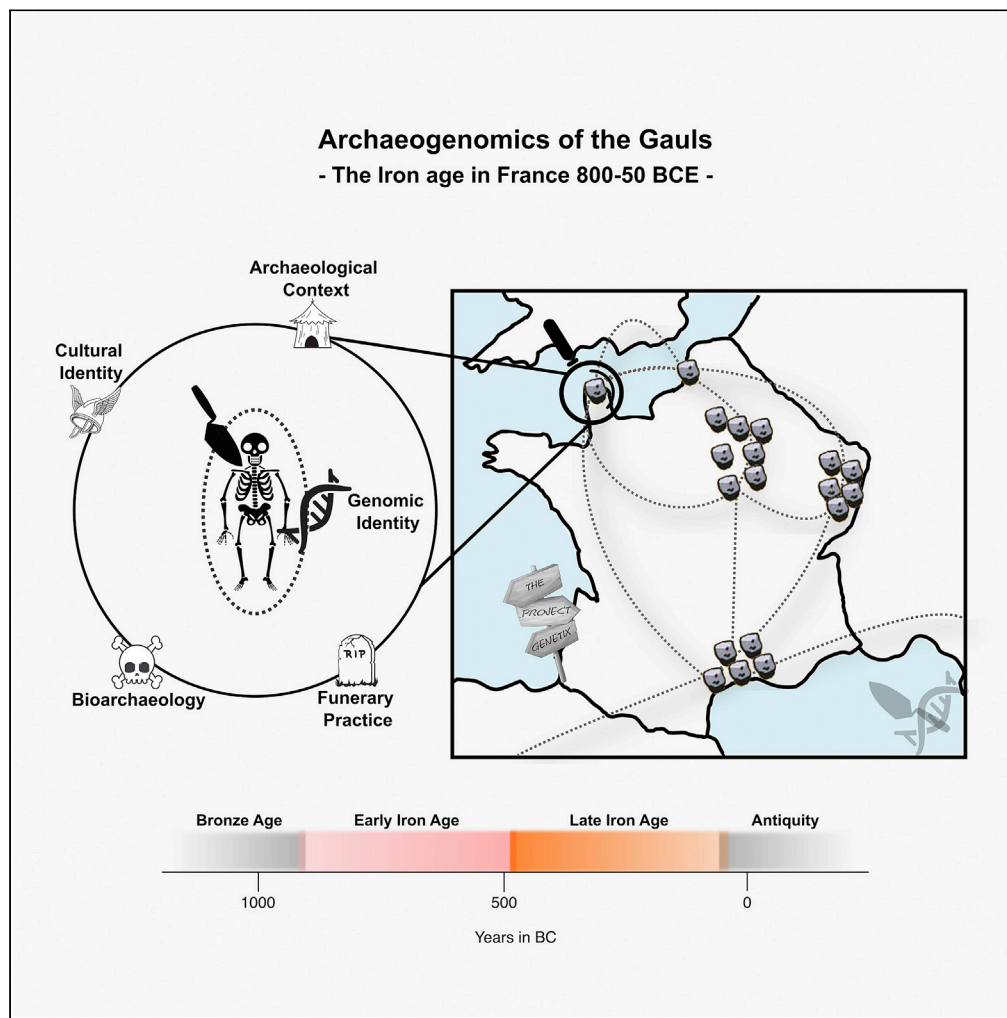


Article

Origin and mobility of Iron Age Gaulish groups in present-day France revealed through archaeogenomics



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Highlights

49 low coverage genomes from 27 sites from France, dated to ≈ 1200–80 years cal BCE

No major migration or population turnover between Bronze and Iron Age in France

A gradual North/South genetic structuration of IA populations

Evidence of individual mobility between regions and neighboring countries

Fischer et al., iScience 25, 104094
April 15, 2022 © 2022 The Author(s).
<https://doi.org/10.1016/j.isci.2022.104094>

Article

Origin and mobility of Iron Age
Gaulish groups in present-day France
revealed through archaeogenomics

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SUMMARY

The Iron Age period occupies an important place in French history because the Gauls are regularly presented as the direct ancestors of the extant French population. We documented here the genomic diversity of Iron Age communities originating from six French regions. The 49 acquired genomes permitted us to highlight an absence of discontinuity between Bronze Age and Iron Age groups in France, lending support to a cultural transition linked to progressive local economic changes rather than to a massive influx of allochthonous groups. Genomic analyses revealed strong genetic homogeneity among the regional groups associated with distinct archaeological cultures. This genomic homogenization appears to be linked to individuals' mobility between regions and gene flow with neighbouring groups from England and Spain. Thus, the results globally support a common genomic legacy for the Iron Age population of modern-day France that could be linked to recurrent gene flow between culturally differentiated communities.

INTRODUCTION

The French Iron Age holds an important place in French history because Gaulish communities are regularly presented to the general public as the direct ancestors of French populations. This major interest has led to an impressive number of archaeological studies describing Iron Age communities through their material culture and funerary practices and questioning their cultural origins and affinities. Despite this interest, questions concerning the cultural and biological processes underlying the emergence and expansion of Iron Age cultures remain intensely debated. Thus, the transition between the Bronze Age (BA) and the Iron Age (IA) was first linked to the rapid shift from bronze to iron technologies between Hallstatt B3 and Hallstatt C (approximately 800 BC). However, this clear cut-off does not appear to reflect the regional archaeological reality that there was a gradual transition to the use of iron instead of a rapid substitution (Verger, 2015). Moreover, the cultural transformations associated with the transition span over two centuries encompassing the late Bronze Age and the first phase of the Iron Age and appear to have followed different rhythms that varied by region (Verger, 2015). Debates also concern the modes of emergence of the Late Iron Age culture *La Tène*, associated with groups generally referred to as 'Celts' and spread over a large part of Europe, spanning from Bohemia to the Atlantic (Roure, 2020). Thus, some authors propose an advent of this cultural entity in Central Europe and Bohemia before its expansion through the migration of groups bringing cultural developments from the northern Alpine area to the rest of Europe (Kruta, 2000; Brun, 2017). Other authors propose a multiregional origin of the *La Tène* culture through the evolution of a mosaic of cultural complexes ('multipolar genesis in networks'; Milcent, 2006) connected by common markers such as art without implying major migration. According to this view, the 'Celts' would be defined as a multitude of related people with different cultural practices (Lejars and Gruel, 2015).

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The great amount of archaeological data available for Iron Age groups from the French territory strikingly contrasts with the near absence of genomic data for the human groups concerned. In the archaeology of death and in the study of ancient human group dynamics, palaeogenetic analyses have led to considerable advances. From the Palaeolithic to the Bronze Age periods, ancient DNA (aDNA) studies have provided arguments complementary to the archaeological evidence to reconstruct the dynamics of the groups at the European macroregional scale (see [Liu et al., 2021](#) for a recent review) and discussions on the social functioning of communities at the local scale (documenting, for example, residence rules or filiation systems; e.g., [Mittnik et al., 2019](#)). Despite the considerable increase in palaeogenomic analyses over the last decade, some territories or periods remain poorly documented. In that respect, the French territory remained neglected in palaeogenomic studies in Europe until the very recent publication of three studies targeting this key crossroad region in Western Europe ([Brunel et al., 2020](#); [Rivollat et al., 2020](#); [Seguin-Orlando et al., 2021](#)). Nevertheless, genetic and genomic data for IA period from French territory remain scarce, with mitochondrial data for 91 individuals and low-coverage genomes for 19 individuals ([Fischer et al., 2018, 2019](#); [Brunel et al., 2020](#)). To date, the underrepresentation of IA populations in palaeogenomic studies, compared with prior periods, can be extended to the European scale, with a total of 44 mitochondrial sequences from Germany, Spain and Italy ([Knipper et al., 2014](#); [Núñez et al., 2016](#); [Serventi et al., 2018](#)) and a total of 27 genomic data points from England ([Martiniano et al., 2016](#); [Schiffels et al., 2016](#)), Bulgaria ([Mathieson et al., 2018](#)), Croatia ([Mathieson et al., 2018](#)), Spain ([Olalde et al., 2019](#)), Hungary ([Gamba et al., 2014](#)), Montenegro ([Allentoft et al., 2015](#)), Estonia ([Saag et al., 2019](#)) and Germany ([Furtwängler et al., 2020](#)).

These observations are particularly frustrating given that only the acquisition of representative palaeogenomic data for French Iron Age groups and their comparison with archaeological data can allow to directly characterize the biological processes potentially involved in the cultural transformations documented between the Bronze Age and Iron Age or between the Early and the Late Iron Age periods. Furthermore, compelling genomic data for these ancient communities provide the only way to test for correlation between the cultural and biological diversities of groups and question modes of exchanges between populations. Finally, genomic data obtained at the local scale can provide major insights into the social organization of communities. For older periods, whether the Neolithic or Bronze Age, palaeogenomic studies have revealed recurrent patrilineal residence rules, patrilineal filiation systems or differences in social level (see, for example, [Lacan et al., 2011](#); [Mittnik et al., 2019](#)). For the Iron Age, the indirect testimonies left by Greeks and Romans (such as *DeBello Gallico* from Julius Caesar, even if they must be considered with caution) mentioned a very hierarchical society characterized by a patrilineal system of filiation. Thus, obtaining genomic data for the Iron Age communities represents a unique opportunity to compare biological, archaeological and textual data.

The outstanding questions presented above and the great potential of the combination of archaeological, textual and genomic data in an attempt to resolve them motivated us to better document the genomic diversity of the Gaulish populations. For this purpose, we targeted 145 individuals from 27 sites spread over the extant French territory and distributed throughout the IA period to optimize our chance of documenting the gene pool of a representative set of French Iron Age individuals. The wide chronological distribution of the dataset permitted us to address questions of origin and evolution of the groups, whereas the wide geographical distribution of the samples allowed us to test for interregional gene flow. Notably, some archaeological evidence highlighted particular exchange networks with the groups from the surrounding areas, such as the example of the necropolis of Urville-Nacqueville, sharing clear archaeological features (roundhouses, Durotrigian burials, etc.) with contemporaneous groups from Britain ([Lefort et al., 2015](#)). Finally, we also targeted sites associated with different funerary practices to better understand the biological identity and potential selection of the individuals buried.

RESULTS

The Iron Age genomic dataset from France

A total of 145 individuals were targeted for palaeogenomic analyses ([Table S1](#)). DNA was extracted, and DNA libraries were built with a partial uracil-DNA glycosylase treatment, allowing for the assessment of postmortem deamination patterns (2%–29%) expected for ancient DNA data. Initial screening via shotgun sequencing of 1 to 2 million reads was used to select libraries with an amount of endogenous DNA above 15%, leading to the exclusion of 92 individuals. For the remaining individuals who passed these quality criteria, we sequenced the libraries to an average depth of 0.178× ([Table S2](#)). We found overall negligible level of contamination in our dataset by testing for heterozygosity of polymorphic sites on the X

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<https://doi.org/10.1016/j.isci.2022.104094>

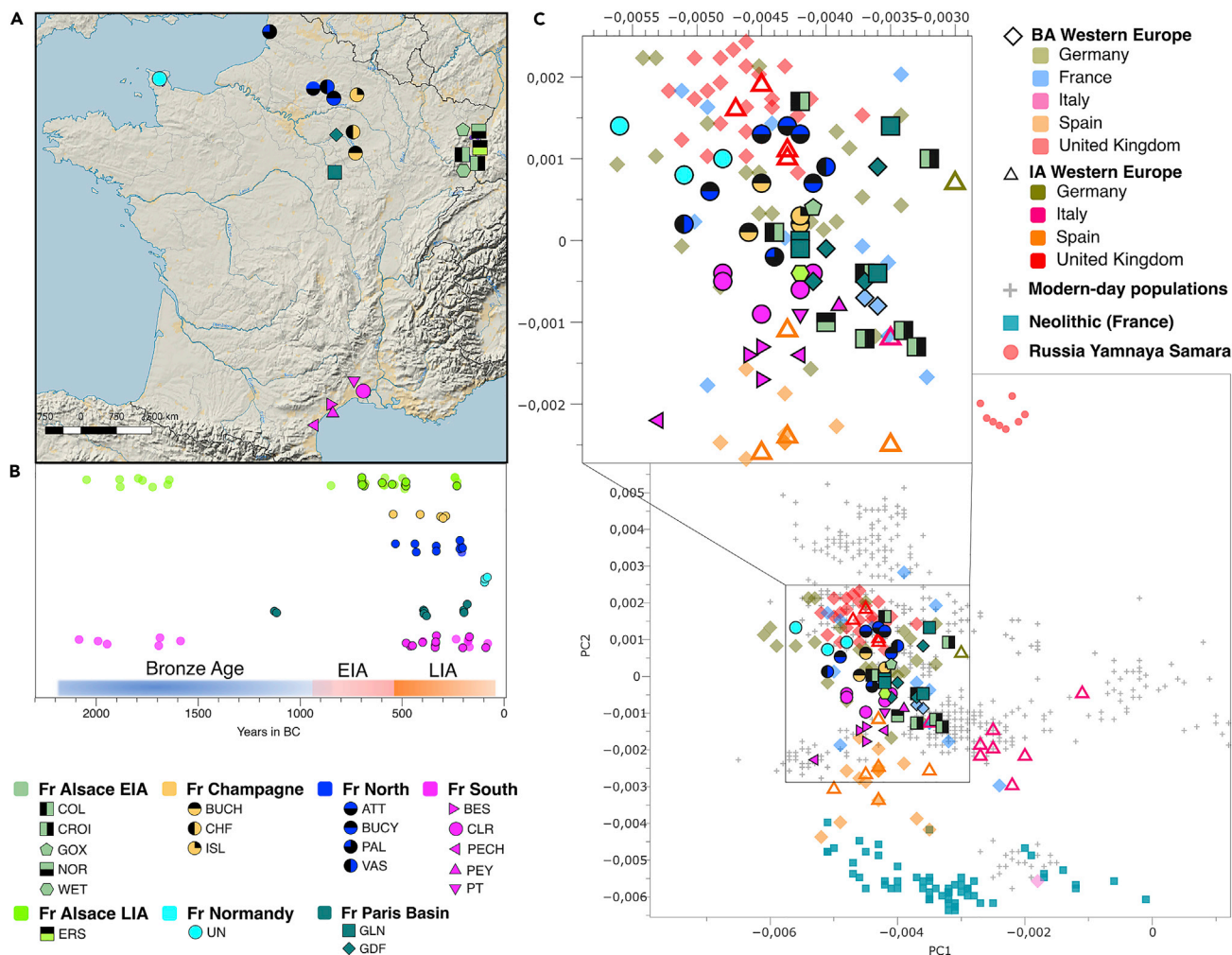


Figure 1. Overview of our dataset

Each colour represents a region, and each symbol represents a site.

(A) Location of samples included in the study.

(B) Timeline of BA and IA individuals with genomic data for the territory corresponding to present-day France. Circles without black outline represent previously published samples.

(C) Principal component analysis of western samples from the Neolithic until the Iron Age projected onto the genomic variability of present-day populations.

chromosome in males (Table S3). The dataset resulting from these successive quality selections encompasses low-coverage genomes for 49 individuals originating from 27 sites, dating from the Bronze Age ($n = 2$) and the Iron Age periods ($n = 47$). We compiled the IA data with 18 low-coverage genomes already published for IA groups from France (Brunel et al., 2020), leading to a total of 65 low-coverage genomes distributed in 6 geographical areas: Alsace ($n = 20$), Champagne ($n = 5$), Normandy ($n = 3$), North ($n = 10$), South ($n = 18$) and Paris Basin ($n = 9$) (see Figure 1A, STAR Methods and Tables S1 and S3). The IA dataset is unbalanced in terms of the chronological distribution of the individuals, with 11 individuals dated to the Early Iron Age and 54 dated to the Late Iron Age period (Figure 1B). This can be partly explained by the funerary treatment and the use of cremation (see, for example, Dedet, 2004 for southern France). The few human remains (from southern or north-western France) available for genomic analyses represent deceased who escaped cremation and benefited from non-ordinary funerary practices. Therefore, the corpus available for genomic analysis may not be representative of the entire population living at the time. For instance, for southern France, genetically analyzed individuals correspond to severed heads (see STAR Methods. site of Le Cailar) or to neonates buried in settlements (see STAR Methods. site Le Plan de la Tour). The dataset is also unbalanced in terms of regional representativeness, with the Normandy

region providing the lowest number of genomes due to the low DNA conservation in the coastal Urville-Nacqueville necropolis targeted (Table S1). Finally, among the 65 individuals, 33 were males and 32 were females, the sex ratio within each region was unbalanced, with notably more females in Alsace and more males in the South (Table S2). With this frame in mind, we analyzed our data with published ancient individuals ($n = 5225$) genotyped on the 1240k panel (Mathieson et al., 2015) as well with modern ($n = 6461$) individuals from a panel of modern-day worldwide populations genotyped on the Affymetrix Human Origins (HO) panel. From the present study's dataset, 65 individuals with more than 20,000 SNPs on the 1240k panel were used for the downstream genome-wide analyses (see STAR Methods and Table S2). We found no first-degree relatives among IA individuals from present-day France allowing us to keep the full dataset for downstream analyses (see STAR Methods, Table S3 and Figure S4).

We first explored our data qualitatively using principal component analysis (PCA) by projecting the ancient genomes onto the genetic variation of an HO set of west Eurasians (Figures 1C and S1). French IA individuals fall within the genomic variability of the modern-day French population. IA samples from Spain and Great Britain also fall within modern-day populations from the same region, highlighting a certain degree of continuity from the Iron Age to modern-day populations in Western Europe, confirming previous results based on mitochondrial DNA (Fischer et al., 2018). The PCA also shows a clinal distribution of our IA French samples according to their latitudinal position: the northern samples are closer to the extant Great Britain population, and the southern samples are closer to the Spanish population (Figure S1). These observations are fully consistent with genomic studies conducted on modern Europeans and highlight a geographically and genomic intermediate position of the French groups between north-western and south-western European populations (Novembre et al., 2008).

To test further the genomic variability of the new IA genomes, we grouped the individuals among different chrono-cultural groups, i.e., according to their region of origin and, when possible, to their dating (Early versus Late Iron Age): EIA_Alsace (from 800 BC to 450 BC), LIA_Alsace (from 450 BC to 50 BC), IA_Champagne, IA_Normandy, IA_North, IA_Paris_Basin and IA_South. We then carried out a *qpWave* analysis iterated overall individuals in the pool, testing for significant evidence of heterogeneity relative to the remaining chrono-cultural group (see STAR Methods and Figure 2). Individuals were considered genomic outliers from the chronological-cultural group from which they originate when the *qpWave* *p* value was <0.05 (Fernandes et al., 2020). This resulted in the identification of six individuals as outliers: BES1248, PECH3 and PEY163 stand as outliers from the IA_South group, CROI11 from the EIA_Alsace group, COL239 from the LIA_Alsace group and GDF1341 from the IA_Paris_Basin group. The analyses at the regional level were consequently conducted separately on these individuals and their chrono-cultural groups. The outlier status of these special individuals will be further discussed.

Genomic continuity from the Bronze Age to the Iron Age

The PCA highlighting Bronze and Iron Age samples from Europe shows relative genomic continuity between groups from the two periods (Figure 1C). To further explore this continuity, we performed a *qpWave* analysis to test whether the regional Bronze and Iron Age groups form a clade. Analyses permitted us to demonstrate an absence of discontinuity (no significant differentiation) between the BA and IA groups in southern France but not in Alsace or in the Paris Basin (Figure 2). The scarcity of data available for the Bronze Age in the Paris Basin ($n = 2$) might not reflect the diversity of the BA population of this region, which could explain this result. Nevertheless, the absence of continuity between both periods in Alsace is documented by a satisfying number of samples and may be linked to notable gene flow in this crossroads region during both periods. Interestingly, the EIA_Alsace and LIA_Alsace groups form a clade, which is consistent with some genetic continuity between periods yet recognizing important cultural transformations (see STAR Methods). Moreover, when looking at the three main ancestral components that contributed to the genomic composition of western European populations, i.e., pre-Neolithic western Hunter-Gatherers (WHG component), Early Neolithic farmers (*Anatolia_N*) and steppe-legacy brought by Bell Beaker groups (*Russia_EMBA*/"*Yamnaya*"; Haak et al., 2015) with *qpAdm* modelling, we observe no significant differences for the last two components between BA and IA populations of southern and northern France (Figures 3A, 3B, and S7) whereas differences exist at the regional level (Figure S8). This indicates the absence of major genetic input from populations with different genetic legacy in the genetic make-up of French IA groups. We then performed *qpAdm* analyses to assess whether the regional Iron Age groups in France could be only explained with the French Bronze Age groups or with supplementary BA groups as sources (see STAR Methods and Table S4). We tested different models and found that all French IA groups could be

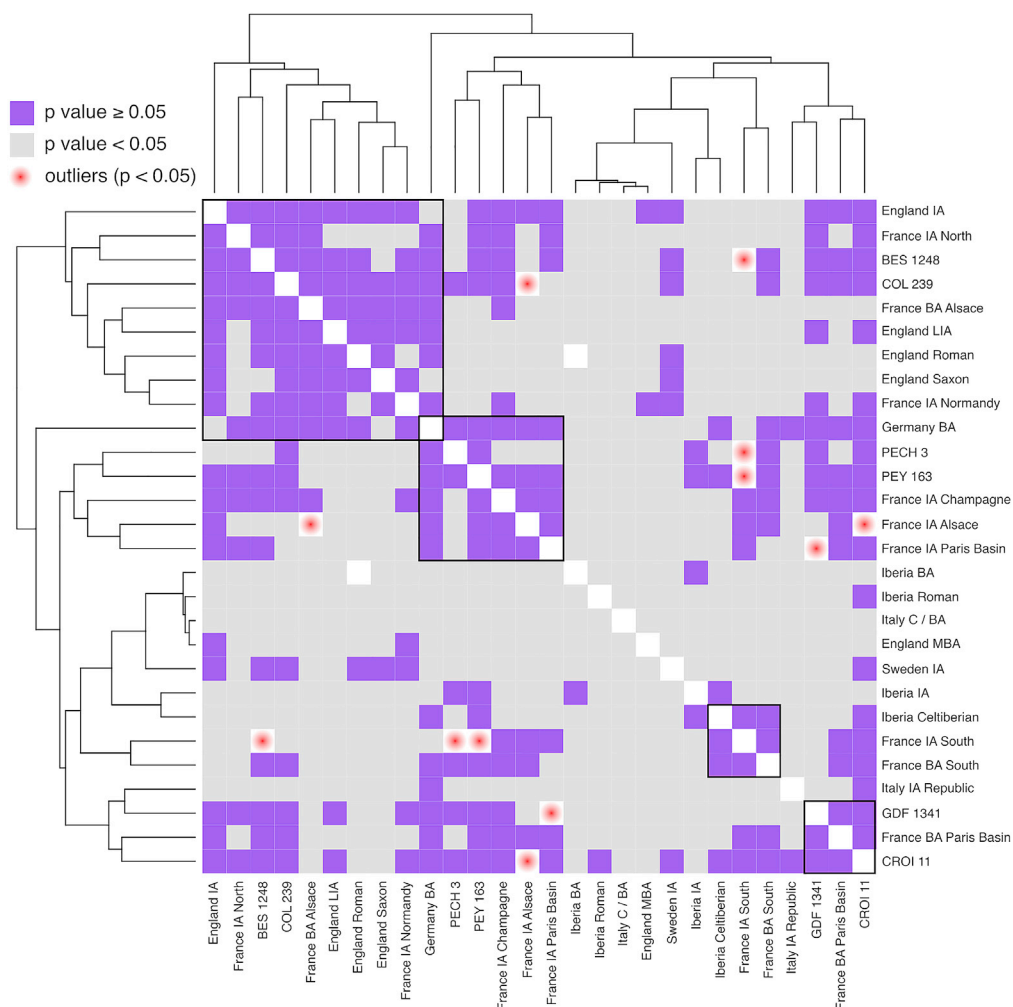


Figure 2. Pairwise qpWave testing to detect outliers

Grey-coloured models have a p value of less than 0.05 and were rejected, pink-coloured models have a p value of more than 0.05. Orange circles represent outliers from the chronological-cultural group from which they originate (p value < 0.05).

explained by one or two French BA groups as sources. Notably, the fact that the EIA_Alsace and LIA_Alsace group gene pools could be explained by a combination of local BA_Alsace and nonlocal BA_South groups as sources reinforces the status of the region as a gene flow crossroad. Although alternative models involving BA sources from other European regions (Table S5) are also statistically possible, following a principle of parsimony, we preferentially suggest that the French IA groups directly derive from the previous French BA groups.

However, when considering uniparental markers, the genetic continuity between the BA and IA groups from French territory can be only partially identified. A total of 86 mitochondrial and 33 Y chromosome lineages were compiled for the French IA sample (see STAR Methods). Despite a great diversity of mitochondrial lineages among French IA groups, nearly 26% of the individuals could be characterized as belonging to haplogroup H. Even though important regional variability in haplogroup frequencies must be pointed out, haplogroup H represents almost 50% of the lineages in IA_North but less than 30% in IA_South groups (Table S3). Notable increases in haplogroup H and J frequencies between French BA and IA can also be highlighted (Figure S2). Regarding the Y chromosome lineages, we observed an increase in Y chromosome diversity in the Iron Age. We identified four major haplogroups in the French IA dataset: haplogroups I1, I2, and G2, which were dominant during the Neolithic in Western Europe, and the overrepresented R1b1a

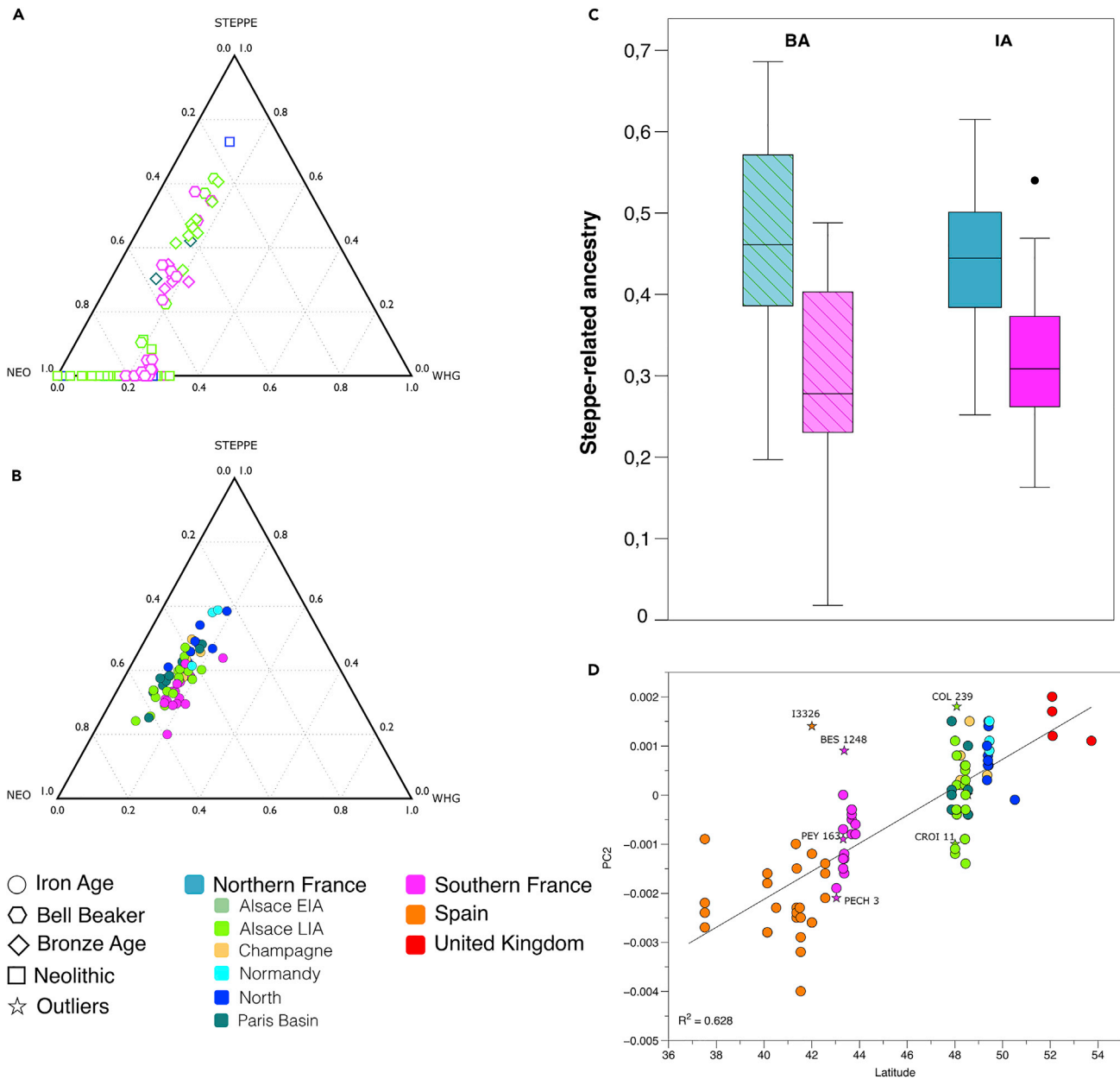


Figure 3. Distribution and average level of ancestral components in IA samples from Western Europe

Each colour corresponds to a region (France) or a country.

(A) Triplot of $qpAdm$ values for the Anatolia Neolithic (NEO), steppe and western Hunter-Gatherer components (WHG) for individuals from the Neolithic to the Bronze Age from France (B) Triplot of $qpAdm$ values for the Anatolia Neolithic (NEO), steppe and western Hunter-Gatherer components (WHG) for individuals from Iron Age from France.

(C) Evolution of $qpAdm$ values for the Steppe related ancestry component in southern and northern French regions, between the Bronze and Iron Age periods.

(D) Relation between the latitudinal position of the archaeological sites where Western Europe IA individuals were found and PC2 values (PCA calculated on the genetic variation of an HO set of west Eurasians).

haplogroup associated with steppe-related migration (69%) (Table S3 and Figure S3) whereas BA males carry only R1b (or R*) haplogroups (Brunel et al., 2020). Nevertheless, it remains to be seen whether the variations observed between BA and IA periods are related to the small amount of data available or to maternal/paternal gene pool shifts linked to specific microevolution processes.

Overall, the compiled results reinforce the archaeological hypothesis that explains the transition from the Bronze Age to the Iron Age by political and economic crisis combined with social changes rather than by major migration or population turnover for the territory of present-day France. These genomic results are in line with more recent archaeological hypotheses that have tended to rule out the involvement of major invasion/migration in the cultural transition (Garcia and Le Bras, 2017). Recent re-evaluations of the archaeological data have indeed indicated progressive cultural evolution during these periods, such as the gradual use of iron (instead of an abrupt substitution from one material to another) or the progressive evolution of pottery (Vergier, 2015). Complicating the scenario, recent archaeological studies have even revealed different cultural evolution rhythms depending on the regions or on the type of material studied (Milcent, 2009; Vergier, 2015). Finally, the genomic data gathered for the Early and Late Iron Age in the Alsace region is consistent with genetic continuity throughout the Iron Age period. Even if the data in hand are restricted to the Alsace region, they support the view that the emergence of the La Tène culture was not necessarily linked to a major influx of populations/genes.

Genomic and cultural diversity among the Gauls

As previously mentioned, the PCA performed on the French IA dataset highlighted a clear latitudinal distribution of individuals (Figure 1C). The genetic differentiation projected on the PC2 axis is positively correlated with the latitudinal position of the sites where were found the samples in France ($r^2 = 0.59$, Pearson). This correlation even increases when adding IA individuals from Iberian Peninsula and England (Figure 3C, $r^2 = 0.628$). However, the f_3 statistic applied in the form $f_3(Mbuti, Ind1, Ind2)$ showed no clear grouping of individuals in relation to their region of origin (Figure S5). To better characterize the genomic variability perceived between French IA individuals, we ran an f_3 statistic in the form $f_3(Mbuti, X, Ind)$, where X represents an ancestral component (WHG, Anatolia_N and Russia_EMBA_Yamnaya). The results clearly pointed out differences between regions of France with a greater affinity between IA_South and the Anatolia_N component, whereas IA groups from northern French (notably Normandy) regions present more affinity with the Steppe-legacy component (Figure S6 and Table S6). To more precisely quantify these gradual affinities, we performed a $qpAdm$ analysis modeling the IA French groups with these three components as source populations (Table S7). The modeling results clearly confirm that a decreasing north to south gradient in the steppe-related component among IA French groups is inversely correlated with an increase in the early farmer component (Figures 3A and S8). The scarcity of data from Bronze Age periods did not allow us to compare these differential affinities at a fine regional scale for this period, but it is worth noting that the distribution of available data into two separated groups, North versus South, permitted us to observe the same tendency (Figures 3B and S7). This steppe-related ancestry gradient is well established for modern-day European populations (Haak et al., 2015) and appears to be well established in French territory, at least since the BA period.

To test for specific genomic affinities between IA French regional groups or individuals, we performed f_3 -outgroup statistics in the form $f_3(Mbuti, Region, Region)$, where Region represents the individuals grouped by geographical affinities (without the previously defined outliers), and in the form $f_3(Mbuti, X, X)$, where X represents individuals from the study (Figure S5). Neithertype of test permitted the detection of any specific affinity between regional groups or individuals. Finally, we also computed f_3 statistics in the form $f_3(Mbuti, Site, Site)$, where Site represents all the individuals discovered within the same archaeological site, and, once again, we did not detect any affinity between funerary groups. We then performed an MDS based on the genetic distance ($1-f_3(Mbuti, Site, Site)$) and did not detect any correlation between genetic and geographical distances between sites. A Mantel test comparing a matrix of genetic distances ($1-f_3$) and a matrix of geographical distances between the sites, with the assumption (H_0) being "there is no correlation between the two matrices", permitted us to confirm the absence of statistical correlation between both types of distances (p value = 0.3931607).

All the statistical tests performed on IA French groups consequently highlighted an extremely gradual genetic structuration of populations distributed across the present-day French territory, complicating the demonstration of statistically significantly differentiated groups. This major observation supports the hypotheses of a common genomic legacy of the populations dispersed throughout this vast area (Collis, 2003; Roure, 2020). This very weak large-scale genomic pattern contrasts with the cultural variability documented among the regions concerned, which remained important enough during the whole Iron Age period for archaeologists to propose to differentiate the Mediterranean, Atlantic, Hallstattian and Latenian Celts (Bouffier and Garcia, 2012; Garcia, 2006). A recent archaeological scenario implies a multipolar

cultural evolution of regional groups sharing some common cultural traits, such as language, religion, and social relations and ornaments and metal furniture (Milcent, 2006, 2009). Combined with the lack of genetic discontinuity pointed out between BA and IA French groups and the global low genetic structuration of groups throughout the IA period, data clearly reinforce a scenario in which regional groups linked through a rich network of cultural and biological exchanges evolve.

Interestingly, biological exchanges between regional French IA groups could be reinforced by the recurrent characterization of genetic outliers, grouping both men and women (see Table S2), which could demonstrate individual interregional mobility. We performed an f_3 statistic analysis in the form $f_3(\text{Mbuti}, \text{Individual}, \text{Region})$ to assess a possible region of origin for each outlier, and we plot the results in Figure 4. The results indeed highlighted genomic affinities between these outliers and French IA groups from other regions representing possible regions of origin of the individuals or possible origins of their direct ancestors. These affinities are also visible through the $qpWave$ analysis (Figure 2). Interestingly, from an archaeological point of view, nothing distinguishes the genetically defined outlier individuals from the others discovered at the same site. This would indicate the full cultural integration of individuals originating from distinct regions. Nevertheless, two exceptions can be pointed out for outlier BES1248 and PECH3. The individual BES1248, from the Bessan site, was found in a single burial, whereas cremation was the predominant funerary practice in southern France during this period (Dedet, 2004). However, the other only adult buried in Bessan (BES1249) does not appear as a genetic outlier and, therefore, impedes drawing any conclusion about the link between outlier status and special funerary features in this case. PECH3 was found in the filling of a ditch associated with the remains of equids contemporary with the ritual phase following the destruction of the Pech Maho site (see STAR Methods for site description). The possibility of an exogenous origin for this individual is particularly interesting as it could reinforce the historical hypothesis of the destruction of the site in relation to the Second Punic War between Rome and Carthage. Interestingly, Olalde et al. (2019) also found a genetic outlier (individual I3326; Figure 3C) in the Ullastret site on the Iberian Peninsula, where many Latenian swords, locally produced, were discovered. This evidence reinforces the idea of important exchanges of goods, people and skills among IA communities. On the other hand, at the Cailar site, where archaeologists have highlighted the practice of severed heads (Ciesielski et al., 2014; STAR Methods; Ghezal et al., 2019), as at Ullastret and Pech Maho, all the individuals analyzed form a genetically homogeneous group and fall within the genetic diversity observed for southern France. Therefore, no genetic element supports the hypothesis of a distant origin for these individuals, whose heads were probably used as trophies. All these specific cases highlight the impressive variability and complexity of interrelation between IA individuals' genetic and archaeological identities.

Interactions between western European contemporaneous groups

Given the gene flow characterized between French IA groups, we tested for equivalent biological interactions with groups from other western European areas. We have seen that PCA and $qpAdm$ analyses highlighted special affinities between IA groups from southern France and Spain and from north-western France and England (Figures 1C and S8). We, therefore, explored these specific affinities through an f_3 statistic in the form $f_3(\text{Mbuti}, \text{IA France}, \text{IA other})$, where *IA other* represents IA groups from regions other than French territory and considering either individuals, sites or regions (see STAR Methods). Unfortunately, palaeogenomic data are still poor or missing for several bordering regions, such as Germany, which limits this part of our study. The performed test did not permit us to significantly differentiate any specific genetic affinities with IA from the European regions documented, surely linked to the very low genomic differentiation of all the groups concerned. $qpWave$ analyses performed between regional groups and other European contemporaneous groups highlight clustering of northern France and north Europe (England and Sweden) IA groups whereas the Gauls of southern France stand out from this cluster but appear closer to the Celtiberians from Iberian Peninsula (Figure 2). Finally, no genetic affinity was found between Greece or Italy and the populations of the IA of the current French territory. Genomic affinities perceived between IA groups from south-western France and northern Spain are expected because they belong to the same cultural entity called ibero-languedocian (Gailledrat, 1997; Py, 1993). Similar types of ceramics and weapons, are indeed found on both sides of the Pyrenees during the IA. Moreover, the settlements and fortifications found in respective regions are quite similar, and some scholars have even proposed the shared use of the Iberian language by the concerned groups (Mullen, 2013). Similarly, the genomic affinity detected between the IA groups from north-western France and England is not surprising. Indeed, the north-western French groups are represented by individuals originating from the Urville-Nacqueville necropolis (Normandy), where specific archaeological features, such as roundhouses, are clearly related to the English IA cultural

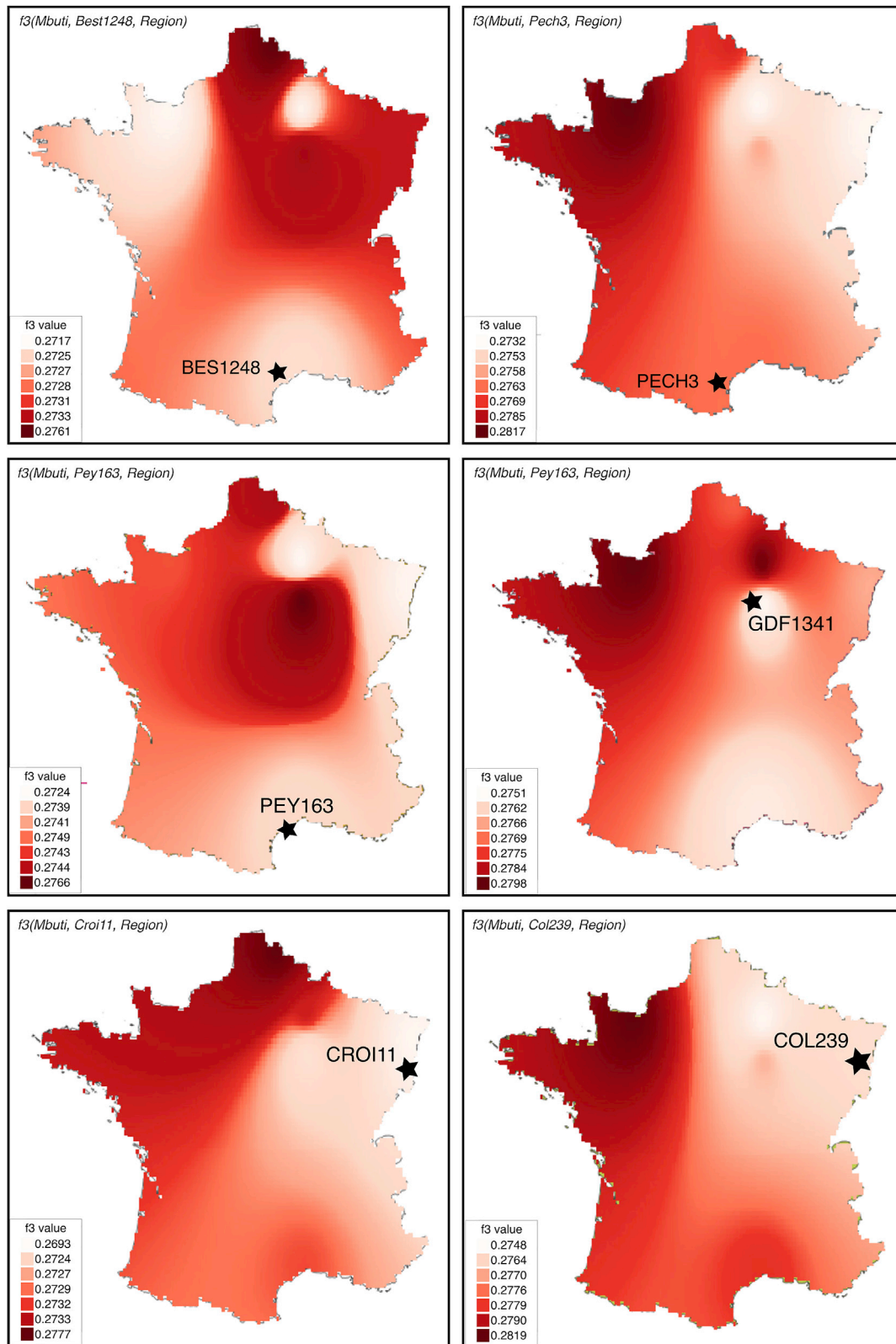


Figure 4. Plot results of the f3-outgroup statistic in the form f3 (Mbuti, Outlier, Region), where “Region” corresponds to Iron Age individuals grouped according to their region of origin

sphere (Lefort et al., 2015). Moreover, Normandy and South England are part of a well-documented network of exchanges during the Bronze Age (Atlantic BA and Manche-Mer du Nord Complex; Marcigny et al., 2017) and the Iron Age periods (part of the medio-Atlantic Iron Age; Milcent, 2006). It is finally worth adding that the three individuals from Urville-Nacqueville that provided genomic data are three males, all buried in the so-called “Durotrigian” position, which is well known in Dorset (Fitzpatrick, 2011; Lefort et al., 2015). Consequently, we cannot exclude that these individuals might originate from southern England and may not be representative of the whole population buried at Urville-Nacqueville (Fischer et al., 2018, 2019). Regardless of the case, the correlation between exogenous material, cultural variations, and gene flow with groups from other regions found in these specific contexts cannot be extended to the full IA archaeological landscape. This is notably the case for the necropolis of Peyrou (southern France, 6th–4th centuries BC), where the funerary treatment of the deceased and the material deposited in the tombs provided clear evidence of the establishment of Greek settlers in the region after an intermittent phase of contacts. However, none of the individuals analyzed ($n=3$, male or female) from the necropolis show peculiar genetic affinity with Greece or the Mediterranean Basin. Interestingly, the individual who stands as an outlier (PEY163) seems to have more affinities with the Paris Basin and did not point out to the Mediterranean area (Figures 2 and 4). As our study is not exhaustive for this site, it is quite possible that the analysis of a larger number of individuals would allow one to highlight the presence of Greek settlers in this necropolis. Indeed, Greek colonies are a very particular environment, where the surrounding native population can live among the Greek settlers (Dedet, 2015). However, this example once again demonstrates that funerary practices and/or exogenous material do not constitute reliable evidence of allochthonous individuals, as already described for other periods/regions (O’Sullivan et al., 2018).

An important result is also the greater dispersion of IA_Alsace individuals in the PCA, overlapping groups from IA_South, IA_North, IA_Paris_Basin or IA_Champagne. This dispersion points to higher genetic diversity within this regional group, which could be evidence of higher gene flow (Figure 1C). The Alsace region is regularly characterized as a “crossroads”, an axis of transit and exchange, because of the presence of the Rhine River, which has constituted a major communication link between western and central Europe through history. Nevertheless, the genetic exchanges characterized for the IA period do not appear to find a special echo in the material culture. During the Early Iron Age, archaeological records suggest material exchanges between southern Alsace and southwestern Germany (Bavaria, Baden-Württemberg), whereas northern Alsace shared contacts with the Lower Rhine Valley. If the arrival during the Hallstatt period of material described as exogenous (amber from the Baltic region, coral from the Mediterranean, Etruscan imports, etc.) could testify to a notable increase in north-south trade, this kind of material remains very occasional. Therefore, even if cultural exchanges with neighbouring regions are verified, contributions from further distances remain apparently limited.

Functioning of communities

To start documenting the local functioning of French IA groups, we performed an analysis to detect long runs of homozygosity (ROH, Ringbauer et al., 2021). We observed that the number of ROHs of 4–8 cM tended to decrease during the Iron Age following the general tendency observed since the Mesolithic, a result explained by a progressive increase in the mating populations and/or an increase in long-distance gene flow (Figure S10). Few archaeological studies have focused on the IA population demography; nonetheless, a study conducted on funerary data by Isoardi (2008) found an increase in population size at the end of the Early Iron Age in the Southern Alps, Provence and probably in the Rhone and Saone valleys, followed by a slight decrease around the mid-4th century BC. They also noted that population size tended to increase during the second half of the 3rd century BC. Various studies have also demonstrated the importance of exchange networks at varying distances during the Iron Age, as evidenced, for example, by the Greek bronze crater in the tomb of Vix (Early Iron Age, Joffroy, 1954) or the Roman amphorae discovered at Urville-Nacqueville (Late Iron Age, Lefort et al., 2015), but we found no evidence of such long-distance exchanges in our data. Such events might have been restricted to a few individuals playing an important role in Iron Age society but having a limited impact on the genetic pool.

Finally, we detected multiple long ROH on different chromosomes in one individual, COL 336 (Alsace), who can be interpreted as the offspring of a first-degree incestuous union (parent-offspring or full siblings) (Figure S11). Intriguingly, the Colmar site includes several burials in pits without any grave goods or weapons accompanying the deceased, whereas the main funerary practice for this period and this region is the inhumation in small funerary complexes of tumuli. Therefore, even if the observed funerary treatment of COL

336 does not differ from that of any other individuals from the site, it could be considered as a relegation-type burial. This could be linked to a rejection by the IA society of this incestuous practice. This is in opposition to what has been described at the site of Newgrange for Neolithic period (Cassidy et al., 2020), where the presence of consanguineous individual on one of the most spectacular Megalithic mound of Europe was interpreted as the evidence of a high hierarchical society with complex chiefdoms. To explore more in details the social organization of IA groups and compare it with historical sources, a more exhaustive genomic dataset would be necessary.

DISCUSSION

In this study, we recovered 49 genomes from BA and IA individuals widely distributed within present-day France. With this valuable dataset in hand, we were unable to detect a genetic discontinuity between the Bronze and Iron Age communities of France, as already mentioned by Brunel et al. (2020). Our dataset also highlighted a north to south gradient for steppe-related ancestry inversely correlated with the Early Neolithic Farmer one. Moreover, the distribution and proportion of these legacy components remain stable between Bronze and Iron Age periods. This is perfectly in line with recent archaeological evidence considering that the transition from the Bronze to the Iron Age was a consequence of social and political changes from the 8th century BC onwards. If no evidence of migration event could be highlighted since the BA, although events of this type involving populations with the same genetic characteristics are hard to perceive, we were able to detect mobility at the individual scale between regions and gene flow with neighbouring groups from England and Spain. Of great interest, these genetic outliers were not always distinguishable from an archaeological perspective, which could mean that they were fully integrated within the community. Although analyses allow us to propose a possible region of origin for these outliers, Sr isotope analyses would be of great interest to complete the individual mobility scenario. Interestingly, these networks are visible not only in the French territory but also on the western European scale. Indeed, we were able to detect specific affinities between northern/north-western France and England IA communities and between southern France and Spain communities. This result is consistent with archaeological evidence, such as the presence of roundhouses and so-called 'Durotrigian' buried in Urville (Normandy) and the definition of the 'Ibero-languedocian' complex in the South. Globally, the results proposed reinforce the idea that 'Celts' derived from local BA populations that evolved progressively between regional groups sharing some common cultural traits and linked through a network of cultural and biological exchanges.

Limitations of study

Due to the extensive use of cremation in the period considered, the highly variable DNA preservation among sites and the unequal distribution of archaeological discoveries among regions, the genomic dataset obtained remains differentially distributed among regions and periods.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
 - Lead contact
 - Materials availability
 - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
 - Archaeological and anthropological information
- METHOD DETAILS
 - DNA extraction and sequencing
 - Read processing, alignment and post-mortem damage
- QUANTIFICATION AND STATISTICAL ANALYSIS
 - Sex determination
 - Contamination estimates
 - Uniparental markers
 - Kinship analyses
 - Inbreeding and diversity estimates
 - Principal component analysis

- Genetic clustering and outlier detection
- f3-outgroup and f4-statistics
- Population modeling
- Data and software availability
- **ADDITIONAL RESOURCES**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104094>.

ACKNOWLEDGMENTS

We thank the Service Régional de l'Archéologie from Normandy, Paris Basin, Occitanie, Grand Est, Hauts-de-France, ANTEA-archéologie, and Institut National de Recherches Archéologiques Préventives (INRAP) for providing bone materials. We are grateful to Olivier Gorgé for making shotgun sequencing possible at Institut de Recherche Biomédicale des Armées in the Division Defense NRBC (Nucléaire, Radiologique, Biologique et Chimique), Département des Services, Unité de Biologie Moléculaire. This work was supported by the Agence National de la Recherche through the Grant ANR15-CE27-0001 (ANCESTRA project, coord. M. Pruvost), by the INRAP through the PAS Ancestra (Coord.MP.Pruvost and F. Maziere), and as part of a program of prospects investments ANR-10-LABX52 (Genetix project, coord. MF. Deguilloux and M. Pruvost, as well as Identi-Fer project, coord. S. Rottier), ANR11-LABX0032-01 ARCHIMEDE (LANGPOP project, coord. F. Maziere and "Des armes et des têtes coupées" project, coord. R. Roure). The study was also financially supported by a PhD research grant from the Ministère de l'Enseignement Supérieur et de la Recherche for CEF. We are grateful to the genotoul bioinformatics platform Toulouse Occitanie (Bioinfo Genotoul, <https://doi.org/10.15454/1.5572369328961167E12>) for providing help, computing and storage resources.

AUTHOR CONTRIBUTIONS

Conceptualisation: CEF, MFD, MP. Data curation: CEF, MHP, ID, MFD, MP. Resources: HBE, AB, EC, BD, SD, FC, EG, SG, AG, AB, GK, FL, AL, AM, FM, SO, CP, EP, SP, IR, MRZ, RR, CT, YT and SR. Formal analysis: CEF, MFD, MP. Funding acquisition: SR, MFD, MP. Writing – original draft: CEF, MFD, MP. Writing – review & editing: all.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: December 14, 2021

Revised: February 17, 2022

Accepted: March 13, 2022

Published: April 15, 2022

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>Biological samples</i>		
osteological remain	this study	Att27
osteological remain	this study	Att3
osteological remain	this study	Att52-2
osteological remain	this study	BES1096B
osteological remain	this study	BES1154
osteological remain	this study	BES1249
osteological remain	this study	BFT228
osteological remain	this study	BLH447
osteological remain	this study	BPV1445
osteological remain	this study	BPV1455
osteological remain	this study	BUCH48-1
osteological remain	this study	BUCH48-2
osteological remain	this study	BUCH82
osteological remain	this study	CHF106
osteological remain	this study	CLR23
osteological remain	this study	CLR24
osteological remain	this study	CLR31
osteological remain	this study	CLR35
osteological remain	this study	CLR44
osteological remain	this study	Col239
osteological remain	this study	Col330
osteological remain	this study	CROI1-4
osteological remain	this study	CROI11
osteological remain	this study	CROI12-2
osteological remain	this study	ERS83-2
osteological remain	this study	GDF1231
osteological remain	this study	GDF1264
osteological remain	this study	GDF1341
osteological remain	this study	GDF1348
osteological remain	this study	GDF1349B
osteological remain	this study	GLN126A
osteological remain	this study	GLN126B
osteological remain	this study	GLN141
osteological remain	this study	GLN29-A
osteological remain	this study	GLN32
osteological remain	this study	Gox287
osteological remain	this study	ISL6950
osteological remain	this study	PAL170
osteological remain	this study	Pech3
osteological remain	this study	Pech9
osteological remain	this study	PEY73

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
osteological remain	this study	PT7
osteological remain	this study	SCPG2
osteological remain	this study	UN129
osteological remain	this study	UN19
osteological remain	this study	UN85
osteological remain	this study	VAS75
osteological remain	this study	VAS79-2
osteological remain	this study	WET429

Chemicals, peptides, and recombinant proteins

Proteinase K 100MG	Sigma Aldrich	Cat# 3115879001
Buffer QG	Qiagen	Cat#19063
OneTaq® 2X Master Mix with Standard Buffer	New England Biolabs	Cat# M0482L
Phusion® High-Fidelity DNA Polymerase	New England Biolabs	Cat# M0530L
NEBNext End Repair Module	New England Biolabs	Cat# E6050L
NEBNext Quick Ligation Module	New England Biolabs	Cat# E6056L
USER Enzyme	New England Biolabs	Cat# M5505L

Critical commercial assays

MinElute PCR Purification kit	QIAGEN	Cat# 28006
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific	Cat# Q32854
NextSeq 500/550 High Output Kit v2.5 (150 Cycles)	Illumina	Cat# 20024907
Agilent High Sensitivity DNA Kit	Agilent	Cat# 5067-4627

Deposited data

Raw and analyzed data	This study	ENA Project: PRJEB50940
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Software and algorithms

READ	Monroy Kuhn et al., 2018	https://bitbucket.org/tguenther/read/src/master/
Admixtools	Patterson et al., 2012	https://github.com/DReichLab/AdmixTools
Yleaf	Ralf et al., 2018	https://github.com/genid/Yleaf
Haplogrep	Dür et al., 2021	https://github.com/seppinho/haplogrep-cmd
ANGSD	Korneliusson et al., 2014	https://github.com/ANGSD/angsd
smartPCA		https://github.com/DReichLab/EIGtree/master/POPGEN
bamUtil		bamUtil 71 https://github.com/statgen/bamUtil
EAGER	Peltzer et al., 2016	https://github.com/apeltzer/eager-gui
AdmixR	Petr et al., 2019	https://github.com/bodkan/admixr

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Melanie Pruvost (melanie.pruvost@u-bordeaux.fr).

Materials availability

Raw sequence data and alignments are available at the European Nucleotide Archive (ENA) under accession number ENA PRJEB50940.

Data and code availability

- Genomic data have been deposited at the European Nucleotide Archive (ENA) and are publicly available as of the date of publication. Accession numbers are listed in the [key resources table](#). All other previously published genomic data used in this study is available at the sources referenced in the [quantification and statistical analysis](#) section.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Archaeological and anthropological information

Nordhouse, lieu-dit "Buerckelmatt" (Bas-Rhin)

Person in charge: S. Plouin, F. Lambach

The necropolis of Nordhouse, formed by six tumuli, is located approximately 20 km south of Strasbourg within the alluvial plain of Ill river. These tumuli are mostly dated from the Late Bronze Age IIIb, with cremation materials deposited in the center of a ditched circular enclosure. Accompanied by rich ceramic items, cremated human remains were placed within a wooden architecture. Characteristic potteries from the same phase were found in the eastern sector of each ditch.

The necropolis of Nordhouse was then occupied between the 7th and 4th centuries BCE (between Hallstatt C2 and La Tène B1). During the first and early Iron Ages, numerous weed burials were deposited in the tumulus, and each monument hosted between 14 and 27 graves. A total of 101 burials were found. An exceptional female tomb stands out for the abundance and richness of its grave goods, including glass, amber, coral, a large shell from the Red Sea and many gold ornaments.

In the present paper we used the genomic results previously published for four individuals ([Brunel et al., 2020](#)), originating from 3 different monuments. Human remains NOR3-15 (an adult female buried with various ornaments) and NOR3-6 (an adult, possible female, buried with an infant and various ornaments) were discovered in tumulus 3, whereas NOR2B6 (an adult male buried with an infant) and NOR4-4 (a young female buried with various ornaments, potentially corresponding to an aristocratic individual) were found in tumulus 2 and 4, respectively.

Author of entry: S. Plouin and F. Lambach

References: in prep

Sainte Croix en plaine (Haut-Rhin)

Person in charge: F. Chenal, Y. Thomas, S. Plouin

On the territory of the commune of Sainte-Croix-en-Plaine, located 11 km south of Colmar (in Alsace, Haut-Rhin), one of the largest funeral complexes discovered in the department extends over more than one hectare. It has been the subject of several preventive excavation campaigns, in 1979–1981, 1997, 1999 and 2005. More than a hundred funerary structures have been unearthed there, mainly within mounds of which only the circular ditches that surrounded them remain today.

The results of these excavations have provided invaluable information on the knowledge of regional funeral practices between the end of the 2nd and the middle of the 1st millennium BC. The alternation of burial and cremation practices and the occasional presence of wooden containers adapted to the morphology of the deceased could be notably pointed out.

The funerary structures included a rich material consisting mainly of ceramics and female bronze ornaments dated between the 7th and early 4th centuries BC. Most of them were produced from bivalve stone moulds, in which the metal was cast. The objects were then embellished with a chiselled or engraved decoration

that may have completely covered them. The most remarkable bracelets are local products and represent major testimonies of Celtic craftsmanship.

In the present study, we propose genomic results from four individuals from this site. Three of them were discovered in the 'Oberes Holzackerfel' location: Croi1-4 (an adult buried with an exceptional iron razor, S1-4), Croi11 (an adult buried in one of the richest burials on the site, deposited with several grave goods such as an amber bead, S11-2) and Croi12-2 (an adult buried with a lignite bracelet, S12-2). Individual SCPG2 was excavated in the 'Oberholtzackerfeld/Echangeur Gendarmerie' location.

These individuals were combined to the Jeb8, originating from the same site and previously published in [Brunel et al., 2020](#).

Author of entry: F. Chenal

References: in prep

Colmar "Jardin des Aubépines" (Haut-Rhin)

Person in charge: M. Roth-Zehner, A. Mauduit.

The settlement of "Jardin des Aubépines" is located in the southwest of the commune of Colmar on a loess layer very favorable for the establishment of pre- and protohistoric deposits. Excavation of the site has established the presence of an imposing ensilage site that begins at Hallstatt B1 and ends at La Tène B1.

Nine individuals (2 women, 2 men, 2 immature and 3 undetermined adults; anthropological study: A. Mauduit) were deposited in silos. Some subjects (ST.336, ST.363) were deposited at the bottom of the structures. In all other cases, the burials were deposited when the silo was already beginning to fill in. Some of them were adorned with bronze or iron fibulae attributable to La Tène B1, bracelets and anklet rings. The silo fillings also delivered ceramics from the same period ([Landolt et al., 2010, 2012](#)).

The selection of the individuals deposited in these special structures does not appear to be governed by biological criteria such as age or gender. When compared to funerary groups recovered in cemeteries, no special features could be observed, whether at the level of health status (they have no more pathologies or stress markers), of the goods accompanying the deceased, or of the positions and orientations of the individuals.

At the proximity of the two silos grouping the human deposits, a silo containing the deposit of approximately ten animals (st. 235), whose skeletons are complete, is noteworthy. The localization of this animal deposit raises the question of its association with the human burials discovered nearby. Among the animals, archaeologists could identify a horse (a small Gallic horse) from which the skull was removed, several dogs, one of which was found in a suit (a large dog), at least one piglet and several lambs. The animals discovered in this structure 235 were deposited with great care and therefore cannot be considered as usual rejects from habitat context.

In the present study, we present genomic data for three individuals: Col239 corresponding to an adult (30–60 years old) male buried with some grave goods, Col330 corresponding to an adult (30–39 years old) male and Col336 corresponding to a young (15–25 years old) female buried with a copper bracelet and presenting a spinal injury that might have been infectious. These individuals were combined to two other individuals, Col153A and Col153i, originating from the same site and previously reported by [Brunel et al., 2020](#).

Author of entry: M. Roth-Zehner

Wettolsheim "Ricoh" (Haut-Rhin)

Person in charge: C. Jeunesse, M. Roth-Zehner

The Hallstattian Necropolis "Ricoh" (1987–1990) is located on loessic land to the east of the Wettolsheim municipality. It covers an area of approximately 1.2 ha and consists of ten burial circles grouping a total of 38 burials and 19 cremations. Half of the tombs are located outside the burial enclosure. The occupation of

the necropolis begins at Hallstatt B2/B3-Hallstatt C and ends at Hallstatt D1. The cremations belong to the initial phase, while the burials mainly date from Hallstatt C2 and D1. The deceased were found placed in wooden containers into large tombs, with a series of vases regularly placed near their feet. Some individuals were adorned with bronze bracelets presenting geometric decorations typical of the Upper Rhine plain, as well as bracelets of lignite, bronze belts, amber and glass beads (Jeunesse, 1988).

In the present paper, we present the results for individual Wet429, a young male.

Author of entry: M. Roth-Zehner

Goxwiller (ZAC PAEI, Bas-Rhin)

Person in charge: S. Goepfert

Goxwiller is located approximately 25 km southwest of Strasbourg and 5 km east of the Vosges foothills, on a terrace made up of loessic wind deposits. The excavation, carried out in 2011, covers a total area of 3.8 ha.

The first indications of occupation date back to the early Bronze Age and correspond to a well and a pit. After a hiatus of several centuries, a final Bronze Age IIIa complex, consisting of seven widely dispersed structures, was established on the right-of-way. These structures belong to a domestic ensemble whose exact nature cannot be established.

The Early Iron Age is the period best represented on the site with 96 structures that can be attributed to the Hallstatt period. Apart from a few miscellaneous excavations (polylobed pits, oblong pits, simple pits), the majority of the structures identified correspond to silos that hosted at least 45 individuals. Three of the silos had a particular filling, indicating that they had been reintervened or excavated in their center. One of them, St.287, yielded the skeleton of a child aged between 7 and 11 years old. The skeleton, which was deposited in the original structure, was only superficially affected by the reintervention, which seems to have been quickly filled in. One of the skeletal bones was radiocarbon dated indicating a range covering the entire Hallstattian period (excluding Ha D3): 791-519 BC to 2 σ (Poz-47205, 2510 \pm 35 BP).

Here, we present data for Gox287, a young individual (7–11 years old).

Author of entry: S. Goepfert

Erstein (Untergasse, Bas-Rhin)

Person in charge: F. Abert

In 2016, a survey archaeological excavation at the Erstein "Untergasse" uncovered an important diachronic site with more than 1,300 archaeological structures.

A first occupation of the Second Iron Age was identified in the western part of the site, encompassing approximately ten burials with exceptional artefacts dated from La Tène B. The burial installation in a rough circle in a restricted space indicates that they were probably originally surmounted by a burial mound. A few nonfuneral structures are also attributable to La Tène, including a silo that provided numerous fragments of ceramics typical of this period and a ditch that crosses the right-of-way from east to west.

The Gallo-Roman period is also represented on the site, particularly in the eastern half of the right-of-way. The structures could only be poorly documented, as most of them have not been excavated. Nevertheless, they have been the subject of a removal of surface material after they had been stripped.

The main occupation of the site dates back to the early Middle Ages. It covers the entire stripped surface and is exceptional in terms of size, density and organisation. It also has the particularity of combining settlement structures with funerary areas. Numerous archaeological structures intersect here, bearing witness to a dense and long-lasting human activity. The settlement contains numerous hut bottoms, storage structures (silos and cellars), wells, post hole drawing plans of buildings and numerous pits. Most of these remains are organised around two or three parallel axes, corresponding to communication routes oriented along a north-south axis.

The funerary structures can be divided into two distinct types. In the western part of the site, isolated burials or graves in groups of two to three graves are present among the habitat remains. To the east, along the northern boundary of the excavation, a small cemetery contains numerous burials, the density of which and some overlaps indicate that they were isolated from the habitat in a reserved space which suggests the existence of a religious building nearby.

Here we present genomic results for Ers83-2, a young individual (approximately 10 years old). This individual was combined to three other samples (Ers1164, Ers86 and Ers88) previously published in [Brunel et al., 2020](#).

Author of entry: F. Abert

Buchères « PLA - secteur 1A » (Aube)

Person in charge: V. Desmarchelier

In 2014, an excavation was carried out in the Aube Logistics Park, in the commune of Buchères, in the south of the Troyes conurbation. This operation was part of the PLA development project, which began with archaeological diagnostics in September 2004. The two stripping operations of 2014 - D41 and D42 - were located to the southeast of this project, in the area known as the "Vignes Neuves" and about 700 m from the Fontaines de Savoie stream, which crosses the Logistics Park from north to south. They are about 100 meters apart and are located on a plateau which also concentrates excavation operations D01 (2005), D37 (2011) and D43 (2015). Overall, over the whole of the PLA, the various operations have revealed occupations of domestic or funerary nature, from the early LBK to the modern period. The 2014 excavation has uncovered structures covering approximately 1 ha, indicating discontinuous occupation from the ancient Mesolithic to the modern period.

The Second Iron Age is the period best represented. Nearly 20 silos - two with burials and two with dog deposits - associated with pits, post holes, a polylobed pit and a hole containing human skeletons are evidence of domestic occupation in this sector ([Desmarchelier, 2020](#)).

Almost all of the silos delivered ceramic that permitted anchoring their use as a dump at the end of La Tène A2 and during La Tène B1. Interestingly, this period is poorly represented at the local scale despite the start of a peak in the intensity of this type of storage during this period in northern France. There is no dating evidence to establish a proven succession of structures over time, all falling within a broad framework of approximately one hundred years at the beginning of the Second Iron Age. However, as no overlap between structures has been observed, a contemporary or chronologically very close use of the structures can be assumed. Mostly consisting of small domestic waste, the assemblages from the silos do not evoke any particular activity other than conversion into domestic dumps or - in the case of dog dumps or burials - for ritual or funerary purposes.

The hole, located to the east of the pickling, was in itself an enigma because of the human skeletons discovered at a considerable depth in its filling. In addition to these deceased, deposited respectively at 3.90 and 5 m deep, this structure also revealed numerous remains of fauna at 7 m deep. Certain clues related to decomposition indicated the presence of localised empty spaces inducing a cover of the structure above the human remains. These men did not appear to have suffered from any pathology, traumatic or infection, apart from a sprained right ankle observed for one individual. There is no evidence to indicate the context of their deposition, whether it was an accidental and fatal fall or a burial with a cover.

Since the first archaeological interventions in the Logistic Park in 2005, 23 individuals have therefore been discovered in 14 silos and one pit, most of which were deposited on the dome of the first fill or closer to the bottom of the structure, between Hallstatt C and Tène C. Likewise, like the deceased from the PLA as a whole, the individuals discovered in the silos and the hole are on the whole lacking in stress indicators and pathologies, with signs of senescence being rare and not very intense.

In the absence of more eloquent evidence, the location of the habitat on which these structures depended remains hypothetical. In fact, even if it can be assumed that it was located close to those that delivered most of the furniture, no characteristic structure was found there. It should be pointed out that, with regard to this

chronological phase, small, lightly built dwellings have little chance of being preserved, as the batteries of silos, due to their depth, are more easily resistant to erosion.

Here we present the genomic results for two individuals: Buch48-1 and Buch48-2 who are two adult males deposited in wells, which is quite rare for the period and the region.

Author of entry: I. Richard

Buchères, Mousse, Saint-Léger-près-Troyes « PLA – D37 et D38 » (Aube)

Person in charge: C. Paresys

In 2011, Inrap carried out an archaeological excavation in the commune of Buchères, in the “Parc Logistique de l’Aube” (Aube, Champagne Ardenne). This excavation, with a total area of 12,395 m², divided into two separate windows, uncovered several sets of structures covering a vast chronological field from the Neolithic to the contemporary period and different functions (land use, funerary, agricultural; Paresys, 2014).

The main occupation of the site is materialised through eleven funerary enclosures of various shapes, nine circular, one quadrangular and one elliptical. Some ceramic fragments were found in their filling, divided into two main periods, the end of the Neolithic and the end of the first Iron Age. Radiocarbon analyses are consistent with these two periods, and three of the oldest enclosures could be chronologically located between the Neolithic and the end of the Bronze Age. In fact, an enclosure dating from the Early Bronze Age was discovered nearby at the time of the diagnosis in 2005.

Burials dated between Hallstatt C and Tène A were found in three of these enclosures, two in the area bounded by the ditch, the last of which intersected the ditch of enclosure D37F0062.

The storage of grain is illustrated by five silos distributed over the two picklings. Four of them then housed six deceased, two of them with metal furniture. Of the deceased deposited in the same structure, two were simultaneous and two others deferred in time. These deceased are more recent than those buried in the enclosures between Tène B and Tène C.

In the present study, we present genomic results for individual BUCH82, an adult discovered in a silo.

Author of entry: I. Richard

Champfleury « RN 51 » (Marne)

Person in charge: S. Culot

The Champfleury “RN 51” (Marne) site is located south of the city of Reims (less than 6.5 km from its ancient center). It is located on the eastern slopes of the Rouillat valley, which slopes slightly from east to west, at altitudes of 107 and 100 m respectively. It is also bordered to the east by one of the many dry valleys encountered in the chalk plain. One of the nearest prominences is less than 1 km to the west, rising to 121 m. Further south, the Montagne de Reims, the flagship of this sector, overlooks the plain from a height of 283 m.

The excavation operation uncovered seven sepulchral pits grouping ten individuals, distributed over three distinct groups (Culot, 2012). These burials probably belong to an even larger necropolis, continuing southwards (Champfleury, “A Mi Champs”, Bonnabel, 2011). The illustrated funerary practice is characteristic of the early Second Iron Age and classic of the Aisne-Marne archaeological culture as defined by J.-P. Demoule (1999). The material associated with the deceased permitted to specify the chronology, ranging from La Tène A (475–400 BC) to La Tène B1 (400–325 BC).

Here we present the genomic results for individual CHF106, an adult male buried with several grave goods.

Author of entry: I. Richard

Isles-sur-Suippe « Les Sohettes » (Marne)

Person in charge: A.-C. Baudry

The Isles-sur-Suippe site is located in the Marne Department, 16 km northeast of Reims. Excavated in 2014 by Inrap (Baudry, 2021), this 4.8 hectare site consists of four sectors covering the chronological periods of the end of the Second Iron Age, Antiquity and the First World War.

The protohistoric occupation consists of an enclosed habitat from the transition between the final La Tène and the beginning of Antiquity. The chronological question is crucial in the case of Isles-sur-Suippe. In fact, approximately fifty architectural units (buildings on posts, palisades) associated with holes, are organised within an enclosure of more than 1.8 ha in surface area. The first elements of dating obtained from the ceramic study and radiocarbon analysis show a relatively short period of occupation of about 150 years, without any apparent chronological hiatus.

One of the pits, pit 6950, was discovered on the edge of the settlement, about 50 m east of the enclosure ditch. It is circular and narrow in cross-section, dug into a compact chalk substrate at least 6 m deep. In its central part, at a depth of 2.5 m, a deposition zone was discovered, containing animal carcasses and the skeleton of a young man. No perennial furniture was found next to this individual except a large ceramic shard. A second deposit was found at the bottom of the shaft, this time consisting of an isolated millstone. The deposit of the animals and the deceased in the pit, because they were very close in time, strongly link the treatment of cattle and humans. It is hypothesised that these deposits were the result of the same operating chain, the purpose of which remains to be elucidated. These practices, which stand outside of usual funerary practices between the 2nd and 1st centuries BC, raise questions about the purpose of this deposit and the practices that may lead to the association of humans and cattle.

Here we present the genomic results for individual ISL6950, the young male recovered in this special structure.

Author of entry: I. Richard

Urville-Nacqueville (Manche)

Person in charge: S. Rottier, A. Lefort

The site of Urville-Nacqueville, Normandy, is an Iron Age coastal settlement and funerary site excavated between 2011–14 and again in 2017. This port, located along the Channel, is characterised by the presence of an artisanal sector and a vast cemetery.

The Iron Age activity focuses on the period 120–80 BC and displays significant links with southern Britain, both in terms of the settlement evidence (e.g. the presence of roundhouses, Kimmeridge shale etc) and burial rites (with some adults individuals buried in a position similar to that seen in the Durotrigian region of southwest England; Lefort et al., 2015).

The excavations of the Urville-Nacqueville necropolis revealed 112 graves (78 burials and 34 cremations) containing the remains of at least 127 individuals (of which 41 were cremated). The inhumations are dominated by subadults, and the cremations concern principally adults. The funerary space is organised through the presence of an enclosure in the northeast. Some unusual funerary practices are observed at Urville-Nacqueville, such as deliberate removal of skulls and extra skulls in some burials. This site is also outstanding for the period and the area for its size. Indeed, for the Second Iron Age in Normandy were mainly found small cemeteries of less than 30 individuals, usually cremated and lacking evidence for subadults (Chanson et al., 2011).

Here, we present genomic results for three adult male individuals buried in the so-called 'Durotrigian' position: UN 129, UN 19 and UN 85.

Author of entry: C-E Fischer

Attichy-Bitry

The La Tène necropolis of Attichy-Bitry “Le Buissonet” is located in the Oise between the towns of Compiègne and Soissons, on the right bank of the Aisne. Partly destroyed by an antiquarian settlement, 11 burials are spread over about 1300 m², grouping 12 individuals, 6 adults, 5 children and 1 individual of undetermined age. The usual funerary practices discovered in the region consist in individual cremated burials. The deceased wore a panoply of ornaments and clothing accessories made of bronze, iron or lignite, with the men also wearing equipment consisting of weapons. In addition to this, there are also toiletries or tools, chariot parts, deposits of meat and ceramic containers for the presentation and consumption of food or liquids. The highest hierarchical rank of this community is expressed in two chariot tombs and a tomb with a circular enclosure where a young child was buried. The funerary occupation of Attichy seems to have extended throughout part of the 3rd century, a period characterised by the abandonment of burial in favor of cremation. The Attichy necropolis can probably be linked to the Aisne-Marne cultural group with a later adoption of cremation for all individuals at the end of the Middle La Tène.

Here we present genomic data for three individuals: Att3, Att27 and Att52-2, that were combined to the individual Att26, previously published in [Brunel et al. \(2020\)](#).

Author of entry: S. Desenne

Bucy le Long

The necropolis of Bucy-le-Long “la Héronnière - la Fosse Tounise” is located on the right bank of the Aisne, 3 km upstream from Soissons in the Aisne. It is located on a sandy prominence of the alluvial terrace, overhanging the river. It was occupied between the 5th and 4th centuries BC. The necropolis covers an area of 2.5 hectares and originally contained around 350 graves, of which 235 have been excavated. Burial is the main mode of deposit and only two cremation graves have been found in the complex. All age groups of adults are present (from 18 to over 80 years old) but the proportion of young adults is important here and the elderly individuals are few. Furthermore, despite the high theoretical infant mortality within this type of population, the bulk of the children are missing. The study of pathologies has not revealed any significant deficiencies or war trauma. The state of health is satisfactory, and the perceived traces of arthrosis and rheumatic pathologies are related to the age of the deceased and the repetition of physical tasks. The funeral practices adopted by this population is dressed burial. The women are distinguished by their finery and the men by their weaponry. The rest of the material consists of toiletries, rare tools, ceramic dishes, and foodstuffs, of which only the bones of the meat remain. The elite individuals are distinguished by a monumental tomb known as a “chariot tomb”. They are buried on a horse-drawn vehicle, a symbol of power.

The necropolis of Bucy-le-Long “Le Fond du Petit Marais”, located on the right bank of the Aisne, 5 km west of Soissons, covers 3,200 m². It comprises 66 burials and 15 monuments organised in a linear fashion along a north/south axis, in two distinct groups separated by at least 30m. Its occupation began at La Tène C1, with the burial ritual, and continued at La Tène C2 and then D1 with the cremation ritual. The deceased are mostly equipped with clothing accessories and food offerings. The most richly endowed tombs are provided with bronze vessels (basins, buckets) and one of them has yielded elements of a chariot.

Here, we present genomic data for two individuals: BFT228, from ‘La Fosse Tounise’ and BLH447, from ‘La Heronniere’. They were added to the individual from ‘Le Fond du Petit Marais’, BFM265, previously published in [Brunel et al., 2020](#).

Author of entry: S. Desenne

La Piece a Liards

Person in charge: S. Oudry

The Etaples necropolis is located along the Channel coast, near the Canche estuary. It is dated to the Middle/Final Hallstatt (Ha D1-D2) and more specifically to the 6th century BC. One of the main features of this necropolis is a specific funerary ritual corresponding to burial in a lateral bent position. Furthermore, it is the only site in the region dating from this period (Henton et al., n.d.). The pits, which are large and structured, are dug out of chalk. Part of them are arranged inside a quadrangular ditch. Comparisons are to be searched about 200 km further south, in Calvados at Basly and Ifs. The convergence of several

funerary features concerning the body suggests strong affinities with the funerary systems in use in the communities settled along the Channel coast at the end of the First Iron Age.

Here we present genomic data for Pal170, an adult buried with a bronze ring.

Author of entry: S. Oudry

Vasseny

The necropolis of Vasseny “au Dessus du Marais”, “Dessus des Groins” is located between the towns of Soissons and Reims, in a meander of the Vesle, a few kilometers from its confluence with the Aisne, on the middle alluvial terrace of the left bank of the river.

The necropolis contains 40 tombs spread over a short period of a century, from the second half of the 5th century to the middle of the 4th century BC. The spatial distribution of the burials shows an irregular grid of four concentrations spread over almost one hectare. The ritual of burial is predominant, although at the turn of the 4th and 3rd centuries BC, cremation seems to develop. Only one case of cremation is recognised at Vasseny among 39 burials. The latter correspond, for the most part, to the graves of adults. The deceased present a panoply specific to their sex and rank. The women wear finery (torque, bracelet, pendants, fibula, earrings, etc.) whereas the men are equipped with weapons (dagger, sword, shield, spears and javelins). To these elements are sometimes added toiletries, tools and more frequently food offerings in the form of pieces of meat and ceramic containers intended for the presentation and consumption of food or liquids.

The highest hierarchical rank of this community is expressed in three graves of individuals, two men and one woman, buried on a chariot.

The Vasseny necropolis is part of the Aisne-Marne cultural group as a whole and shows a high degree of homogeneity in the funerary practices of this period. This medium-sized site is part of a geographical network organised on three levels of social integration perceptible within the Aisne-Marne cemeteries.

Here we present genomic data for two individuals: Vas75 and Vas79-2.

Author of entry: S. Desenne

Barbuise ‘Les Grèves deFrécul’ and ‘La Saulsotte’ (Aube)

Person in charge: S. Rottier

The site of Barbuise, located in the department of Aube, on the right side of the Seine, is composed of a group of localities discovered as early as the XIX^e century. In 1832, an inventory of the megaliths was carried out by the Société Académique de l’Aube and several menhirs and dolmens at Courtavant and La Saulsotte were surveyed. However, it was not until 1937 that the Latenian cemetery known as “Les Grèves deFrécul” was discovered and partially excavated by Henri Lamarre.

The site “Les Grèves deFrécul” was occupied during the Iron Age, between the 5th and the 3rd centuries BC.

Several excavations were carried out from 1937 to 1975 and revealed 150 burials in five enclosures. The site was then part of rescue excavations during the 1990s and was reexcavated in 2000. During the last excavation, more than 100 burials were counted and almost all of them were associated with a funerary enclosure. However, it is important to note that among all these graves, only 15 are considered to be intact, i.e. not excavated by H. Lamarre in the 1930 and 1940s (Rottier and Piette, 2001).

Here we present genomic results for five individuals from the Iron Age as well as two samples from the Bronze Age. From the Iron Age period, GDF1231 (enclosure E17), GDF1264 (enclosure E8) and GDF1341 (enclosure E19) are females whose the age at death could not be estimated. Two other individuals originating from enclosure E19 were analysed and correspond to an immature individual (GDF1348), and an adult female (GDF1349-A). From the Bronze Age period, genomic data were obtained for individuals BPV1445 and BPV1455.

Author of entry: C-E Fischer

Gurgy 'Les Noisats' (Yonne)

Person in charge: S. Rottier

Gurgy's site 'Les Noisats', occupied between 3rd and 1st centuries BC, is located in the department of Yonne (89), on the right side of the river Yonne. This relatively short period of occupation represents about 7 generations (one generation corresponding to 28 years; [Moorjani et al., 2016](#)).

This deposit was first investigated in 1997 by S. Collet and F. Müller. The authors mentioned a few poorly dated structures of minimal interest, but pointed to the presence of an important funerary site from Early and the Late Iron Age associating a circular enclosure and a quadrangular enclosure, comparable to those discovered at the Gurgy 'La Picardie' site, located a few hundred meters away. The funerary complex was excavated by S. Rottier in 2004. It consists in a mound of around 700 m² which yielded 40 individuals spread among 35 graves. It should be noted that the mound has been levelled and that only the deepest and latest graves remain ([Mordant and Rottier, 2004](#)), so the number of individuals does not represent the group as a whole.

Here we present genomic data for four individuals originating from this mound. Sample GLN29-A corresponds to a male between 7 and 13 years old, GLN32 corresponds to an adult male with a missing skull, whereas GLN126 and GLN141 correspond to two adult females buried in the northern part of the tumulus.

Author of entry: C-E Fischer

Oppidum du Plan de la Tour (Gailhan, Gard)

Person in charge: B. Dedet

The settlement of Plan de la Tour (Gailhan, Gard) is located in eastern Languedoc, in the foothills of the Cevennes. It was occupied between the 5th and 4th centuries BC. The excavation delivered the burials of more than twenty subadults. These deceased were not incinerated, while cremation is the rule in this region for adults admitted to the village cemetery. They were buried in a small pit of the size of the body inside the houses. The bodies are not swaddled, as shown by the observation of the position of the limbs.

Here, we present genomic results for one perinatal individual PT7 (sepulture B2, inhumation W18-23-2). This individual was combined to individual PT2, previously published in [Brunel et al., 2020](#).

Oppidum de Pech Maho (Sigeac, Aude)

Person in charge: E Gailledrat

Pech Maho (Sigeac, Aude) is a small fortified trading post founded at the middle of the 6th century BC and abandoned at the end of the 3rd century BC.

The settlement acted as a place of exchange and meeting between native populations and Mediterranean merchants (Greek, Etruscans, Iberians). Domestic levels yielded some graves of very young children.

Here we present genomic results for two individuals: sample PECH3 that was found in a deposit (Sep. 71289, obj-71289-1) and corresponds to an adult male, and sample PECH9 that corresponds to a perinatal found on dumping ground.

They were combined to two other individuals, previously published in [Brunel et al. \(2020\)](#): sample PECH5 originating from an isolated grave (Sep. 46101, obj-46101-2) and corresponding to a young male (15–18 years old) and sample PECH8 also originating from an isolated grave (Sep. 47003, obj-47003-1) and corresponding to an adult male.

La nécropole du Peyrou 2 (Agde, Hérault)

Person in charge: B. Dedet

The Peyrou site at Agde (Hérault), groups an incineration necropolis functioning during the second half of the 7th century BC (Peyrou 1) and a necropolis hosting 35 burials revealing very different funerary practices (Peyrou 2) and used from the end of the 5th century to the middle of the 2nd century BC. Among the sub-adults, 8 perinatals or infants were found buried in a vase. Adults were buried only with objects relating to the mortuary toilet (perfume vases) or a symbolic tribute with no difference according to the sex of the deceased. These practices are identical to the Greeks' rituals, very different from those of the surrounding region during the Iron Age. Ancient texts attest to the existence of a trade settlement created by the Greek colonists of Marseilles in this place at this time.

Here we report genomic data for individual PEY73, an adult female buried with a pouring vase. These results were combined to those previously published in [Brunel et al. \(2020\)](#) for two other samples: PEY53, an adult female buried with some grave goods, and PEY163, an adult male buried without any grave goods.

La Monédière (Bessan, Hérault)

Person in charge: A Beylier

La Monédière (Bessan, Hérault) site yielded a small collection of burials dated from the 2nd century BC.

The site of La Monédière is the place of a Gallic fortified settlement occupied between the early 6th century and the end of the 5th century BC. Covering an area of nearly 4 ha, this settlement occupies a slight relief on the right bank of the Hérault river. Its foundation appears closely linked to very early contacts made in this part of the Gulf of Lyon with the Mediterranean societies and the establishment, at the mouth of the Hérault, of the Agde littoral counter which is roughly 6 km away. The trading activities, indicated by the abundance of products imported from the Greek or Etruscan world, are generally at a level higher than that observed in neighboring establishments. The quantity of amphora reveals the involvement of this site in trade networks uniting the coast and the interior, as well as its role in the redistribution of the products transported from the Mediterranean within the framework of Mediterranean trade, by land or waterways. Benefiting from a favorable geographical position, La Monédière stands as an essential marketplace and a privileged meeting place between natives and Greeks, to such an extent that the question of the *in situ* installation of a Hellenic community was raised. Its occupation was interrupted towards the end of the 5th century BC at the time of the foundation of the colony of Agde/Agathè by Marseilles. In the course of the 2nd century BC, the site was revisited. At this time, a funeral complex was established, yielding a small collection of burials associated with a very poorly known settlement, which could be an integral part of the chôra of the Agathe colony.

Here we present the genomic results for two immatures and one adult. Samples BES1096B and BES1154 are dated to the 5th century BC and correspond to two immatures buried in the settlement, a funerary practice common for the area and the period. The last sample, BES1249 corresponds to an adult found in the cemetery dated to the 2nd century BC, whose burial is not representative of the funerary gestures for the area and period, where adults are mostly cremated. These individuals were combined to BES1248, another adult previously published in [Brunel et al., 2020](#).

Le Cailar (Cailar, Gard)

Person in charge: R. Roure

The site of Le Cailar, south of Nîmes, is an important lagoon harbour of the Iron Age. It has been studied since the 2000s and archaeological excavations have shown that the settlement has been occupied since the 6th century BC and was involved in the exchanges with Greek Marseille and all of the Mediterranean. The protohistoric and ancient occupation of Le Cailar lies at the confluence of Vistre and Rhône. During the whole 3rd century BC, many severed head and metal weapons were displayed on a large public place near the fortification. About 2700 fragments of human bones were recorded during ten excavation campaigns, almost all belonging to the skull. Chemical analyses proved that those heads were embalmed ([Ghezal et al., 2019](#)).

Here we present genomic results for five individuals represented by skull deposits: CLR23, CLR24, CLR31, CLR35 and CLR44. All samples were males and were recovered from the public place, within the walls.

METHOD DETAILS

DNA extraction and sequencing

All experiments were performed in the ancient DNA facilities at the PACEA laboratory (CNRS, University of Bordeaux, France). aDNA was preferentially extracted from petrous bones but for some individuals, teeth or even intact long bones were selected (see [key resources table](#)). Bone surface was decontaminated before extraction, *ie.* scraped, cleaned with diluted bleach and exposed to ultraviolet (UV) light for each side for 20 min. After soft surface abrasion with a drill, sampling was performed using a clean drill into the denser regions around the cochlea of the petrous bone or into the cortical part of the long bones. Teeth were completely ground to fine powder.

Between 100-250 mg of bone/tooth powder was used for each extraction. aDNA was extracted using a two-steps procedure adapted from [Damgaard et al. \(2015\)](#), and purified by a silica based method on a MinElute column (QIAGEN) (see [Brunel et al., 2020](#)).

For all DNA extract, double-stranded libraries were built from 10 to 25 μ L of DNA template, following a protocol proposed by [Meyer and Kircher \(2010\)](#) and using unique index pairs ([Kircher et al., 2012](#)). A partial uracil-DNA-glycosylase (UDG half) treatment was applied to remove deaminated cytosines except for the final nucleotides at the 5' and -3' reads ends to preserve part of the damage pattern characteristic for ancient DNA ([Rohland et al., 2015](#)).

We first screened all indexed libraries via shotgun sequencing targeting around 1 million reads. Libraries were pooled and sequenced on an Illumina NextSeq 500 (2x75bp reads) at Institut de Recherches Biomédicales des Armées (Paris, France). Reads were analysed with EAGER ([Peltzer et al., 2016](#)) to process the raw data and to select satisfying libraries for deeper sequencing. Selection criteria included sufficient endogenous DNA proportion (>15%), complexity of the library and presence of damage patterns characteristic for aDNA (see [Table S1](#) for shotgun screening results). Selected libraries were then more deeply sequenced on the same platform in order to obtain low-coverage genomes of at least 0.1 \times ([Table S2](#)).

Read processing, alignment and post-mortem damage

Raw sequence data were processed using EAGER ([Peltzer et al., 2016](#)) with the following steps. Reads were trimmed for adaptor sequences and collapsed into single reads using ClipandMerge software. Reads were mapped against the Human Reference Genome hs37d5 with BWA (Burrows-Wheeler Aligner) v0.7.12 ([Li and Durbin, 2010](#)), and duplicate reads with the same orientation and start and end positions were removed using DeDup v0.12.1. Reads with a mapping quality phred score <30 were excluded. MapDamage v.2.0.6 was used to observe characteristic aDNA damage patterns, before trimming two bases at the ends of each read to remove residual deaminations with BamUtil (https://genome.sph.umich.edu/wiki/BamUtil:_trimBam). A summary of quality statistics is given in [Table S3](#)

QUANTIFICATION AND STATISTICAL ANALYSIS

Sex determination

Genetic sex was calculated using the methods described in [Skoglund et al. \(2013\)](#) estimating the fraction of reads mapping to Y chromosome out of all reads mapping to either X or Y chromosome (see [Table S2](#)).

Contamination estimates

We used the ANGSD (Analysis of Next Generation Sequencing Data) package to test for heterozygosity of polymorphic sites on the X chromosome in male individuals, applying a contamination threshold of 5% ([Table S2](#)). Contamination estimates were extremely low and permitted us to keep all samples for further analyses.

Uniparental markers

To process mitochondrial DNA data, reads were mapped to the revised Cambridge Reference Sequence (rCRS- GenBank Accession Number NC_120920.1). VCF files were built using bcftools mpileup and then submitted to HaploGrep 2 ([Weissensteiner et al., 2016](#)) in order to determine mitochondrial haplotypes ([Table S2](#)). Reliable characterization of maternal lineage could be conducted for 43 individuals. The combination of our mitochondrial dataset with the maternal genomes published by [Brunel et al. \(2020\)](#) permits to confirm the prominence of haplogroups H (25,58%), J (20,93%), K and U (both at 15,12%) among French

IA groups. Large chronological groups, from the Palaeolithic to the Bronze Age period, were constituted to figure out the diachronic evolution of maternal lineages frequencies on the French territory by combining our dataset with previous published data (Brunel et al., 2020; Fischer et al., 2018, 2019; Fu et al., 2016; Olalde et al., 2018; Rivollat et al., 2020, Figure S2).

Y chromosome haplotypes were called using Yleaf statistical package for each male individual (Ralf et al., 2018). All the 29 male individuals identified in our dataset had sufficient coverage for Y haplotype assessment (see Table S2). Most individuals were found to carry haplogroup R1b1a (69%), other males belonging to haplogroups G2a2 (17,24%), I1 and I2 (both at 6,9%). We compiled our dataset with previous published data (Brunel et al., 2020; Fu et al., 2016; Olalde et al., 2018; Rivollat et al., 2020) to study the evolution of the frequencies of Y chromosome haplogroups from the Palaeolithic to the Iron Age (Figure S3).

Kinship analyses

We estimated the degree of genetic relatedness between our individuals by applying Relationship Estimation from Ancient DNA (READ; Monroy Kuhn et al., 2018). Of the 61 French Iron Age individuals for which low coverage genomes are available (Figure S4), we identified only three individuals presenting a 2nd degree of relatedness at Attichy-Bitry (North): individual ATT27, individual ATT3 and individual ATT52-2. The other individual from Attichy (ATT26) shares no biological link up to the 3rd degree with the others.

Inbreeding and diversity estimates

To detect potential inbreeding among the French IA groups and individuals, we calculated length of runs of homozygosity (ROH; Ringbauer et al., 2021) using the software HapROH on individuals carrying more than 300,000 SNPs. We performed hapROH to the pseudo haploid data of 1240k SNPs to detect runs of homozygosity longer than 4 centimorgan for all IA individuals.

Of the individuals providing suitable coverage for this analysis, only individual COL336 from the Iron Age site of Colmar "Jardin des Aubépines" (Haut-Rhin) showed evidence for long ROH greater than 50 cM. The sum and length distribution of ROH measured for COL336 suggest their parents were first-degree relatives (Figure S11), i.e., parent-offspring or full siblings (whose offspring will have a quarter of their genome in ROH). This individual was found deposited in a silo without any particular care. The overall absence of long ROH for other French IA individuals or groups indicate that the groups considered were sufficiently large or with controlled union rules to avoid inbreeding (Figure S10).

Principal component analysis

PCA analysis was run with the Human Origins reference panel for 592,998 autosomal genotypes in 796 west Eurasian modern individuals using smartpca v10210 (EIGENSOFT) with the options lsqproject:YES and shrinkmode: YES, using 51 modern populations to calculate eigenvectors on which aDNA samples were projected. Genotypes were downloaded from David Reich's website (dataset v44.3.1240K_HumanOrigins, available at <https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data>) and merged with our dataset. Projections on the first two PCs are provided in Figures 1C and S1.

Genetic clustering and outlier detection

To prepare our dataset for analyses, we divided individuals in six regions and, when possible, in chronological sub-groups: EIA_Alsace (from 800 BC to 450 BC), LIA_Alsace (from 450 BC to 50 BC), IA_Champagne, IA_Normandy, IA_North, IA_Paris_Basin and IA_South (see Table S2).

We carried a qpWave from the ADMIXTOOLS package (<https://github.com/DReichLab>) iterated overall individuals in the pool, testing for significant evidence of heterogeneity relative to a sub-pool of all individuals in the main cluster (except the test individual when it was part of the main cluster). The right set panel A was composed of: Mbuti.DG, Ethiopia_4500BP_published.SG, CHG, Russia_EHG, Russia_Ust_Ishim_HG_published.DG, Czech_Vestonice16, Russia_MA1_HG.SG, Israel_Natufian, Jordan_PPNB_published, Iberia_EIMiron, Anatolia_N_published, Morocco_LN.SG, WHG, Iran_GanjDareh_N, and Russia_EBA_Yamnaya_Samara. We identified outliers from the main cluster individuals when the qpWave p-value was $p < 0.05$ (Fernandes et al., 2020).

We found six individuals spending as outliers from the chronological and regional group to which they belong:

- Bes1248 stands as an outlier from the South_IA group
- Croi11 stands as an outlier from the Alsace_IA_1 group
- Col239 stands as an outlier from the Alsace_IA_2 group
- GDF1341 stands as an outlier from the Paris_Basin_IA group
- Pech3 stands as an outlier from the South_IA group
- Pey163 stands as an outlier from the South_IA group

Therefore, these individuals were not included in the analyses at the regional level.

We run *qpWave* analyses with the same reference panel comparing BA and IA groups from the French territory and Europe to test for statistically significant differentiation. We created a similarity matrix, which was then used to generate the heatmap using the *heatmap.2* function of the R-package *gplots* (Warnes et al., 2019) (Figure 2).

f3-outgroup and f4-statistics

Outgroup *f3*-statistics were calculated using *qp3Pop* and *f4*-statistics using *qpDstat* with the *f4* mode from ADMIXTOOLS (<https://github.com/DReichLab>). We used the 1240K panel to optimize the number of SNPs covered by the ancient individuals and get more resolution in the statistic tests. Standard errors were calculated with the default block jackknife.

To test for specific genomic affinities between IA individuals, we performed a *f3*-outgroup statistics in the form *f3* (*Mbuti*, *Ind*, *Ind*). The results were plotted in a heatmap (Figure S5) using the *heatmap.2* function of the R-package *gplots* (Warnes et al., 2019). The heatmap shows no specific affinities between individuals from the same site or region (ATT27 and ATT52-2 were removed from this analysis as they present 2nd-degree genetic relatedness between them and with ATT 3).

To determine if a specific ancestral legacy could explain the outlier status of the individuals, we performed *f3*-outgroup statistic in the form *f3* (*Mbuti*, *X*, *Outliers/Region*) with *X* being either one of the three major genetic components of the European population: *WHG*, *Anatolia Neolithic* and *Russia_Samara_EBA_Yamnaya*. The results show that some individuals (Bes1248, Col239) seem to have excess in the Yamnaya component compared to their group of origin (Figure S6). We also performed a *f3* (*Mbuti*, *Outlier*, *Region*) for each outlier and each region. The results were plotted on maps using QGIS v.3.10 (<https://www.qgis.org/en/site/>, Figure 4).

To test if the Yamnaya component distribution permitted any significant differentiation between IA groups from UK, French regions (North, Alsace, the Paris Basin, Champagne, the South) and the Iberian Peninsula, we also performed a *f4* statistical analysis, in the form *f4* (*Mbuti*, *Russia_Samara_EBA_Yamnaya*; *PopA*, *PopB*), where *PopA* and *PopB* represented Iron Age groups. The only IA groups found to be genetically differentiated from other groups according to the steppe component are the groups from United Kingdom and Normandy (for which this component is the most important) versus all other IA groups. Interestingly, the *f4* test *f4* (*Mbuti*, *Russia_Samara_EBA_Yamnaya*; *IA Normandy*, *IA UK*) is not significant, showing similar proportions for this component between the UK and Normandy groups (Table S6).

Population modeling

We used *qpAdm* from the ADMIXTOOLS package (<https://github.com/DReichLab>) to estimate admixture proportions for all French IA individuals. To assess the percentage of the three major components *WHG*, *Anatolia Neolithic* and *Russia_Samara_EBA_Yamnaya* into each individual, we performed a *qpAdm* analysis with the reference panel B composed of: *Mbuti.DG*, *Ethiopia_4500BP_published.SG*, *CHG*, *Russia_EHG*, *Russia_Ust_Ishim_HG_published.DG*, *Czech_Vestonice16*, *Russia_MA1_HG.SG*, *Israel_Natufian*, *Jordan_PPNB_published*, *Iberia_EIMiron*). Results are presented in the Figure 3A and detailed in Figure S9 and in Table S7. In a second model, we performed *qpAdm* analyses to assess if the various

Iron Age groups in France could be modelled with ancestry represented by Bronze Age groups using different combinations of BA groups from different regions of France, England, Germany, Italy and Spain with the reference panel A ([Tables S4](#) and [S5](#)).

Data and software availability

Raw sequence data and alignments are available at the European Nucleotide Archive (ENA) under accession number ENA: PRJEB50940.

ADDITIONAL RESOURCES

Our study has not generated or contributed to a new website/forum and it is not part of a clinical trial.