CLASSIFICATION

BIOLOGICAL SCIENCES: Population Biology

TITLE (112; 135 CHARACTERS MAX.)

Ancient genomes from North Africa evidence prehistoric migrations to the Maghreb from both the Levant and Europe

SHORT TITLE (48; 50 CHARACTERS MAX.)

Paleogenomics of Moroccan Neolithic populations

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KEYWORDS

ancient DNA; paleogenomics; Neolithic; North Africa

ABSTRACT (249; 250 WORDS MAX.)

The extent to which prehistoric migrations of farmers influenced the genetic pool of western North Africans remains unclear. Archaeological evidence suggests the Neolithization process may have happened through the adoption of innovations by local Epipaleolithic communities, or by demic diffusion from the Eastern Mediterranean shores or Iberia. Here, we present the first analysis of individuals' genome sequences from early and late Neolithic sites in Morocco, as well as Early Neolithic individuals from southern Iberia. We show that Early Neolithic Moroccans are distinct from any other reported ancient individuals and possess an endemic element retained in present-day Maghrebi populations, confirming a long-term genetic continuity in the region. Among ancient populations, Early Neolithic Moroccans are distantly related to Levantine Natufian hunter-gatherers (~9,000 BCE) and Pre-Pottery Neolithic farmers (~6,500 BCE). Although an expansion in Early Neolithic times is also plausible, the high divergence observed in Early Neolithic Moroccans suggests a long-term isolation and an early arrival in North Africa for this population. This scenario is consistent with early Neolithic traditions in North Africa deriving from Epipaleolithic communities who adopted certain innovations from neighbouring populations. Late Neolithic (~3,000 BCE) Moroccans, in contrast, share an Iberian component, supporting theories of trans-Gibraltar gene flow. Finally, the southern Iberian Early Neolithic samples share the same genetic composition as the Cardial Mediterranean Neolithic culture that reached Iberia ~5,500 BCE. The cultural and genetic similarities of the Iberian Neolithic cultures with that of North African Neolithic sites further reinforce the model of an Iberian migration into the Maghreb.

SIGNIFICANCE STATEMENT (119; 120 WORDS MAX.)

The acquisition of agricultural techniques during the so-called Neolithic revolution has been one of the major steps forward in human history. Using next-generation sequencing and ancient DNA techniques, we directly test if Neolithization in North Africa occurred through the transmission of ideas or by demic diffusion. We show that Early Neolithic Moroccans are

composed of an endemic Maghrebi element still retained in present-day North African populations and distantly related to Epipaleolithic communities from the Levant. However, late Neolithic individuals from North Africa are admixed, with a North African and a European component. Our results support the idea that the Neolithization of North Africa might have involved both the development of Epipaleolithic communities and the migration of people from Europe.

INTRODUCTION

One of the greatest transitions in human history was the transition from hunter-gatherer to farming lifestyle. How farming traditions expanded from their birthplace in the Fertile Crescent has been a matter of contention. Two models were proposed: one involving the movement of people and the other based on the transmission of ideas. Over the last decade, paleogenomics has been instrumental in settling long-disputed archaeological questions[1], including those surrounding the Neolithic revolution[2]. Compared to the extensive genetic work done on Europe and the Near East, the Neolithic transition in North Africa, including the Maghreb, remains largely uncharacterized. Archaeological evidence suggests that some of the major innovations associated with the Neolithic, such as farming and pottery production, could have been introduced into northern Morocco through sea voyaging by people from Iberia or the central Mediterranean as early as *ca*. 5400 BCE[3,4]. In fact, some of the Neolithic pottery recorded in North Africa strongly resembles that of European cultures like the Cardial Early Neolithic, the Mediterranean early farmer culture located in Iberia[5]. However, other innovations such as some pottery traditions and bone and lithic technical customs could be the result of *in situ* development from Epipaleolithic communities, indicating a strong continuity in the local population since the Late Pleistocene[6-10].

Genetic data from present-day populations[11-13] suggests that North African ancestry has contributions from four main sources: 1) an autochthonous Maghrebi component related to a back migration to Africa ~12,000 years ago from Eurasia; 2) a Middle Eastern component probably associated with the Arab conquest; 3) a sub-Saharan component derived from trans-Saharan migrations; and 4) a European component that has been linked to recent historic movements. Paleogenomic studies have begun to provide insights into North African Prehistory[14-16]; however, no research to date has tested whether the Neolithic transition in the Maghreb was driven by local populations who adopted cultural and technological innovations or the migration of people. Here, we perform genome-wide analysis of remains from the Early Neolithic site of Ifri n'Amr or Moussa (IAM; ~5,200 BCE, n=7) and the Late Neolithic site of Kelif el Boroud (KEB; ~3,000 BCE; n=8) (Supplementary Note 1). To test possible migrations through the Strait of Gibraltar, we also analyse human remains from the

108 southern Iberian Early Neolithic site of El Toro (TOR; ~5,000 BCE; n=12) (Figure 1). This
109 Iberian Early Neolithic culture bears similarities with early Maghrebi pottery decoration, as
110 well as bone and lithic tool production traditions which suggest an African influence[17]
111 (Supplementary Note 1). Including these southern Iberian samples in our analysis enables a
112 direct test of this hypothesis.

113 [Figure 1 here]

114 **RESULTS AND DISCUSSION**

115 We sequenced 38 Illumina pair-end libraries from 27 individuals, and selected the best-
116 conserved libraries for subsequent analyses. Endogenous DNA content was generally low
117 (2.88% on average) (Supplementary Note 2). Depth of coverage was consistently improved
118 when enriching using baits targeting specific sites for the Multiethnic Genotyping Array
119 (MEGA) (~100X), compared to whole-genome capture (~15X) (Supplementary Note 2).
120 Following enrichment, we generated thirteen low-coverage genomes (five from IAM, four
121 from KEB and four from TOR), with MEGA coverage ranging from 0.04X to 1.72X depth,
122 and genome-wide coverage ranging from 0.01X to 0.74X depth (Table 1). All samples
123 considered in this study met the standard aDNA authentication criteria, including observation
124 of DNA fragmentation (~46 bp average read length) and damage patterns due to cytosine
125 deamination toward the 5′ ends of molecules (Supplementary Note 3).

126 Mitochondrial DNA and Y-chromosome haplogroups obtained for IAM (Moroccan Early
127 Neolithic) and KEB (Moroccan Late Neolithic) suggest either a population replacement or an
128 important genetic influx into Morocco between 5,000−3,000 BCE. IAM samples belong to
129 the mtDNA haplogroups U6a and M1—both of which are associated with the back migration
130 to Africa from Eurasia in Upper Palaeolithic times[18,19]—while KEB samples belong to
131 haplogroups K1, T2 and X2, prominently found in Anatolian and European Neolithic
132 samples[2,20] (Supplementary Note 4). Regarding the paternal lineages, IAM individuals carry
133 Y chromosomes distantly related to the typically North African E-M81 haplogroup, while

134 the Y chromosome from KEB belongs to the T-M184 haplogroup; though scarce and broadly
135 distributed today, this haplogroup has also been observed in European Neolithic individuals[16]
136 (Supplementary Note 5). Both mtDNA and Y-chromosome lineages (K1, J2 and T2
137 haplogroups, and G-M201 haplogroup, respectively) for samples from TOR (Iberian Early
138 Neolithic) are similar to those observed in Europe during Neolithic times[20].

139 [Table 1 here]

140 When projected on a Principal Components Analysis (PCA) space built using sub-Saharan
141 African, North African, European and Middle Eastern population of the Human Genome
142 Diversity Project (HGDP) dataset genotyped with MEGA, IAM samples are placed close to
143 Mozabites, while Iberian Neolithic samples fall close to southern European populations
144 (Supplementary Note 6). As suspected from the mtDNA and Y-chromosome data, KEB
145 samples do not cluster with IAM and are placed in an intermediate position between IAM
146 and TOR. We further explored the genetic structure of these samples using the program
147 ADMIXTURE[21] (Figure 2). At K=5, we observe sub-Saharan African (red), early European
148 Neolithic (green), North African (yellow), Middle Eastern (violet) and eastern European
149 components (orange). Congruently with PCA results, TOR is composed of the early
150 European component, clustering with Sardinian samples, and IAM is composed of the North
151 African component, clustering with Mozabites. Finally, KEB is placed in an intermediate
152 position, with ~50% of both early European Neolithic and North African ancestries. It is
153 worth mentioning that, compared to current North African samples, IAM and KEB do not
154 show any sub-Saharan African ancestry, suggesting that trans-Saharan migrations occurred
155 after Neolithic times. This is in agreement with the analysis of present-day genome-wide
156 data from Morocco, which estimated a migration of western African origin into Morocco
157 only ~1,200 years ago[11].

158 West Eurasian populations can be modelled as admixture of four different ancestral
159 components[2]: Eastern and Western European hunter-gatherers, and Iranian and Levantine
160 Neolithic. We explored the placement of Moroccan and Southern Iberian Neolithic samples

161 in this context, and compared their genetic affinities to ancient and present-day West
162 Eurasian and Levant populations in the Human Origins panel. Interestingly, PCA reveals that
163 IAM individuals are different from any aDNA sample studied to date (Figure 2;
164 Supplementary Note 6). When projected, IAM samples are close to modern North Africans,
165 in the Levantine corner of the PCA space (Figure 2). Southern Iberian Neolithic individuals
166 from TOR cluster with Sardinians and with other Anatolian and European Neolithic samples.
167 Moreover, KEB samples are placed halfway between the IAM and Anatolian/European
168 farmer clusters, in close proximity to Levant aDNA samples and also to Guanche samples[16],
169 the indigenous population of the Canary Islands known to have a Berber origin[22]. When
170 compared using ADMIXTURE (See Supplementary Note 7 for details), IAM samples
171 possess ~100% of a component partially shared by aDNA samples from the Middle East and
172 Levant at low K values. At K=6, this IAM-like component is observed mainly in modern
173 North Africa, following a west-to-east cline, and in the Guanches. TOR and other Early
174 Neolithic samples from Iberia cluster together with farmers from Anatolia, the Aegean area
175 and Europe. At K=8, the Early Neolithic individuals from Iberia differentiate from the
176 Anatolian, Aegean and European Early Neolithic samples, and share their main component
177 (purple) with Middle Neolithic/Chalcolitic samples (Figure 2). Finally, at low K values, KEB
178 can be explained as having both IAM-like and European Neolithic components, suggesting
179 an admixture process between IAM-like people and early farmers. Nevertheless, at K=8, the
180 European component in KEB is predominantly "purple," with some "green" component.
181 This "green" component is also present, at a low frequency, in Natufians and other ancient
182 Levantine populations. The substantially larger contribution of the "purple" component,
183 when compared with the "green", suggests a significant genetic contribution of ancient
184 Iberians in Morocco (Figure 2). The same admixture profile is observed in Guanches, but the
185 amount of IAM ancestry is consistently higher in all the samples. Given that the Guanches
186 could have had originated in a different area of the Maghreb, this result might suggest that
187 the European Neolithic impact in North Africa was heterogeneous.

188 [Figure 2 here]

189 To compare our samples directly to the genomes of ancient and modern populations, we
190 calculated pair-wise F_{ST} distances, which, unlike PCA and global ancestry analyses, are
191 insensitive to the inclusion of large numbers of individuals from modern populations. F_{ST}
192 values indicate that the IAM samples are as differentiated from all other populations as
193 Yoruba are from non-Africans (Supplementary Note 9), with the sole exception of KEB and,
194 to a lesser extent, the Guanches and modern North African populations. Given the relatively
195 low heterozygosity and high identity-by-descent proportions observed in IAM
196 (Supplementary Note 8), this differentiation could be driven by isolation and genetic drift.
197 IAM is divergent from the other populations, with the exception of populations that likely
198 received genetic influx from them. This raises the possibility that IAM was isolated in North
199 Africa since Palaeolithic times, when a back migration from Eurasia brought mtDNA
200 haplogroups M1 and U6 to the Maghreb[18]. Although IAM is clearly more similar to KEB
201 than to any other population, the converse is not true. KEB has lower F_{ST} distances with any
202 Anatolian, European (excluding European hunter gatherers), Levantine and Iranian
203 population, rather than with IAM. In the modern DNA reference panel, KEB is similar to
204 North African, European and Middle Eastern populations. Among the ancient populations,
205 TOR is more similar to Middle Neolithic/Chalcolithic Europeans, and, among modern
206 populations, to populations from Spain, North Italy and Sardinia.

207 To further investigate the genetic affinities of IAM, KEB and TOR samples, we conducted
208 outgroup f3-statistic analysis[23]. Results indicate that, when KEB and Guanches are excluded,
209 IAM shares more drift with ancient Levantine populations, such as Natufians (Epipaleolithic)
210 and Pre-Pottery Neolithic individuals (Figure 3; Supplementary Note 10), than with any
211 other ancient population. To explore further the connection between IAM and Levantine
212 populations, we performed an f4-statistic analysis to test whether IAM shares more alleles
213 with any other population in the Human Origins panel[2,24] than with ancient populations from
214 the Levant (Supplementary Note 10). Consistently, and also with the exception of KEB and
215 Guanches, all comparisons indicated higher similarity with Natufians and Levantine farmers.
216 This suggests that most of IAM ancestry originates from an out-of-Africa source, as IAM
217 shares more alleles with Levantines than with any sub-Saharan Africans, including the

218 4,500-year-old genome from Ethiopia[14]. To further test the hypothesis that IAM is more
219 closely related to out-of-Africa populations, we determined if we could detect Neanderthal
220 ancestry in IAM, which is typical of non-African populations. A signal of Neanderthal
221 ancestry has been detected in modern North African populations[25]. A lack of Neanderthal
222 ancestry in IAM would imply that the signal observed today is a product of more recent
223 migration into North Africa from the Middle East and Europe in historical times. When
224 compared to the Neanderthal high coverage genome sequence from Altai[26] and the low-
225 coverage sequence from Vindija Cave[27], and using the S-statistic[23], we detected a
226 Neanderthal introgression signal into IAM, suggesting derivation from the same event shared
227 by non-African populations. All these results together indicate that the origin of IAM was
228 outside Africa, most probably from the Levant. However, it is important to take into account
229 that the number of ancient genomes for comparison is low and future sampling can provide
230 further refinement in the origin of IAM.

231 Both F_{ST} and outgroup-f3 statistic analyses indicate that KEB shares ancestry with IAM, but
232 also more genetic drift with Neolithic and Chalcolithic populations from Anatolia and
233 Europe, with the highest shared genetic drift appearing in Iberian Early Neolithic samples
234 (Figure 3; Supplementary Note 10). This pattern and the result from ADMIXTURE could be
235 explained if the KEB population was a mixture between IAM-related and European
236 Neolithic groups. To formally test this hypothesis, we used an admixture-f3 test[23], using
237 KEB as the test population, IAM as a reference population and one of the Anatolian and
238 European Neolithic and Chalcolithic populations as the second reference population. All
239 comparisons produced negative values of the f3-statistic, which suggests the KEB population
240 can be modelled as a mixture of IAM and Anatolian/European Neolithic.

241 TOR has more shared ancestry with Iberian Early Neolithic samples and other Neolithic and
242 Chalcolithic populations from Europe. Archaeological studies have suggested that there was
243 an Andalusian Early Neolithic culture with North African influences before the Cardial
244 expansion into the Western Mediterranean basin[28]. However, we observe that TOR samples

245 have a similar genetic composition to that of Cardial individuals from Iberia, evidencing a
246 common origin, and ruling out an Andalusian Early Neolithic distinct from Cardial Culture.

247 [Figure 3 here]

248 Finally, although limited by low coverage, phenotypic predictions based on genetic variants
249 of known effects agree with our estimates of global ancestry. IAM people do not possess any
250 of the European SNPs associated with light pigmentation, and most likely had dark skin and
251 eyes. IAM samples present ancestral alleles for pigmentation-associated variants present in
252 SLC24A5 (rs1426654), SLC45A2 (rs16891982) and OCA2 (rs1800401 and 12913832)
253 genes. On the other hand, KEB individuals exhibit some European- derived alleles that
254 predispose individuals to lighter skin and eye colour, including those on genes SLC24A5
255 (rs1426654) and OCA2 (rs1800401) (Supplementary Note 11).

256 **CONCLUSION**

257 Genetic analyses have revealed that the population history of modern North Africans is quite
258 complex[11]. Based on our aDNA analysis, we identify an Early Neolithic Moroccan
259 component that is restricted to North Africa in present-day populations[11], and that is the sole
260 ancestry in IAM samples. We hypothesize that this component represents the autochthonous
261 Maghrebi ancestry associated with Berber populations. This Maghrebi component is
262 different from those of any ancient samples studied so far and is distantly related to that of
263 Epipaleolithic people from the Levant. Our data suggests that the IAM population was
264 isolated in the Maghreb since the Upper Palaeolithic back migration, although it is
265 impossible to be certain without paleogenomic data from North African Palaeolithic samples.
266 An expansion in Early Neolithic times followed by strong genetic drift might also be
267 plausible.

268 Our hypothesis is in agreement with archaeological research pointing to the first stage of the
269 Neolithic expansion in Morocco as the result of a local population who adopted some
270 technological innovations, such as pottery production or farming, from neighbouring areas.

271 By 3,000 BCE, a continuity in the Neolithic spread brought Mediterranean-like ancestry to
272 the Maghreb, most likely from Iberia. Other archaeological remains, such as African
273 elephant ivory and ostrich eggs found in Iberian sites, confirm the existence of contacts and
274 exchange networks through both sides of the Gibraltar strait at this time. Our analyses
275 strongly support that at least some of the European ancestry observed today in North Africa
276 is related to prehistoric migrations, and local Berber populations were already admixed with
277 Europeans before the Roman conquest. Furthermore, additional European/Iberian ancestry
278 could have reached the Maghreb after KEB people; this scenario is supported by the
279 presence of Iberian-like Bell-Beaker pottery in more recent stratigraphic layers of IAM and
280 KEB caves. Future palaeogenomic efforts in North Africa will further disentangle the
281 complex history of migrations that forged the ancestry of the admixed populations we
282 observe today.

283 **MATERIAL AND METHODS**

284 Measures to avoid and monitor contamination from modern DNA were applied, at all times,
285 during sample manipulation. Ancient DNA was extracted from teeth or bone, built into
286 double-stranded indexed libraries and sequenced on an Illumina NextSeq 500
287 (Supplementary Note 2). Due to the environmental conditions of the burial sites, we
288 expected to recover low proportions of endogenous DNA from these ancient remains. To
289 overcome limitations due to DNA degradation, we applied two different capture methods to
290 enrich for human reads (Supplementary Note 2): one targeting the whole genome[29] and one
291 targeting the variants of the MEGA array (Illumina Inc.).

292 Reads were trimmed and adapters removed using AdapterRemoval[30], and then mapped to the
293 human reference genome (hg19) using BWA[31]. Low quality (MAPQ<30) and duplicate reads
294 were removed using SAMtools[32]. MapDamage[33] was used to visualize misincorporation and
295 fragmentation patterns, and to rescale the quality of bases likely affected by post-mortem
296 damage. Confidence intervals of sex determination were calculated following Skoglund et
297 al.[34]. MtDNA haplogroups were determined using HaploGrep[35]. Y-chromosome haplogroup
298 inference was carried out as in Schroeder et al.[36]. As the reference panel, we used both the

299 Human Origins panel[2] and the HGDP dataset genotyped with MEGA-ex (Illumina Inc.). For
300 principal component analysis, we projected the aDNA samples on the PCA space built with
301 the modern dataset, using smartpca[37] and LASER[38]. Admixture estimations were done using
302 ADMIXTURE software[21]. F_{ST} distances were calculated using smartpca[37]. Identity-by-
303 descent proportions were estimated using PLINK[39], and heterozygosity estimations using a
304 newly developed method for low-coverage genomes (Supplementary Note 8). f-statistics
305 estimates were calculated using admixtools software[23]. All plots were prepared using R
306 software[40]. Detailed information about methods is included in the Supplementary Notes.

307

308 **Acknowledgements** C.D.B and R.F. were funded by a grant from the National Science Foundation (1201234).
309 R.F. was funded by Fundación Canaria Dr. Manuel Morales fellowship. A.E.R.S. was funded by Ciencia sem
310 Fronteiras fellowship - CAPES, Brazil. B.S. and J.K. were funded by a grant from the Gordon and Betty Moore
311 Foundation (GBMF-3804).

312 **Author Contributions** R.F. and C.D.B conceived the idea for the study, D.M.S, M.D.C.M, J.S., J.M, Y.B.,
313 F.J.R.S, A.M. and A.T.M assembled skeletal material and provided archaeological background for the samples,
314 R.F., A.E.R.S., J.K. and A.S. performed work in the wet laboratory, F.M. developed methods for data analysis,
315 M.R. developed methods for DNA capture, R.F., F.M., M.A.A, P.A.U, G.W., analysed data, C.D.B and B.S.
316 supervised the study, R.F., F.M. and C.D.B. wrote the manuscript and supplements with input from all co-
317 authors.

318 **Author Information** Sequence data are available through the European Nucleotide Archive (PRJEB22699).
319 Consensus mtDNA sequences are available at the National Center of Biotechnology Information (Accession
320 Numbers MF991431-MF991448). The authors declare no competing financial interests. Correspondence and
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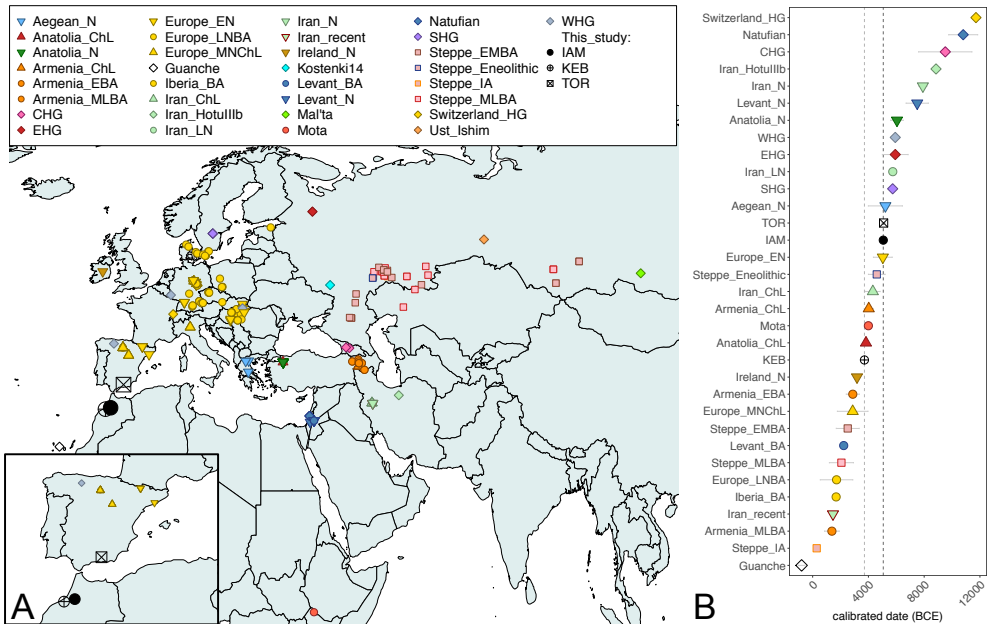
428

432 **FIGURES**

433 Figure 1. Geographical location (A) and calibrated radiocarbon date (B) of the
434 samples included in this study, as well as other ancient DNA samples from the
435 literature.



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437

Figure 2. Ancestry inference in ancient samples from North Africa and the Iberian Peninsula. (A) PCA analysis using the Human Origins panel, (B) ADMIXTURE analysis using the HGDP-MEGA dataset (K=5), (C) ADMIXTURE analysis using the Human Origins dataset for modern and ancient populations (K=8), and (D) detailed ADMIXTURE analysis for European Neolithic samples (K=8).

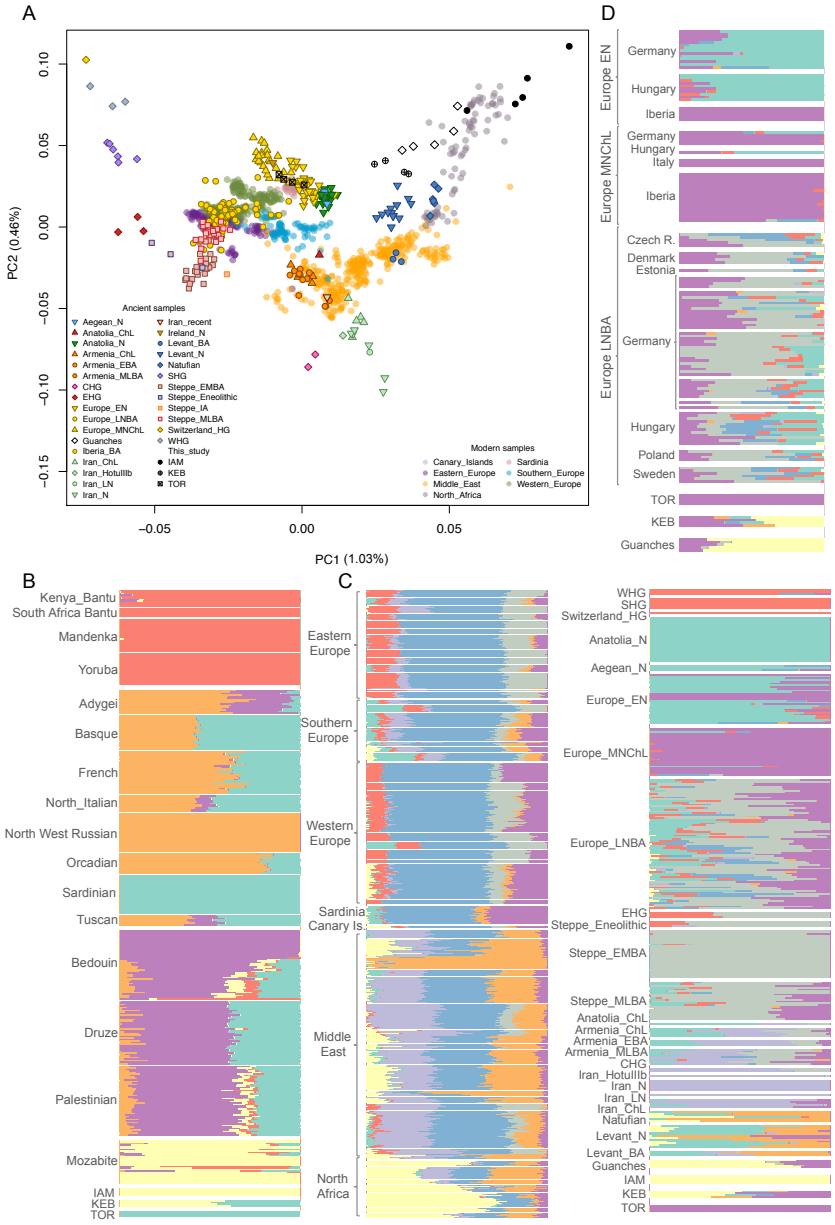
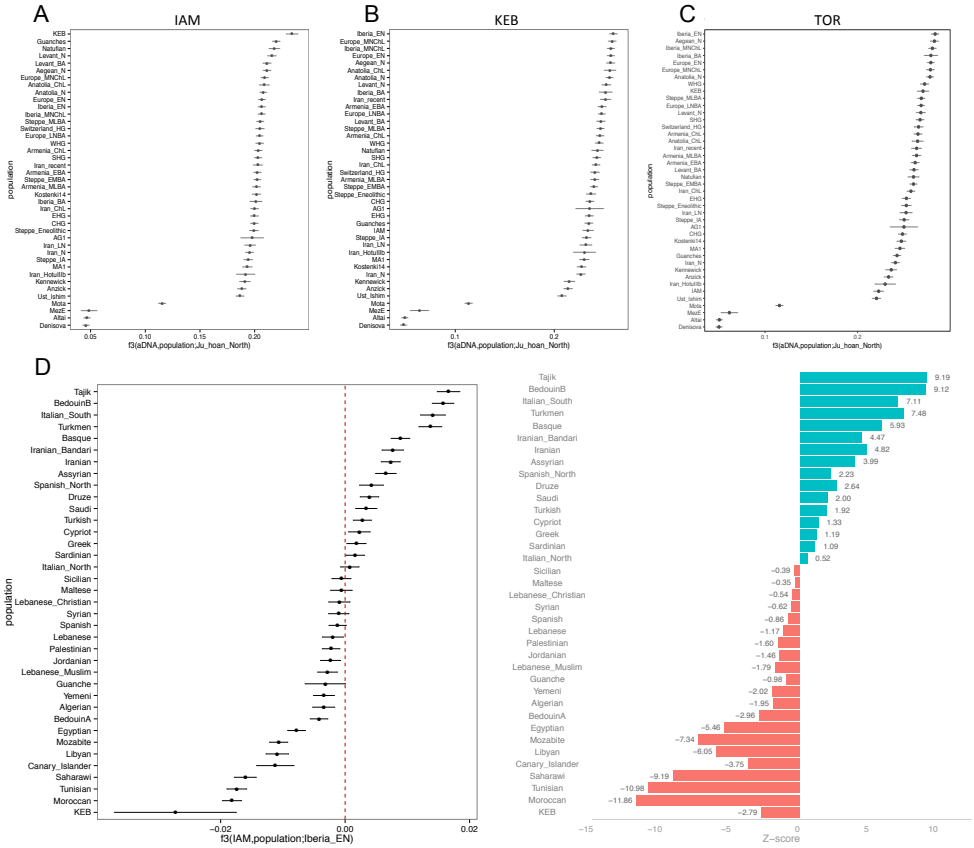


Figure 3. Outgroup f3-statistic for IAM (A), KEB (B) and TOR (C), and admixture f3-statistic for KEB (D).



448 Table 1 - Summary statistics for North African and Iberian samples.

Sample	Origin	2 sigma calibrated radiocarbon date (BCE)	MtDNA coverage	MtDNA haplogroup	Contamination estimation	Molecular sex	Y-chromosome haplogroup	SNPs in MEGA dataset	SNPs in Human Origins dataset	MEGA coverage	Genome-wide coverage
IAM.3	Ifri n'Amr or Moussa	5325 - 5210	5X	M1b1	6.42% (21.49% - 1.90%)	XX	-	26,798	5,353	0.04X	0.01X
IAM.4	Ifri n'Amr or Moussa	5215 - 5005	52X	U6a1b	4.66% (6.64% - 3.26%)	XY	E-L19*	62,729	17,093	0.12X	0.02X
IAM.5	Ifri n'Amr or Moussa	5199 - 5176 5066 - 4942	82X	U6a1b	3.68% (5.26% - 2.62%)	XY	E-L19*	408,744	208,661	1.72X	0.74X
IAM.6	Ifri n'Amr or Moussa	5290 - 5265 5230 - 5195 5180 - 5060	21X	U6a7b2	5.28% (8.48% - 3.02%)	XX	-	90,226	18,285	0.17X	0.02X
IAM.7	Ifri n'Amr or Moussa	5000 - 4840	129X	U6a3	2.35% (3.39% - 1.67%)	XY	-	177,041	45,134	0.38X	0.06X
KEB.1	Kelif el Boroud	3780 - 3650	18X	X2b	3.03% (6.31% - 1.23%)	XX	-	96,946	39,312	0.16X	0.08X
KEB.3	Kelif el Boroud		20X	K1a1b1	3.16% (5.73% - 1.72%)	-	-	-	-	-	-
KEB.4	Kelif el Boroud		135X	K1a1b1	2.49% (3.27% - 1.89%)	XX	-	304,607	77,964	0.64X	0.12X
KEB.6	Kelif el Boroud		14X	K1a4a1	3.77% (6.72% - 1.98%)	XY	T-M184	95,152	52,694	0.16X	0.14X
KEB.7	Kelif el Boroud		12X	T2b3	1.44% (4.31% - 0.31%)	XY	-	-	-		-
KEB.8	Kelif el Boroud		23X	X2b	3.03% (6.23% - 1.27%)	XX	-	34,385	18,974	0.06X	0.05X
TOR.1	El Toro	5280 - 4780	170X	T2c1d	1.42% (2.07% - 0.98%)	-	-	-	-	-	-
TOR.5	El Toro	5040 - 4850	18X	J2b1a	4.47% (8.76% - 1.96%)	XY	G-M201	-	-	-	-
TOR.6	El Toro	5280 - 4780	53X	T2b3	4.15% (5.89% - 2.94%)	XX	-	318,110	101,698	0.67X	0.17X
TOR.7	El Toro		126X	T2b3	7.06% (8.36% - 5.88%)	XX	-	115,652	24,719	0.18X	0.03X
TOR.8	El Toro		234X	K1a1	3.95% (4.80% - 3.26%)	XX	-	212,370	46,364	0.38X	0.06X
TOR.11	El Toro		124X	K1a2a	3.88% (5.00% - 2.98%)	XX	-	80,687	17,306	0.15X	0.03X
TOR.12	El Toro		174X	J2b1a	1.50% (2.30% - 0.93%)	XY	-		-	-	-

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