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Multi-scale archaeogenetic study of two French Iron Age communities: from internal social- to broad-scale population dynamics

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Key words:

Late Iron Age; ancient DNA; social dynamics; phylogeography; French territory

Highlights:

- Multidisciplinary study of Late Iron age groups from Northern France
- Population-scale DNA-based approach permitted the documentation of communities' internal social dynamics
- Gallic groups were characterised by patrilocality residence and patrilinearity rules
- Distinct necropolis welcomed distinct biological and social groups

24 • Local-scale communities' features were essential to address broad-scale phylogeographical
25 issues

26

27

Abstract:

Ancient DNA (aDNA) research can address anthropological questions at both the broad continental scale to provide elements of discussion regarding ancient human population dynamics and the very small spatial scale to document population social functioning. In the present study, we propose an original approach combining local- and broad-scale issues, applied to two contemporaneous groups dated from the Late Iron Age period and originating from Northern France. Our analyses targeted uniparental markers (mitochondrial DNA (mtDNA) and the Y chromosome) and permitted the characterization of 43 maternal and 17 paternal lineages for the Urville-Nacqueville necropolis (UN) as well as 27 maternal and 19 paternal lineages for the Gurgy 'Les Noisats' (GLN) tumulus. Data in hand provided a unique opportunity to document Gallic communities' genetic diversities and discuss their internal social dynamics as well as their genetic affinities with other ancient European groups. At the local scale, our study first highlighted patrilocal residence rules and patrilinearity for these Gallic groups, in concordance with textual sources and archaeological data. Second, the gene pool characterization indicated that the UN necropolis welcomed a more cosmopolitan community than did the GLN tumulus and that the funerary space organization in the UN was correlated to the genetic structuration of the community. These local scale characteristics were put into perspective for broad-scale discussions and highlighted different genetic affinities of the UN and GLN communities with ancient European groups. Both communities appeared inserted in distinct exchange networks that may have been responsible for the slight differentiation of their maternal gene pools. Finally, we demonstrated that the sub-groups encountered in the UN necropolis (maternally differentiated) presented very distinct genetic affinities with ancient or contemporaneous human groups from other Western European regions. Consequently, our study reinforces the idea that the identification of genetically differentiated groups within archaeological sites and discussion concerning their specific composition constitute a clear prerequisite before addressing broad-scale phylogeographical issues.

54 Introduction

55 Over the past decade, ancient DNA (aDNA) research has addressed archaeological and
56 anthropological questions, whether at the broad continental scale or at the very small spatial scale.
57 The first continental scale has provided major elements of discussion regarding ancient human
58 population migrations, admixture or replacements (for a recent review see Harris, 2017). At the very
59 local scale, the palaeogenetic approach has shed light on specific populations' social functioning, with
60 an emphasis on kinships and matrimonial systems (see for example Keyser-Tracqui et al., 2003 and
61 Haak et al., 2008). The genetic constitution of human populations can be very complex due to social
62 structuration, specific matrimonial or filiation systems. The considerable variability of population
63 gene pools, due to these specific internal social dynamics, implies that the use of a small number of
64 samples to represent largely dispersed archaeological cultures may lead to erroneous interpretation
65 of data. Moreover, the genetically analysed ancient human communities generally originate from
66 specific funerary sites whose access may have been limited to a peculiar group who may not be fully
67 representative of the community. Therefore, considerable variability in the genetic diversity of
68 ancient groups can even be encountered within cemeteries. The precise understanding of both the
69 funerary site functioning and the constitution of the "dead community", addressed through
70 archaeological, anthropological and small-scale aDNA studies, thus constitutes a preliminary process
71 necessary to the appropriate use of the group gene pools for the reconstruction of broad-scale
72 population dynamics (Johannsen et al., 2017; Veeramah, 2018).

73 Thus far, aDNA studies addressing both scales and thus combining local communities' social
74 functioning and phylogeographical issues remain rare (Beau et al., 2017; Knipper et al., 2017; Rivollat
75 et al., 2015; Veeramah, 2018). For the Iron Age, our period of interest, an original study combined
76 biological (osteology, isotopes, DNA) and archaeological data obtained on individuals from a
77 "princely site" in Germany to investigate the social structuration of the local community (Knipper et

al., 2014). While the data obtained allowed to discuss individuals' maternal relationships, the small gene pool recovered could not be used to address phylogeographical issues. In the present study, we propose an original approach combining local- and broad-scale issues, applied to two groups dated from the Late Iron Age period and originating from Northern France.

Our study targeted two communities corresponding to the famous French "Gauls", according to the Classical and ancient texts, and originating from the second part of the Late Iron Age sites of Urville-Nacqueville (120- 80 BC, Manche) and Gurgy 'Les Noisats' (300-100 BC, Yonne) (Fig 1 and 2). It is important to note that the communities targeted were associated to distinct Celtic Gallic cultures according to texts and historical data: Urville-Nacqueville was associated to the Unelles group whereas Gurgy "Les Noisats" was located in Senons' territory.

Figure 1 : Location of the Urville-Nacqueville site and spatial distribution of the mitochondrial (mtDNA). Individuals sharing a mtDNA haplotype are linked by a line.

Figure 2 : Location of the Gurgy ‘Les Noisats’ site and spatial distribution of the mitochondrial (mtDNA). Individuals sharing a mtDNA haplotype are linked by a line.

Urville-Nacqueville (UN) is a port site located along the Channel seashore which is characterized by the presence of an artisanal sector and a vast cemetery (Lefort et al., 2015). The funerary space, organized around a square enclosure, included 112 identified burials and at least 120 individuals. The necropolis of Gurgy Les Noisats is located in the Parisian Basin within the Senon’s territory. Many archaeological sites dated to the Iron Age have been described in the region (Baray, 2016; Duval, 1976; Hure, 1931). Although numerous habitats could be identified in the region for the period, none could be associated to the Gurgy necropolis (Delor, 2013). Gurgy ‘Les Noisats’ (GLN) consists of a tumulus approximately 30 metres in diameter that received 40 individuals spread over 35 graves (unpublished). To document the biological diversity of the UN and GLN groups, our aDNA study targeted uniparental genetic markers (mitochondrial DNA, mtDNA and the Y chromosome), permitting the characterization of maternal and paternal lineages. At the very local scale, special

attention was paid to the combination of archaeological, anthropological and genetic data (i) to discuss funerary groups' diversity and constitution, (ii) to test for the potential presence of groups genetically differentiated within each necropolis and (iii) to address each community-specific social functioning. The comparison of the results obtained for the partially contemporary sites of UN and GLN permitted us to question potential general social dynamics for the French Late Iron Age context. Interestingly, the European Iron Age is the most ancient archaeological period for which textual documentation is available. The textual sources describing the Gauls and their social habits were not written by the Gauls themselves but by contemporary Romans and Greeks and must consequently provide a distorted view of the French Iron Age groups' cultural diversity or habits. Whatever the case, texts such as the writings of Strabon, Poseidonios d'Apamée or Caesar (*De Bello Gallico*) have survived over time and constitute a unique opportunity to combine archaeological, biological and textual data to better understand Gallic societies. Finally, the elements of discussion obtained at the local scale were put into perspective to address broad scale issues, including (i) the biological affinities of distinct Gaul communities and (ii) the genetic legacy and dynamics of European Iron Age populations.

1. Materials and methods

1.1 Archaeological samples

The excavations of the Urville-Nacqueville necropolis permitted the discovery of 112 graves (78 burials and 34 cremations) containing the remains of at least 127 individuals (of which 41 were cremated) (Lefort and Rottier, 2014). All aDNA analyses were conducted on individuals recovered from burials (*i.e.*, not cremated) and targeted 51 individuals distributed throughout the entire necropolis. The sample included all individuals published in Fischer et al. (Fischer et al., 2018) plus 6 newly analysed individuals (S1 Table). To obtain a clear picture of the genetic diversity of sub-groups corresponding to distinct necropolis sectors or funerary practices, we targeted individuals from both outside (N=19) and inside the enclosure (N=32), as well as several individuals from the same small

groups of burials. The sample was thus supposed to addressing the genetic variability of intra- and extra-enclosure groups and to test for potential groupings of individuals according to maternal or paternal kinship.

Contrary to the Urville-Nacqueville necropolis, which is outstanding for the period and the region, the Gurgy 'Les Noisats' (GLN) site is typical of the necropolises known for the Paris Basin during the Late Iron Age. This site corresponds to a tumulus that grouped a total of 35 graves, containing the remains of at least 40 individuals. Among the graves discovered, 8 correspond to plural tombs, and 5 of them group an adult and an immature. The limited number of individuals allowed to submit all of them to palaeogenetic analyses (S2 Table). The diameter of the tumulus (approximately 30 m) as well as the absence of selection of the deceased according to age or sex or the majority of burials over cremation appeared consistent with necropolises observed for the same context (Baray, 2003; Marion et al., 2009). The combination of these characteristics with a short duration of use of the tumulus (200 years, based on metallic material, unpublished) allowed us to assume that the population recovered in the GLN tumulus was representative of the whole local community.

Since both necropolises were used during a short period (less than two hundred years) and because biological studies conducted on both sites have shown the absence of selection of the dead according to age or sex, we expect that the funerary groups were representative of the local communities as a whole. For the specific case of the UN, a change in the funerary treatment could be highlighted as related to age at death since children were usually buried while adults were burnt (Lefort and Rottier, 2014). If burnt adults were not accessible for our aDNA study, the absence of age or sex selection of individuals having access to the necropolis suggests that the gene pool recovered for the buried children group may reflect the population gene pool. Consequently, we consider that the sampling strategy designed in our study was adequate to unravel entire communities' gene pools and to question the presence of groups genetically differentiated within each necropolis.

1.2 Ancient DNA analyses and descriptive analyses

Most UN samples were collected during excavations (from 2012 to 2014) with all precautions against contaminations. Teeth were then preferentially collected for adults, whereas intact long bones were selected for immature individuals. All sampling performed after 2015 targeted petrous bones according to the recent discovery of the exceptional DNA conservation in this anatomical element (Pinhasi et al., 2015). The GLN human remains were collected for genetic analyses in 2016, 12 years after the excavations (unpublished). We targeted for all GLN individuals, in order of preference, petrous bones then teeth and, finally, bones. Since GLN human remains had been collected without any precautions against contamination and regularly manipulated during post-excavation studies, they were systematically decontaminated before extraction *i.e.*, scraped, cleaned with bleach and subsequently exposed to UV radiation for 20 minutes for each side.

The genetic analyses targeted the uniparental markers permitting the characterization of maternal (mitochondrial DNA, mtDNA) and paternal lineages (Y-chromosome). Our approach combined the analysis of 18 mtDNA and 10 Y-chromosome SNPs (single nucleotide polymorphisms) and the sequencing of the mtDNA Hyper Variable Sequence (HVS-1). Details of all analyses can be found in Rivollat *et al.* (2015). Y-chromosome SNP analyses were conducted on adult individuals identified as males through osteological analyses (determined by the Bruzek (Bruzek, 2002) and DSP (Murail et al., 2005) methods) as well as adults with undetermined sex and all immature individuals.

The genetic diversities of the GLN and UN groups were measured through the computation of Nei genetic diversity (h^2) using Arlequin software (version 3.5.1.2) (Excoffier and Lischer, 2010). Fisher's exact tests were used to test the statistical significance of genetic diversity differences measured between groups or DNA conservation variability observed between sites. The tests were performed with BiostaTGV (<https://biostatgv.sentiweb.fr/?module=tests/fisher>). Resampling analyses were performed with R 3.6.0 (R Core Team, 2019) taking into account sample size heterogeneity between groups for the comparison of Nei's diversity indices (see R script in SI).

Adjusted bootstrap confidence interval (Zieffler, Harring, & Long, 2011) were computed for the differences in Nei's indices between the groups under study, using 10000 bootstrap replicates.

To discuss the genetic affinities of UN and GLN communities with ancient European human groups, we ran a series of descriptive analyses using the **datas** published by Fischer and colleagues, which includes 1433 mitochondrial sequences dating from the Palaeolithic to the Iron Age (42,500-300 cal. BC), divided into 29 chronological, geographical and cultural groups (Fischer et al., 2018) (S3 Table). The proportions of shared mtDNA haplotypes and population-specific pairwise genetic distances (FST) were computed using Arlequin software (version 3.5.1.2)(Excoffier and Lischer, 2010). A principal component analysis (PCA) based on mtDNA haplogroup frequencies was carried out, taking into account the major European haplogroups identified and distinguished in aDNA literature targeting European populations: H, HV, I, J, K, N, T, U4, U5a, U5b, U, V, W, X and "others". The PCA was performed using the R package FactoMineR (Lê et al., 2008).

2. Results and Discussion

2.1 DNA conservation variability among necropolises

Mitochondrial lineages reported for the UN site combined those already published in Fischer *et al.* (2018) and updated (some SNP profiles and HVS-I sequences could be completed), plus those obtained on 6 newly analysed individuals. In the end, 42 mitochondrial haplogroups (determined through SNP analysis) could be characterized over 51 targeted individuals (80.7% success rate), and 43 complete and authenticated HVS-1 sequences (mtDNA haplotypes) could be obtained (82.7% success rate) (S1 and S4a Tables). Concerning the Y chromosome, 17 partial or complete SNP profiles were obtained, permitting us to corroborate the sex of 3 adults identified as males through osteological analyses and to unravel the existence of male individuals in the immature group (N= 15) (see details in S4b Table). The analyses conducted on the 40 individuals originating from the GLN necropolis permitted the characterization of 27 mitochondrial haplogroups (67.5% success rate) and

23 complete HVS-1 sequences (57.5% success rate). A total of 19 Y-chromosome SNP profiles, complete or partial, were obtained and confirmed the morphological sex of 6 male adults and provided new clues for males identification for 11 individuals (see details in S1, S4c and S4d Table).

A higher success rate of HVS-1 sequence recovery could be observed for UN individuals when compared to GLN individuals (UN: 82.7%; GLN:57.5%, statistically significant; p value = 0.00846). The excellent conservation of mitochondrial DNA has already been highlighted for the UN necropolis (Fischer et al., 2018) and could be explained by its location on the edge of the Channel foreshore.

Moreover, immature UN individuals were regularly discovered in benches made of plants, confirming the very exceptional conservation parameters in UN. Moreover, this organic material appears to have been responsible for the formation of a crust on the bones that may have protected them from various degradations.

Since our sampling targeted petrous bones, teeth and other bones, we tested for differential mitochondrial DNA conservation among these anatomical elements (S1. Figure). Fisher's exact tests revealed significant mtDNA conservation differences among anatomical elements for UN samples, with an easier mtDNA recovery for the teeth compared to other skeletal elements (p value = 0.045). This result is consistent with the recent report of a higher mtDNA/nuclear DNA ratio for teeth compared to petrous bones (Furtwängler et al., 2018). Given the lower number of samples, especially for bones ($N=2$), we did not perform statistical tests to compare GLN samples. No significant differences in mtDNA recovery could be highlighted between teeth and bones (other than petrous bones), even after merging the UN and GLN results to increase the number of samples. Finally, contrary to that for teeth and bones, we observed a similar mtDNA recovery from GLN and UN petrous bones, whereas the taphonomic environments were very different. This point is in line with studies that demonstrated that DNA was less subject to environmental variations within petrous bones (Gamba et al., 2014; Hansen et al., 2017).

Finally, when samples from both sites were merged to increase sample size, mitochondrial DNA appeared to be more preserved for adults than immature individuals. Even if the difference was not

significant (p value = 0.478), this observation could be explained by skeletal conservation variation related to age at death. Indeed, it has been clearly shown that the anatomical conservation index (ICA) of immature individuals was lower than that measured for adults, highlighting differential macroscopic conservation according to age at death (Bello et al., 2002).

2.2 French Iron Age communities' internal social dynamics

A noticeable variety of mtDNA haplogroups could be characterized for the UN group (N individuals = 43), including K1, J1, H, H1, H2, H3, H5, H6/8, H11, U4, U5a, U5b, I, V, T1 and T2 major lineages (Fig 1). A total of 16 sub-haplogroups and 27 distinct haplotypes could be determined among the UN sample. Among the mtDNA haplotypes discovered, 9 were shown to be shared between at least two distinct individuals, indicating possible maternal kinship (S1 Table). However, several of these shared maternal lineages corresponded to mtDNA haplotypes very frequent within ancient European groups, and their interpretation in terms of kinship determination may be erroneous. This is the case for H (CRS), H1 (CRS), J1 (16069-16126), U4 (163556) and V (16298) lineages shared by 2 to 3 individuals inside the necropolis. More interestingly, some individuals shared infrequent lineages that may thus be more informative in terms of maternal kinship, as this is the case for K1a (16093-16172-16224-16311), K1b1a (16093-16224-16311-16319), H2a2b (16235-16291), H6/8 (16362) and H1 (16213) haplotypes carried by 2 to 3 individuals (Fig 1). It is worth noting that individuals sharing these maternal lineages were discovered both inside and outside the enclosure.

The smaller GLN group (N individuals = 27) is unsurprisingly characterized by a smaller number of mtDNA sub-haplogroups (N=9) encompassing lineages H, H1, H3, HV, J1, J2, K, U5a and T2. A total of 13 distinct haplotypes could be determined among the GLN sample, of which 3 were shown to be shared between at least 2 individuals. However, maternal lineages shared between GLN individuals all corresponded to very frequent mtDNA haplogroups (H (CRS), H1 (CRS) and T2a1b (16126-16294-16296-16324) haplotypes), impeding any discussion in terms of maternal kinship (Fig 2).

For both UN and GLN communities, a striking homogeneity of the Y-chromosome lineages could be observed, all of them corresponding either to R* or R1b (M343) haplogroups. However, we must highlight that the SNP markers used to characterize the individual paternal haplogroups were very low in number and did not permit deep exploration inside the Y-chromosome phylogeny. Furthermore, numerous artefactual alleles regularly appeared in the SNP profiles, complicating lineage determination. The paternal uniformity unravelled for French Iron Age groups is consequently in part the result of the genotyping design. Regardless of the case, we consistently found in our Iron Age samples R* / R1b paternal lineages that are linked to the massive migration from the steppes and dated to the Late Neolithic-to-Bronze Age transition (Haak et al., 2015). This migration was responsible for an impressive genetic turnover in the European populations, with Neolithic haplogroups being replaced by new paternal (R1a and R1b) lineages originating from the eastern regions (see below).

The Nei genetic diversity was calculated for each Iron Age group based on maternal markers and provided clues for high maternal diversity for both UN and GLN communities (0.969 and 0.885, respectively). A nonparametric adjusted bootstrap 95% percentile interval was computed for this difference, yielding the range of [-0.2377 ; -0.0026]. This range of values indicates likely candidates for the true value of genetic diversity difference between these two groups. Since this interval does not include the null value, it provides moderate evidence for the hypothesis that there is a higher diversity in the UN group. We propose that the high maternal genetic diversities measured and the variation of diversities observed between our French Iron age communities can be explained by (i) the groups' residence rules and (ii) the constitution of the populations buried in the necropolises.

2.2.1 A patrilocal matrimonial system?

The gene pools of societies practising patrilocal residence rules are characterized by a series of specific features, including a low paternal lineage diversity (linked to low mobility of men) combined with a high maternal diversity (related to important mobility of women among groups,

introducing new lineages in the recipient population) (Dudar et al., 2003; Oota et al., 2001). Both features could be unravelled for UN and GLN sites and suggested that these communities practised patrilocal residence rules. It is worth noting that aDNA studies have regularly provided arguments permitting the identification of patrilocal residence rules for ancient European groups (Haak et al., 2008; Knipper et al., 2017; Lacan et al., 2011). For example, a study conducted by Knipper et al. (2017) on Bell Beaker and Early Bronze Age societies from Central Europe and based on aDNA and isotopic data highlighted patrilocal residence system as well as exogamy for women. In this specific case, a high mitochondrial diversity ($h^2 = 0.9924$) could be calculated. Because the maternal diversity calculated by Knipper et al. covered 80 individuals spread over 7 distinct archaeological sites dated between 2500 and 1700 BC (representing approximately 27 generations), we believe that the high maternal diversities measured for GLN and UN communities, composed of individuals originating from a unique site and distributed among only 7 generations (approximately 200 years) for GLN and only 3 generations (less than 100 years) for UN, constitute a clear argument for patrilocality.

For both UN and GLN necropolises, even if some individuals shared the same mitochondrial lineages, no group of tombs related to maternal lines could be detected (Fig 1 and 2). The absence of visible structuration of the funerary spaces according to maternal affinities may provide argument in favour of patrilinearity. In that case, the paternal homogeneity characterized inside both necropolises may be consistent with a structuration of the necropolises in link with paternal kinships. If it is obvious that more precise genomic data are necessary to confirm this hypothesis, it is nevertheless interesting to note that patrilocality and patrilinearity have both been proposed for Gaulish societies according to textual and archaeological data. In that respect, ancient texts, such as *De Bello Gallico* or Strabon's writings have provided arguments to consider that Gallic societies had practised patrilocal residence rules, with inter-ethnic alliances linked to the exchange of women between *civitas* (the *civitas* being a political entity that included several *pagi*, i.e., group of tribes with a specific ethnic identity; Lewuillon, 1990). In the same way, according to the work of Lewuillon (1990), Gallic societies may have been built on the fundamental and dominant principle of

patrilinearity, with a very strong impact of paternal heredity on communities' internal social structuration. Palaeogenetic data obtained for Late Iron Age UN and GLN communities are thus in line with archaeological or textual arguments. Since the UN group revealed a maternal diversity statistically higher than the one measured for the GLN group, we confronted at the very local scale funerary and genetic traits available to discuss the constitution of each "dead community".

2.2.2 Different funerary groups' constitution?

Numerous arguments have permitted the identification of the UN site as an important place of exchange with Southern England. Indeed, the site is located along the Channel coast and faces two port sites of Southern England: Hengistbury Head and Poole Harbour. Exchanges with this region are mirrored by the presence in the UN of cultural elements shared on the both side of the Channel. This is notably the case for circular buildings, the use of lignite as a raw material for the manufacture of bracelets or the position of some buried adults, which evokes the one encountered among the Durotriges group (a contemporary group based in Dorset; Lefort et al., 2015). Obviously, the interpretation of the UN site as a place of human circulation and gene flow along the Channel and the Atlantic coast (as already discussed in Fischer et al., 2018), could explain the higher maternal diversity measured for the community.

Since the UN funerary space was clearly organized in different sectors (inside and outside an enclosure), we explored whether this funerary space organization was linked to any genetic differentiation. The observed maternal diversity (h^2) was found to be higher for the group discovered outside the enclosure (0.983) than for the group buried inside the enclosure (0.976). This might suggest that the enclosure constituted a funerary sector that welcomed a special group of deceased. Given the relatively small sample sizes (respectively $N=19$ inside and $N=32$ outside the enclosure), the adjusted bootstrap confidence interval, including the null value as a credible candidate for the difference in genetic diversity between these two groups, failed to provide a strong evidence in favor of this hypothesis (extensive results available as Supporting Information online) however, this

337 observation was reinforced by the apparent exclusion of several maternal lineages (notably lineages
338 J1 and T) from the enclosure group (Fig 1). Consequently, we can propose the hypothesis that the
339 enclosure was dedicated to a specific group of the community, characterized by lower genetic
340 diversity. This hypothesis resonates with the propositions offered by archaeological studies. In some
341 necropolises dated to the Late Iron Age, the presence of an enclosure was attributed to the
342 appropriation of the territory by a dominant family, who gathered its deceased members in a
343 dedicated funerary space. The structuration of the funerary space would then reflect the social
344 hierarchy of the community members (Lambot, 2000; Verger, 2009). In the specific case of the UN
345 necropolis, the enclosure may thus have received the members of a dominant family, characterized
346 by lower genetic diversity than the rest of the community, due to its smaller effective size and/or
347 endogamy. The fact that some individuals sharing the same maternal lineage were distributed both
348 inside and outside the enclosure (Fig 1) seems counterintuitive with the hypothesis of a funerary
349 space organized according to family structure. However, since textual sources suggest that social
350 status was inherited through paternal lines (Lewuillon, 1990) and that our genetic data would be in
351 accordance with societies practising patrilinearity, the funerary space should be rather structured
352 alongside Y-chromosome lineages. Unfortunately, our low-resolution analysis of paternal lineages
353 does not permit us to test this point. An alternative hypothesis concerning the constitution of the
354 dead groups having access to the different UN sectors may include chronological evolution from an
355 initial group. Indeed, some archaeological arguments may indicate that the enclosure was used
356 before the rest of the necropolis. Notably, individuals UN-4 and UN-108 were found in graves dug
357 across the enclosure ditch and must therefore have been buried after the enclosure disappearance.
358 This could indicate that the enclosure and the individuals buried in it were more ancient than the
359 individuals found outside. In such a case, the older funerary group found in the enclosure could have
360 corresponded to a smaller founding group (with a lower diversity), which may have been at the origin
361 of a larger community found outside the enclosure or have been replaced by this more recent group.
362 Nevertheless, the chronological succession of genetically differentiated groups must have occurred

over only a hundred years *i.e.*, the duration of use of the UN necropolis. Whatever the case, if the processes implied in the genetic differentiation of the groups found inside vs. outside the enclosure in Urville-Nacqueville remain to be determined, the presence in the site of two genetically differentiated groups must be taken into account when addressing broad scale issues including groups' biological affinities, genetic legacies and dynamics.

As already mentioned, the combination of these characteristics with a short duration of use of the tumulus (200 years) allowed us to assume that the population recovered in the GLN tumulus was representative of the whole local community. The lower maternal genetic diversity measured for the GLN group could be explained by the fact that the necropolis welcomed the members of a more local community than the cosmopolitan population found in the UN site. According to Gerritsen (Gerritsen, 2006, 2003), a local community includes people who interact daily in a reduced social context in space, such as hamlets or a group of farms, and who can share a common burial place and could therefore be considered a group of affiliated individuals. For other authors, the extended family group is a unit of production, consumption and social interaction in which people participate in collective decisions and defend their own interests and those of their members (Fernández-Götz, 2014). For our study, we preferred to use the term local community in the sense defined by Gerritsen.

Interestingly, several studies have permitted a better understanding of the local organization of Gaul societies. Notably, Brun's work has permitted to show that, in a rural context, territories were organized into isolated farms distributed every 3 to 5 km (Brun, 2011). This spatial organization of agricultural communities appears to have been established during the second part of the Bronze Age and persisted during the Iron Age. More recent studies have estimated the number of *vici* and *aedificia* composing a *civita* (Lejars and Gruel, 2015; Verger, 2009). These terms refer to various political, spatial and social entities proposed for the Gallic communities. Thus, a *civitas* was a political entity that included several *pagi*, *i.e.*, groups of tribes characterized by specific ethnic identities. *Civitas* were composed of *vici* grouping themselves within several *aedificia* (Fernández-Götz, 2014).

The *aedificia* corresponded to both the farm buildings and their inhabitants and could be compared to what Fernandez-Götz called "households" (Fernández-Götz, 2014). For some authors, these households represent the "locus of everyday life and producer of most of the activities and things that archaeologists recover" (Earle and Kristiansen, 2010) and must therefore be distinguished from the nuclear family concept. In this special context, different theoretical types of tomb and habitat configurations have been proposed (Brun, 2011). Among them, the model "grouped tombs and dispersed habitat", *i.e.*, a common funerary site used for several households scattered around the necropolis could correspond to what we found in GLN. While no habitat area is currently known and associated with the GLN tumulus, the moderate number of burials and the lower mitochondrial diversity observed prompt us to propose the hypothesis of the use of the tumulus by a local community distributed among few *aedificia*.

The data available for UN and GLN necropolises thus indicated (i) that they welcomed different types of communities (more cosmopolitan for UN vs. more local for GLN) and (ii) that the structuration of the funerary space in UN was linked to a genetic structuration of the community. Consequently, it is necessary to take into account these specificities for the reconstruction of broad-scale population dynamics.

2.3 French Iron Age communities' affinities and dynamics

2.3.1 A common maternal legacy but distinct exchange networks

We first conducted descriptive analyses taking into account the gene pools of whole UN and GLN groups. A principal component analysis (PCA) based on mtDNA haplogroup frequencies was computed after the merging of the UN and GLN gene pools with those of available ancient groups dated from the Palaeolithic to the Iron Age (database published in Fischer *et al.* 2018). Since potential maternal kinship within both UN and GLN necropolises has been previously noted (which may bias haplogroup frequency estimations), we carried out PCA either considering all the

414 individuals (Fig 3) or only one individual per haplotype (S2 Fig). As the exclusion of potentially
415 maternally related individuals had no significant effect on the PCA structure, we decided in the main
416 text to present the PCA obtained for the entire UN and GLN groups.

Figure 3: Principal component analysis (PCA) on the ancient mtDNA dataset. A) PCA performed on haplogroup frequencies, built with PC1 and PC2 B) PCA performed on haplogroup

frequencies, built with PC1 and PC3. HGCN: Hunter-gatherers from Central Europe; HGW: Hunter-gatherers from Western Europe; HGS: Hunter-gatherers from Southern Europe; CAR: Cardial; EN: Early Neolithic; MN: Middle Neolithic; LN: Late Neolithic; UN: Urville-Nacqueville; GLN: Gurgy 'Les Noisats'.

The PCA built with PC1 and PC2 (23.58% and 13.24%, respectively) (Fig 3a) confirmed that most of the variations in mtDNA haplogroup frequencies lies between ancient European hunter-gatherers, Neolithic and Bronze Age groups. In this PCA, the UN and GLN samples grouped with other Iron Age groups from Spain and Germany, and this western European Iron Age cluster showed clear affinities with Bronze Age groups, indicating global maternal continuity between groups from both periods. The first important outcome of our analyses was then the demonstration of a coherent western European Iron Age group (composed of populations from Germany, France and Spain), notably characterized by a high proportion of haplogroup H (UN = 38.63%, GLN = 58.6%, IA_Spain = 37.5% and IA_Germany = 36.36%)(S6 Table). Such proportions of haplogroup H are close to the values observed for current European populations. Since it has been demonstrated that the major genetic ancestral components of current European populations were introduced during the Bronze Age (Allentoft et al., 2015; Haak et al., 2015), the resemblance between Iron Age groups and extant European populations was expected. The second important outcome of our analyses was directly linked to the previous one and corresponded to the characterization of a clear maternal steppe legacy in both UN and GLN communities. Distinct lines of evidence supported this point. First, the UN and GLN position in the PCA appeared linked to high frequencies of maternal lineage H and a noticeable proportion of haplogroups U4 (for UN group) and I, three characteristics shared with European Bronze Age groups bearing an important steppe legacy (S6 Table). Second, clear affinities between our French Late Iron Age communities and Yamnaya pastoralist groups or groups presenting a major steppe-ancestry component (*i.e.*, groups linked to Corded Ware Culture (CWC), groups associated with Bell Beaker in Germany (BBC_GER), Great Britain (BBC_GB) or Spain

(BBC_Spain), and Early Bronze Age groups from Great Britain (EBA_GB)) could be consistently supported through (i) the study of shared informative haplotype frequencies (S7 Table) or (ii) the measurement of close genetic distances (F_{ST} ; S8 Table). Lastly, the partial data obtained for Y-chromosome lineages in UN and GLN revealed a striking majority of haplogroups R*/R1b that were mainly associated with the arrival of steppe migrants in central Europe after 3000 BC (Allentoft et al., 2015; Haak et al., 2015; Mathieson et al., 2015).

If shared maternal legacy could be unravelled for both French Late Iron Age groups, we could identify some divergences in terms of genetic affinities with distinct regional ancient groups. The second PCA, computed with PC1 and PC3 (respectively 23.58% and 11.70%) (Fig 3b), tended to associate groups dated from the Neolithic to the Iron Age, found within each European region. In this PCA, it is worth noting that the GLN group held a specific position close to the Middle Neolithic group from the same location (MN_France; Rivollat et al., 2015) and to ancient groups from Spain associated with Bell Beaker culture or dated to the Early Bronze Age. The maternal affinities between GLN and ancient groups from Spain were also supported by low F_{ST} values (all in the range of 0.02; S8 Table) as well as noticeable shared haplotype rates with the Spanish group associated with the Epicardial (6.67%) or dated to the Bronze Age (6.67%) (S7 Table). If similar maternal affinities could be identified between the UN group and groups from Spain (through the study of both shared informative haplotype frequencies (S7 Table) or genetic distances (S8 Table)), they were always found in combination with affinities with groups from Great Britain, associated with Bell Beaker culture or dated to the Early Bronze Age. For the UN group, these maternal links with Great Britain and Spain may be explained by genetic exchanges along the Atlantic coast either during the Neolithic or the Bronze Age periods (as already discussed in Fischer et al. 2018). In sum, the different geographical positions of the UN and GLN communities, inserted in distinct exchange networks, may have been responsible for the slight differentiation of their maternal gene pools and affinities.

2.3.2 Distinct funerary groups - distinct maternal legacy ?

472 Because we could demonstrate that the Urville-Nacquerville funerary group was composed of two
473 maternally differentiated sub-groups (corresponding either to two socially distinct groups and/or to a
474 chronological evolution from an initial group), we tested for potential distinct maternal legacy. We
475 consequently performed all the same descriptive analyses (PCA, FST, shared haplotypes), separating
476 groups buried inside the enclosure ("UN_intra_enclos") vs. groups found outside the enclosure
477 ("UN_extra_enclos"). Interestingly, the results of these analyses revealed different maternal affinities
478 depending on the group considered. Notably, we observed in PCA computed with either PC1 and PC2
479 (23.03% and 13.5%) or PC1 and PC3 (23.03% and 11.72%) that "UN_intra_enclos" and
480 "UN_extra_enclos" groups were systematically placed in very distinct positions. In the first PCA, the
481 group originating from the enclosure notably held a very special outlier position, whereas the group
482 buried outside the enclosure was found to be very close to the GLN group. This first analysis may
483 indicate a very special maternal constitution of the group having access to the UN enclosure.
484 However, the results obtained through subsequent analyses were more difficult to interpret. Indeed,
485 the second PCA (S3 Fig) indicated affinities between the group buried outside the enclosure and
486 Neolithic groups of Germany, Spain and Great Britain, while the group found inside the enclosure
487 was determined to be closer to the groups from Spain dated from the Late Neolithic or the
488 Chalcolithic periods. The FST calculated for the distinct funerary groups also pointed out different
489 affinities, with the group from the enclosure being closer to the Channel / Atlantic coast groups
490 (EBA_GB = 0.01625; Cardial = 0.01823; BBC_Spain = 0.01848; CH_Iberia = 0.02099) while the group
491 outside the enclosure presented more affinities with French groups from the Paris basin dated from
492 the Neolithic and Iron Ages (MNF = 0.00865; GLN = 0.01039) as well as with various groups from
493 Central Europe (EN_MN_Germany_Alsace = 0.01716; EN_transDanubia = 0.0863 and IA_Germany =
494 0.01963) (Table S9). Finally, affinities regarding eastern groups differentiated the two UN funerary
495 groups. Notably, maternal affinities with the Yamnaya pastoralist groups were stronger for the group
496 outside the enclosure in regard to the group buried inside the enclosure (FST 0.02755 and 0.04687,
497 respectively; shared haplotypes 14.634% and 2.439%). All data in hand consequently tended to show

that the group having access to a specific funerary structure in the UN necropolis, an enclosure, was characterized by a special maternal gene pool, showing affinities with ancient groups distributed along the Channel or the Atlantic region (Spain). In contrast, the group buried outside the enclosure may have resembled the typical French Gaul community found in GLN. These elements of discussion would reinforce the idea of a social structuration linked to the funerary organization found in the UN necropolis and/or support the hypothesis of a funding group, partly originating from an allochthonous region, establishing the necropolis and having access to the enclosure. Then, this group may have opened this matrimonial system and this funerary space to some populations, which are more similar to the one observed at GLN. Although these hypotheses need to be tested through more precise palaeogenomic analyses, the results obtained highlight the fact that genetically differentiated groups can be encountered within cemeteries and that these groups may present very distinct genetic affinities with ancient or contemporaneous human groups from other regions. The identification of these genetically differentiated funerary groups and discussions concerning their specific composition constitute a clear prerequisite before addressing broad-scale phylogeographical issues.

3. Conclusion

The ancient DNA results obtained for two human groups of the French Late Iron Age are in total accordance with textual sources or archaeological data describing both paternal residence rules and patrilinearity for Gaulish societies. Beyond these common internal social dynamics, the comparison of genetic, osteological and archaeological data permitted us to note that the necropolises analysed welcomed communities with distinct compositions. Indeed, the Urville-Nacqueville necropolis, located along the Channel seashore and linked to a port, appeared to host a more cosmopolitan community than the Gurgy 'Les Noisats' tumulus that may have received the deceased of a local community. Moreover, the fine-scale analysis of both communities revealed that the organization of the funerary space in the UN necropolis was directly linked to a genetic structuration of the funerary

group. Indeed, data in hand permitted us to propose that the enclosure discovered in this site may have either received the members of a dominant family or a smaller funding group that may have been at the origin of a larger community found outside the enclosure. Finally, we demonstrated that genetically differentiated groups found within the UN necropolis presented very distinctive signals concerning genetic affinities with contemporary or more ancient groups. These results highlight how fine-scale analyses of ancient groups permit a better understanding of community establishment or internal social functioning necessary for a correct assessment of broad-scale genetic affinities.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Data Availability Statement:

The sequences were deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>; accession numbers MH051192 – MH051228 and MK612040 – MK612073).

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716 **Supplementary data**

717 **S1 Percentage of mitochondrial DNA recovery depending on sites and skeletal elements**

718 **S2 Fig. PCA taking into account potential kinship between individuals**

719 **S3 Fig. PCA considering UN as two groups**

720 **S1. Script. R-Script used for genetic diversity computation as well as resampling tests (.txt)**

721 **S1 Table. Biological and genetic data compiled for Urville-Nacqueville individuals targeted in our**
722 **study. (XLSX)**

723 **S2 Table. Biological and genetic data compiled for Gurgy ‘Les Noisats’ individuals targeted in our**
724 **study. (XLSX)**

725 **S3 Table. Details and references of the ancient populations dataset. (XLSX)**

726 **S4 Table. Details of the mtDNA and Y-chromosome SNPs analysed on Urville-Nacqueville and Gurgy**
727 **‘Les Noisats’ human remains. (XLSX)**

728 **S5 Table. Genetic diversity indices measured on ancient groups. (XLSX)**

729 **S6 Table. MtDNA haplogroups frequencies for ancient groups (XLSX)**

730 **S7 Table. Mitochondrial haplotypes shared between UN and GLN communities and ancient**
731 **European groups. (XLSX)**

732 **S8 Table. Fst values measured between ancient European groups (XLSX)**

733 **S9 Table. Mitochondrial haplotypes shared between UN and GLN communities and ancient groups**
734 **and Fst values measured between ancient European groups if UN is considered as two groups**
735 **(XLSX)**

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Figure S1 : Success rate in recovering mitochondrial DNA, according to site and anatomical elements. In blue UN: Urville-Nacquerville and in orange GLN: Gurgy 'Les Noisats'

Figure S3 : Principal component analysis (PCA) on the ancient mtDNA dataset, taking into account potential maternal kinship. A) PCA performed on haplogroup frequencies, built with PC1 and PC2 B) PCA performed on haplogroup frequencies, built with PC1 and PC3. HGCN: Hunter-gatherers from Central Europe; HGW: Hunter-gatherers from Western Europe; HGS: Hunter-gatherers from Southern Europe; CAR: Cardial; EN: Early Neolithic; MN: Middle Neolithic; LN: Late Neolithic; UN: Urville-Nacqueville; GLN: Gurgy 'Les Noisats'.

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Figure S3 : Principal component analysis (PCA) on the ancient mtDNA dataset, considering

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UN as two groups. A) PCA performed on haplogroup frequencies, built with PC1 and PC2 B) PCA

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performed on haplogroup frequencies, built with PC1 and PC3. HGCN: Hunter-gatherers from

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Central Europe; HGW: Hunter-gatherers from Western Europe; HGS: Hunter-gatherers from

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Southern Europe; CAR: Cardial; EN: Early Neolithic; MN: Middle Neolithic; LN: Late Neolithic;

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UN_intra: Urville-Nacqueville intra enclosure; UN_extra: Urville-Nacqueville extra-enclosure ; GLN:

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Gurgy 'Les Noisats'.