

1 **Late Upper Palaeolithic hunter-gatherers in the Central Mediterranean: new archaeological**
2 **and genetic data from the Late Epigravettian burial Oriente C (Favignana, Sicily).**

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24 **Abstract**

25

26 Grotta d’Oriente, a small coastal cave located on the island of Favignana (Sicily, Italy) is a key site

27 for the study of the early human colonization of Sicily. The individual known as Oriente C was

28 found in the lower portion of an anthropogenic deposit containing typical local Late Upper

29 Palaeolithic (Late Epigravettian) stone assemblages. Two radiocarbon dates on charcoal from the

30 deposit containing the burial are consistent with the archaeological context and refer Oriente C to a

31 period spanning about 14,200-13,800 cal. BP. Anatomical features are close to those of Late Upper

32 Palaeolithic populations of the Mediterranean and show strong affinity with Palaeolithic individuals

33 of San Teodoro. Here we present new ancient DNA data from Oriente C. Our results, confirming

34 previous genetic analysis, suggest a substantial genetic homogeneity among Late Epigravettian

35 hunter-gatherer populations of Central Mediterranean, presumably as a consequence of continuous
36 gene flow among different groups, or a range expansion following the Last Glacial Maximum
37 (LGM).

38
39 Keywords: Mediterranean, Late Epigravettian, Hunter-gatherers, LGM, ancient DNA, Grotta
40 d’Oriente

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42 **1.Introduction**

43 In the last few years, developments in sequencing techniques have enabled the generation of an
44 unprecedented amount of genomic data from past populations. In particular, ancient genomes from
45 Upper Palaeolithic and Mesolithic periods have made it possible to explore the early genetic
46 makeup of hunter-gatherers of the (Jones et al., 2015; Fu et al., 2016; Hofmanová et al., 2016;
47 Posth et al., 2016; Modi et al., 2017; Mathieson et al., 2018). Given its geographic location and the
48 presence of Upper Palaeolithic and Mesolithic human remains belonging to at least 3 individuals
49 (Oriente A, B and C: Mannino et al., 1972; Lo Vetro and Martini, 2006; Di Salvo et al., 2012;
50 Mannino et al., 2012; Martini et al., 2012a), Grotta d’Oriente, on the island of Favignana (SW
51 Sicily), is a key site for the study of the human colonization of Sicily during the Upper Palaeolithic
52 (Lo Vetro and Martini, 2012). Paleogenetic and morphological studies on the Mesolithic individual
53 Oriente B indicated a close proximity with Late Epigravettians of the Italian Peninsula (D’Amore et
54 al., 2010; Mannino et al., 2012), while genome-wide single nucleotide polymorphism (SNP) data
55 showed that the Upper Palaeolithic Oriente C clusters closely with other Western European Hunter-
56 Gatherer (WHG) populations from Mesolithic and Late Palaeolithic Western Europe (Mathieson et
57 al., 2018), confirming previous morphological analysis (Henke, 1989; Brewster et al., 2014).
58 Here we generated new genome-wide data in order to refine the genetic affinities of Oriente C to
59 other European hunter-gatherer populations. Our results and additional population genetic analyses
60 provide insights into the origin and population structure of the hunter-gatherers that inhabited
61 Europe during the Late Upper Palaeolithic and Mesolithic.

62 **2. Archaeological setting**

63 *2.1. The site and its setting*

64 Grotta d'Oriente is a small coastal cave located on the island of Favignana, the largest (~20 km²) of
65 a group of small islands forming the Egadi Archipelago, ~5 km from the NW coast of Sicily (Fig. 1
66 A-B). The cave opens on the north-eastern slope of Montagna Grossa, at ~40 m.a.s.l. (Fig. 1 C-D)
67 The cave is formed of two distinct areas: a small chamber at the left of the entrance and a large
68 gallery on the right (Martini et al., 2012b) (Fig. 1 E). Early excavations were conducted, without a
69 strict methodology, in the small chamber in 1972 (Mannino, 1972; 2002). New excavations were
70 performed in 2005 as a part of an interdisciplinary project carried out by the University of Florence
71 and Museo e Istituto Fiorentino di Preistoria (Colonese et al., 2011, 2014, 2018; Craig et al., 2010;
72 Lo Vetro and Martini, 2006; Martini et al., 2012a,b). A new trench was opened in 2005 next to the
73 trench excavated in the 1970s and accurate recovery of materials and a microstratigraphic approach
74 were followed. During the new excavations, a well-detailed archaeological sequence was
75 documented (Fig. 1 F): the deposit, investigated up to a depth of about 2m, consists of 8 main
76 sedimentological units (layers); five of which contain evidence of human frequentation of the cave
77 during prehistory: Late Upper Palaeolithic (Layer 7), Early Mesolithic (Layer 6), Late Mesolithic or
78 Early Neolithic (Layer 5) and Bronze Age (Layers 4-3). These cultural deposits were further
79 divided into sublayers each corresponding to different paleosurfaces which are often characterized
80 by hearths (more or less structured) and pits, and abundant artefacts and faunal remains (both
81 terrestrial and marine). These sublayers (AMS radiocarbon data are in Table 1) are attributable to
82 short-term episodes of human frequentation.

83 Below this sequence is a layer (Layer 8), containing Pleistocene fauna with no evidence of human
84 activity. Above it are two historical levels (Layers 1-2), with scarce pottery remains, which have
85 been largely reworked. In addition to the Palaeolithic burial Oriente C, which is the object of this
86 study and was brought to light during the excavations in 2005 (Lo Vetro and Martini, 2006; Martini
87 at al., 2012a), two other burials were discovered at Grotta d'Oriente in 1972: Oriente A (most likely

88 a Late Palaeolithic adult male) and Oriente B (Mesolithic adult female) (Mannino, 2002; Mannino
89 et al., 2012 and references therein).

90

91 *2.2. The burial Oriente C*

92 The Oriente C funereal pit opens in the lower portion of layer 7, specifically sublayer 7D. Two
93 radiocarbon dates on charcoal from the sublayers 7D (12149 ± 65 uncal. BP) and 7E, 12132 ± 80
94 uncal. BP are consistent with the associated Late Epigravettian lithic assemblages (Lo Vetro and
95 Martini, 2012; Martini et al., 2012b) and refer the burial to a period between about 14200-13800
96 cal. BP, when Favignana was connected to the main island (Agnesi et al., 1993; Antonioli et al.,
97 2002; Mannino et al. 2014). Several attempts at direct radiocarbon dating on Oriente C were made
98 by CEDAD laboratories (University of Lecce) but were unfortunately all unsuccessful.

99 The burial is located at the SW corner of the small chamber at the cave entrance, close to the rock
100 wall. The skeleton is completely devoid of the lower limbs, large part of the pelvis, and the hands,
101 because the burial was partly disturbed by two events: 1) during antiquity (in the Early Mesolithic),
102 there was a small pit that originated from the top of the Layer 7 that partially affected the burial,
103 perhaps removing the lower left skeletal elements; 2) in the early 1970's, the trench excavated by G.
104 Mannino intercepted the skeleton at the level of the pelvis as is clearly visible in Fig. 2.

105 In addition to these two events, a third one must be taken into account: a very peculiar feature of
106 this burial is the presence of a femur (left) placed between the shoulders of Oriente C, on the thorax.

107 The taphonomic data do not allow us to detect post-depositional disturbance of the skeleton that
108 could have occurred in case of a reopening of the grave; the dislocations of some bones could
109 therefore be attributed to post-burial movements inside the funerary pit. For this reason the femur
110 could have been deposited during the interment and could have belonged to another individual
111 (perhaps a relic).

112 However since the femur is compatible with the rest of the articulated skeleton (see below) we also
113 need to consider the possibility that the femur belonged to Oriente C and resulted in a disturbance

114 of the grave during the Palaeolithic which caused a dislocation of the lower bones after the
115 decomposition of the corpse. This hypothesis could explain why G. Mannino did not notice the
116 presence of Oriente C burial, which he would have to intercept with his excavation trench, because
117 the skeleton was already devoid of the lower limbs (Mannino, 2002; Lo Vetro and Martini, 2006). If
118 this event happened, it would have preceded the disturbance in the Mesolithic (the small pit opened
119 at the top of layer 7A) as the grave was closed again after the deposition of the femur on the
120 individual's chest and sealed by the Palaeolithic deposit (layer 7C).

121 Beyond many disturbances and severe diagenetic phenomena, the preservation of the human
122 remains at the site was very poor. To allow the recovery of human remains avoiding irreparable
123 damage, during excavation it was necessary to consolidate them several times with abundant
124 quantities of Paraloid B72, dissolved in acetone. Subsequently, the remains were removed and
125 restored by gluing the parts after careful cleaning of the surfaces with acetone in order to remove
126 the Paraloid film. Before any consolidation and restoration, during the burial excavation numerous
127 bone fragments were recovered, and later determined by morphological examination to be most
128 likely human.

129 Oriente C laid in dorsal decubitus oriented from South (the skull's position) to North. The head
130 rested on a large limestone chip with the face was turned slightly to the left. The right upper limb
131 was extended on the side of the trunk, while the left one was flexed (about 120°) with its lower end
132 placed on the lower abdomen. The bones of Oriente C curated in the Museo Fiorentino di Preistoria
133 in Florence are the following: skull cap with fragmentary base; fragments of mandibular rami; Lt.
134 M³; dental fragments comprising a fragment of an upper molar larger than the M³. Rt. humerus:
135 fragmentary lower third diaphysis; fragments of lo. epiphysis. Lt. Humerus: lower epiphysis. Rt.
136 Radius: head. Lt. radius: up. epiphysis; fragments of diaphysis. Rt. ulna: upper 2/3 of diaphysis. Lt.
137 ulna: up. and lo. epiphysis missing styloid process; fragmentary diaphysis. Lt. iliac bone: fragment
138 comprising the ant. sup. Iliac spine. Lt. femur: missing lo. epiphysis. Lt. 3rd metatarsal. All of these

139 bones show the same colour (red-brownish) and degree of fossilization except the left ulna which is
140 darker and the left radius which is nearly black because of a small fire lit in the grave (see above).
141 The skull and mandible and left elbow were slightly displaced, upper right limb long bones were
142 articulated, and the left iliac blade partially covered the flexed left forearm. These bones certainly
143 belong to a single intentionally buried individual. A left femur was laid transversely above the upper
144 part of the articulated skeleton, with the upper epiphysis on the left humerus (Fig. 2). No cut-marks
145 or other traces linked to defleshing are evident on the femur. It is possible but not proven that the
146 femur belongs to the same individual represented by the articulated bones (Lo Vetro and Martini,
147 2006). Several other human bones, often fragmentary, curated in the Museo Archeologico
148 Regionale “Antonino Salinas” in Palermo (Mannino et al., 2012) were found in the Oriente cave
149 during '70 excavations: some of these bones could belong to Oriente C, especially the hand bones,
150 but it is not possible at the moment to establish this.

151 The left humerus found in trench B does not belong to Oriente C as both humeruses are among the
152 articulated bones curated in Florence; the distal left radius and right ulna from trench B are not
153 represented in Florence and they could belong to Oriente C.

154 Age at death could not be accurately determined because of the lack of suitable anatomical parts.
155 Nevertheless, we observe that rgw exocranial sutures are not fused and there is a beginning of
156 fusion on the endocranial aspect of the obelic suture; the lt. M3 and the fragment of upper molar (an
157 M1 or M2) are unworn, the six preserved long bone epiphysis (inferior right and left humerus;
158 upper right and left radius; upper and lower left ulna) are completely fused to the diaphysis and do
159 not show traces of osteoarthritis. We conclude that the individual probably was a young adult,
160 maybe 25-30 years old. Oriente C lacks the diagnostic parts of the hip bones, but the long bone
161 midshaft and epiphysis measurements – commonly used in sex determination of fragmentary human
162 remains –indicated that the individual was most likely female (see Table S1 and Figure S1 in
163 Supplementary material). This determination was later confirmed genetically (Mathieson et al.,

164 2018). Stratigraphic and taphonomic features suggest that the funerary ritual of Oriente C consisted
165 of a sequence of steps that can be summarized as follows:

166 *1- Excavation of the funerary pit.* The pit originates in the lower part of the layer 7 (sub-layer
167 7D) and affects the base of the layer 7 (sub-layer 7E) and the underlying layer 8 (sterile
168 yellowish sands); It is shallow (about 25 cm) and has a flat bottom. The original mouth of
169 the pit may have been obliterated in the case of a subsequent reopening of the pit (see step
170 5)The North portion of the pit was removed during the excavations in the 1970s:

171 *2- Deposition of the body.* The individual was placed into the grave, his skull resting almost on
172 the western edge of the pit;

173 *3a- Burning action 1.* After the deposition, when the soft tissues of the body were probably still
174 present, a low-heat fire was lit at the bottom of the grave, in direct contact with the body, in
175 the area of the lower left hemithorax. The short and weak combustion left traces on the left
176 forearm and deposited charcoal and ashy soil at the bottom of the pit. Following the
177 hypothesis that the femur was placed on the shoulders of the deceased during the interment,
178 this event must have occurred after the fire was already extinguished, since the femur lay on
179 a thin layer of soil covering the charcoal and there are no traces of burning on the femur.

180 *3b- Burning action 2.* A second low-heat fire was lit to the right of the skull.

181 *4- closing of the grave.* The individual was definitively interred

182 *5-Possible reopening of the grave and deposition of a femur (if the femur belonged to Oriente*
183 *C individual).* A femur was placed between the shoulders of the body after the reopening of
184 the grave. In this case the original mouth of the grave may had been partially obliterated and
185 pit limits detected during the excavation may refer to the reopening of the burial. The
186 reopening, if there was any, took place during the Late Upper Palaeolithic (top of layer 7D)
187 since layer 7C, which covers the mouth of the pit, still refers to the Late Epigravettian.

188 *6-Deposition of stones.* Along the eastern edge of the grave, and also inside it, limestone blocks
189 were deposited. Some of these blocks protruding from the pit were probably placed as a
190 marker for the identification of the location of the burial.

191 The anatomical features of Oriente C are close to those of Late Upper Palaeolithic populations of
192 the Mediterranean and show strong affinity with other Palaeolithic individuals of Sicily. As
193 suggested by Henke (1989) and Fabbri (1995) the hunter-gatherer populations were
194 morphologically rather uniform. This interpretation is further supported by the low or negligible D^2
195 distance demonstrated by D'Amore et al. (2009) in the comparison between San Teodoro
196 (individuals 1-2-3-5-7) craniofacial morphometrics and other Upper Palaeolithic individuals.
197 Like other Late Epigravettian burials in Sicily and Italy (Palma di Cesnola, 2006), Oriente C is a
198 simple burial with little or no grave goods and personal ornaments. The only items in the pit were a
199 pierced shell of *Cerithium* sp. (perhaps a clothing ornament) and a few small lumps of red ochre,
200 next to the skull and the femoral head. Stable isotope analysis suggested a largely terrestrial diet
201 with low-level consumption of marine foods which is comparable to other Late Upper Palaeolithic
202 individuals from Sicily and Italy (Craig et al., 2010; Mannino et al., 2012).

203

204 **3. Genetic analysis**

205 A previous attempt at mitochondrial DNA analysis on a rib fragment of Oriente C, performed in
206 2006 by University of Rome “Tor Vergata” (Lo Vetro and Martini, 2006), was unsuccessful. The
207 current ancient DNA analysis was done on a single long bone fragment that was not exposed to the
208 substances used for consolidation and restoration (see above section 2.2). Sample preparation, DNA
209 extraction and library construction were carried out in dedicated ancient DNA facilities in Boston as
210 described in (Mathieson et al., 2018). To increase coverage compared to the previously reported
211 data, we generated two additional libraries from the same extract, performed in-solution enrichment
212 (“1240k”) and sequenced the product on an Illumina NextSeq500 using v.2 150 cycle kits for 2×76
213 cycles and 2×7 cycles. We merged these data with the data from the original library and made

214 pseudo-haploid calls by selecting a single sequence at each single nucleotide polymorphism (SNP).
215 The resulting dataset contained information on 288,223 SNPs covered at least once, compared to
216 61,547 in a previous publication (Mathieson et al.,2018), allowing for higher resolution analysis.
217 To investigate the genetic affinities of Oriente C, we used the *qp3Pop* program from
218 ADMIXTOOLS (Patterson et al., 2012) to compute f_3 -statistics and to estimate the amount of
219 shared genetic drift between Oriente C and 98 published Mesolithic and Late Palaeolithic hunter-
220 gatherers (with coverage at a minimum of 20,000 of 1240k positions) from 30 sites across Europe
221 (Gamba et al.,2014; Gonzales-Fortes et al.,2017; Gunther et al.,2018; Haak et al.,2015;Jones et
222 al.,2015; Lazaridis et al.,2014; Lipson et al.,2017; Mathieson et al.,2015,2018; Olalde et al.,2014).
223 We used the *qpDstat* program to estimates D -statistics to test whether pairs of populations form a
224 clade. The statistic $D(\text{outgroup}, A, B, C)$ is zero if A is an outgroup to the clade (B,C), positive if A
225 is closer to C, and negative if it is closer to B. For D - and f_3 -statistics, we estimated standard errors
226 using the default block jackknife procedure implemented in ADMIXTOOLS (Patterson et al.,
227 2012).
228 We confirmed the originally reported mitochondrial haplogroup assignment of U2'3'4'7'8'9. This
229 haplogroup is present in both pre- and post-LGM populations, but is rare by the Mesolithic, when
230 U5 dominates (Posth et al.2016). We further confirmed that the new genome-wide data was
231 consistent with the original data by computing D -statistics (Patterson et al.,2012) of the form D
232 (*Mbuti, X, Original Oriente data, Merged Oriente data*). None of these statistics were significantly
233 non-zero when X ranged over other European Mesolithic hunter-gatherers (maximum $|Z| = 1.8$ in 34
234 tests), and present-day French ($Z = -0.35$) and Sardinian ($Z = -0.13$) populations.
235 Lipson et al. (2018) (their supplementary Figure S5.1) and Villalba-Mouco et al. (2019) (their
236 Figure 2A) showed that European Late Palaeolithic and Mesolithic hunter-gatherers fall along two
237 main axes of genetic variation. Multidimensional scaling (MDS) of f_3 -statistics shows that these
238 axes form a “V” shape (Fig. 3). At the point of the “V” lie the individuals that have been described
239 as belonging to the “Western hunter-gatherer” (WHG) population, clustering closely with the 8,000

240 BP Loschbour individual (Lazaridis et al.2014). One arm represents a cline of ancestry that links
241 WHG with “Eastern hunter-gatherer” (EHG) populations who carry ancestry related to the “Ancient
242 North Eurasian” (ANE) population represented by the 24,000 BP Mal’ta individual (Raghavan et
243 al., 2014). Along this cline lie Eastern European hunter-gatherer populations such as those from the
244 Balkan Peninsula, present-day Ukraine (Mathieson et al., 2018), the Baltic (Jones et al., 2017;
245 Mathieson et al., 2018) and Scandinavia (Haak et al., 2015, Gunther et al., 2018). The other arm of
246 the “V” is a cline containing Late Upper Palaeolithic and Mesolithic individuals from Iberia (for
247 example the individuals from El Mirón and La Braña), and Late Upper Palaeolithic individuals
248 from Central Europe (Goyet and Hohle Fels). As shown by Fu et al. (2016), this cline reflects an
249 ancestry contribution from a population related to the 35,000 BP Aurignacian Goyet Q116-1
250 individual. In this analysis, Oriente C falls at the tip of the “V”, at the extreme end of the WHG
251 grouping.

252 Focusing further on Oriente C, we find that it shares most drift with individuals from Northern Italy,
253 Switzerland and Luxembourg, and less with individuals from Iberia, Scandinavia, and East and
254 Southeast Europe (Fig. 4A-B). Shared drift decreases significantly with distance (Fig. 4C) and with
255 time (Fig. 4D) although in a linear model of drift with distance and time as a covariate, only
256 distance ($p=1.3\times 10^{-6}$) and not time ($p=0.11$) is significant. Consistent with the overall E-W cline in
257 hunter-gatherer ancestry, genetic distance to Oriente C increases more rapidly with longitude than
258 latitude, although this may also be affected by geographic features. For example, Oriente C shares
259 significantly more drift with the 8,000 year-old 1,400 km distant individual from Loschbour in
260 Luxembourg (Lazaridis et al.,2014), than with the 9,000 year old individual from Vela Spila in
261 Croatia (Mathieson et al.,2018) only 700 km away as shown by the D-statistic (Patterson et
262 al.,2012) D (Mbuti, Oriente C, Vela Spila, Villabruna); $Z=3.42$. Oriente C’s heterozygosity was
263 slightly lower than Villabruna (14% lower at 1240k transversion sites), but this difference is not
264 significant (bootstrap $P=0.12$).

265 **4. Discussion**

266

267 Sicily falls within the area of expansion of the Epigravettian model widespread after the LGM in
268 Mediterranean Europe, from Provence to the eastern Balkan border up to the Black Sea and the SW
269 Anatolia (Fontana et al. in press). This “cultural province” is characterized by peculiar features
270 which concern not only lithotechnics but also artistic production and burial customs. The
271 Epigravettian (about 21.0-11.5 cal. ka BP) is a homogeneous cultural phenomenon despite the
272 paleo-environmental differences occurring in a wide territory and some differentiations in resources
273 exploitation strategies and human-environment interactions which were, perhaps, responsible for
274 the appearance of regional variants. This homogeneous structure is also recognizable in Sicily
275 despite the fact that the Late Epigravettian culture in the island presents a very specific local aspect,
276 especially in lithic productions. The occurrence of cosmopolitan expressions and behaviours
277 (iconographic languages, funeral rite) make Sicily the most continental of the Mediterranean islands
278 with respect to material culture at the end of the Upper Palaeolithic.

279 The large number of archaeological sites related to the human frequentation of Sicily in the Late
280 Upper Palaeolithic reveal that Late Epigravettian hunter-gatherer groups inhabited intensively the
281 island during the Late Glacial period (Lo Vetro and Martini, 2012). The robust record of
282 radiocarbon dates proves that they reached Sicily not before 15-14 ka cal. BP, several millennia
283 after the LGM peak. In our opinion, in fact, the hypothesis about an early colonization of Sicily by
284 Aurignacians (Laplace, 1964; Chilardi et al., 1996) must be rejected, on the basis of a recent re-
285 interpretation of the techno-typological features of the lithic industries from Riparo di Fontana
286 Nuova (Martini et al., 2007; Lo Vetro and Martini, 2012; on this topic see also Di Maida et al.,
287 2019).

288 The Late Upper Palaeolithic burial Oriente C is a simple burial, and its sober ritual and the modality
289 of deposition fit very well in the context of the Late Epigravettian burials of Sicily and Central-
290 Southern Italy (Palma di Cesnola, 2006). Regarding the funerary ritual, an interesting issue

291 concerns the occurrence of a femur placed on the shoulders of the individual and its possible
292 belonging to the skeleton found in place.

293 Many sources of evidence indicate that the LGM may have had a major role in shaping the genetic
294 and phenotypic variation of Upper Palaeolithic populations. A recent study based on complete
295 mitochondrial genomes has revealed a genetic homogeneity between European hunter-gatherers. A
296 significant predominance of the U lineage was detected with most of the sequences belonging to
297 U5 haplotypes (Posth et al.2016). The finding of the haplogroup U2'3'4'7'8'9 in the Oriente C
298 individual, previously recovered in the Upper Palaeolithic humans from Grotta Paglicci (Posth et
299 al.,2016) provides additional evidence for the hypothesis that Epigravettian culture might have
300 reached Sicily during the migration of Upper Palaeolithic groups from Southern Italy around the
301 LGM (Palma di Cesnola, 2006; Lo Vetro and Martini, 2012; Mannino et al., 2012), which accords
302 with the morphological similarity of Late Upper Palaeolithic and Early Mesolithic populations in
303 the region (Henke, 1989; Brewster et al., 2014). The find of genetic similarity of Oriente C with
304 Late Upper Palaeolithic and Mesolithic individuals from Northern Italy (i.e. Villabruna) and Central
305 Europe (i.e. Bichon, Loschbour) (Fig. 3) is also in line with previous studies according to which
306 Sicilian hunter-gatherers were found to be morphologically closely related to Late Epigravettians of
307 the Italian Peninsula and continental Europe (Fabbri, 1995; D'Amore et al., 2010).

308

309 **5.Conclusion**

310 These analyses have implications for understanding the origin and diffusion of the hunter-gatherers
311 that inhabited Europe during the Late Upper Palaeolithic and Mesolithic. Our findings indicate that
312 Oriente C shows a strong genetic relationship with Western European Late Upper Palaeolithic and
313 Mesolithic hunter-gatherers, suggesting that the “Western hunter-gatherers” was a homogeneous
314 population widely distributed in the Central Mediterranean, presumably as a consequence of
315 continuous gene flow among different groups, or a range expansion following the LGM.

316 The DNA study of Oriente C is particularly relevant to studying the peopling of the Central
317 Mediterranean by Anatomically Modern Humans after the LGM. The data support the hypothesis
318 that hunter-gathering groups arrived in Sicily from the Italian peninsula, confirming results derived
319 from anatomical studies on human fossil remains of Grotta di San Teodoro and from the stone
320 assemblages whose features fit in the panorama of the Late Epigravettian of Southern Italy.

321

322 **Data accessibility:** The 1240k capture sequencing data for Oriente C (merged new and existing
323 data) has been deposited in the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under
324 accession number PRJEB33231.

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326

327 **Acknowledgments:** The authors thank and remember with affection their friend and colleague
328 Sebastiano Tusa, prematurely died in the plane crash of the Ethiopian Airlines in 2019. Dr. Tusa did
329 so much for understanding the prehistoric heritage of Sicily.

330

331

332 **Authors' Contributions:** GC and DLV designed the paper, DLV and FM conducted the excavation
333 at Grotta d'Oriente, PFF conducted the anthropological study of the human remains, DLV, FM, PFF
334 provide information about the taphonomy of the burial, LS collected the sample, IM, SM, NR and
335 DR conducted the DNA analysis, GC, IM, DLV, PFF wrote the manuscript with input from all co-
336 authors.

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338

339 **Funding:** The excavation of Grotta d'Oriente in 2005 was funded by the *Regional Operational*
340 *Programme for Sicily 2000/2006, II, 2.0.1.* (European Commission), and the permission of the
341 Soprintendenza ai Beni Culturali e Ambientali (Regione Siciliana, Assessorato ai Beni Culturali e
342 Ambientali). The study of Oriente C individual was part of the MIUR-PRIN 2010–2011 action
343 (EPIC Project: Biological and cultural heritage of the central-southern Italian population through 30
344 thousand years. Grant ID: 2010EL8TXP). DR is an Investigator of the Howard Hughes Medical
345 Institute.

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494 **Table 1.** Grotta d’Oriente, radiocarbon age for the stratigraphic sequence. ¹⁴C ages are reported as
495 conventional and calibrated years BP IntCal13 (Reimer et al., 2013) in OxCal v4.3.

Layer	Material	¹⁴ C yr BP	¹⁴ C yr cal BP (68%)	¹⁴ C yr cal BP (95%)	Cultural period	Reference
5A	Charcoal	7040 ± 55	7940 – 7829	7969 – 7741	Late Meso-Early Neolithic	Lo Vetro and Martini, 2006 Martini et al., 2012
6B	Charcoal	8619 ± 65	9660 – 9530	9762 – 9485	Early Mesolithic	Lo Vetro and Martini, 2006 Martini et al., 2012
6C	Charcoal	8608 ± 65	9658 – 9526	9737 – 9480	Early Mesolithic	Lo Vetro and Martini, 2006 Martini et al., 2012
6D	Charcoal	8699 ± 60	9732 – 9551	9888 – 9542	Early Mesolithic	Lo Vetro and Martini, 2006 Martini et al., 2012
7D	Charcoal	12149 ± 65	14136 – 13932	14195 – 13791	Upper Palaeolithic	Unpublished
7E	Charcoal	12132 ± 80	14107 – 13853	14198 – 13765	Upper Palaeolithic	Lo Vetro and Martini, 2006 Martini et al., 2012

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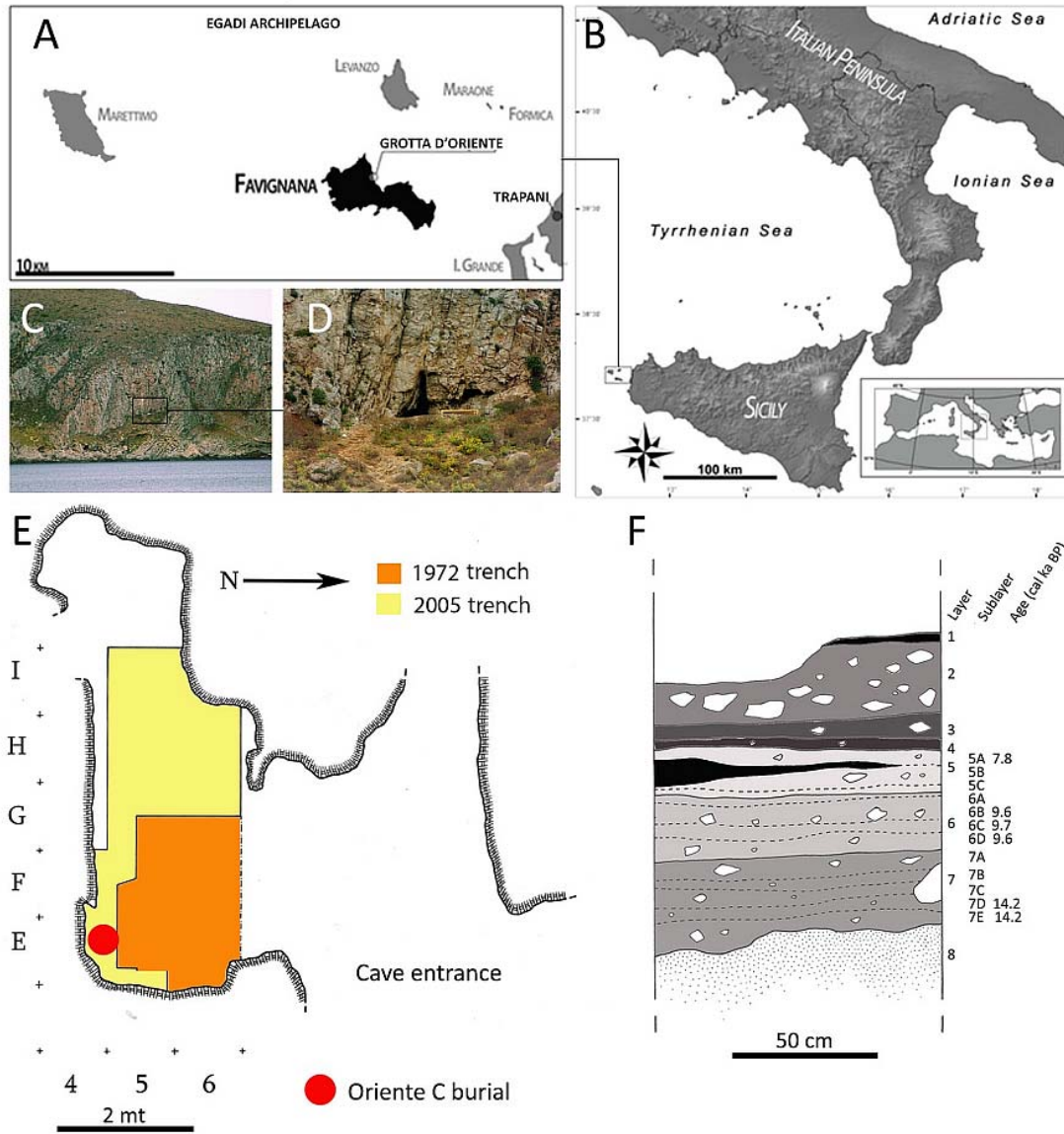
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516 **Figure 1.** A-B) Geographic location of Grotta d'Oriente; C-D) the cave entrance on the slope of
517 Montagna Grossa; E) excavation areas; F) stratigraphic sequence showing the layers and sublayers.

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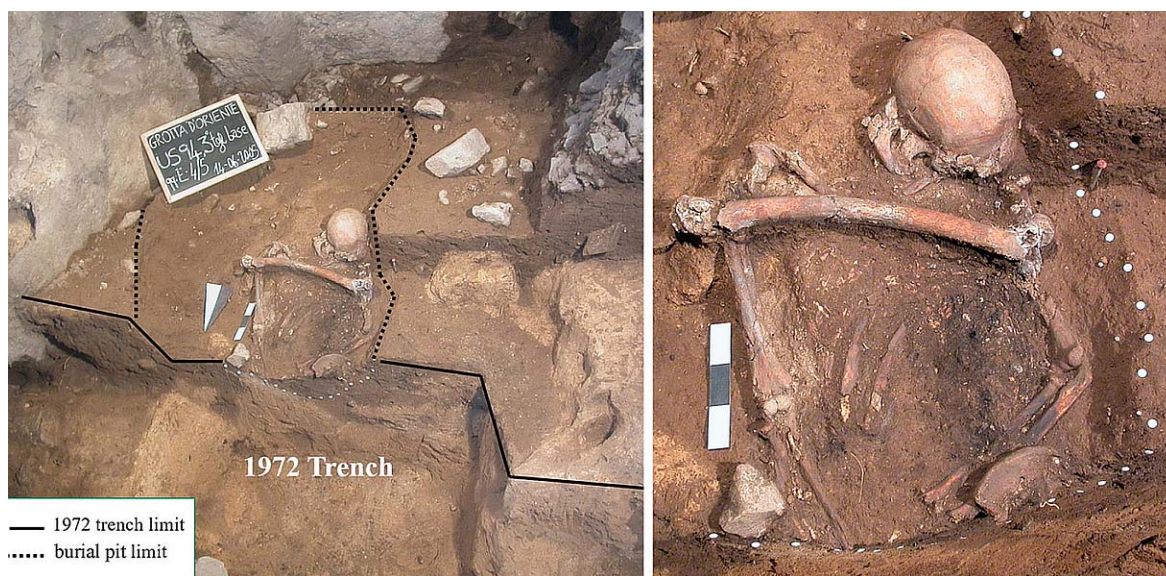
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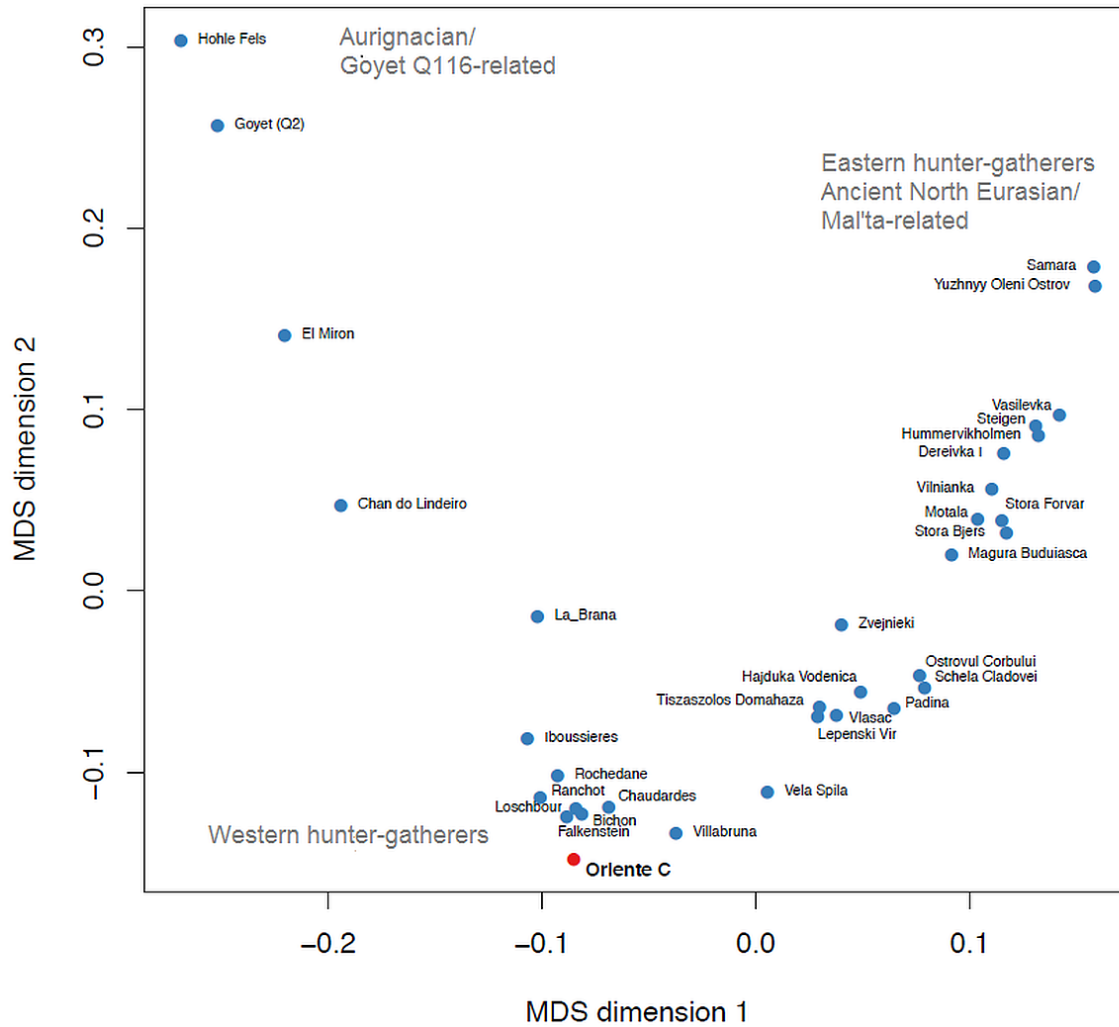
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527 **Figure 2.** The burial Oriente C during the excavation: a wide view of the burial on the left, a close-
528 up of the skeleton on the right.

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Figure 3. Multidimensional scaling of outgroup f_3 -statistics for Late Upper Palaeolithic and Mesolithic hunter-gatherers.

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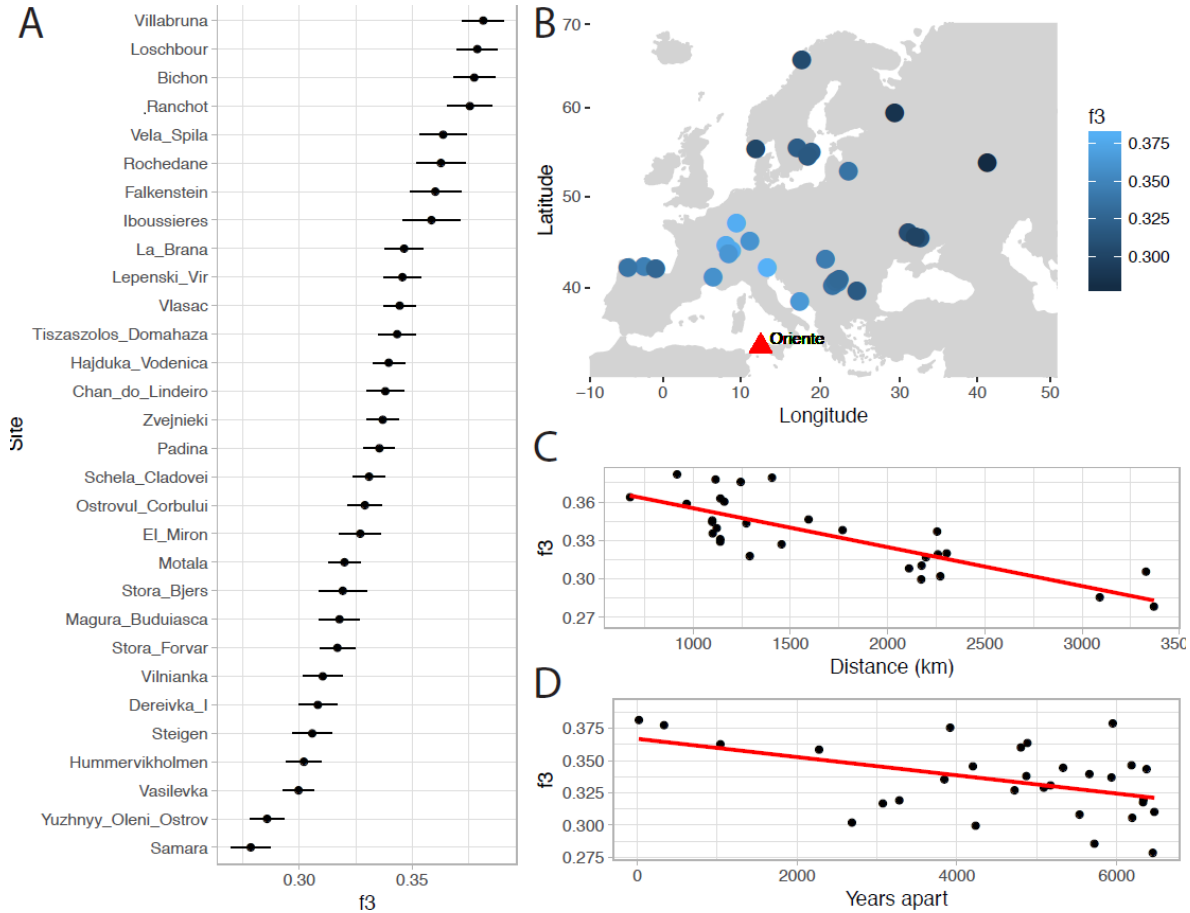
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545 **Figure 4.**A) Shared drift, estimated using f_3 -statistics between Oriente C and 98 Mesolithic and
546 Late Palaeolithic hunter-gatherers from 30 sites; B) The same statistic as in A plotted with
547 geographic position; C) Decay of shared drift with distance from Oriente C; D) Decay of shared
548 drift with absolute difference in date from Oriente C.

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