



Paleogenomic insights into the origins of French grapevine diversity

Jasmin Ramos-Madrigal, Anne Kathrine Wiborg Runge, Laurent Bouby, Thierry Lacombe, José Alfredo Samaniego Castruita, Anne-Françoise Adam-Blondon, Isabel Figueiral, Charlotte Hallavant, José Miguel Martinez-Zapater, Caroline Schaal, et al.

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1 **Palaeogenomic insights into the origins of French grapevine**

2 **diversity**

3 Jazmín Ramos-Madrigal¹, Anne Kathrine Wiborg Runge^{1,2}, Laurent Bouby³,
4 Thierry Lacombe⁴, José Alfredo Samaniego Castruita¹, Anne-Françoise Adam-
5 Blondon⁵, Isabel Figueiral⁶, Charlotte Hallavant⁷, José M. Martínez-Zapater⁸,
6 Caroline Schaal⁹, Reinhard Töpfer¹⁰, Bent Petersen^{1,11}, Thomas Sicheritz-
7 Pontén^{1,11}, Patrice This⁴, Roberto Bacilieri⁴, M. Thomas P. Gilbert^{1,12,*}, Nathan
8 Wales^{1,2,13,14*}

9
10 ¹ Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350
11 Copenhagen, Denmark. ² BioArCh, Department of Archaeology, University of York, Wentworth
12 Way, York YO10 5DD, UK. ³ ISEM - UMR 5554, CNRS-IRD-EPHE-Université Montpellier, Place
13 Eugène Bataillon, CC 065, 34095 Montpellier Cedex, France ⁴ UMR AGAP, Université Montpellier,
14 CIRAD, INRA, Montpellier SupAgro, 2 Place Viala, 34060 Montpellier, France. ⁵ URGI, Unité de
15 Recherche Génomique-Info, UR1164, INRA, Université Paris-Saclay, Route de Saint-Cyr 78026
16 Versailles, France. ⁶ Inrap, Méditerranée and ISEM - UMR 5554, CNRS-IRD-EPHE-Université
17 Montpellier, Place Eugène Bataillon, CC 065, 34095 Montpellier Cedex, France. ⁷ Bureau d'
18 études Hadès, laboratoire TRACES - UMR 5608 (pôle Terrae) - UT2J, 5 allées A. Machado, 31058
19 Toulouse Cedex 9, France. ⁸ Instituto de Ciencias de la Vid y del Vino (CSIC-UR-Gobierno de La
20 Rioja), Ctra. de Burgos km 6, 26007 Logroño Spain. ⁹ GéoArchEon Sarl, Laboratoire Chrono-
21 Environnement - UMR 6249, Université de Franche Comté, 16 route de Gray, 25000 Besançon,
22 France. ¹⁰ Julius Kühn-Institut Bundesforschungsinstitut für Kulturpflanzen, Institut für
23 Rebenzüchtung, Geilweilerhof, D-76833 Siebeldingen, Germany. ¹¹ Centre of Excellence for
24 Omics-Driven Computational Biodiscovery, Faculty of Applied Sciences, AIMST University, Kedah,
25 Malaysia. ¹² NTNU University Museum, 7491 Trondheim, Norway. ¹³ Department of Plant and
26 Microbial Biology, University of California, 111 Koshland Hall, Berkeley, CA 94720, USA. ¹⁴
27 Laboratoire d'Anthropobiologie Moléculaire et d'Imagerie de Synthèse, CNRS UMR 5288,

28 Université Paul Sabatier, 31000 Toulouse, France. * e-mail: nathan.wales@york.ac.uk or
29 tgilbert@snm.ku.dk

30

31 **The Eurasian grapevine (*Vitis vinifera*) has long been important for wine**
32 **production and a food source. Despite being clonally propagated, modern**
33 **cultivars exhibit great morphological and genetic diversity, with thousands**
34 **of varieties described in historic and contemporaneous records. Through**
35 **historical accounts, some varieties can be traced to the Middle Ages, but the**
36 **genetic relationships between ancient and modern vines remain unknown.**
37 **We present target-enriched genome-wide sequencing data from 28**
38 **archaeological grape seeds dating to the Iron Age, Roman era, and**
39 **medieval period. When compared to domesticated and wild accessions, we**
40 **found the archaeological samples were closely related to Western**
41 **European cultivars used for winemaking today. We identified seeds with**
42 **identical genetic signatures present at different Roman sites, as well as**
43 **seeds sharing parent-offspring relationships with varieties grown today.**
44 **Furthermore, we discovered one seed dated to ~1100 CE was a genetic**
45 **match to ‘Savagnin Blanc’, providing evidence for 900 years of**
46 **uninterrupted vegetative propagation.**

47

48 Since its domestication in Southwestern Asia more than 6000 years ago ¹⁻³, the
49 Eurasian grapevine (*Vitis vinifera* L.) has become one of the world’s most widely
50 produced and economically valuable fruit crops. Although grapevine products
51 are widely consumed as table grapes, dried raisins, fruit preserves, and cooked
52 leaves, both archaeological and historical evidence indicates that wine has been

53 its primary use ^{4,5}. A key unresolved question in ancient viniculture is the origin
54 and proliferation of vegetative propagation ⁶. Like many other fruit crops,
55 grapevine is grown almost exclusively as clonal lineages, wherein favored
56 varieties are maintained through horticultural techniques like grafting, layering,
57 and planting of shoots ^{7,8}. These methods take advantage of its natural ability to
58 reproduce asexually under certain conditions, and ultimately enable the
59 establishment of genetic clones of valuable cultivars. With vegetative
60 propagation, viniculturists can consistently harvest berries with a desired flavor
61 profile, and with relatively limited effort, have the potential to expand cultivars
62 to new vineyards and distant regions. The alternative approach of sowing seeds
63 is unreliable because grapevine genomes are highly heterozygous and
64 individuals grown from seed are highly diverse in quality, yield, phenotype, and
65 phenology ⁸. Moreover, winemakers have to wait from three to five years until
66 vines reach maturity ⁹, before it is possible to assess berry quality and yield.
67 Thus, clonal lineages of high-quality vines have become indispensable in modern
68 viniculture. Discovering the antiquity of vegetative propagation technologies and
69 the unique histories of individual grapevine varieties will mark a major
70 advancement in our understanding of ancient viniculture, provide a means to
71 investigate longstanding local agricultural traditions, and generate pertinent
72 information for future development of breeding schemes (*e.g.* through better
73 understanding why some varieties have been more successful than others, or
74 adding historical value to present-day cultivars).

75

76 The history of winemaking in France provides a useful model to explore how
77 vegetative propagation helped establish ancient vineyards, and how those

actions ultimately shaped the economy and landscape of one of the world's most esteemed winegrowing countries. Written sources and archaeological records indicate vineyards were first planted at the Greek colony of *Massalia*, present day Marseille, during the 6th century BCE ^{10,11}. Winemaking subsequently spread along the Mediterranean coast ¹², but it was not until end of the first century BCE that Romans greatly increased wine production across southern France ¹⁰. Roman authors, including Pliny the Elder in the first century CE (¹³: Book XIV), discussed grafting and grapevine varieties, thereby demonstrating their proficiency in vegetative propagation techniques. While Pliny describes varieties, it is currently impossible to link Roman names to modern grapevines; however, it is frequently speculated that some living varieties were grown by the Romans, and that those genetic clones have been maintained for two millennia ⁹. After the fall of the Roman Empire, winemaking traditions continued in France, and by the Middle Ages, contemporary variety names appear in written records ¹⁴. Even though historic names are still used today, it remains unknown whether the same genetic clone has been maintained, or if names have been assigned to other lineages.

Archaeobotanical remains, in particular seeds, have the potential to shed new light on the legacy of French grapevine varieties, and more generally on the history of viniculture. Using morphometric analyses of seed shape, researchers have shown seeds from most domesticated grapevines (*V. vinifera* subsp. *vinifera*) can be distinguished from those produced by wild vines (*V. vinifera* subsp. *sylvestris*) ^{15,16}. With this approach, Bouby *et al.* ¹⁰ determined that early Roman sites in Southern France (50 BCE–225 CE) contained greater numbers of

103 morphologically wild seeds than the following period (225–600 CE), raising the
104 question of whether Romans collected and cultivated wild berries for
105 winemaking. Through this time series, seed shapes tended toward domesticated
106 morphotypes, a finding the authors hypothesize represents a combination of
107 continued selective pressures with a sporadic incorporation of native varieties
108 through sexual reproduction. While these interpretations are thought provoking,
109 the authors also recognize critiques that some wild and domesticated vines
110 produce morphologically indistinguishable seeds.

111

112 One of the most promising avenues of research for ancient viniculture is
113 palaeogenomic (or ancient DNA, aDNA) analysis of well-preserved
114 archaeological pips^{17–19}. For example, Wales *et al.*²⁰ demonstrated that many
115 waterlogged grape seeds contain high proportions of endogenous DNA that
116 could be interrogated with state-of-the-art, high-throughput aDNA sequencing.
117 With the establishment of genomic databases for hundreds of modern cultivars
118 and wild grapevines²¹, we sought to examine how DNA recovered from
119 archaeological samples could sidestep some of the challenges of conventional
120 archaeobotanical methods and reveal relationships between ancient samples
121 and modern varieties, thereby providing otherwise unachievable insights on past
122 implementation of vegetative propagation and the antiquity of some of the
123 world's most produced grapevine varieties.

124

125 **Results and discussion**

126

127 **Successful enrichment of SNP loci in archaeological pips**

128 We performed targeted enrichment and shotgun sequencing of 10,000 SNP loci
129 in 28 archaeological grape seeds. The pips were recently excavated from
130 waterlogged features (wells, latrines, ditches, and pits) at 9 French sites
131 (Supplementary Fig. 1), and based on archaeological context, date as early as the
132 Iron Age (510–475 BCE) and as late as the medieval period (1050–1200 CE)
133 (Table 1). SNP loci were selected from the GrapeReSeq panel, a DNA microarray
134 that was developed to authenticate varieties for breeding and germplasm
135 management ²¹. This reference panel provides data for 783 domesticated
136 varieties (*V. vinifera* subsp. *vinifera*), 112 wild (*V. vinifera* subsp. *sylvestris*)
137 accessions collected from Eurasia and North Africa, and 11 other *Vitis* species.
138 We obtained a 4- to 400-fold enrichment at the targeted SNP sites, leading to an
139 on-target depth of coverage of $1\text{--}25.7\times$ (Supplementary Table 1 and
140 Supplementary Fig. 2). Nucleotide misincorporation patterns observed in the
141 sequencing data and read length distributions were consistent with those
142 expected for degraded DNA ²² (Supplementary Figs. 3, 4 and 5a) .

143

144 **Archaeological seeds related to European winemaking lineages**

145 We employed multidimensional scaling (MDS) to investigate whether
146 archaeological samples were more closely related to wild accessions or
147 domesticated varieties. Samples were compared to the GrapeReSeq panel
148 following the random allele sampling strategy described in *bammds* ²³, to
149 account for varying depth of coverage in the archaeological samples.
150 Additionally, we expanded our reference dataset with publicly-available whole-
151 genome sequencing data from 27 wild and domesticated grape accessions ^{24–26}
152 (Supplementary Table 2). The MDS plots showed all 28 archaeological samples

153 fall within the variability of domesticated grapevines, suggesting none of the
154 seeds originated from truly wild vines (Fig. 1a). While it is plausible that samples
155 near the boundary of the domesticated and wild clusters could represent F₁
156 hybrids between domesticated varieties and wild grapevines (*e.g.* specimen R-
157 LLE_09), we find no evidence for large-scale collection of wild berries by Romans
158 or medieval people at these sites. Likewise, the oldest sample, from the Iron Age
159 site of La Cougourlude dating to 510–475 BCE, also falls within the MDS space
160 composed of cultivated grapevines. These findings support Bouby *et al.*'s¹⁰
161 hypothesis that even though many pips from Roman and medieval sites exhibit
162 wild morphotypes they in fact originate from domesticated varieties.

163

164 Once we determined that archaeological seeds likely originated from
165 domesticated grapevines, we repeated the MDS analysis without wild accessions
166 to achieve a more refined picture of the relationships to regional varieties and
167 types of berries (*i.e.*, predominantly used in winemaking or as table grapes). The
168 majority of the archaeological pips were most closely related to wine cultivars
169 from West and Central Europe (Fig. 1b), although the three Early Roman samples
170 from the Mas de Vignoles XIV site had a closer affinity to wine grapes from the
171 Balkans and the Iberian Peninsula. Overall, this analysis shows that the
172 archaeological seeds are predominantly related to Western European varieties
173 that are used for winemaking, and not grapevines that are today grown further
174 east for wine or table grapes. These data suggest that 2000 years ago cultivated
175 vines in the modern territory of France were distinct from their Near Eastern
176 ancestors and well on their way to founding the germplasm of modern varieties
177 used in Western European winemaking. We also verified that the patterns

178 observed in the MDS analysis using the GrapeReSeq panel were consistent with
179 those obtained from a whole-genome (WG) reference panel (Supplementary
180 Table 2 and Supplementary Fig. 6).

181

182 We further explored the genetic structure of the archaeological seeds with a two-
183 step model-based clustering analysis. First, ADMIXTURE ²⁷ was used to infer the
184 ancestry proportions within the samples in the reference panel, and then
185 FastNGSAdmix ²⁸ was used to estimate the ancestry proportions in the
186 archaeological samples (Fig. 1c and Supplementary Fig. 7). The results were
187 consistent with the MDS analysis, showing that most archaeological seeds were
188 related to wine grapes from Western Europe.

189

190 As there is evidence for gene flow with local wild grapevines in Western Europe
191 ¹, we explored the wild ancestry components identified through the clustering
192 analysis. Since these proportions are estimated on the GrapeReSeq SNPs they do
193 not necessarily represent whole-genome ancestry proportions. However, this
194 allowed us to: 1) compare the proportions between present-day varieties and
195 the archaeological seeds at these diagnostic sites, and 2) identify the potential
196 source of the wild grape ancestry in the archaeological seeds. Wild grapevines
197 carry four main ancestry components when assuming 8 clusters (Fig. 1c). While
198 American and Asian *Vitis* species (yellow) and Eurasian wild grapes from the
199 Caucasus and Turkey (light blue) separate into individual clusters that do not
200 contribute significant ancestry to any other group, wild grapes from the African
201 and Western European populations display two ancestry components (dark and
202 light green) that are found in some domesticated grapes. All archaeological

203 samples except for the most recent (M-LM_22) show evidence of genetic
204 contributions from wild grapevines (Fig. 1c and Supplementary Fig. 7), and these
205 wild ancestries are primarily associated with Western and Central European
206 vines. While these data provide the first clues on the timing of genetic
207 introgression from local vines into domesticated lineages, the amount of wild
208 ancestry does not follow a consistent pattern related to sample age. For example,
209 the oldest sample (La Cugourlud, 510–475 BCE) shows some of the highest
210 levels of wild ancestry (~45%), while other early samples from Mas de Vignoles
211 XIV (2nd–1st century BCE) have marginal amounts of wild ancestry (3.5–4.5%),
212 and five samples from La Lesse-Espagnac (175–225 CE) range from ~10–38%. In
213 fact, these proportions of wild ancestry are similar to those observed in modern
214 French varieties, suggesting that the admixture with wild grapevines took place
215 at the earliest stages of viniculture in France, and potentially before other
216 cultivated lineages were introduced to France (*i.e.*, from Greece or the Italian
217 Peninsula). Together, these results suggest that the local wild gene pool played
218 an early role for domesticated varieties, with the gene flow between wild
219 grapevines and domesticated cultivars occurring at least 2500 years ago.

220

221 **Ancient use of vegetative propagation**

222 The availability of genotype data for hundreds of cultivars in the GrapeReSeq
223 panel, allowed us to explore relationships between archaeological pips excavated
224 from individual sites and across different regions of France. We estimated
225 kinship coefficients among pairs of samples using *KING*²⁹ and *NgsRelate*³⁰. Pairs
226 of samples were classified based on the kinship coefficients and the proportion
227 of sites with ‘zero alleles Identical by State’ (IBS0)²⁹, into the following

228 categories: identical clones, parent-offspring, highly-related/full-siblings or
 229 unrelated ²¹ (Supplementary Table 3). We found six instances of genetically
 230 identical pairs or groups of seeds (Fig. 2a). Additionally, we identified first-
 231 degree relationships (parent-offspring and highly-related /full-siblings) and
 232 unrelated varieties (Fig 2b). However, since grape seeds that have been cross-
 233 fertilized contain paternal derived DNA³¹ which could affect the relatedness
 234 analyses, we explored whether the archaeological seeds contained maternal DNA
 235 only (as expected from empty seeds), or both paternal and maternal DNA. To do
 236 so, we generated sequencing data from three seeds and a wood sample of the
 237 same plant and conducted a simulation study, in an attempt to estimate the
 238 parental contribution in the archaeological samples (Supplementary Fig. 8;
 239 Supplementary Section 16). We found that data from all archaeological seeds,
 240 except R-TDM_06, R-TDM_08, R-HW71_03 and M-MDV12_09, were consistent
 241 with a paternal DNA contribution of $\leq 10\%$ (Supplementary Figs. 8 to 11).
 242 Moreover, we studied the dependence of the relatedness analyses on such
 243 contribution and found that $\leq 10\%$ paternal DNA does not significantly affect the
 244 results (Supplementary Fig. 12). Therefore, we consider that clonal and parent-
 245 offspring relationships are not affected in most samples. On the other hand, full-
 246 sibling relationships could derive from multiple scenarios if the samples
 247 involved contain paternal DNA (Supplementary Fig. 12c), and thus we classified
 248 pairs of samples with this type of relationship as 'highly related'.
 249 Grape seeds have been found to follow a degradation process of the two tissues
 250 that contain paternal DNA, the endosperm and embryo, resulting in empty seeds
 251 (*e.g.* in up to 30% of the cases for 'Chardonnay' variety ^{32,33}). Our results
 252 suggest that the observed clonal clusters among archaeological samples

253 represent empty seeds with only maternal tissue, either produced by the same
254 plant, such as might occur at one archaeological site, or by one grapevine variety
255 spread through vegetative propagation (Fig. 2 and Supplementary Table 3). Five
256 of these clonal clusters consist of two or three seeds from a single stratigraphic
257 context: an Early Roman ditch at Mas de Vignoles XIV near Nîmes city (2nd–1st
258 century BCE), a Roman well at Mont Ferrier, Tourbes (1st century CE), a Roman
259 well at La Lesse-Espagnac (ca. 200 CE), a Roman well at Terrasses de Montfau,
260 Magalas (4th century CE), and an early medieval well at Mas de Vignoles XIV (ca.
261 800 CE). Given that bunches of grapes might have been pressed for juice and
262 discarded *en masse*, these genetically identical specimens may well represent
263 seeds from single plants. The other genetic cluster consists of three seeds from
264 Horbourg-Wihr in Alsace and one seed from La Lesse-Espagnac in
265 Mediterranean France (Fig 2b); while all four samples date to the 2nd century CE,
266 these genetic clones suggest that Romans transported grapevine across long
267 distances (>600 km), most likely as cuttings.

268 Five archaeological sites in Southern France demonstrated the presence of
269 multiple genotypes within a single temporal stratum, providing genetic evidence
270 that multiple lineages or varieties were maintained at individual vineyards. For
271 example, we identified six different genotypes at Mas de Vignoles XIV near
272 Nîmes, three of which shared first-degree relationships and three of which were
273 unrelated (Fig. 2b). Overall, these relationship data indicate that vegetative
274 propagation, long-distance transportation of varieties, and multivarietal
275 cultivation have been practiced in France since the Roman era, consistent with
276 historic accounts ⁴.

277

278 **The antiquity of modern French varieties**

279 We lastly investigated the relatedness between archaeological and modern
280 varieties, by computing kinship coefficients and the proportion of IBS0 sites
281 between pairs of archaeological samples and samples present in the GrapeReSeq
282 panel using *KING* ²⁹ (Fig. 3 and Supplementary Tables 4 and 5). Our results
283 confirm long-held beliefs that Roman and medieval viticulturists maintained
284 ancient lineages using vegetative propagation ¹³, and that modern French
285 viniculture is in large part a product of these traditions. One archaeological
286 sample from La Madeleine (Orléans), dating to 1050–1200 CE, was an identical
287 clone of ‘Savagnin Blanc’ (VIVC17636), a variety today cultivated for wine
288 production in Northeastern France and other countries from Central Europe
289 (*kinship coeff.* = 0.496; *IBS0* ~ 0.0001; Identity of 99.7% and 99.9% for the
290 GrapeReSeq and WG panels, respectively) (Supplementary Tables 4 and 5).
291 Several researchers previously identified ‘Savagnin Blanc’, also known as ‘
292 Traminer Weiss’, as a recurrent parent of many commercially important
293 European varieties ^{1,34,35}, and written accounts document the appellation as early
294 as 1539 CE ³⁶. Our findings extend the presence of this variety in France by
295 hundreds of years and furthermore, suggest that either ‘Savagnin Blanc’ or its
296 direct relatives have been cultivated in France since the 1st century CE, since
297 archaeological seeds from Mont Ferrier, Tourbes have a parent-offspring
298 relationship with ‘Savagnin Blanc’ (Figs. 2b and 3).

299

300 Several archaeological seeds were closely related to ‘Mondeuse Blanche’
301 (VIVC7919), a French variety characteristic of the Northern French Alps that has

302 been suggested to have acted as a key progenitor ^{35,37}. We found that four
303 genetically identical 2nd century CE seeds from Horbourg-Wihr and La Lesse-
304 Espagnac have a parent-offspring relationship with ‘Mondeuse Blanche’,
305 indicating that just one reproductive cycle has taken place in this lineage in the
306 past 1800 years (Fig. 3). This finding presents an exciting consilience of genetic
307 and archaeobotanical data; using morphometric analysis, Terral *et al.* ¹⁶ also
308 found evidence for ‘Mondeuse Blanche’ among 1st–2nd century CE pips from the
309 Rec-de-Ligno site, which lies less than 10 km from La Lesse-Espagnac. We also
310 observed that ‘Mondeuse Blanche’ is highly related (full-sibling or similar
311 relationship) to an archaeological seed from Colletiere, dating to circa 1000 CE,
312 close to the region where ‘Mondeuse Blanche’ is still grown today (Savoie, Ain)
313 (Fig. 3). Interestingly, the medieval seed is also highly related to ‘Tressot’
314 (12640) (cited since 1396 in France ³⁸) and ‘Servanin’ (VIVC11526), both French
315 varieties that are rarely cultivated today.

316

317 In addition to ‘Mondeuse Blanche’, four other Roman seeds from Southern
318 France provided parent-offspring relationships to modern Alpine varieties: three
319 1st century CE seeds from Mont Ferrier are highly related to ‘Arvine’
320 (VIVC664) and ‘Amigne’ (VIVC425) and one 1st–3rd century CE seed from
321 Roumeges is a first-degree relative to ‘Humagne Blanc’ (VIVC5450) (Fig. 3).
322 All three are Swiss varieties used for white wine, and the former two are
323 recorded in Switzerland by the 17th century CE ³⁹. Tradition holds that the
324 Romans brought ‘Amigne’ to Switzerland as a variety they referred to as
325 ‘*Aminea*’; however some researchers have suggested the connection is primarily

etymological, with the retained usage of the Latin word *amoenus* for “delicious”
⁴⁰. Our findings suggest there indeed is a close genetic link between the varieties
grown by the Romans and some modern Swiss cultivars, including ‘Amigne’.
Moreover, these data suggest that modern Alpine varieties may have been
cultivated in a more widespread geographic region during the Roman period,
thus posing an important question on their origin and the adaptation of modern
grapes. The approaches established here can be applied to other archaeological
pip assemblages with the aim of detecting when regional and economically
important lineages first appeared and how they were maintained.

Impact of cultural changes in the viniculture of France

Specimens from the Mas de Vignoles XIV site in Nîmes provide one final
observation on the changing nature of viniculture in France. This site allowed us
to investigate a transect of three time periods: 2nd –1st century BCE in the early
Roman period, 417–515 CE in the Late Roman period when viticulture was fully
established in the region, and 731–851 CE in the early medieval period. While
cultivars from the most recent period were found to share first-degree
relationships with modern French varieties, no relationship was found between
cultivars from the Roman period and the modern varieties (Fig. 3). Our results
from Mas de Vignoles XIV suggest a change in grapevine diversity from Roman to
Medieval times. This transition can also be observed in the MDS analyses (Fig.
1b); the three seeds from the early Roman period (R-MDV14_04/07/09) are
placed closer to East European and Iberian grape varieties, while Late Roman
and early medieval seeds are more similar to West Europe varieties. These
results show the relatively high diversity of grapes cultivated in this region

351 during this period, as well as replacement and incorporation of new varieties
352 through time.

353

354 **Concluding remarks**

355

356 Palaeogenomic analysis of archaeobotanical remains has helped reveal the
357 evolutionary histories of annual crops like barley ⁴¹ and maize ^{42,43}, but this
358 project represents the first nuclear aDNA study of a vegetatively propagated fruit
359 crop. Our results highlight the utility of state-of-the-art palaeogenomic methods
360 in the study of ancient viniculture through space and time. While previous
361 studies on ancient chloroplast DNA ²⁰, microsatellites ^{18,19,44}, and proteins ¹⁸ have
362 provided insights into the history of grapevine cultivation, their resolution is
363 limited. With the availability of a nuclear DNA diversity panel, we interrogated
364 genome-wide data from archaeological grape seeds, identified relationships
365 between ancient pips and modern varieties, observed connections between
366 distant sites, and traced the history of vegetative propagation in France. Future
367 palaeogenomic research on archaeological grape seeds holds great potential in
368 identifying the links between past and present grape varieties, and especially for
369 refining our knowledge of the pace of domestication and improvement under
370 vegetative propagation ⁴⁵.

371

372 **Materials and methods**

373

374 **Archaeological sample description**

375 Grape seeds were collected from nine archaeological sites in France during
376 excavations of wells, latrines, pits, and ditches (Supplementary Fig. 1; see
377 Supplementary Section 1 for a description of the archaeological sites). Sediment
378 samples were systematically collected and immediately isolated to prevent
379 contamination and stored in cool conditions (4° C). The sediment samples were
380 processed at the Institut des Sciences de l' Evolution (ISEM) in Montpellier,
381 France. To prevent contamination with modern material, seeds were isolated in
382 a clean room separate from the archaeobotanical laboratory. Additionally,
383 surfaces and tools were cleaned with bleach prior to handling. Most of the
384 samples included in this study were photographed inside the clean room, with
385 specific equipment in order to carry out morphological analyses. Archaeological
386 samples were dated either by association with archaeological artifacts found in
387 the same stratigraphic units, dendrochronology, or radiocarbon dating. The age
388 of the samples ranged from the Iron Age (510–475 BCE) to the medieval period
389 (1050–1200 CE) (Supplementary Fig. 1 and Table 1).

390

391 **Archaeological samples processing**

392 Archaeological samples were processed in dedicated aDNA facilities at the
393 University of Copenhagen following standard measures to prevent
394 contamination. Individual seeds were decontaminated with 10% bleach, rinsed
395 with molecular biology grade water, and pulverized. DNA was extracted from the
396 resulting powder following standard protocols standardized for
397 archaeobotanical remains ⁴⁶. DNA extracts were converted into double-stranded
398 Illumina libraries using the NEBnext DNA Library Prep Mast Mix Set 2 (E6070L,
399 New England BioLabs) with modifications described in Wales *et al.* ⁴⁷ (see

Supplementary Section 4 for a description of the protocol). Resulting Illumina libraries were enriched for a set of genomic loci present in the GrapeReSeq reference panel ²¹ (Supplementary Section 5). This panel covers genomic sites known to be informative for identification of grape cultivars. Libraries were captured following the MYbaits protocol as described in Supplementary Section 6. Finally, pre- and post-capture libraries were sequenced on an Illumina 2500 HiSeq platform in SR100 mode. Sequencing reads obtained from the pre-captured libraries were used to assess the capture efficiency only.

408

409 **Sequencing data processing**

AdapterRemoval2.0 ⁴⁸ was used to remove Illumina adapter sequences, low quality stretches and ambiguous bases from the read ends. Resulting reads ≥ 30 base pairs were mapped to the grape nuclear reference genome 12X.2 ⁴⁹, chloroplast ⁵⁰ and mitochondrial ⁵¹ genomes using *bwa aln* (0.7.5a) ⁵²; seeding was disabled (-l was set to 1000) to improve the mapping sensitivity of aDNA reads ⁵³. Reads with mapping quality below 30 or ambiguously mapping were discarded, PCR duplicates were removed using *MarkDuplicates* (<http://picard.sourceforge.net>), reads were realigned around indels using *GATK* ⁵⁴ and the MD-tag was recalculated using *samtools* 1.2 ⁵⁵. Finally, we excluded 5 bases from the 5' and 3' ends of each read from subsequent analyses to reduce the proportion of bases with aDNA damage. Genotype calling was performed in the resulting alignments using a combination of the *HaplotypeCaller* and *UnifiedGenotyper* algorithms from *GATK* ⁵⁴ on sites with a minimum coverage of 10 \times as described in Supplementary Section 12. To evaluate the genotyping pipeline, we generated sequencing data for two modern grape cultivars using the

425 SNP capture protocol. These two varieties are present in the GrapeReSeq panel,
426 thus provide a direct comparison between our method and the GrapeReSeq
427 microarray. We found a concordance of 99.4% and 99.3%, between the called
428 genotypes and their corresponding genotypes in the GrapeReSeq panel.

429

430 **Ancient DNA authentication**

431 The authenticity of the aDNA data was assessed on the basis of the length
432 distribution and the nucleotide misincorporation patterns observed in the
433 sequencing data. We used *bamdamage*²³ to estimate per base nucleotide
434 substitutions in the mapped reads. Reads with mapping quality lower than 30
435 and base quality lower than 20 were discarded. Archaeological samples
436 displayed increased C-to-T and G-to-A substitutions as well as short reads
437 (Supplementary Figs. 3 and 4), consistent with aDNA data²².

438

439 **Reference datasets**

440 We used two reference datasets to compare the archaeological grape seeds to
441 present-day grape varieties (see Supplementary Section 11 for a detailed
442 description of the reference panels used). 1) The GrapeReSeq panel consists of
443 783 modern grape cultivars (*V. vinifera* subsp. *vinifera*) and 112 wild grape
444 individuals (*V. vinifera* subsp. *sylvestris*) representative of the genetic diversity
445 found in Europe (81 accessions), as well as from North Africa (18 accessions)
446 and the Caucasus (13 accessions) genotyped for 10,000 diagnostic SNPs²¹. 2) We
447 assembled a whole-genome (WG) reference panel incorporating sequencing data
448 from 27 publicly available wild and domesticated grape accessions^{24–26}. Raw
449 reads were obtained from the NCBI SRA, mapped and processed using similar

parameters as the archaeological samples. To avoid ambiguities due to
synonymy the VIVC number ⁵⁶ is assigned to cultivars as indicated in
Supplementary Table 4.

Genetic structure analyses

We explored the genetic relationships between the archaeological grape seeds
and the samples in the two assembled reference panels using multidimensional
scaling as implemented in *bammds* ²³ (Figs. 1a and 1b, and Supplementary Fig.
6). Samples with an on-target depth of coverage $\geq 3\times$ were included to the
reference panel by sampling a random allele from the called genotypes; while the
six low coverage samples were incorporated from a majority count consensus
sequence (Supplementary Table 1). After filtering low-quality SNPs, the final
datasets consisted of 9,896 and 3,076,549 sites for the GrapeReSeq and WG
panels, respectively. Note that, for analyses using the GrapeReSeq panel we did
not exclude transition sites. However, data from the genotype calls and majority
count consensus sequences obtained for the archaeological samples showed
error rates comparable to those of modern grape samples (Supplementary Fig.
5), suggesting that the aDNA derived error is unlikely to have a substantial effect
in the analyses.

We used the model-based clustering approaches implemented in *fastNGSadmix*
²⁸ and *ADMIXTURE* ²⁷ to estimate ancestry proportions in the archaeological
samples (Fig. 1c and Supplementary Fig. 7). First, ADMIXTURE was run on the
GrapeReSeq panel assuming 2-8 populations/clusters ($K=2-8$). We obtained
1,000 independent replicates for each value of K and kept the one with the best
likelihood. Then, we estimated genotype likelihoods for the archaeological

475 samples using the *samtools* model implemented in *ANGSD* v1.9 ⁵⁷ at the sites
476 included in the GrapeReSeq panel. Finally, we obtained maximum likelihood
477 estimates of the ancestry proportions for the archaeological samples using the
478 genotype likelihoods and the *ADMIXTURE*-inferred allele frequencies for each
479 value of *K* using *fastNGSadmix*. Figure 1c shows the results for the *K*=8, which
480 resulted among the lowest cross-validation errors (Supplementary Fig. 7b).

481

482 **Relatedness analyses**

483 To explore the relationships among pairs of archaeological samples and between
484 the archaeological samples and samples in the reference panels, we estimated
485 kinship coefficients using two approaches: the called genotype-based approach
486 implemented in *KING* ²⁹ and the genotype likelihood-based approach
487 implemented in *NgsRelate* ³⁰ (Supplementary Tables 3 to 5).

488 *KING* was run assuming non-homogeneous population structure for the two
489 reference panels and using called genotypes for the archaeological samples. Pairs
490 of samples were classified based on the kinship coefficients and the proportion
491 of sites with ‘zero alleles Identical by State’ (IBS0), as suggested in
492 Manichaikul *et al.* ²⁹, in the following categories: identical clones ($K \geq 0.49$ and
493 $IBS0 \leq 0.001$), parent-offspring ($0.177 < K < 0.354$ and $IBS0 \leq 0.001$), highly
494 related/sibling ($0.177 < K < 0.354$ and $IBS0 \leq 0.25$) or unrelated (Supplementary
495 Tables 3 to 5). These values have been shown to be reliable in discerning known
496 first-degree relationships among grape cultivars ²¹.

497 *NgsRelate* was used as a complementary method to validate the results obtained
498 using *KING* and to include low coverage samples for which it was not possible to
499 call genotypes. To run *NgsRelate*, we first estimated genotype likelihoods for the

500 archaeological samples using the *samtools* model (-gl 1) implemented in *ANGSD*
 501 v1.9 ⁵⁷. Reads with mapping quality lower than 30 and bases with quality lower
 502 than 20 were discarded. We then estimated allele frequencies for the two
 503 reference panels using *PLINK 1.9* ⁵⁸. These frequencies together with genotype-
 504 likelihoods were used to obtain kinship coefficients and the proportion of sites
 505 sharing 0, 1 or 2 alleles identical by descent (IBD) between pairs of samples
 506 (Supplementary Table 3). These results were evaluated together with the
 507 obtained from the genotype-based approach to assign relationships between
 508 pairs of archaeological samples.
 509 In Supplementary Section 16, we explore the possibility of paternal DNA present
 510 in the archaeological seeds through a simulation study and comparing the
 511 archaeological seeds data with that obtained from fresh seeds (Supplementary
 512 Figs. 8 to 11 and Supplementary Table 6). While most of the archaeological seeds
 513 were found to be consistent with data derived from a single individual, our
 514 analyses indicate four seeds contain $\geq 10\%$ of paternal DNA (Supplementary Fig.
 515 11). Additionally, we evaluate potential effects of paternal DNA in the
 516 relatedness analyses and found that: 1) clonal relationships can only be detected
 517 from true identical individuals even in the presence of paternal DNA, 2) parent-
 518 offspring relationships are only ambiguous when the sample contains $>10\%$
 519 paternal DNA, and 3) apparent full-sibling relationships can result from multiple
 520 scenarios, thus pairs of samples with this type of relationship were classified as
 521 'Highly related pairs' (Supplementary Fig. 12). Relationships between
 522 archaeological seeds and modern grapes were evaluated based on the
 523 conclusions from Supplementary Section 16.

524 We further explored the effect of sequencing depth and panel ascertainment in
 525 the robustness of the relatedness inferences (Supplementary Section 17). The
 526 results indicate that the metrics used to identify relationships between the
 527 samples are reliable for samples with an on-target depth of coverage of $\geq 2 \times$
 528 when using genotypes, and $1 \times$ when using genotype likelihoods
 529 (Supplementary Table 7). Additionally, we confirmed that samples identified as
 530 identical clones display an IBS distance < 0.0001 both in the sites overlapping the
 531 GrapeReSeq panel and in off-target sites (Supplementary Fig. 13; Supplementary
 532 Section 18).

533

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684

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703

704 **Corresponding authors**

705 Correspondence to Nathan Wales and M. Thomas P. Gilbert.

706

707 **Author contributions**

708 The project was conceived by N.W., M.T.P.G., R.B., and L.B., and headed by N.W.
709 and M.T.P.G.; J.A.S.C., A.K.W.R, R.B. and N.W. designed experimental enrichment
710 methodology with input from J.M.M.Z., R.T. and A.F.A.B.; A.K.W.R. processed
711 ancient DNA with input from N.W.; J.R.M., A.K.W.R., R.B. and N.W. designed
712 analysis strategy; J.R.M. performed bioinformatic analysis with assistance from
713 B.P. and T.S.P. and input from N.W, M.T.P.G. and R.B.; J.R.M., N.W., M.T.P.G., R.B.,
714 L.B., T.L. and P.T. interpreted the results; L.B., I.F., C.S. and C.H., curated

715 archaeological material; J.R.M., N.W. and M.T.P.G. wrote the manuscript with
716 input from R.B., L.B. and T.L. and the other authors. All authors revised, edited
717 and accepted the manuscript. Primary funding acquired by M.T.P.G.

718

719 **Competing interest**

720 The authors declare no competing interests.

721

722 **Data availability**

723 Sequencing data produced in this study are available at the NCBI SRA under the
724 reference PRJNA489970. Genotype data are available in the *figshare* repository
725 under the following DOI: 10.6084/m9.figshare.7610987.

726

727 **Figure legends**

728 **Figure 1. Genetic affinities between archaeological grape seeds and**

729 **modern *Vitis vinifera* accessions.** a. Multidimensional scaling plot (MDS)

730 including archaeological samples, wild *V. vinifera subsp. sylvestris* accessions, and

731 domesticated varieties. b. MDS plot restricted to archaeological samples and

732 domesticated varieties. Colors correspond to the main ancestry clusters

733 identified in Laucou *et al.* ²¹. *Archaeological samples that were incorporated to

734 the dataset by sampling a random allele from a majority count consensus

735 sequence instead of called genotypes c. Model-based clustering analysis of the

736 GrapeReSeq panel assuming K=8 clusters. Vertical bars represent individual

737 accessions, colors represent the inferred ancestry components, and the fraction

738 of each color corresponds to the estimated ancestry proportion. Archaeological

739 samples are sorted by age, and by sample identification within a stratigraphic
740 context. Samples that were identified as identical clones are grouped with black
741 lines and capital letters (A-F) at the bottom.

742 **Figure 2. Geographic distribution and relationships between the distinct**
743 **genetic types of archaeological samples.** a. Relatedness among pairs of
744 archaeological samples. Kinship coefficients were estimated using *NgsRelate*
745 between pairs of samples for SNP loci present in the GrapeReSeq panel. Capital
746 letters (A-F) on the left indicate genetically identical clones, *i.e.*, putative ancient
747 and historical varieties. *Archaeological seeds that were found consistent with
748 carrying >10% paternal DNA. b. Map displaying the distribution of genetic types
749 (circles) in each archaeological site. Capital letters (A-F) on the circles indicate
750 clusters of genetically identical seeds represented by more than one seed.
751 Shading of the circles indicates sample age. In red is shown the genetic type that
752 was found in more than one archaeological site. Lines connect pairs of samples
753 that are related as parent-offspring (solid lines) or highly-related/full-sibling
754 (dotted lines). Note that, since in the presence of paternal DNA full-sibling
755 relationships could derive from multiple scenarios, we classified samples
756 consistent with full-sibling relationships as ‘highly-related’ (see Supplementary
757 Section 16).

758 **Figure 3. Genetic origins of ancient and historic French grapevine varieties.**
759 Relationships identified between archaeological samples and modern cultivars
760 included in the GrapeReSeq panel. Solid lines represent parent-offspring
761 relationships and dotted lines represent pairs of highly related (full-sibling or
762 similar) samples. Sibling relationships involving pairs of modern cultivar are not
763 displayed for simplicity. *Archaeological seeds that were found consistent with

764 carrying >10% paternal DNA. The VIVC (<http://www.vivc.de>) and GrapeReSeq

765 identifiers for the modern cultivars can be found in Supplementary Table 4.

766

767

768 **Table 1.** Description of the archaeological grape seeds used in the study.

769

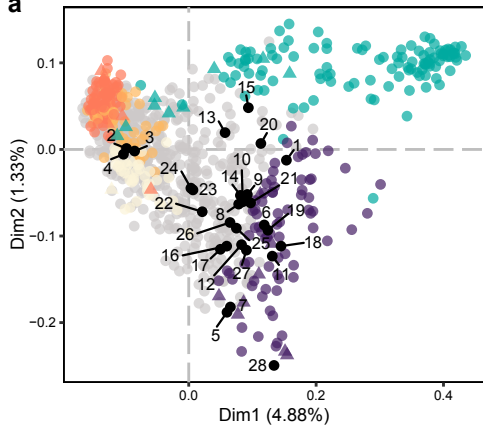
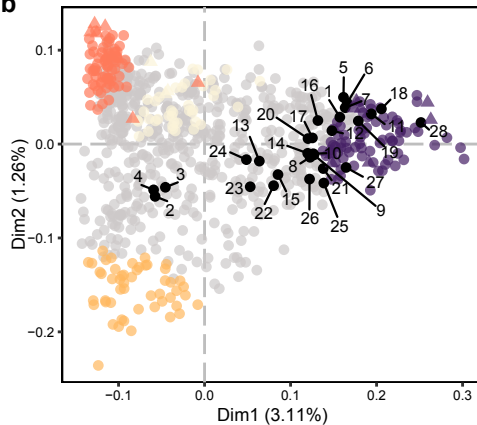
#	Sample ID	Geographic coordinates	Archaeological site	Stratigraphic unit	Structure	Age	Dating method	Period	GC †
1	IA-LC_01	43.573639, 3.914750	La Cougourlude, Lattes	US 31084	Ditch FO 30277	510-475 BCE/2480 ± 30 BP (769- 417 cal BCE)	Archaeological artifacts/C14	Iron Age	
2	R-MDV14_04	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 14152	Rural ditch FO 14194	2nd-1st c BCE	Archaeological artifacts	Early Roman	A
3	R-MDV14_07	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 14152	Rural ditch FO 14194	2nd-1st c BCE	Archaeological artifacts	Early Roman	A
4	R-MDV14_09	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 14152	Rural ditch FO 14194	2nd-1st c BCE	Archaeological artifacts	Early Roman	A
5	R-MF_21	43.432556, 3.394222	Mont Ferrier, Tourbes	US 2076	Well PT 2052	1st c CE	Archaeological artifacts	Roman	B
6	R-MF_23	43.432556, 3.394222	Mont Ferrier, Tourbes	US 2076	Well PT 2052	1st c CE	Archaeological artifacts	Roman	
7	R-MF_25	43.432556, 3.394222	Mont Ferrier, Tourbes	US 2076	Well PT 2052	1st c CE	Archaeological artifacts	Roman	B
8	R-HW70_18	48.080500, 7.399194	Horbours-Wihr	N.D.	Pit ST7054	2nd c CE	Dendrochronology / Archaeological artifacts	Roman	C
9	R-HW71_03	48.080500, 7.399194	Horbours-Wihr	N.D.	Pit ST7172	2nd c CE	Archaeological artifacts	Roman	C
10	R-HW71_17	48.080500, 7.399194	Horbours-Wihr	N.D.	Pit ST7172	2nd c CE	Archaeological artifacts	Roman	C
11	R-R_09	43.471306, 3.670139	Roumeges, Poussan	US 5007(12/13)	Well PT 5001	1st-3rd c CE	Archaeological artifacts	Roman	
12	R-R_14	43.471306, 3.670139	Roumeges, Poussan	US 5007(12/13)	Well PT 5001	1st-3rd c CE	Archaeological artifacts	Roman	
13	R-LLE_02	43.300806, 3.239917	La Lesse- Espagnac, Sauvian	US 3019	Well PT 3005	175-225 CE	Archaeological artifacts	Roman	
14	R-LLE_08	43.300806, 3.239917	La Lesse- Espagnac, Sauvian	US 3019	Well PT 3005	175-225 CE	Archaeological artifacts	Roman	C
15	R-LLE_09	43.300806, 3.239917	La Lesse- Espagnac, Sauvian	US 3019	Well PT 3005	175-225 CE	Archaeological artifacts	Roman	
16	R-LLE_13	43.300806, 3.239917	La Lesse- Espagnac, Sauvian	US 3019	Well PT 3005	175-225 CE	Archaeological artifacts	Roman	D
17	R-LLE_14	43.300806, 3.239917	La Lesse- Espagnac, Sauvian	US 3019	Well PT 3005	175-225 CE	Archaeological artifacts	Roman	D
18	R-TDM_06	43.472806, 3.223000	Terrasses de Montfau, Magalas	US 4015	Well PT 4000	4th c CE	Archaeological artifacts	Roman	E
19	R-TDM_08	43.472806, 3.223000	Terrasses de Montfau, Magalas	US 4015	Well PT 4000	4th c CE	Archaeological artifacts	Roman	E
20	R-TDM_10	43.472806, 3.223000	Terrasses de Montfau, Magalas	US 4015	Well PT 4000	4th c CE	Archaeological artifacts	Roman	
21	M-MDV13_07	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 13525	Well PT 13319	1605 ± 35 BP (417-515 CE)	C14	Late Roman/ Medieval	

22	M-MDV12_02	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 12111	Well PT 12024	1220 ± 30 BP (731-851 CE)	C14	Early Medieval	
23	M-MDV12_04	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 12111	Well PT 12024	1220 ± 30 BP (731-851 CE)	C14	Early Medieval	F
24	M-MDV12_05	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 12111	Well PT 12024	1220 ± 30 BP (731-851 CE)	C14	Early Medieval	
25	M-MDV12_07	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 12111	Well PT 12024	1220 ± 30 BP (731-851 CE)	C14	Early Medieval	
26	M-MDV12_09	43.808222, 4.368222	Mas de Vignoles XIV, Nîmes	US 12111	Well PT 12024	1220 ± 30 BP (731-851 CE)	C14	Early Medieval	F
27	M-C_27	45.436417, 5.520306	Colletiere, Charavines	N.D.	Cultural layer, rubbish deposits	1006-1040 CE	Dendrochronology	Medieval	
28	M-LM_22	47.900472, 1.884333	La Madeleine, Orléans	US 15126	Cesspit F 1517	1050-1200 CE	Archaeological artifacts	Medieval	

770 † Genetic clusters composed of identical clones. The genetic cluster was assigned
771 according to the relatedness analyses described in the results section.

772

773

a**b**

● Wine grapes – West and Central Europe
 ● Wine grapes – Iberian Peninsula and Northwest Africa
 ● Wine grapes – Balkans and East Europe
 ● Table grapes
 ● Other domesticated grapes
 ● *V. vinifera* subsp. *sylvestris*

● Grapereseq panel and ancient

▲ Whole-genome reference data (Table S2)

Iron Age:	9. R-HW71_03*	20. R-TDM_10
1. IA-LC_01*	10. R-HW71_17	
	11. R-R_09	Medieval Period:
Roman Period:	12. R-R_14*	21. M-MDV13_07
2. R-MDV14_04	13. R-LLE_02	22. M-MDV12_02
3. R-MDV14_07*	14. R-LLE_08	23. M-MDV12_04
4. R-MDV14_09	15. R-LLE_09	24. M-MDV12_05
5. R-MF_21	16. R-LLE_13	25. M-MDV12_07
6. R-MF_23	17. R-LLE_14	26. M-MDV12_09
7. R-MF_25	18. R-TDM_06*	27. M-C_27
8. R-HW70_18	19. R-TDM_08*	28. M-LM_22

c