque population per se, are characterized by numerous low-frequency autochthonous haplogroups, each explaining ~2%–6% of the region's contemporary maternal ancestry, along with other H haplogroups that present a pan-European distribution and are observed at even lower frequencies. However, the autochthonous haplogroups are clearly more frequent within contemporary Basque speakers. Second, the identification of these autochthonous haplogroups attests

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Table 2. Time Estimates of the Six Autochthonous Haplogroups

Haplogroup	N ^a	Percentage	Rho	Standard Error	Age Estimate (in Years)	95% Confidence Interval (in Years)
Coalescence Age						
H1j1	52	12.4%	1.86	0.49	4845	2324 – 7408
H1t1	34	8.1%	1.94	0.97	5057	99 - 10176
H2a5a1	22	5.2%	1.33	0.65	3422	118 - 6800
H1av1	17	4.0%	1.24	0.52	3213	567 - 5906
H3c2a	14	3.3%	1.27	0.37	3291	1403 - 5204
H1e1a1	12	2.9%	1.23	0.72	3187	-464 - 6927
Splitting Age						
H1j1	52	12.4%	2.86	1.11	7514	1764 - 13470
H1t1	34	8.1%	2.94	1.39	7730	554 - 15227
H2a5a1	22	5.2%	2.33	1.19	6094	-6 - 12434
H1av1	17	4.0%	2.24	1.13	5854	65 - 11860
H3c2a	14	3.3%	2.27	1.07	5934	443 - 11619
H1e1a1	12	2.9%	5.23	2.13	14011	2729 - 26000

^aNumber of individuals.

However, our data show that the postglacial period also left some genetic footprints in the source populations of such major population expansions. This is clearly demonstrated by the various H haplogroups, including H1j1, H1t1, H2a5a1, H1av1, H3c2a, and H1e1a1, which are today restricted to the Franco-Cantabrian region and, more specifically, to Basque-speaking populations. These autochthonous haplogroups are shared, in some cases, with their immediate neighbors, suggesting gene flow between Basques and non-Basque-speaking peoples of the Franco-Cantabrian region and/or that the geographic extension of the Basque-speaking peoples used to be larger than thought today. We anticipate, however, that the same level of resolution used in the case study of the Basques at the European scale will reveal the presence of low-frequency autochthonous haplogroups in other regions of Europe, such as the case of the U5b3a1a clade that distinctively characterizes the people of Sardinia,⁴⁸ shedding further light on the demographic history of the European continent. However, our results are particularly meaningful in the case of the Basques because they are concordant with a genetic continuity since postglacial times to the present day, as a possible vehicle for the preservation of the unique Basque language.

In conclusion, our study has identified six autochthonous haplogroups, which explain 36% of the contemporary variation of haplogroup H in the region, restricted to Basque-speaking peoples and their immediate neighbors and virtually absent in the rest of Europe. In light of this, our data provide support for the hypothesis of a partial genetic continuity of contemporary Basques *latu sensu*—the historical Basque Country—with the earlier settlers of their homeland since pre-Neolithic times.

Supplemental Data

Supplemental Data include the complete list of Genographic Consortium members, six figures and four tables and can be found with this article online at <http://www.cell.com/AJHG/>.

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Web Resources

The URL for data presented herein is as follows:

mtPhyl, <http://eltsov.org/mtphyl.aspx>

Accession Numbers

The GenBank accession numbers for the 420 complete mtDNA sequences reported in this paper are JQ324516–JQ324935.

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