Reshaping the *Hexagone*: the genetic landscape of modern France

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Abstract

Unlike other European countries, the human population genetics and demographic history of Metropolitan France is surprisingly understudied. In this work, we combined newly genotyped samples from various zones in France with publicly available data and applied both allele frequency and haplotype-based methods in order to describe the internal structure of this country, by using genome-wide single nucleotide polymorphism (SNP) array genotypes. We found out that French Basques are genetically distinct from all other populations in the *Hexagone* and that the populations from southwest France (namely the Gascony region) share a large proportion of their ancestry with Basques. Otherwise, the genetic makeup of the French population is relatively homogeneous and mostly related to Southern and Central European groups. However, a fine-grained, haplotype-based analysis revealed that Bretons slightly separated from the rest of the groups, due mostly to gene flow from the British Isles in a time frame that coincides both historically attested Celtic population movements to this area between the 3th and the 9th centuries CE, but also with a more ancient genetic continuity between Brittany and the British Isles related to the shared drift with hunter-gatherer populations. Haplotype-based methods also unveiled subtle internal structures and connections with the surrounding modern populations, particularly in the periphery of the *Hexagone*.

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42 **Introduction**

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44 Located in the center of Western Europe, Metropolitan France has historically acted as a
 45 bridge connecting Northern Europe to the Mediterranean and the Iberian spaces. The
 46 geographical position of France strongly affected the history of the settlement of the
 47 different parts of the territory, whose continuous fragmentation through time is attested
 48 by the large number of populations and cultures that settled this area. Greeks, Romans
 49 and Celtic tribes from central Europe shaped a first internal structure between the 6th
 50 and the 1st centuries BCE, while waves of barbarian invasions (Alamanni, Burgundians,
 51 Visigoths, Franks, and Celts) strongly impacted the population landscape of France
 52 during the 5th century CE¹. During the 9th and 10th centuries CE, foreign invasions
 53 from all sides also influenced the territory: Muslims and Saracens from North Africa
 54 coming through Iberia, Hungarian Magyar from the east, and Vikings (*Northmen*) from
 55 the north¹. Nowadays, France is a cosmopolitan country whose society is shaped by a
 56 plurality of lifestyles and truly different ethno-cultural diversity. Without any doubt,
 57 the impact of political refugees throughout the 20th century, or of the immigration
 58 from colonized countries to mainland France, such as the migration of Arabs and
 59 Berbers from Algeria which was the most extensive of all colonial migrations to
 60 Western Europe before the 1960s², enriched the modern genetic landscape of the
 61 French territory. However, it is beyond our intention to explore this plethora of recent
 62 genetic contributions here, which can be quantified much more precisely with
 63 demographic analyses. Instead, we can apply genomic tools to excavate a deeper and
 64 ancient genetic background.

65 At the light of this complex past, the genetic landscape of France has been poorly
 66 analyzed, especially in recent times. The first studies with classical markers defined a
 67 general heterogeneous pattern considering different geographical arrangements such as
 68 military districts, historical provinces, and regions^{3,4}. With his synthetic maps, Cavalli-
 69 Sforza proposed that this heterogeneity was a consequence of differential Neolithic
 70 influences between northern and southern France, and also pointed out a differentiation
 71 for Brittany and Gascony⁵. More recently, studies on mitochondrial DNA highlighted a
 72 general homogeneity when the samples were distributed among the 22 regions
 73 established in 1982 and historic provinces^{6,7}. Generally, the mtDNA haplogroup

composition of French people did not differentiate neither internally, nor from the surrounding European genetic landscape^{6,7}. On a microgeographical scale, Brittany showed affinity with Scandinavia and Britain, while French Basques stood out for a high frequency of haplogroup H, suggesting a link with the Neolithic diffusion in Europe^{6,7}. In agreement with the homogeneity described by mtDNA studies, the Y-chromosome diversity strongly pointed out a lack of differentiation between the distinct groups when samples were organized on a regional scale. Even in this case, Brittany represented an exception, showing a lower Y-chromosome diversity that was interpreted as consequence of a possible founder effect, plus an isolation process⁸. Based on autosomal variants, a genome-wide study on Western France did not find any differentiation among the distinct groups organized on a regional geographical distribution⁹. Even in this case, the only outlier was Brittany, whose higher linkage disequilibrium suggested a lower effective population size, thus supporting the hypothesis of isolation inferred by the outcomes of the Y-chromosome analyses. Furthermore, in agreement with mitochondrial studies, Bretons were found to be admixed with individuals from the British Isles⁹. In this work, we present a comprehensive genome-wide study on France, using both allele frequency and haplotype-based methods, to determine the minimal meaningful geographic unit of genetic differentiation within France, describe the geogenetical landscape patterns within France, and trace the historic and ancient sources of gene flow into the *Hexagone*.

Material and Methods

Dataset arrangement and genotypes

In this study, informed consent was obtained from 331 individuals from different French departments. Internal Review Board approval for this work was granted by CEIC-PSMAR ref. 2016/6723/I. These samples were compiled by the Institute of Forensic Sciences, University of Santiago de Compostela, and most of them were first reported in an analysis of Y-chromosome markers in ref.⁸. As specified in the latter work, all the subjects and their parents were born in mainland France and bore a French surname. DNA was extracted from blood samples as described in Ramos-Luis *et al.*⁸. A

total of four Axiom ® Genome-Wide Human Origins Arrays (~629 K SNPs)¹⁰ were genotyped at the Centro Nacional de Genotipado - Universidade de Santiago de Compostela facility. Genotype calling was performed running four different batches according to the Affymetrix Best Practices Workflow implemented in the software Axiom™ Analysis Suite 2.0. Out of 331 samples, 52 failed the genotyping process and a total of 279 samples were retained. Three additional samples were removed following an Identity-by-descent analysis (IBD) since they displayed a Proportion IBD value $\geq$ 0.125 (minimum threshold for removing relatedness equal or higher than a third degree). Eventually, 276 samples were retained. To complete the French dataset, 79 additional samples from a public source¹¹ and 60 from unpublished data (from an ongoing study on the Basque Country and the Franco-Cantabrian region; samples are subset from those in ref.¹²) were added to the original 276, leading to a total of 415 samples. In a preliminary part of this work, 20 out of the 276 samples were identified as outliers and removed from the study (see Supplementary Figure 1 and caption). Thus, the complete dataset included 256 newly genotyped samples, plus 139 additional ones, for a final group of 395 samples (Dataset A) distributed among 20 different French departments (see Supplementary Figure 2 for the geographical distribution). For the allele frequency analyses, as comparison with external populations, a total of 333 samples were added to Dataset A, forming Dataset B. This external group included 218 samples among Germany, Norway, Spain, Italy, England, Ireland, and Scotland¹¹, together with 107 samples from the Spanish autonomous communities of Catalonia, Valencian Community, and Balearic islands¹³, and 8 additional samples from South Italy (Naples) newly genotyped with Axiom ® Genome-Wide Human Origins Arrays (~629 K SNPs). Further 799 samples from external populations¹¹ were added to the previous ones when applying haplotype-based methods (Dataset C). Lastly, in the analysis with ancient data, 282 ancient samples¹¹ were added to the previous dataset, with the only exclusion of the 122 sub-Saharan African samples (Dataset D) since their presence would have reduced the resolution for the distribution of the rest of the samples in the PCA, masking signals of admixture in the dedicated analyses (see Supplementary Figure 3 for the geographical distribution of the modern samples from Datasets B and C, and Supplementary table 1 for a summary of the different dataset composition).

140 Data Quality Control

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 142 Data were prepared using PLINK1.9¹⁴. Uniparental markers and X-chromosome
 143 variants were excluded. For the French dataset, a preliminary set of filters were applied
 144 to each group separately before the merging process. We filtered out all variants with
 145 missing call rates greater than 5%, those that failed Hardy-Weinberg test at $p < 10^{-5}$,
 146 and samples with more than 10% missing genotype data. After merging, only variants
 147 common to the three datasets were retained and SNPs with a minor allele frequency
 148 (MAF) below 5% were excluded, resulting in a final 343,884 variants used for
 149 haplotype-based methods (Dataset A). For the analyses that needed a set of independent
 150 markers, SNPs were pruned setting a pairwise linkage disequilibrium maximum
 151 threshold of 0.5, a window of size 200 and a shift step of 25. Eventually, the pruned
 152 data retained 142,803 variants (Dataset A). In the analyses that included the external
 153 populations, only the pruned dataset, consisting in 154,889 SNPs, was used for the
 154 allele frequency analyses (Dataset B), while a set of 380,697 variants was retained in the
 155 haplotype-based methods (Dataset C). Regarding Dataset D, a set of 163,631 SNPs was
 156 retrieved after pruning (See Supplementary table 1 for a summary).

157

158 **Statistical analyses**

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160 Eigenvectors were computed using the SmartPCA program in Eigenstrat software
 161 package (v. 13050)¹⁵. For Dataset D, we used the option lsqproject:YES when
 162 projecting ancient on top of the modern samples. Results were plotted in R (v 3.0.1).

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164 The F_{ST} fixation index was computed using the SmartPCA tool (v. 13050) from the
 165 Eigenstrat software package. Results were produced in Rstudio¹⁶ using R version 3.4.4
 166¹⁷. The F_{ST} matrix was used together with a geographic distance matrix produced with
 167 The Geographic Distance Matrix Generator (v. 1.2.3, available from
 168 http://biodiversityinformatics.amnh.org/open_source/gdmg) in order to perform a
 169 Mantel test correlation using the ade4¹⁸ library in R. Results were displayed using
 170 ggplot2¹⁹ and reshape²⁰ libraries.

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172 Based on different hierarchical levels (within Departments, Between Departments
 173 within Areas/Regions, Between Areas/Regions; see Supplementary Figure 4 for a visual
 174 representation of the used Areas and Regions), AMOVA was performed using the

175 *poppr.amova* function in R package *poppr* (v. 2.8.1) ^{21,22} and significance was tested
 176 with the *randtest* function implemented in R package *ade4*. For every percentage of
 177 variance, a p-value was calculated based on 1000 permutations.
 178 Patterns of population structure were explored, in both Dataset B and D, using
 179 ADMIXTURE ²³ testing from K=2 to K=10 ancestral clusters and using 10 independent
 180 random seeds. Results were represented using the software *pong* ²⁴. For Dataset B,
 181 admixture was formally tested with f3 statistics computed using the *qp3Pop* function
 182 implemented in *Admixtools* ¹⁰, while outgroup-f3 statistics were tested for Dataset D in
 183 the form of f3(Ancient, X; Mbuti), where 3 Mbuti samples from ref. ¹¹ were added to
 184 Dataset D (1690 total samples, same variants as in Dataset D).

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186 **EEMS (Estimated Effective Migration Surface)**

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188 EEMS ²⁵ analysis was run using Dataset A (142,803 variants from the pruned file).
 189 With a matrix of average pairwise genetic dissimilarities calculated using the internal
 190 program *bed2diffs*, a sample coordinates file, and a habitat coordinates file generated
 191 using Google Earth Pro (v. 7.3.2.5495), we performed 10 pilot runs of 6 million MCMC
 192 iterations each, with 3 million burn-in, and a thinning interval of 30,000. A second set
 193 of 5 runs was then performed restarting the chain with the highest likelihood with 4
 194 million MCMC iterations, 1 million burn-in, and thinning interval of 10,000. The
 195 density of the population grid was set to 300 demes, and random seeds were used for
 196 each one of the runs. We used the default hyperparameter values but tuned some of the
 197 proposed variances to improve convergence in the second set of runs. Results for the
 198 chain with the highest likelihood were displayed using *eems.plots* function in the R
 199 package *rEEMSplots*.

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201 **Haplotype-based analysis**

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203 Two different analyses were performed: one on the internal French population only
 204 (Dataset A), and one also including external populations (Dataset C). In both cases,
 205 phasing was performed using the software *Shapeit* (v. v2.r837) ^{26,27}. When running
 206 *ChromoPainter* ²⁸, all samples were used as both recipients and donors, ²⁸without any
 207 population specification (-a option) and not allowing self-copying. First, the parameters
 208 for the switch rate and global mutation probability were estimated with the EM

algorithm implemented in ChromoPainter using the parameters -i 15 -in -iM for chromosomes 1, 7, 14, and 20 for all the samples. This step allows to estimate the two parameters that will be then averaged for all chromosomes. The outcome for the average weighted values for the global mutation probability and the switch rate parameters were respectively 0.000745 and 266.67196 for Dataset A, and 0.000586 and 237.50784 for Dataset C. In a second step, ChromoPainter was run for all chromosomes using the two fixed parameters. Later, the final coancestry matrices for each chromosome were combined using the tool Chromocombine. The latter also estimates the C parameter which is needed for the normalization of the coancestry matrix data when we run fineSTRUCTURE in order to identify the population structure. The MCMC of fineSTRUCTURE was run using 1000000 burn-in iterations (flag -x), 2000000 iterations sampled (flag -y), and thinning interval of 10000 (flag -z). Eventually, the fineSTRUCTURE tree was estimated running three different seeds and using the flags -X -Y -m T that allow to build the sample relationship tree. In the analysis on Dataset C, the work was then divided in two phases. In the first one, ChromoPainter and fineSTRUCTURE were rerun, this time silencing France in order to define the external groups only. In the second phase, fineSTRUCTURE was rerun using the “force file” option (-F), using “continents” as donor groups (represented by the external groups defined in the first phase); -F is a function that allows to exclude the donor representation in the building tree phase and focus on the distribution of the recipient groups, represented by the French samples only. We then applied the non-negative-least-squares (nnls) function from GLOBETROTTER ²⁹ in order to describe the ancestry profiles for the French groups we detected with the “force file” option. We then used GLOBETROTTER in order to describe admixture events, sources and dates. More details about the usage of GLOBETROTTER are reported in Supplementary note 1.

Results

Internal genetic structure in France

In order to define the best geographical partitioning of genetic differentiation, a hierarchical analysis of molecular variance (AMOVA) was performed with areas or

regions as major grouping factors. We determined first the proportion of genetic variation partitioned among geographic areas, among departments within geographic areas, and within departments. We next tested the proportion of genetic variation partitioned among regions (considering the 13 regions established in 2016), among departments within regions, and within departments. A further AMOVA was performed only testing the proportion of genetic variation partitioned among and within departments. As shown in Table 1, in all cases the main contribution to the genetic variance was found at the lowest hierarchical level (variation within departments), while differences among regions resulted in a negative value that could be interpreted as zero, meaning absence of any structure at this level. Conversely, differences among areas displayed positive values, supporting the role of areas as more reliable grouping factors of genetic variations when considering wider sample distributions. Finally, the results for the variation between departments, also supported by significant p-values in all the AMOVA analyses, pointed to the fact that this level of stratification might be a better representation for the minimal unit of genetic differentiation. Based on these results, samples were distributed on the map according to the departmental locations (Supplementary Figure 2) and all the subsequent analyses considered this grouping factor, although, given their known cultural and genetic identity, we retained Basque-speakers as a separate group in the Pyrénées-Atlantiques department. A first Principal Component Analysis (PCA) showed two distinct groups separated along the first PC (Figure 1A): the Basque samples on the right part of the plot, against most of the rest of the samples on the left one, within which a structure cannot be defined. These two major groups are connected by a “bridge” of samples represented by non-Basque-speaking individuals from the Gascony region in the southwestern corner of France. When we averaged the eigenvalues for the first two PCs and represented the same PCA, together with standard deviation (SD) values for each group, no evident pattern could still be discerned beyond the separation of Basques and Gascons (Supplementary Figure 5A). When we removed both Basque and Gascon samples from the analysis (Figure 1B), the resulting PCA showed some internal pattern of differentiation, more clearly defined by the average PCA (Supplementary Figure 5B), in which samples from the departments belonging to the northwestern region of Brittany seem to form a cluster on the left part of the plot.

Patterns of gene flow within France

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277 In the genetic variation computed with the F_{ST} analysis, a general homogeneous pattern
 278 was found, with fine scale values of differentiation between some departments. The
 279 southwestern samples (Basques and Gascons) showed the highest values of
 280 differentiation with the northwestern departments reaching scores between 0.008 and
 281 0.009 for the Basque-speaking samples, and between 0.004 and 0.006 for the non-
 282 Basque-speaking ones (Supplementary Figure 6A, left), followed by lower values of
 283 differentiation with the northern and northeastern departments. Without the
 284 southwestern samples, the main differentiation was recorded between the northwestern
 285 departments and the southeastern corner of the country, with a highest value of
 286 differentiation around 0.002 between the southeastern department of Bouches-du-Rhône
 287 (BdR) and the northwestern Breton department of Côtes-d'Armor (CdA)
 288 (Supplementary Figure 6B, left). Lower levels of differentiation were locally found
 289 among the departments in the northwest, and among those in the north together with the
 290 northeastern ones. A Multidimensional Scaling analysis (MDS) based on the F_{ST}
 291 matrices clearly showed how the southwestern samples separate from the rest of the
 292 groups (Supplementary Figure 6A, right), and how the Breton departments do the same
 293 once the Gascon and Basque samples are removed (Supplementary Figure 6B, right). A
 294 Mantel test of isolation by distance (IBD) between the F_{ST} values and the geographical
 295 distances showed a positive and statistically supported correlation ($R^2=0.332$, $P=0.001$)
 296 (Supplementary Figure 7A), moving to even more positive values when the
 297 southwestern samples were removed ($R^2=0.432$, $P=0.001$) (Supplementary Figure 7B).
 298 Next, we used the EEMS analysis, a method for visualizing genetic diversity patterns,
 299 and found that the resulting effective migration surface mirrors the outcomes of genetic
 300 differentiation detected by the F_{ST} analyses (Figure 2); a higher effective migration was
 301 locally found in northern, northeastern and northwestern France among departments
 302 belonging to the same geographical areas, while a major barrier was discovered along
 303 the western side of France.

304

305 **Haplotype sharing patterns within France**

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307 Using haplotype-based methods (Dataset A), we looked for patterns of haplotype
 308 sharing, illustrating relations between departments. In this first step, cutting the
 309 fineSTRUCTURE tree at the very base, allowed us to describe a fine scale haplotype

310 sharing distribution on a departmental scale; the outcome is a picture of the haplotype
311 configuration within France (Figure 3). The resulting map shows finer-grained detail:
312 we can define at least four distinct groups, plus a more widespread component. In the
313 southwestern corner, the Basque samples clearly separate from the Gascon ones. In the
314 northwestern vertex, the Breton departments exhibit their very own haplotypic
315 signature, in agreement with the lower level of differentiation detected with the F_{ST}
316 analysis and the higher internal effective migration rate detected with EEMS. The same
317 was found for the northern and northeastern departments that display a clearly shared
318 haplotypic configuration. The southwestern department of Haute-Garonne (HG) and the
319 southeastern one of Bouches-du-Rhône (BdR) present higher frequencies for some local
320 haplotypes that in other departments reached only lower frequencies. Otherwise, a more
321 generally spread French haplotypic background is found on the north-south axis.

322

323 **Sources of gene flow into France**

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325 When we added external sources from the surrounding populations (yellow dots in
326 Supplementary Figure 3) to describe allele-based genomic components with
327 ADMIXTURE (Figure 4), the configuration observed pointed to a general
328 homogeneous picture. The only exception was represented by the samples belonging to
329 the Breton departments whose configuration was more alike to that in the Irish, Scottish,
330 and English groups. Moving through the different K ancestral components, this
331 behavior clearly characterizes the northwestern departments, separating them from the
332 rest of the French groups since the very first K ancestral components (Figure 4). Thus,
333 we formally tested for admixture events using the f3-statistics with the test groups being
334 the different departments, and the external surrounding populations as sources. We only
335 retained the negative f3 values for those departments represented at least by two
336 individuals. Results are shown in Supplementary Table 2 where only significant Z-scores
337 < -3 are reported, while results for those departments passing all the requested filters but
338 with higher Z-score values are shown in Supplementary Table 3. Notably, in 9
339 departments, a combination of sources that was highly significant was Ireland-Southern
340 Italy.

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343 **Haplotype sharing patterns with external sources**

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 345 Based on the haplotype sharing with external sources it was possible to redefine the
 346 French haplotype configuration. After merging the 395 French samples with the 1132
 347 external ones (Dataset C), we first defined the external groups by silencing France when
 348 rerunning ChromoPainter and fineSTRUCTURE. The result was represented by 35
 349 different external groups (Supplementary Figure 8a). Secondly, focusing on our target,
 350 we redefined the French internal clusters using the 35 external ones as “continents”
 351 when running fineSTRUCTURE (Supplementary Figure 8b). The 13 different clusters
 352 we found within France were then represented as separate maps (Figure 5); each map in
 353 the figure is a heatmap showing the number of samples falling in the different
 354 departments. Out of 13 groups, 10 satisfied the conditions of having at least 10
 355 individuals and a major geographical area with a number of subjects corresponding to
 356 more than 50% of the entire cluster. These conditions allowed us to name each cluster
 357 based on the fact that a specific area was more represented than others in terms of
 358 sample size. The exclusion of three clusters did not impact the analysis, since only
 359 8.35% of the French samples were then not included as target in the following analyses
 360 with GLOBETROTTER. As in the analysis described in the previous paragraph, even in
 361 this case France appeared to be organized in few major areas of interest. As shown in
 362 Figure 5, the Northwest presented two main groups (B1 and B2), the Southwest divided
 363 in Basque (Bas) and Gascon (G1 and G2) groups, the Northern (CN) and Northeastern
 364 (NE) areas, the Southeast (SE), and a central/southwestern part of France (CSW1 and
 365 SW). These ten main areas represented the targets for the GLOBETROTTER analysis
 366 that we used to describe the ancestry profiles, the admixture events, and their dates.

367

368 **Ancestry profiles and dating admixture events**

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370 The results from the application of the nnls algorithm are displayed in Figure 5; on both
 371 sides of each target the ancestry profiles are represented as doughnut charts (on the left
 372 the results from the NM analysis, on the right the ones for the M one). The different
 373 colors represent proportions of haplotype sharing with specific sources (only
 374 contributions above 2.5% are shown). In the NM analysis, it is possible to appreciate
 375 how the haplotype sharing with other French sources (brown color) represents the
 376 highest proportion for all the different targets. When masking the French component,
 377 more refined patterns of contributions from external sources are detected. With the only

exception of the southwestern targets (G1, G2, and Bas), the remaining ones show a higher contribution from north Italy and Great Britain. Apart from these common signal, it is possible to highlight contributions from those neighboring populations that are more geographically close to specific areas within the French territory. The southwestern targets (G1, G2, and Bas) received more from the Spanish side, the northwestern targets (B1 and B2) share more with the external cluster source named *Irish_Scottish* (with a proportion of 23.91% and 18.32% for the B1 and B2 targets respectively), the northeastern target (NE) is more connected to the external cluster source representing central and eastern European countries (receiving 17.64% from the source we named *Central_Eastern_EU*), as also from the *NorthernEurope* cluster source (which contributes 7.35% and 5.78% to the NE and CN targets, respectively). The southeastern target (SE) is mostly connected to the Italian sources and other Mediterranean countries, and the central/southwestern target (CSW1) clearly received more from both Spain and Italy.

As explained in Supplementary note 1, GLOBETROTTER provided evidence of admixture for 8 out of 10 targets, and for 5 of them we could also describe the dates and the sources of admixture as shown in Supplementary Figure 9. For three targets GLOBETROTTER gave *one-date* as result, while for the remaining two *one-date-multiway* was detected. In each case, only one date of admixture was detected; for the *one-date* groups a single admixing couple of sources was described, while two couples of sources were presented in the case of *one-date-multiway*. For a better interpretation of the results, consider the caption from Supplementary Figure 9.

Relations with ancient populations

In the analysis with Dataset D, we first explored the position of France in the context of other modern populations, and then we focused on the relation with a set of ancient samples from different periods. In Supplementary Figure 10, panel A shows the PCA with the modern samples; France (white circles) is located in a position that mirrors its geographical situation, in between British, Irish, Mediterranean, central and eastern European samples. In panel B, a set of ancient samples was projected into the modern genetic space. In this second PCA, most of the French individuals are close to the Steppe and the Late Neolithic Bronze Age (LNBA) European samples, with some

412 subjects connecting with the Anatolian Neolithic and the Early Neolithic European
 413 groups, and few others with the Europe Middle Neolithic and Chalcolithic
 414 (Europe_MNChL) samples. Results from the ADMIXTURE analysis are reported for
 415 the lowest cross-validation error detected (K=4 in Supplementary Figure 11). At this
 416 level, four ancestral components are clearly visible: the hunter-gatherer (HG) ancestry
 417 (principally represented by the Scandinavian HG, in pink), Neolithic (mostly Anatolian
 418 and then European, in green), the Iran Neolithic (black), and Natufian (purple). Again,
 419 the proportion of these components in France is intermediate between those in Southern
 420 and Central European groups. It is especially the Natufian component that seems to act
 421 as a discriminant factor, not only inside France where it is virtually absent with few
 422 exceptions on the Mediterranean side, but mostly among the various modern groups.
 423 Outgroup f3-statistics in the form of $f_3(\text{Ancient}, X; \text{Mbuti})$ allowed us to quantify for
 424 each X modern group the amount of shared drift with different ancient populations.
 425 Figure 6 shows the outcome for these statistics, with a focus on the shared drift with the
 426 three main European ancestral components: Western, Eastern, and Scandinavian hunter-
 427 gatherers, European Neolithic farmers, and the European Bronze Age steppe
 428 component. In most cases, French populations fit the expected pattern of distribution in
 429 the wider panorama of the European area. However, the European Neolithic component
 430 seems to be higher in the SW of France, while Brittany carries a proportion of HG
 431 ancestry that is higher than elsewhere in France but closer to the values in the British
 432 Isles.

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437 Discussion

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439 We have used both allele frequency and haplotype-based methods in order to describe
 440 the internal structure of pre-20th century Metropolitan France. While the first yielded a
 441 more homogeneous landscape, the latter unveiled patterns of local differentiation with
 442 some connections with the surrounding European populations. Furthermore, we
 443 explored patterns of genetic continuity with ancestral populations, contextualizing
 444 France in the wider European panorama. In previous works about France, samples were

445 differently arranged into the geographical space and no consensus had been reached on
 446 what subdivision was more appropriate; apart from the peculiar military districts³,
 447 historical provinces^{4,6} and old regions⁸ are the most used so far. Thus, our first goal
 448 was to search for the best geographical level of genetic stratification before arranging
 449 our samples on a map. After the French Revolution in 1790, in order to weaken the old
 450 loyalties, the ancient provinces of France were subdivided into departments, whose
 451 overall configuration has been mostly conserved so far³⁰. Furthermore, in 1982, a
 452 system of 22 regions was established by grouping different departments into wider areas
 453³¹. However, in 2016, the number of the regions was reduced to 13, with the consequent
 454 rearrangement of the departments³². Given this background, our AMOVA results
 455 provide evidence that regions, as a new internal reorganization, are not a suitable model
 456 for the genetic compartmentalization and point to the absence of any contribution to the
 457 total genetic variation, possibly implying that regions are separating genetically similar
 458 departments into different groups. On the other hand, departments, as result of a more
 459 conserved internal geographical structure, represent the best minimal unit of genetic
 460 stratification.

461

462 **Dissecting the *Hexagone***

463 Principal component analysis on allele frequencies revealed the expected Basque
 464 differentiation, adding Gascons in SW France as a population closely related to them,
 465 while the rest of France appeared relatively homogeneous. However, EEMS results
 466 pointed to the existence of other barriers to gene flow, particularly between NW France
 467 (Brittany) and the rest, while other areas acted as corridors, in central France and along
 468 the N and NE borders (Figure 2). It should not be excluded, though, that unsampled
 469 regions caused some possible artifacts³³. It was with fineSTRUCTURE that we could
 470 really define a fine scale internal subdivision of France (Figure 3). A general
 471 widespread French haplotypic background moving through the north-south axis was
 472 detected; possibly the overall homogeneity found with the principal component analysis
 473 can be linked to the fact that, on an allele frequency scale, such widespread pattern may
 474 represent a confounding factor. Indeed, only the two Southwestern groups (Basques and
 475 Gascons) were not reached by this common French haplotypic background. Particular
 476 haplotype sharing patterns could also be observed along the north and northeast of
 477 France, in the southeast, and among the northwestern departments.

478

479 In order to understand whether these internal patterns of differentiation are due to recent
480 events or whether they reflect a more ancient history, we relied on different analyses
481 obtaining distinct information. On the one hand, we looked at the relation with modern
482 external populations, exploring both allele-frequency (ADMIXTURE and f3-statistics)
483 and haplotype-based methods (using GLOBETROTTER, we described the ancestry
484 profiles for 10 different French targets, defined by the haplotype sharing with external
485 sources, and provided a date of admixture events for 5 of them). On the other hand, we
486 looked for the continuity between modern France and ancestral populations from
487 different times.

489 **France, *carrefour* of Europe**

490 An ADMIXTURE plot (Figure 4), and a PCA with reference populations
491 (Supplementary Figure 10A) place most French populations as similar to their
492 geographic neighbours, namely the British Isles, Central Europe, Spain and Italy, in
493 accordance with the general observation in Europe of geographic distance as the main
494 predictor of genetic distance^{34,35}. This may explain an apparently surprising outcome of
495 our work: 9 out of 22 distinct targets in f3 statistics we tested against different external
496 sources gave significant results with the lowest Z-scores detected for the same couple
497 represented by the South Italian and Irish sources. Z-scores lower than -3 indicate that
498 our test populations are admixed from sources not necessarily identical but related to the
499 sources we used in the analysis¹¹. Interestingly, these results found support in the
500 outcome from the ancestry profiles we carried out with the Dataset C. The ancestry
501 profiles described in Figure 5 are informative of differential migratory patterns³⁶ into
502 each of the ten French genetic targets. The ancestry profiles are a way to describe the
503 genome of each one of the ten French target as a mixture of the genomes from other
504 groups, without inferring any particular admixture event³⁷. With this analysis, each
505 target is described as a composition of different proportions of haplotype sharing with
506 other sources, excluding the contribution of the group that we want to explain (no self-
507 copying allowed). Following the previous results from the f3-statistics, in the M
508 analysis we found that 7 out of the 10 targets we tested were mostly described by high
509 proportions of haplotype sharing with both Italy and the British Isles. Furthermore, the
510 NM analysis highlighted the presence of a very strong shared French component,
511 possibly reflecting the result of a higher intermixing between individuals from the
512 different parts of modern France.

513

514 An additional dimension to the central genetic position of France in Western Europe is
515 given by the comparison with a time transect of ancient samples. The ADMIXTURE
516 results for dataset D (Supplementary Figure 11), as well as the projected PCA
517 (Supplementary Figure 10B) place France again as intermediate between Southern and
518 Central Europe. However, this pattern is locally nuanced, as discussed below. Thus, it
519 appears that France has been operating as a crossroads for human migration in Western
520 Europe since, at least, the Early Neolithic.

521

522

523 **Basques and Gascons**

524 These groups clearly differentiated from the rest of France both with allele frequency
525 and with haplotype-based methods. It is interesting to notice that the presence of two
526 distinct groups in the Southwestern region stressed the outcome of the isolation the
527 Basque-speaking group experienced, splitting from their non-Basque-speaking
528 neighbors from the very same department (PA and PAB groups). This finding is in
529 agreement with their recognized distinct cultural entity ³⁸ and their genetic outlier
530 position in the European landscape ³⁹, as also with the lower internal levels of
531 differentiation we detected with the F_{ST} analysis, and the low effective migration rates
532 evidenced by EEMS, resulting in a barrier to migration in the southwestern corner of
533 France.

534 The ancestry profile for French Basques (Figure 5) reflects an almost exclusive
535 component from Spanish Basques, with some minor contribution from two other source
536 clusters in the Iberian Peninsula. Quite often, Spanish populations are modelled as the
537 result of a Basque background plus external admixture ⁴⁰, so it is not surprising that
538 haplotypes found in Basques are also present in Spain. French and Spanish Basques, as
539 well as other populations in NE Iberia, share also an increase in shared drift with Early
540 Neolithic ancient samples (Figure 5D). The Basque singularity has often been explained
541 as due to the persistence of an ancient gene pool, as old as the Late Glacial ⁴¹, or as the
542 Pre-Neolithic ⁴², or as the Neolithic ⁴³ (as our results seems to suggest), but a recent
543 analysis of a large number of ancient Iberian samples ⁴⁴ points to a more recent
544 divergence, probably in the Iron Age, of the Basque population.

545

Gascons have been shown to be intermediate between French Basques and other French populations by PCA (Figure 1), and to carry a sizeable proportion of Basque ancestry (Figure 5). This could be the result of the postulated contraction of the Basque-speaking lands since the late Antiquity. Place names may indicate that Basque or languages similar to it may have been spoken in Aquitaine (SW France) south of the Garonne river⁴⁵.

The Celtic connection

As shown by EEMS (Figure 2), a barrier to gene flow delineates the northwestern corner of France, indicating the presence of another distinct group represented by the Breton departments. This group was firstly detected, on a coarser scale, with the removal of the Southwestern samples (Basques and Gascons) from the first PCA, and its outstanding position is in agreement with different studies on both uniparental and autosomal markers⁶⁻⁹. However, based on the fineSTRUCTURE results, in our work we detected a stronger evidence of differentiation based on haplotypic data. ADMIXTURE showed a connection to the Irish samples (Figure 4), which is also indicated by the ancestry profiles of the B1 and B2 targets, which showed higher proportions for the *Irish_Scottish* cluster source (Figure 5). The GLOBETROTTER analysis for determining the admixture dates pointed to some interesting results (Supplementary Figure 9). B2, the largest Breton target, gave signals of admixture around 700 CE, in the time frame of the British Celtic migrations (from Cornwall and south-west Britain) into Gaulish Armorica (then renamed Brittany) from the 3rd to 9th centuries CE, with a higher flow between the 5th and the 6th centuries CE⁴⁶. This completely agrees with previous findings⁷⁻⁹. Historical migrations from Ireland to Brittany are well recorded since the 4th century CE⁴⁷, as well as the emigration of Irish people during the War of Ireland (1641-1651) into the present day departments of Finistère (FI) and Côte d'Armor (CdA), within which a higher integration of the Irish immigrants is proved by records of marriage, birth and death certificates⁷. Furthermore, a Celtic root for the Breton language links the Breton departments to the Insular Celtic languages from the British Isles⁴⁸. Still, the connection may be more ancient. In Figure 6, we explore the three main European ancestral components⁴⁹: the pre-Neolithic hunter-gatherers, the European Neolithic farmers, and the European Bronze Age steppe. Observing the shared drift with

the three hunter-gatherer groups (panels A, B, and C), it is possible to notice how the northwestern departments are mirroring the values shown by the British Isles, the Central-Eastern countries, and Northern Europe. Brittany is thus showing a signal of continuity with the British Isles which could be ascribed to a period older than the later Celtic migration. Always Brittany is acting as an outlier in the case of the shared drift with the Steppe Early and Middle Bronze Age group. In Figure 6 (panel E) it is possible to see how Brittany breaks the northeast-to-southwest decreasing gradient of shared drift. Even in this context, Brittany shows a continuity with the British Isles. Actually, this is consistent with the archaeological records and the development of a late Megalithic culture that characterized Ireland, Britain and Brittany in a period when other parts of Europe were experiencing the advent of metallurgy⁵⁰.

Borderlands

The northeastern rim of France, and the Mediterranean southeastern region represent areas in the perimeter of the *Hexagone* that may have received particular genetic influences. In the ancestry profiles (Figure 5), the NE and SE targets exhibit the most complex genetic make-ups, with a diverse array of sources. The *Central_Eastern_EU* cluster source is mostly represented in the NE target, which includes the departments of Bas-Rhin and Moselle; this area recalls the long history of the Alsace-Lorraine territory: a fuzzy border between France and Germany for a long time, and only recently retroceded to France in 1945⁵¹.

The SE target (most abundant in the Bouches-du-Rhône department) copied from several Mediterranean sources (thus representing the target with more complexity). This area has been a corridor and a landing place for different Mediterranean peoples, since 600 BCE when Greeks established a colony on the Mediterranean coastline of France in the city of Massalia (present-day Marseille)⁵². However, this Mediterranean connection may be older, since the late Epipaeolithic Natufian component (Supplementary Figure 11), which is found almost exclusively in the Mediterranean populations, is found in France in the highest frequency in the Bouches-du-Rhône department.

Conclusions

614

615 In conclusion, according to our results, France is a genetic intermediate between
616 Central, Eastern, and Northern Europe, with some influences from the Mediterranean
617 countries on the southeastern coast. Analyses with both modern and ancient groups
618 pointed to a clear separation of the southwestern groups (Basques and Gascons) and of
619 Brittany from the rest of the French areas. The application of haplotype-based methods
620 allowed us to look beyond the more homogeneous French haplotypic background,
621 discovering connections with the neighbouring populations (e.g., French northeastern
622 departments with central and eastern Europe), while analyses with ancestral populations
623 strengthened the historical connection between Brittany and the British Isles.

624

625 **DATA AVAILABILITY**

626 The genotypes of the samples typed for this manuscript can be downloaded from
627 https://figshare.com/articles/France_Dataset/10008689
628 and https://figshare.com/articles/Naples_Dataset/10008731

629

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639

640 **AUTHOR CONTRIBUTIONS**

641 SAB and FC designed the study; SAB carried out the analyses and interpretations,
642 which were discussed with FC and DC; ERL and DC provided samples and unpublished
643 genotypes. SAB wrote a first draft of the manuscript, with contributions from FC and
644 DC. All authors read and approved the last version of the manuscript.

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770 Main Figures and Tables

771

	Groupings	% Total variance	Φ -statistics	p
A	Variations Between Areas	0.02	$\Phi_{ST} = 0.0026$	0.4605
	Variations Between Departments Within Areas	0.23	$\Phi_{ST} = 0.0023$	0.0009
	Variations Within Departments	99.73	$\Phi_{ST} = 0.00027$	0.0009
B	Variations Between Regions	-0.054	$\Phi_{ST} = -0.0005$	0.7362
	Variations Between Departments Within Regions	0.3	$\Phi_{ST} = 0.003$	0.0009
	Variations Within Departments	99.74	$\Phi_{ST} = 0.0025$	0.0009
C	Variations Between Departments	0.26	$\Phi_{ST} = 0.0026$	0.0009
	Variations Within Departments	99.73		

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773

774 **Table 1.** Hierarchical analysis of molecular variance (AMOVA). Results for percentage of total variance,
775 Φ -statistics, and p-values are reported for the three distinct analyses. **A)** proportion of genetic variation
776 partitioned among geographic areas, among departments within geographic areas, and within
777 departments; **B)** proportion of genetic variation partitioned among regions, among departments within
778 regions, and within departments; **C)** proportion of genetic variation partitioned among departments and
779 within departments

780
781
782 **Figure 1.** Principal Component Analysis of French samples (dataset A) with **A)** Basque and Gascon samples, and **B)** without them. Colors correspond to distinct geographic
783 areas, while different symbols with the same color represent distinct departments in each area (See map distribution). However, Basques are colored differently than the non-
784 Basque-speaking samples from that same area, but symbols recall the departments they share with the non-Basque-speaking groups.

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787 **Figure 2.** EEMS plot based on 395 French samples (Dataset A). Different shades of the same color
788 represent differential levels of high (blue) or low (red) effective migration rates. The zero value indicates
789 the average effective migration rate. Geographical locations for the different departments are averages of
790 the coordinates among samples.

791

Figure 3. Pie charts showing the spatial distribution of haplotypes inferred by the fineSTRUCTURE tree. Each pie chart is a department, while colors correspond to the clusters described in the tree above the map. See Figure 1 for department names. Asterisks indicate departments with only one sample.

Figure 4. ADMIXTURE results from K=2 to K=10 for the 395 French samples (Dataset A) divided in nine major groups, and 12 groups representing external sources from surrounding countries; the lowest cross-validation error was found with K=2.

Figure 5. Ancestry profiles for 10 French targets. Each map is a target defining a specific major area of the French territory. On the left of each map, the donut chart is representing the ancestry profile for the not masked analysis (NM); on the right the same analysis has been masked (M). The different colors represent proportions of haplotype sharing with a specific source (only contributions above the 2.5% are shown); sources are defined in supplementary Figure 8. In the NM analysis, the brown color refers to contributions coming from other French groups (cumulative value). Target names stand for: B1 and B2, Brittany; NE, NorthEast; CSW1, Central-SouthWest; SE, SouthWest; Bas, Basques; G1 and G2, Gascons; CN, Central-North; SW, SouthWest.

Figure 6. Maps showing the distribution of the shared drift between different ancestral populations and the modern ones (X in the f3 statistics). Panels: **A**) f3(Western Hunter Gatherers,X;Mbuti), **B**) f3(Eastern Hunter Gatherers,X;Mbuti), **C**) f3(Scandinavian Hunter Gatherers,X;Mbuti), **D**) f3(Europe_Early Neolithic ,X;Mbuti), **E**) f3(Steppe Early Middle Bronze Age,X;Mbuti). In France, departments with less than two individuals are not shown.

Supplementary Data

Supplementary Figure 1. PCA with 415 French samples highlighted the presence of outliers clearly skewing the global distribution of the samples (A). We assessed the origin of those samples using ChromoPainter and fineSTRUCTURE in the context of external references from three worldwide populations (CEU, YRI, CHB) from the 1000 genomes project⁵³ and North African samples from published data¹¹. Four clusters were defined (B), assigning the majority of our samples (395) to the European cluster. The remaining 20 were outliers mainly belonging to the North African cluster (16 samples), 2 samples each were instead assigned to the Asian and the Sub-Saharan African clusters.

Supplementary Figure 2. Map showing sample distribution among the different departments.

Geographical coordinates are averages among samples. Different colors define the three datasets used in this work (blue dots correspond to the 256 samples genotyped for this work; yellow dots correspond to the 79 samples from Lazaridis et al., 2016; red dots correspond to the 60 samples from unpublished data).

Sample size and acronyms for the departments are: PdD, Puy-de-Dôme (33); CR, Creuse (25); CdO, Côte-d'Or (1); AI, Aisne (1); NO, Nord (47); PdC, Pas-de-Calais (4); PAR, Paris (22); YO, Yonne (1); MO, Moselle (8); BR, Bas-Rhin (48); IeV, Ile-et-Vilaine (45); CdA, Côtes-d'Armor (3); FI, Finistère (5); SM, Seine-Maritime (2); LA, Loire-Atlantique (1); BdR, Bouches-du-Rhône (21); LAN, Landes (10); HG, Haute-Garonne (43); PA, Pyrénées-Atlantiques (15); PAB, Pyrénées-Atlantiques Basque (31); HP, Hautes-Pyrénées (29).

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858 **Supplementary Figure 3.** External group distribution. Average geolocation points for the 79 external
859 populations are displayed. Yellow dots refer to the 333 samples included in the allele frequency analyses.
860 Yellow and red points together represent the 1132 samples used in the haplotype-based analyses

Supplementary Figure 4. Higher hierarchical levels used in the AMOVA analysis for **A)** Regions and **B)** Areas. Grey vertical lines highlight unsampled zones. Acronyms for the Areas are: NW, Northwest; N, North; NE, Northeast; W, West; CN, Central North; E, East; C, Center; SW, Southwest; SE, Southeast.

861

Supplementary Figure 5. Averaged Principal Component Analysis with **A**) Basque and Gascon samples, and **B**) without them. Color and symbol codes are the same as in main Figure 2. For each group, each averaged eigenvalue is represented along with standard deviation bars for the two PCs.

Supplementary Figure 6. On the left: heatmap and dendrogram based on F_{ST} matrices **A)** with the Basque and Gascon samples and **B)** without them. On the right: Multidimensional scaling (MDS) based on F_{ST} values **A)** with the Franco-Cantabrian samples and **B)** without them.

871 **Supplementary Figure 7.** Mantel test of isolation by distance between the genetic (F_{ST}) and geographic
872 (in Km) distances **A**) with the Basque and Gascon samples and **B**) without them. R^2 scores and p-values
873 are within each figure.

Supplementary Figure 9. Dating results for 5 French targets according to the M analysis in GLOBETROTTER. In the left panel, squares refer to one-date, circles to one-date-multiway. The internal color refers to the highest surrogate's value of the major source, while the color of the CI bars corresponds to the highest surrogate's value of the minor source. Sources are represented as horizontal bars on the right side and are separated by a white space (together the sources account for the 100% of the values). In the one-date-multiway cases, two different sets of sources are presented and, where needed, both colors are represented for major and minor sources. Dates have been calculated as $1950 - (g \cdot N)$ where $g=28$ years and N is the calculated number of generations in the GLOBETROTTER analysis.

Supplementary Figure 10. Principal component analysis with dataset D. **A)** Only modern samples; **B)** Projection of ancestral populations from different periods on top of the modern samples (grey dots; among the modern populations, only France is distinguishable as white circles).

Supplementary Figure 11. ADMIXTURE results for K=4 ancestral components using dataset D. Results for the ancient samples are on the top of the figure. Below, modern samples are organized according to major geographical groupings.

Dataset	Samples	Analysis	N° of SNPs
A	395	Allele frequency / Haplotype-based	142,803 / 343,884
B	728 (395 + 333)	Allele frequency	154,889
C	1527 (728 + 799)	Haplotype-based	380,697
D	1687 (1527 - 122 + 282)	Allele frequency	163,631

880 **Supplementary Table 1.** Summary of the dataset composition; both number of samples and number of
881 variants are reported according to the analysis the dataset was used for.

882