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LRA participated in data analysis, design of the study and manuscript writing. DC and GH contributed with manuscript writing and design of the study. All authors gave final approval for publication.

# **Dissecting human North African gene-flow into its western coastal surroundings**

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## **Keywords**

North Africa; Canary Islands; Iberian Peninsula; gene-flow; haplotype-based methods; fineSTRUCTURE

## **Abstract**

North African history and populations have exerted a pivotal influence on surrounding geographical regions, although scant genetic studies have addressed this issue. Our aim is to understand human historical migrations in the coastal surroundings of North Africa. We built a refined genome-wide dataset of North African populations to unearth the fine-scale genetic structure of the region, using haplotype information. The results suggest that the gene-flow from North Africa into the European Mediterranean coast (Tuscany and the Iberian Peninsula) arrived mainly from the Mediterranean coast of North Africa. In Tuscany, this North African admixture date estimate suggests the movement of peoples during the fall of the Roman Empire around the 4th century. In the Iberian Peninsula, the North African component likely reflects the impact of the Arab expansion since the 7th century and the subsequent expansion of the Christian Kingdoms. In contrast, the North African component in the Canary Islands has a source genetically related to present-day people from the Atlantic North African coast. We also find sub-Saharan gene-flow from the Senegambia region in the Canary Islands. Specifically, we detect a complex signal of admixture involving Atlantic, Senegambian and European sources intermixing around the 15<sup>th</sup> century, soon after the Castilian conquest. Our results highlight the differential genetic influence of North Africa into the surrounding coast and show that specific historical events have not only had a socio-cultural impact but additionally modified the gene pool of the populations.

## 31 Introduction

32 North Africa is a genetically diverse region from a human population perspective. North African  
33 populations show a complex and heterogeneous genetic structure that has been described as an  
34 amalgam of at least four different ancestral components: Middle Eastern, sub-Saharan African,  
35 European, and autochthonous North African (Henn et al., 2012). Most of the genetic studies about  
36 North Africa have focused on the inner relationships among populations, or the gene-flow from  
37 nearby populations (Arauna et al., 2017; Henn et al., 2012). However, there are scant studies that  
38 have focused on North African gene-flow into neighboring regions (Botigué et al., 2013). It is well  
39 known that the surrounding coast has been historically influenced by North African peoples (Plaza  
40 et al., 2003; Secher et al., 2014); however, the demographic impact of those contacts has not been  
41 properly addressed. Our aim is to assess the North African demographic and genetic influence in  
42 nearby regions outside the African continent by assessing the gene-flow in three geographical  
43 neighboring regions with documented contacts with North Africa: the Canary Islands, the Iberian  
44 Peninsula, and Tuscany.

45 The Canary Islands, located in the Atlantic coast of North Africa, have been inhabited since  
46 approximately 1,000 BCE (Navarro Mederos, 1997; Secher et al., 2014). The islands were known by  
47 the Phoenicians, Greeks, and Romans; however, it is thought that there was no contact with the  
48 autochthonous settlers of the islands since the 4<sup>th</sup> century until the Castilian conquest in the 15<sup>th</sup>  
49 century\_ (Peña, 2013). By the time of this European conquest of the Islands, the aboriginal  
50 population size has been estimated around 100,000 individuals (Rodríguez-Martin and Martín-Oval,  
51 2009). A Northwest African origin of the first settlers of the islands is consistent with patterns of  
52 uniparental and classical genetic markers in modern and ancient samples (Fregel et al., 2009a;  
53 Maca-Meyer et al., 2004). In particular, the presence of haplogroups in the Canary Islands that are  
54 only found in individuals of North African descent, such as mitochondrial (mtDNA) haplogroup U6  
55 (Maca-Meyer et al., 2003) and Y chromosome haplogroup M81 (Solé-Morata et al., 2017), among  
56 some others considered founder lineages, support the North African origin of the islanders. The  
57 frequencies of these haplogroups in the extant population of the Canary Islands show a clear sexual  
58 bias: the percentage of the maternal North African component estimated through the analysis of  
59 mtDNA lineages is high, between 42 and 74% (Maca-Meyer et al., 2004); while the paternal  
60 component analyzed through the study of Y-chromosome lineages is lower, between 5 and 16%  
61 (Fregel et al., 2009a). Additionally, Botigué and colleagues (2013), analyzing genome-wide data,  
62 showed a higher IBD sharing between individuals from the Canary Islands and North Africa  
63 compared to individuals from continental Europe, suggesting a higher gene-flow from the African  
64 continent to the Islands. Finally, genome-wide analysis with ancient DNA from the Canary Islands  
65 have corroborated the North African origin of the autochthonous component and its presence in  
66 current Canary Islanders (Rodríguez-Varela et al., 2017). However, the exact dates of admixture  
67 from Europe and the precise geographical origin of the North African component in the Islands

68 have not been addressed.

69 The most well documented contact between North Africa and the Iberian Peninsula is the Arab  
70 expansion, which crossed the Mediterranean and arrived to Gibraltar in the 8<sup>th</sup> century. However,  
71 genetic studies, mainly genetic studies based on uniparental markers, together with archaeological  
72 and anthropological evidence, have suggested previous contacts across the Gibraltar Strait that  
73 date back to prehistoric times (Bosch et al., 2001; Currat et al., 2010; Maca-Meyer et al., 2003;  
74 Plaza et al., 2003; Secher et al., 2014). Recently, ancient DNA studies have supported prehistoric  
75 migrations from North Africa into the Iberian Peninsula since around 4,000 ya (years ago)  
76 (González-Fortes et al., 2019; Olalde et al., 2019; Valdiosera et al., 2018). Moreover, mtDNA,  
77 Y-chromosome, and STRs studies (Adams et al., 2008; Capelli et al., 2009; Casas et al., 2006; Plaza  
78 et al., 2003; Regueiro et al., 2015), as well as genome-wide and ancient DNA analyses (Botigué et  
79 al., 2013; Moorjani et al., 2011; Olalde et al., 2019), have also shown gene-flow in historical times.  
80 Moorjani et al. (2011) dated African gene-flow into Southern Europe around 55 generations ago,  
81 with the highest proportions in Iberia:  $3.2 \pm 0.3\%$  in Portugal, and  $2.4 \pm 0.3\%$  in Spain, which was  
82 related to a demographic impact either in Roman or Arab periods. Botigué et al. (2013) showed  
83 that the inclusion of North African populations in their analyses increased those estimated  
84 percentages of gene-flow, suggesting a higher North African gene-flow in Iberia, and that the  
85 sub-Saharan gene-flow detected entered with the North African wave, challenging the  
86 interpretation of a direct sub-Saharan influence in Southern Europe. Additionally, the North African  
87 gene-flow in the Iberian Peninsula was dated to 6-10 generations ago, although previous gene-flow  
88 was not discarded. In a large study of human populations admixture, Hellenthal and colleagues  
89 (2014) described a complex scenario with continuous gene-flow during the past 2,000 years in  
90 Iberia with North and sub-Saharan Africans. In sum, although all studies agree on the genetic  
91 influence of North Africa in Iberia, there is no clear consensus in the pattern of gene-flow and the  
92 estimated dates of the North African admixture.

93 The presence of the Etruscans, in what it is nowadays referred to as the Tuscany territory in the  
94 Italian Peninsula, has been largely documented. However, the genetic footprint of the Etruscans in  
95 current populations has been only claimed in some isolated populations, but not in the Tuscan  
96 general population (Ghirotto et al., 2013). Moreover, although a Middle Eastern or Anatolian origin  
97 has been hypothesized for the Etruscans (Achilli et al., 2007), recent studies analyzing  
98 mitochondrial DNA have rejected an origin outside Italy (Belle et al., 2006; Ghirotto et al., 2013).  
99 Recently, an exhaustive genome-wide study of the Italian population (Fiorito et al., 2016) has dated  
100 different admixture events in Italy coming from different sources, including old events dated  
101 around 3,000 ya that involved Caucasus, Middle Eastern, and Central Italian populations; whereas  
102 other more recent admixture processes involved gene-flow from North-Central Europe around the  
103 collapse of the Roman Empire, a period which has been associated with extensive human  
104 movements. This continuous gene-flow in multiple directions at different times has yielded a  
105 complex genetic structure in the Italian Peninsula shown in both uniparental and genomewide

106 analyses (Boattini et al., 2013; Fiorito et al., 2016), and traces of North African influences have also  
107 been detected, although the amount and timing of such contributions to Italy have not been  
108 assessed (Busby et al., 2015; Fiorito et al., 2016).

109 Our aim is to assess the impact of gene-flow from North Africa to surrounding populations for  
110 which there is documented evidence of contact between the populations, in particular the Canary  
111 Islands, Iberia, and Tuscany. Previous North African – European gene-flow analyses (Botigué et al.,  
112 2013; Moorjani et al., 2011) were limited by the scant geographic distribution of North African  
113 samples available. In order to overcome these issues, we use the largest genome-wide dataset of  
114 North African samples available, including different Berber groups that have not been included in  
115 previous studies of North African gene-flow, which allows us to describe detailed and complete  
116 genetic scenarios for North African admixture into the surrounding areas. The application of  
117 haplotype-based methods to a large dataset of samples and autosomal markers might refine our  
118 knowledge on the i) estimated dates of the admixture events; ii) the specific geographic sources of  
119 the gene-flow; and, iii) the quantification of the amount of gene-flow in the three targeted  
120 populations.

## 121 **Material and methods**

### 122 **Building the dataset**

123 We built a dataset of more than 1,200 samples that includes European and sub-Saharan African  
124 samples from the 1000 genomes project (The 1000 Genomes Project Consortium, 2015); Iberian,  
125 Basque and Canary Islands populations from Botigué et al. (2013); and a large and diverse dataset  
126 of North African populations (which includes both Arab and Berbers and covers a wide geographic  
127 extension) from Henn et al. (2012) and Arauna et al. (2017) (see Table S1 and Figure S1). For some  
128 of the Iberian samples from the 1000 genomes project, no geographical coordinates are available;  
129 and, therefore, for some analyses they were assigned as “Iberian” without specifying the location.  
130 Both Plink versions 1.07 and 1.9 have been used depending on the analyses (Chang et al., 2015;  
131 Purcell et al., 2007). SNPs missing in more than 10% of the individuals, those that failed  
132 Hardy-Weinberg test at 0.01 significance threshold, and those with a minor allele frequency (MAF)  
133 below 0.05 were discarded. After filters, 267,475 SNPs remained for analyses. Individuals sharing  
134 more than 85% of their genome identity by state (IBS) were removed, and remaining individuals  
135 with more than 10% of missing SNPs were also excluded. For the analyses that required linkage  
136 equilibrium, SNPs were pruned using a pairwise linkage disequilibrium maximum threshold of 0.5  
137 using a windows size of 50 a shift step of 5, removing 149,956 SNPs.

### 138 **Haplotype-based Methods**

#### 139 Phasing

140 The phasing of SNPs was performed with SHAPEIT (O'Connell et al., 2014), using the  
 141 population-averaged genetic map from the HapMap phase II (The International HapMap  
 142 Consortium, 2003) and the 1000 genomes dataset as a reference panel (The 1000 Genomes Project  
 143 Consortium, 2015). This phasing step was performed after an alignment with the reference panel  
 144 and the removal of SNPs that did not align.

#### 145 ChromoPainter

146 ChromoPainter (Lawson et al., 2012) was run to infer the genome-wide number and proportion of  
 147 haplotype segments for which each individual shared with every other individual, without  
 148 population specification (i.e. using all sampled individuals as both recipients and donors, -a mode).  
 149 We followed the protocol analogous to that outlined in Hellenthal et al (2014). In particular, first  
 150 the global mutation probability and the switch rate parameters were estimated using the EM  
 151 algorithm implemented in ChromoPainter with the following parameters: -i 10 -in -iM, in  
 152 chromosomes 1, 7, 14 and 20 for all individuals. The mutation probability and the switch rate  
 153 parameters estimated were averaged across these four chromosomes, weighting by the number of  
 154 SNPs per chromosome. The average weighted values were 0.00017 and 208.30557 for the global  
 155 mutation and switch rate, respectively. ChromoPainter was run afterwards for all chromosomes  
 156 using these fixed global mutation and switch rates values. The final co-ancestry matrices (i.e.  
 157 \*.chuncklengths.out and \*.chunckcounts.out files) were summed across chromosomes.

#### 158 FineSTRUCTURE

159 We used ChromoCombine to estimate the fineSTRUCTURE C parameter (c=0.264). Then, following  
 160 Leslie et al (2015), FineSTRUCTURE v.2.0.4 (Lawson et al., 2012) was run using 2 million iterations of  
 161 MCMC, sampling values every 10,000 iterations following 1 million "burn-in" iterations (i.e. -x  
 162 1000000 -y 2000000 -z 10000). Finally, the FineSTRUCTURE tree was inferred using default  
 163 parameters (i.e. -m T). Three seeds were estimated in order to check robustness of the analyses.  
 164 Based on the FineSTRUCTURE results, we established genetic clusters to use as "populations" for  
 165 subsequent Globetrotter analyses.

#### 166 Globetrotter

167 We applied GLOBETROTTER to identify and date admixture events in each of our target populations  
 168 using genome-wide linkage disequilibrium decay patterns, under a model that assumes  
 169 instantaneous admixture involving 2 or more groups at 1 or 2 times in the past, followed by  
 170 random mating among individuals from the admixed population. To do so, we followed the  
 171 protocol of Hellenthal et al 2014. In particular, after defining genetic clusters based on  
 172 fineSTRUCTURE results (see FineSTRUCTURE clusters in Figure 2 and Table S1), we performed a  
 173 separate run of ChromoPainter painting each cluster using all other clusters as donors (i.e.  
 174 disallowing "self-copying" from other members of the own cluster). The clusters are assigned  
 175 geographical names in order to facilitate the comprehension, however the detailed information of

**Commented [MOU1]:** Reviewer: Page 4 Line 134. It is not clear if the samples present in the dataset were discarded from the Imputation panel. If not, is that expected to produce any bias? Authors should discuss about it and/or remove the samples from the imputation panel.

I don't understand what he means with: "samples present in the dataset", he means the reference panel? If he refers to that they are not included in any moment with our data, just used as a reference, but this is standard procedure I don't think we need to specify that.

176 the distribution of the samples contributing to each cluster can be found in table S4 and figures S2  
177 and S4. Then, we ran Globetrotter (Hellenthal et al., 2014) using the copy vectors (i.e.  
178 \*chunklength.out file) from the first ChromoPainter run used in FineSTRUCTURE (i.e. that painted  
179 each individual using all other sampled individuals) and the painting (i.e. \*samples.out files) from  
180 the second ChromoPainter run (i.e. that painted each individual using all other individuals outside  
181 of their cluster). The null.ind parameter was set to 1 for all the Globetrotter analyses, as  
182 recommended, to account for decay in linkage disequilibrium that may not be attributable to  
183 genuine admixture signals. Four different target groups were tested separately for admixture:  
184 Canary\_Islands, Iberian\_Peninsula, Tuscany, and Basque. For each of the four targets, other  
185 clusters were used as surrogates, except that the Canary\_Islands cluster was not included as a  
186 surrogate when testing the Iberian\_Peninsula cluster for admixture, due to the relatively high  
187 genetic similarity between Iberian\_Peninsula and the Canary Islands. We performed 100 bootstrap  
188 iterations to infer confidence intervals for date estimates, for both one- and two-date models of  
189 admixture. As a result, for each target cluster we have 200 estimates of the fit of the model,  
190 combined across one and two dates of admixture, and the estimated dates for each bootstrap. We  
191 assumed a generation time of 25 years (Laval et al., 2010).

192 Admixture between more than two sources at a given time is inferred by GLOBETROTTER as  
193 multiway admixture, and described as two events that each involve two sources (where each such  
194 source may comprise some unknown mixture of the genuine admixing groups). To better interpret  
195 these events, in these multiway cases we manually reviewed the coancestry curves generated for  
196 each pair of surrogate populations to establish the sources participating in the admixture process,  
197 as illustrated in Fig S7. In all these cases we found evidence for three distinct sources intermixing.  
198 In particular we assumed the three surrogates (or groups of surrogates) demonstrating the  
199 patterns in Fig S7 represented three distinct admixing sources. We represent the genetic make-up  
200 of each of these three sources in Table S3 by decomposing the GLOBETROTTER proportions  
201 estimation considering only two sources and recalculating those proportions considering the three  
202 manually inferred sources.

## 203 **Results**

204 We have compiled a dataset of more than 1,200 samples that includes a large and diverse dataset  
205 of North African populations to study the influence of North African gene flow in neighboring  
206 populations. Principal Components Analysis (PCA) of all populations in the dataset differentiates  
207 sub-Saharan and European populations along the first PC (PC1) (Figure 1). The North African  
208 samples are widely spread along the first PC reflecting high heterogeneity, in accordance with the  
209 previously described differential admixture of the subpopulations (Arauna et al., 2017; Henn et al.,  
210 2012). PC2 further differentiates North African samples and highlights the genetic diversity within  
211 North Africa. On the first two PCs, the Canary Islands samples are placed close to the Iberian



212 samples but shifted towards the Middle East and North Africa. When focusing on the European  
213 samples (inner PCA in Figure 1), three largely non-overlapping clusters can be observed: the Finns;  
214 Northern and Western Europeans (Great Britain and CEU); and Southern Europeans (Tuscany,  
215 Iberia, Basque Country, and also the Canary Islands).

216 We used haplotype-based methods to dissect the genetic structure of the studied populations and  
217 understand their genetic relationships. We performed FineSTRUCTURE analyses (Figure 2) and  
218 identified three major splits separating our data: North Africa, Europe, and sub-Saharan Africa.  
219 Within these major geographical clusters, several sub-clusters can be identified that suggest a finer  
220 resolution of genetic structure. For example, within the European cluster, six sub-clusters are found  
221 that correlate with geography: Iberian Peninsula, Tuscany, Basque, Canary Islands, Northwest  
222 Europe, and Finland (Figure S1). 13 Syrian samples clustered together with the Canary Islands  
223 populations and were removed from further analyses. Similarly, within sub-Saharan African  
224 samples we find four sub-clusters that correspond closely with sampling locations: Luhya (from  
225 Kenya), sub-Saharan Atlantic (GWD and MSL), Guinean Gulf (YRI and ESN), and North  
226 Africa\_sub-Saharan\_ancestry, which is composed of North African samples with substantial  
227 sub-Saharan admixture (as previously described in Arauna et al., 2017). In contrast, sub-clusters  
228 within North Africa do not show as precise a correlation with geography, with several sub-clusters  
229 containing individuals that span broad geographical areas: East, West, Central, Atlantic,  
230 Mediterranean, Tunisia Chenini, and Tunisia Sened (the last two have been already described as  
231 drifted populations that show high levels of relatedness (Arauna et al., 2017; Henn et al., 2012))  
232 (Figures 2 and S2). Finally, a dissection of the Iberian Peninsula sub-cluster shows four minor  
233 clusters: NorthWest\_Iberian, South\_Iberian, and two clusters without clear geographic structure  
234 (Iberian\_Peninsula1 and Iberian\_Peninsula2) (Figure S3). One Iberian individual was an outlier (did  
235 not cluster), and therefore this individual was not included in further analyses.

236 We identified and dated admixture events with GLOBETROTTER using the clusters defined in Figure  
237 2 (Figure 3). We focused on Tuscany, Iberia, and the Canary Islands, three populations that  
238 surround North Africa for which there is documented contact with North Africa (Boattini et al.,  
239 2013; Brett and Fentress, 1997; Camps, 1995; Camps and Vela i Aulesa, 1998; Fiorito et al., 2016;  
240 Naylor, 2009), in order to dissect possible admixture events between these geographical areas. We  
241 also tested admixture in the Basque population, but no admixture was detected. Assuming a single  
242 date of admixture per group, different times of admixture were inferred for the three populations:  
243 in Tuscany the mean estimated admixture time after 100 bootstrap iterations was  $485 \pm 19$  CE; in  
244 the Iberian Peninsula the estimated gene-flow was dated to  $1,000 \pm 9$  CE; and, finally, in the Canary  
245 Islands the estimated date of admixture with North Africa was  $1,555 \pm 7$  CE (Figure S4 and Table S2).  
246 However, while the data strongly supports a single event of North African admixture in Tuscany; in  
247 the Canary Islands and the Iberian Peninsula a history of multiple episodes of gene-flow cannot be  
248 ruled out, according to the goodness of fit test for two admixture events (Figure S5). The

249 GLOBETROTTER manual notes that the program concludes “multiple dates” of admixture when its  
250 goodness of fit score for two dates relative to the fit of one date is above 0.35 which are based on  
251 simulation results –(Hellenthal et al., 2014). In our dataset, 7% and 3.5% of the bootstraps exceed  
252 0.30 for the Canary Islands and Iberian Peninsula, respectively (Fig S5).

253 The sources inferred in the admixture events are also different in each of these three populations.  
254 In Tuscany, GLOBETROTTER concludes a simple admixture event between two sources (Figure 3).  
255 The major source is inferred to be related to present-day European groups, with the largest  
256 component being Iberian-like but with an additional Northwestern European-like component. The  
257 minor contributing source inferred for Tuscany relates genetically to individuals from the  
258 Mediterranean shore of North Africa, though this minor source also contains an Iberian  
259 component. In contrast, in the Iberian Peninsula we detected a more complex pattern of gene-flow  
260 of a three-way admixture between a North African-like source from the Mediterranean shore, a  
261 Basque-like source, and a European-like source with Northwest and South (Tuscany) components,  
262 possibly at different times as noted above. Finally, in the Canary Islands, admixture is detected  
263 between a European-like source, mainly related to people from the Iberian Peninsula but with  
264 some relatedness to Northwest Europeans and Tuscans, and a second source of admixture  
265 representing a composite of present-day North Africans from the Atlantic and sub-Saharan Africans  
266 from the Senegambia region.

267 Since the Iberian Peninsula analysis showed a complex pattern of gene-flow that could be  
268 attributed to the presence of genetic substructure, we analyzed the genetic subclusters within  
269 Iberia. Four different minor genetic clusters could be identified, as described above. The analysis of  
270 these four minor clusters allowed us to dissect the sources and dates of admixture within the  
271 Iberian Peninsula (Figure 4). GLOBETROTTER infers a single pulse of admixture for each of the  
272 Iberian\_Northwest and Iberian\_Peninsula2 minor clusters, with overlapping dates of gene-flow  
273 related to North African sources occurring around the 8<sup>th</sup> century (717-759 CE and 734-778 CE  
274 respectively, 95% CI). In the Iberian\_Peninsula1 minor cluster, the inferred date of North African  
275 related admixture is around the 11<sup>th</sup> century (1027-1058, 95% CI), while for the Iberian\_South  
276 minor cluster, GLOBETROTTER dates admixture to the second half of the 14<sup>th</sup> century (1330-1356,  
277 95% CI). However, in the last two cases, again multiple episodes of gene-flow cannot be ruled-out  
278 (Figure S6), and thus Figure 4 may reflect dates of more recent gene-flow and mask older  
279 gene-flow. In all Iberian clusters, GLOBETROTTER infers a North African-like source that mainly  
280 relates to our Mediterranean cluster. However, Iberian\_Northwest and Iberian\_Peninsula2 (which  
281 are the clusters for which GLOBETROTTER infers older, single pulses of admixture), also show a  
282 North African West-like component (Table S3).

283 In summary, the North African gene-flow detected in the three geographical areas analyzed  
284 (Tuscany, Iberia, and the Canary Islands) differ not only in the estimated dates of admixture, but  
285 also in the sources of admixture and amount of DNA inherited for each source. In particular

286 Tuscany and Iberia show admixture from a Mediterranean-like source, while the Canary Islands  
287 show admixture from an Atlantic North African-like source (Figure 2, Table S2).

## 288 Discussion

289 The aim of our study was to dissect gene-flow from North Africa to three surrounding coastal areas  
290 that have been documented to have had historical contact with North Africans: Tuscany, Iberia,  
291 and the Canary Islands. We applied haplotype-based methods on a large sample set using  
292 genome-wide markers in order to refine our knowledge of the gene-flow between these  
293 geographical areas, focusing on the following: i) the estimated dates of the admixture, ii) the  
294 geographical origins of the sources of the admixture events, and iii) the proportions of the  
295 gene-flow. The extensive dataset and the use of haplotype-based methods allowed us to estimate  
296 precise and narrow confidence intervals for admixture dates which we correlated with historical  
297 processes. Different estimated times, sources, and proportions of admixture were detected in each  
298 of the three populations analyzed.

299 While all three populations show evidence of admixture between European-like and North  
300 African-like source groups, the geographic characterization of the North African source varies  
301 across populations. In particular, the North African source in the Canary Islands is more genetically  
302 similar to populations along the Atlantic coast, while the North African source in Iberia and Tuscany  
303 is more genetically similar to populations along the Mediterranean Coast.

304 In the Canary Islands, our date of admixture corresponds to the time of the Castilian conquest (15<sup>th</sup>  
305 century). The European contribution is mainly Iberian, but it also shows a small amount of  
306 Northwest European genetic influence, which might be related to the presence of Normans  
307 involved in the first steps of the conquest (Reverón, 1944). The African source shows both a North  
308 African component from the Atlantic and a sub-Saharan component from Senegambia.

309 The mixture of the Atlantic and Senegambia components in the Canary Islands could be explained  
310 by admixture at different times prior to European contact. Our data suggest that the initial settlers  
311 of the Islands may have already been a composite of these two components. This scenario is  
312 supported by the presence of sub-Saharan mitochondrial lineages (i.e. L haplogroups) (Fregel et al.,  
313 2009b, 2015; Maca-Meyer et al., 2004; Ordóñez et al., 2017) in ancient Canary samples.  
314 Alternatively, admixture between the Atlantic and the Senegambia components could have  
315 occurred by gene-flow from Senegambia at different times after the initial settlement of the Islands  
316 and before their admixture with Europeans. However, the sub-Saharan gene-flow into North Africa  
317 is high and has been continuous through time, which makes it difficult to discern whether the  
318 Senegambia component was already present in North Africa before the first colonization of the  
319 Islands or whether it arrived later on. Moreover, the initial colonization of the Islands was very  
320 recent, making it difficult to ascertain how much of the North African component may be

321 attributable to the initial settlers versus potential gene-flow from North Africa after the initial  
322 colonization. Future studies including ancient DNA from North Africa could help resolve these  
323 issues.

324 Both the dates and the origin of the gene-flow from the North African Mediterranean coast suggest  
325 a genetic impact of the Arab expansion in the Iberian Peninsula. The Northwest of the Iberian  
326 Peninsula shows our oldest estimated date of North African admixture and is consistent with a  
327 single pulse of admixture around the time of the early arrival and conquest of Iberia by the Arabs.  
328 In contrast, our results suggest that the South of the Iberian Peninsula experienced more recent  
329 admixture and perhaps continuous gene-flow. In this case, the admixture is dated to the last  
330 periods of the Arab rule in the Peninsula in the second half of the 14<sup>th</sup> century. ~~In 1212, when the~~  
331 Christian Kingdoms became allies in the Battle of *Navas de Tolosa* and conquered all Southern  
332 territories except the Nasrid Kingdom of Granada, ~~which was conquered at the end of the 15<sup>th</sup>~~  
333 ~~century~~. The inferred continuous gene-flow suggests that contact between the Arab and Southern  
334 Iberian populations was not limited to that time period, and the estimated dates represent an  
335 upper bound on centuries of admixture (figures 4, S5 and S6). Collectively, we can identify at least  
336 two different gene-flow events in the Iberian Peninsula for which the inferred dates correlate with  
337 Arab rule in the territory: an early concentrated event in the Northwest of the Peninsula, and a  
338 continuous and more recent event in the South. Moreover, the North African populations that  
339 settled in the Peninsula during the Arab conquest may have had different origins (both in time and  
340 in geography), which could be indicative of different migration waves (table S3).

341 In three of the four minor genetic clusters identified for the Iberian Peninsula (Iberian\_Peninsula1,  
342 Iberian\_Peninsula2 and Iberian\_South), three-way admixture was detected between European-like  
343 (mainly Iberian), North African-like, and Basque-like sources. Alternatively, in the case of the other  
344 minor cluster, Iberian\_NorthWest, only two sources of admixture (North African-like and  
345 Iberian-like) were detected. This is in agreement with different admixture events occurring at  
346 different moments and in which different populations were involved. The fact that in the  
347 Northwest of Iberia the admixture does not involved a Basque-like component, while it  
348 participated in the admixture events detected in the rest of the Iberian Peninsula, suggests  
349 different Iberian populations participated in geographically separated admixture events. This may  
350 reflect different waves of the Christian Kingdoms expansion.

351 The genome-wide study of Fiorito et al., (2016) performed admixture analyses in a large-scale  
352 Italian dataset, and highlighted more complex events of admixture than the one described herein  
353 in Tuscany. Specifically, they described continuous gene-flow from different sources since 3,000 ya,  
354 which could be the result of their more geographically diverse sample set relative to our  
355 geographically localized sample of Tuscany. Perhaps because of this, we infer only a single pulse of  
356 admixture which coincides with the movement of people during the fall of the Roman Empire,  
357 which was just one of the multiple events detected by Fiorito and colleagues (2016). Nonetheless,

358 our focus on North African populations has allowed us to propose a more precise origin for the  
359 North African gene-flow into Tuscany, with our best surrogate group being comprised of  
360 present-day people living on the Mediterranean shores of North Africa.

361 Our study highlights the importance of including an extensive and diverse North African dataset in  
362 genetic studies. North Africa is a very heterogeneous region, with ample sociological, historical, and  
363 genetic diversity. Our use of an extensive dataset and the use of population clusters based on  
364 genetic homogeneity allowed us to detect and describe events of admixture with more precision  
365 than previous studies investigation the influence of North African gene-flow into surrounding  
366 regions. Recent methods based on haplotype information, such as those presented here, will  
367 illuminate the finer structure and genetic history of Iberian populations, particularly as sampling  
368 increases both in terms of numbers and geographic regions encompassed (Bycroft et al., 2018). In  
369 the case of the Canary Islands, ancient DNA studies might also help to better understand the origin  
370 of the first settlers of the islands and identify its influence in modern populations. (Fregel et al.,  
371 2019).

## 372 **Competing interests**

373 The authors declare no competing interest.

## 374 **Author's contributions**

375 LRA participated in data analysis, design of the study and manuscript writing. DC and GH  
376 contributed with manuscript writing and design of the study. All authors gave final approval for  
377 publication.

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## 524 **Figure captions**

525 **Figure 1.** Principal Component Analysis (PCA). The larger PCA shows all the samples included in the  
526 study, whereas the inner PCA only includes the European samples.

527 **Figure 2.** FineStructure clustering shown as a dendrogram and its correspondence in a map. The  
528 filled rectangles are the North African samples, and the proportion of individuals from each of the  
529 clusters in each geographical sampled population is shown in pie-charts. Clusters containing  
530 European and sub-Saharan African individuals are denoted by non-filled rectangles colored blue  
531 and yellow, respectively, and are labeled primarily according to geography.

532 **Figure 3.** Globetrotter admixture results for the three geographical regions analyzed (Tuscany,  
533 Iberia, and the Canary Islands). The mean admixture date and confidence intervals for each  
534 admixture event are shown above the graphs. The geographical locations of surrogates that  
535 contribute more than 2.5% are colored in the maps, with circle sizes showing the proportion of  
536 contribution. Colored areas boundaries are defined by the genetic clusters' geographic distribution.  
537 Each different shade of grey corresponds to a different admixing source group, with the surrogates

538 representing that source group linked via a continuous or dashed line. The pie in each graph shows  
539 the proportion inferred from each admixing source for the given target population (Tuscany, Iberia,  
540 or the Canary Islands, respectively).

541 **Figure 4.** Density plot for the admixture dates estimates after 100 bootstrap iterations of  
542 Globetrotter. The x-axis shows the date of admixture in years. On the top left the FineStructure  
543 dendrogram and the geographical distribution of minor clusters for the Iberian samples are shown,  
544 with each pie showing the proportion of individuals from that sampling location that were assigned  
545 to each of the four minor clusters (colors). The size of each circle corresponds to the number of  
546 sampled individuals. One cluster was formed by only one individual and therefore is not  
547 considered.

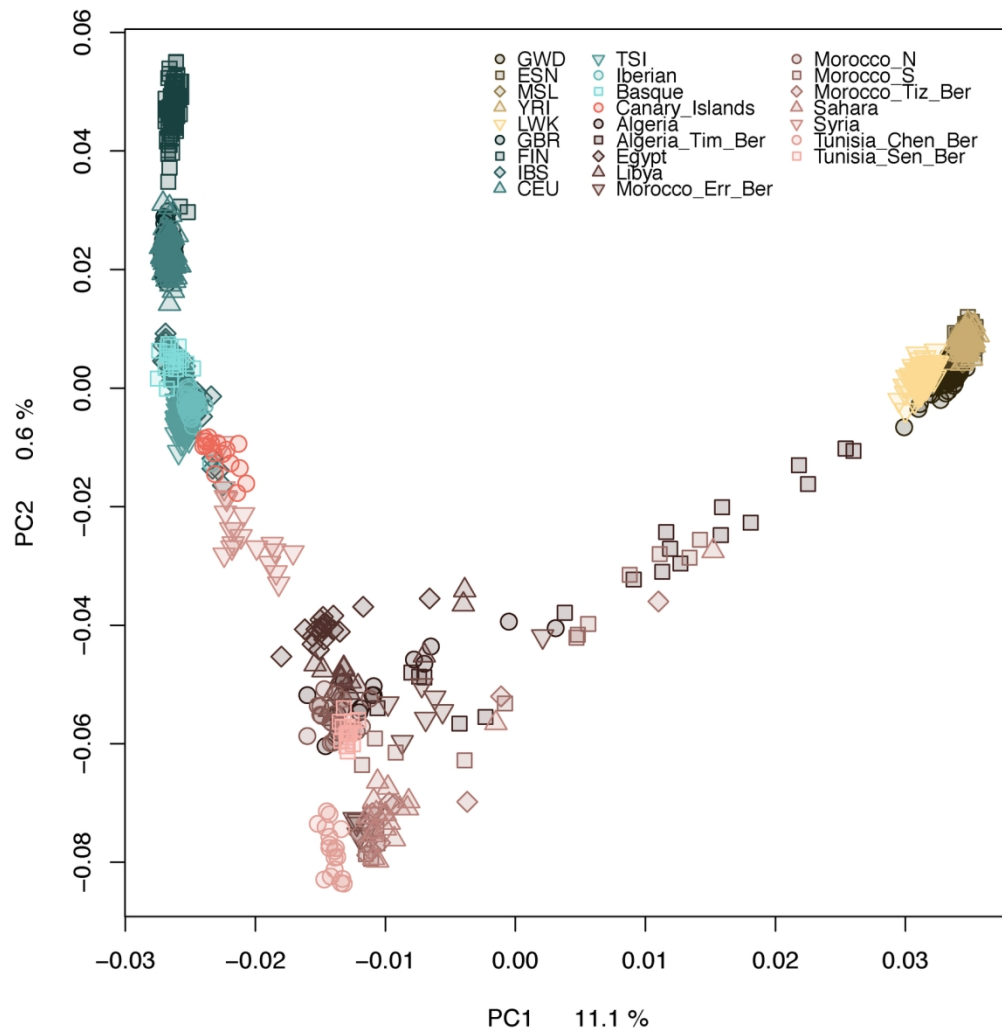


Figure 1. Principal Component Analysis (PCA). The larger PCA shows all the samples included in the study, whereas the inner PCA only includes the European samples.

153x157mm (300 x 300 DPI)

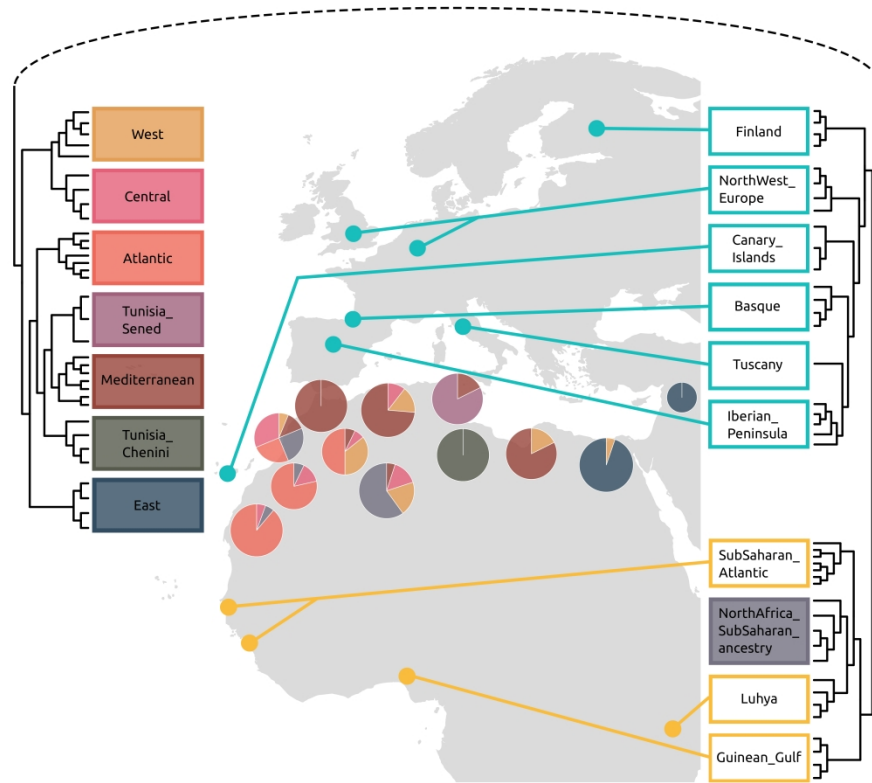


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496x512mm (299 x 299 DPI)

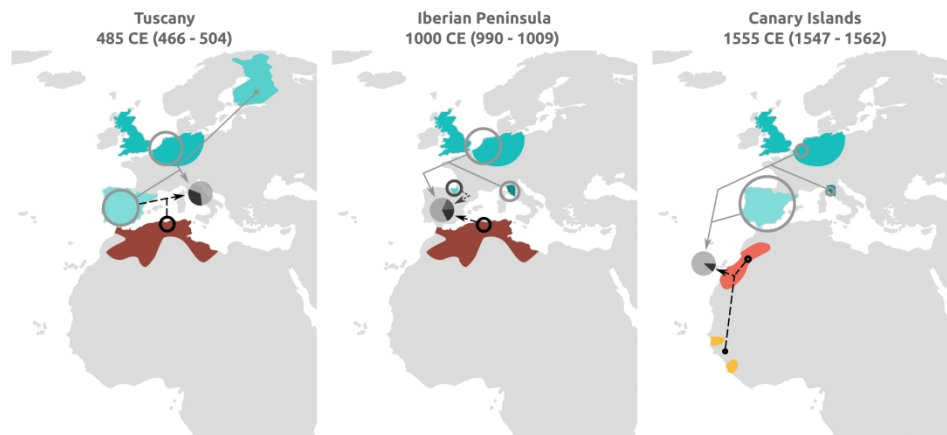


Figure 3. Globetrotter admixture results for the three geographical regions analyzed (Tuscany, Iberia, and the Canary Islands). The mean admixture date and confidence intervals for each admixture event are shown above the graphs. The geographical locations of surrogates that contribute more than 2.5% are colored in the maps, with circle sizes showing the proportion of contribution. Colored areas boundaries are defined by the genetic clusters' geographic distribution. Each different shade of grey corresponds to a different admixing source group, with the surrogates representing that source group linked via a continuous or dashed line. The pie in each graph shows the proportion inferred from each admixing source for the given target population (Tuscany, Iberia, or the Canary Islands, respectively).

199x100mm (600 x 600 DPI)

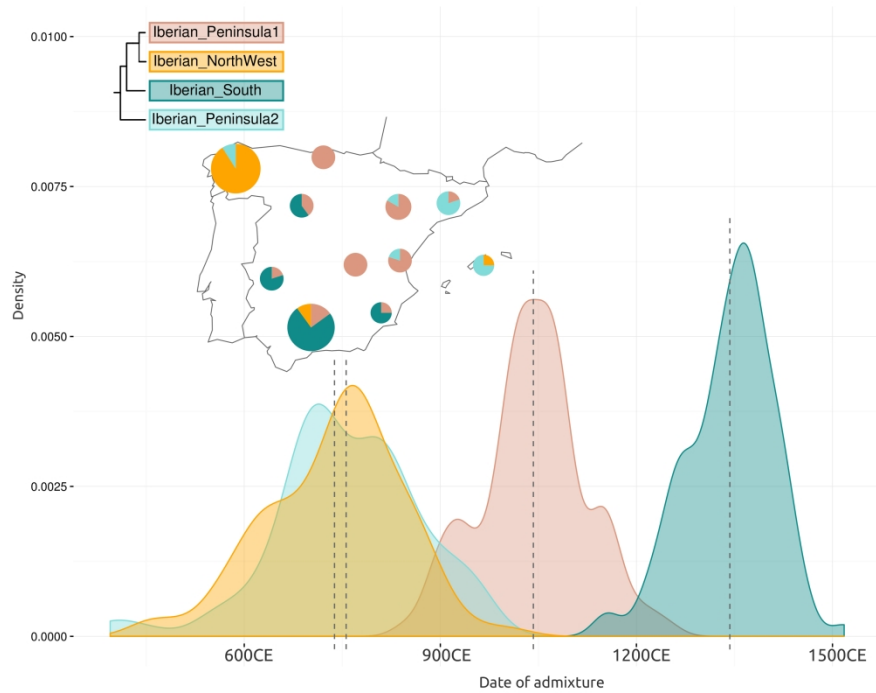


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296x209mm (300 x 300 DPI)