

Supplementary Information

Ancient DNA reveals 4,000 years of grapevine diversity, viticulture and clonal propagation in France

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Supplementary note: archeological sites and sample information

- **L1 Vistre, Nîmes (Gard)¹**

Archaeological surveys conducted by Inrap (directed by M. Bovagne) along a 4 km stretch of Vistre river, in the communes of Nîmes, Milhaud, Aubord, and Bernis, were undertaken in the context of river recalibration works. These excavations provided an exceptional opportunity to trace the evolution of the river course over the past seven millennia, and to assess its impact on the human communities that settled along its banks.

Evidence of human sedentary occupation was recorded across several sectors of the survey area, spanning from the 5th millennium BCE to the 2nd century BCE. Sediment sampling yielded a remarkably rich archaeobotanical assemblage, largely composed of well-preserved waterlogged remains. Charred seeds were rare, though charcoal was abundant in some structures. The richest concentrations of waterlogged seeds were found in Sectors 3 (Early Bronze Age) and Sector 4 (Neolithic and Antiquity). The taxonomic composition of the assemblages reflects diverse ecological habitats, with a notable abundance of aquatic species (*Alisma plantago-aquatica*, *Baldellia ranunculoides*, *Ceratophyllum submersum*, *Chara* sp., *Ranunculus aquatilis* agg), and riverbank plants (*Helosciadium nodiflorum*, *Schoenoplectus lacustris*, various *Cyperaceae*, *Lycopus europaeus*).

Grape pips of *Vitis vinifera* subsp. *sylvestris* were particularly abundant in stratigraphic units 1559 (Sector 3, Early Bronze Age) and 1530 (Sector 4, Antiquity). One of the waterlogged pips from Sector 3 was radiocarbon dated (Poz-135412: 3735±35 BP; 2282–2028 BCE) and is included in the present study.

- **L2 Clos de Roque & Saint Jean, Saint Maximin-la-Sainte-Beaume (Var)^{2,3}**

A large-scale rescue excavation was carried out by Inrap (directed by M. Taras-Thomas) in 2017-2018 in the quartier Clos de Roque at Saint-Maximin-la-Sainte-Beaume, ahead of a major development project covering more than 30,000 m². The investigations revealed a long sequence of human occupation from the Early Neolithic through the Iron Age. The Iron Age remains were particularly significant due to the diversity of structures and richness of associated artifacts².

The site includes the remains of a modest rural establishment, with features such as pits, silos, wells, and kilns. Additionally, a separate area interpreted as a possible feasting zone was uncovered, characterized by 30 aligned fireplaces and two refuse pits. Anthropological analysis of charcoal from the fireplaces suggests a deliberate selection of firewood for these events, with a notable over-representation of evergreen oak (*Quercus ilex*)³.

One of the wells (PT 2003) yielded well-preserved waterlogged plant remains in its lower stratigraphic units, notably including buds, seeds, and fruits. The assemblage was dominated by wild taxa, though a small number of grapevine pips were present. One pip was radiocarbon dated to 770-423 BCE (Poz-135337: 2475±30 BP). Ceramic material indicates that the construction and use of the well occurred between the late 7th and the late 6th centuries BCE².

- **L3 Place Jules Verne, Marseille (Bouches-du-Rhône)⁴⁻⁷**

Rescue excavations were carried out by Afan (directed by A. Hesnard and M. Pasqualini) in 1992-1993 at Place Jules Verne, in the centre of Marseille, near the “Vieux Port” (Hesnard 1994, Morhange et al. 1996, Hermaty et al. 1999). The excavated area covered 3,700 m² and revealed port installations and subaquatic infilling layers from the ancient harbour of Greek and Roman Massalia.

Founded in the early 6th century BCE by Phocian Greeks from Asia minor, Massalia developed rapidly into a major Mediterranean trading hub. The Place Jules Verne excavations provided extensive insights into the functioning of the harbour and the adjacent urban area from the foundation of the city through the end of the Roman period. Over the centuries, large quantities of sediment and refuse from surrounding human activity

accumulated in the harbour basin. The anaerobic conditions of the submerged environment providing optimal conditions for the preservation of organic material.

The abundant ceramic assemblage enabled fine chronological resolution of the infilling layers. Analysis of waterlogged seeds and fruits offers a detailed perspective on dietary practices in the city and their evolution over nearly a millennium⁴. Fruits, legumes, and condiments characteristic of the Greek and Roman diet (e.g., *Cucumis* sp., *Ficus carica*) are well represented. Grape pips and pedicels (*Vitis vinifera*) are especially abundant, interpreted as evidence for wine production in Massalia, which exported wine in distinctive amphorae from as early as the second third of the 6th century BCE.

The grape pips analyzed in the present study derive from two organic infill layers attributed to the first phase of use of the harbour (6th-5th century BCE), dated based on associated artefacts. One of the two assemblages has also been confirmed supported by AMS radiocarbon dating of a grape seed (Poz- 135410: 2440±30 BP; 751-408 BCE).

- **L4 L'île, Martigues (Bouches-du-Rhône)**⁸⁻¹⁰

The “île” of Martigues is a small Iron Age settlement uncovered through urban archaeological excavations led by the Archaeological Department of the town of Martigues (directed by J. Chausserie-Laprée), primarily between 1977 and 1989, with additional work conducted in 2001. The settlement occupied a strategic location at the eastern end of the Caronte channel, which links the Étang de Berre to the Mediterranean Sea, approximately 30 km from the Greek city of Massalia (Marseille). Founded in the mid-5th century BCE, the settlement persisted until the end of the 2nd century BCE.

The agglomeration consisted of modest house dwellings ranging from 5 to 20 m², constructed from stone and mudbrick, organized into elongated blocks, separated by streets, and enclosed by a substantial fortification wall. The site experienced repeated destruction fire, followed by reconstruction. These conflagrations preserved large quantities of charred seeds and fruits originally stored within domestic contexts, particularly during the 4th century BCE. This material provides valuable insight into the subsistence strategies and daily life of a farming community⁸. The local inhabitants cultivated cereals, particularly *Hordeum vulgare*, as well as several pulses (*Lathyrus cicera/sativus*, *Vicia ervilia*). Grape pips also occur regularly in the assemblages, and one charred context containing pips, pedicels, stalks and crushed berries strongly suggests grape pressing, likely for wine production.

The grape pip analyzed in the present study was not charred but waterlogged. It was recovered from a refuse deposit rich in grape remains (8901 – 137 Nord), preserved beneath the water table and sealed by the floor of a house. Based on associated archaeological material, the deposit dates to the 3rd century BCE. This chronology is further supported by AMS radiocarbon dating of another waterlogged grape pip from the same sample (Poz-135408: 2265±30 BP; 396-207 BCE).

- **L5 Lattara–Saint Sauveur, Lattes (Hérault)**¹¹⁻¹⁹

Extensive archaeological excavations have been conducted at the site of Lattara–Saint Sauveur over more than 25 years since the 1980s, by a CNRS research team successively directed by M. Py, T. Janin, and P. Garmy^{12,13}. Lattara was a major Mediterranean port city situated at the mouth of the Lez river and on the edge of a coastal lagoon, approximately 7 km from present-day Montpellier. Founded *ex nihilo* in the late 6th century BCE, the settlement initially played host to Etruscan merchants. By around 475 BCE, it had been burned and evolved under increasing Greek influence, within a broader context heavily shaped by indigenous cultures.

The city was enclosed by a fortification wall and covered an area of approximately 3.5 hectares. From at least the 4th century BCE onwards, the interior part was densely built up with houses—typically one or two-room dwellings—aligned along two main streets.

Over the course of the excavations, large-scale archaeobotanical investigations were undertaken, leading to the analysis of hundreds of samples¹⁴⁻¹⁶. While most of the plant macroremains were charred, certain 5th century BCE layers were preserved below the water table, very near the ground surface in this coastal environment. This yielded substantial

quantities of waterlogged seeds and fruits. Archaeological and archaeobotanical evidence from the site has provided significant insights into early viticulture and wine production in the region. These practices appear to have begun as early as the 5th century BCE and intensified in the 3rd century BCE^{11,15-19}.

The three waterlogged grape pips analyzed in this study were recovered from three separate contexts: one from an outdoor refuse deposit adjacent to a domestic structure (US69164; dated to 475-450 BCE), and two others from distinct layers of the same well (PT69247), specifically US69218 and US69243 (dated to 500-475 BCE). These contexts were precisely dated on the basis of associated archaeological material, with two contexts further confirmed by AMS radiocarbon dating of two individual grape seeds.

- **L5 Lattara–Chenal, Lattes (Hérault)²⁰⁻²⁷**

Excavations at Lattara, ongoing since the 1980s, focused up until 2015 on several intra-mural sectors of the city. In 2017, a new research program was launched under the direction of G. Piquès (CNRS), targeting the Roman-era harbour previously investigated by H. Prades²⁰ and D. Garcia^{21,22} along the southern edge of the city rampart. To date, nearly two hectares extending southwest of the harbour have been explored.

The recent excavations have clarified the southern boundary of the city and uncovered several buildings—primarily commercial in nature— while also confirming the presence of a canal that once provided access to the harbour. This canal, likely constructed by reusing an earlier palochannel²³, was in operation across multiple phases from the end of the 3rd century BCE to the 3rd century CE²⁴.

Extensive archaeobotanical research is currently underway²⁵, and recent publications have begun to shed light on the environment and plant-based food consumption during the Roman occupation^{26,27}. Most of the plant macroremains are waterlogged and derive from food processing waste, especially residues from winemaking as well as seeds, wood and leaves from the surrounding vegetation.

Numerous archaeological layers have yielded thousands of grape pips, pedicels, stalks and even entire fruits, some with a greenish colour. The waterlogged grape pips analyzed in this study were recovered from different filling layers associated with successive phases of canal use:

- US205031 (200 to 150 BCE), corresponding to the initial construction phase, when a stone ramp was built for water access and a wooden wattle fence was installed to stabilize the banks. The date was confirmed by AMS radiocarbon dating of a grape pip;
- US204097 (125 to 25 BCE), associated with the construction phase, during which it was fully operational measured 22 meters in width.

- **L6 9bis, Chemin de la Planho, Vieille Toulouse (Haute-Garonne)^{28,29}**

Rescue excavations at La Planho (commune of Vieille-Toulouse, Haute-Garonne) were carried out in 2017 by Hades (directed by J. Robert) across an area of 1,730 m²²⁸. The site is located on the western edge of La Planho plateau, within the core of the Iron Age agglomeration of Vieille-Toulouse, overlooking the Garonne river.

More than 200 archaeological features were recorded, including pits, hearths, postholes, ditches, and wells, mostly dated to the 2nd–1st centuries BCE. From 125 BCE onward, the density of occupation increased. Although the state of preservation varied, the presence of craft activities alongside residential dwellings was identified, including at least buildings constructed in earth and timber.

The waterlogged grape pip analyzed in the present study was recovered from well PT74, located to the northern part of the excavation, away from the main inhabited area. The well, dug around 125 BCE, served as a water source before being gradually filled through both natural deterioration and intentional refuse dumping. The fill included ceramics, faunal remains (including human bones), and well-preserved plant material. The pip was retrieved from a lower layer of the well, likely corresponding to its primary phase of use. Although a few charred cereal remains were identified, the archaeobotanical assemblage was

dominated by waterlogged seeds of wild species, particularly weeds. Fruit remains were scarce but included fig and grape²⁹.

- **L7 50 Grand Rue, Horbourg-Wihr (Haut Rhin)³⁰**

Excavations at the site known as 50 Grand Rue in Horbourg-Wihr were conducted under the supervision of M. Roth-Zehner (Archéologie Alsace) between 2016 and 2021. Horbourg-Wihr is situated on the Rhine Plain in the Haut-Rhin department, near the river Ill, approximately ~1 km east of Colmar. The excavations covered an area of 1,344 m², and focused on a district of the Roman town of Horbourg-Wihr, located about ten km from the Roman military camp at Biesheim.

Although initial settlement at the site began around the start of the 1st century CE, intense urban development occurred in the latter half of the century and continued until the early 3rd century CE. During this period, the excavated area was occupied by a street flanked by two residential zones. From the final quarter of the 1st century CE to the second half of the 3rd century CE, a series of timber-lined pits were constructed on the site. Owing to the high water table, the wooden structure and the organic content of these pits are generally well preserved. Similar features—typically quadrangular and measuring about 2.5x1.5 meters—are known from other contexts in Horbourg-Wihr, with 71 pits documented beyond the excavation site at 50 Grand Rue. While their original function remains uncertain (and possibly associated with craft activity), their later use is clearer: they were ultimately repurposed as latrines and/or rubbish pits. One such example is provided by pit CV203. Its basic fill layer (US1217) contained abundant vegetable food waste, which remains to be studied by an archaeobotanist. The three grape seeds analyzed in the present study originate from this layer. Ceramic evidence indicates that pit CV203 was filled in and abandoned at the end of the 1st century CE.

- **L8 Rue Emile Jamais, Nîmes (Gard)^{31,32}**

Archaeological excavations conducted in 2014 in the city of Nîmes (Gard), under the direction of B. Houix (Inrap), revealed traces of the site's agrarian past. During the 2nd to 1st centuries BCE, the area was used for viticulture, as attested by the presence of grapevine plantation pits. By the end of the 1st century BCE, this rural landscape had been transformed into an artisanal area, featuring kilns and hearths. Further transformation occurred in the 1st century CE, when the site evolved into a residential quarter. In the northern part of the excavation, a domus was identified with a courtyard and wells, while in the south, a group of buildings, including one equipped with a hypocaust, was later repurposed into a metallurgical workshop.

One of the wells (PT2263), located within this residential complex, yielded an assemblage of well-preserved organic remains, including charcoal, waterlogged seeds and fruits, and everyday artefacts, such as lamps, amphorae, and pottery. The archaeobotanical material recovered from the stratigraphic unit 2591 includes a range of economic species commonly found in Roman contexts of southern Gaul, such as *Vitis*, *Ficus*, *Pyrus/Malus*, *Cucumis melo/sativus*, *Juglans*, *Rubus fruticosus*, and *Rubus idaeus* (Cabanis in Houix 2016). This well was sealed during the 3rd century CE.

- **L9 Font de Mauguio, Mauguio (Hérault)³³⁻³⁵**

La Font de Mauguio 3 is a small Roman-era rural estate specialized in viticulture, excavated in 2018 by Mosaïques Archéologie over a surface of 6,630 m²³³. The site lies on the coastal plain at the eastern edge of the modern town of Mauguio, near the Étang de l'Or. While the location of the domestic living quarters remains unclear, the excavations uncovered an agricultural complex featuring grape-processing installations, including a crushing area and wine press, as well as a wine cellar with a capacity of 70 *dolia*, corresponding to an estimated volume of 930 to 1308 hectolitres. This building was surrounded by vineyard plots and was likely constructed toward the end of the 1st century BCE or the beginning of the 1st

century CE. However, viticulture in the area probably predates the structure, with the initial planting of vines tentatively placed between the 2nd and 1st centuries BCE.

Two wells situated to the south and east of the agricultural building were remarkably preserved, with their organic content maintained protected by the water table. The archaeobotanical analysis of charcoals, wood, seeds, and fruits recovered from these contexts provided strong evidence for the economic importance of grapevine cultivation at the site. Other taxa—some of which were also likely cultivated locally—include *Juglans regia*, *Olea europaea*, and possibly *Prunus avium/cerasus*^{34,35}.

The two waterlogged pips analyzed in this study come from a single layer (US2085) located at the base of the fill of well PT2008. This context, interpreted as the functional surface associated with the well's use, is dated to the 1st-2nd centuries CE. The archaeobotanical assemblage from this layer was dominated by grape pips, indicating a massive and possibly deliberate discard event—perhaps the residue of wine production—rather than the gradual accumulation of domestic waste³⁵.

- **L10 Place de la Libération, Troyes (Aube)**³⁶⁻³⁹

Rescue excavations were carried out between 2004-2005 and 2007-2010, by Inrap (directed by P. Kuchler) in Place de la Libération, in the centre of the city of Troyes (Aube), in the lower Seine valley. The high water table at the site ensured excellent preservation of organic materials and allowed archaeologists to document a long chronological sequence of human occupation, from the Palaeolithic to the 19th century CE. The Roman phases revealed the development of an urban district from the early 1st to the mid-3rd century CE, offering valuable information on urban planning, domestic architecture, and local economic and dietary practices³⁶.

Numerous waterlogged structures—including basins, pits, cesspits, and wells—were excavated below the water table. Their wooden linings permitted precise dendrochronological dating, and the preservation of botanical remains enabled a large-scale carpological study³⁷. Most of the identified seeds and fruits represented food waste (cereals, pulses, legumes, condiments, fruits), with grape remains particularly abundant. Geometric morphometric analysis of grape pips recovered at the site showed a mixed assemblage of morphotypes, some resembling wild grape (*Vitis vinifera* subsp. *sylvestris*), and others aligning with domesticated varieties, including forms with southern affinities³⁸.

The grape pips analyzed in the present study originated from a cesspit (Fo1548; 2 pips), a basin or vat (US3140; 1 pip), and two wells: PT3159 (2 pips, 1st century CE), and PT3209 (3 pips, 2nd century CE). Well PT3209 also contained a large assemblage of grape remains, including numerous pips, pedicels, and grape-skin fragments. This concentration suggests on-site grape processing, possibly for winemaking, during the 2nd century CE³⁹.

- **L11 Reg de Ligno, Valros (Hérault)**⁴⁰

As part of the construction of the A75 motorway between Pézenas and Béziers, preventive archaeological excavations were carried out by Inrap in 2006-2007 (directed by J.-B. Chevance and C. Jung). Investigations focused on several sectors, notably Sectors 3 and 4, collectively referred to as *Aire de Repos de Valros*. These revealed traces of Roman occupation dated to the 1st and 2nd centuries CE.

One of the sites within this area, *Reg de Ligno*, included a well (PT2005) interpreted as being associated with a nearby funerary and cultic complex. This complex was located in immediate proximity to a vine-growing and wine-making establishment. The extent of viticultural activity is by the presence of grapevine plantation pits over 4 hectares.

Although the well could only be partially excavated for safety reasons, a significant number of grape pips were recovered from the fill, alongside numerous fragments of *dolia*. Five waterlogged grape pips from stratigraphic unit US2593, dated to the 2nd century CE by associated material culture, were included in the present study.

- **L12 Lattara–Chenal, Lattes (Hérault)²⁰⁻²⁷**

Same site as L5 Lattara–Chenal, Lattes (Hérault), but the pips come from a different archaeological layer, US205083 (150–200 CE), corresponding to the final major phase of use before the canal was gradually infilled and abandoned at Lattara.

- **L13 Rue de la Croix Verte, Limoges (Haute Vienne)^{41,42}**

In 2015 and 2017, rescue excavations were undertaken by the Hades archaeological firm (directed by R. Macario) on two adjacent plots in the historical centre of Limoges, covering a combined area of approximately 4,000 m²⁴¹. These investigations focused on a district of the ancient city of *Augustoritum*, located on the right bank of the Vienne river. The excavations revealed a range of urban features, including streets, residential buildings, and areas dedicated to craft and commercial activities, and documented their development from the 1st to the late 4th century CE.

Several wells were uncovered during excavation, two of which were subjected to archaeobotanical analysis⁴². The waterlogged grape pip analyzed in the present study was retrieved from the basal layer (US2645) of well PT258. This well, located in an area directly accessible from the street, was likely for public use. Pottery found in the basal layer of the well dates the deposit to between 170 and 240 CE.

The carpological assemblage revealed a concentration of food waste, dominated by fruit remains, including *Corylus avellana*, *Cucumis* sp., *Juglans regia*, *Castanea sativa*, *Mespilus germanica*, various *Prunus* species, and more.

- **L14 Pré aux Pêcheurs, Antibes (Alpes-Maritimes)⁴³⁻⁴⁵**

Rescue excavation work (Inrap) conducted by Inrap at Pré aux Pêcheurs (Antibes, Alpes Maritimes), under the supervision of I. Daveau, were carried out in advance of the construction of an underground car park. These investigations uncovered significant remains of the ancient Greek trading post of Antipolis, founded by the Phocaeen individuals who established Marseille. Benefiting from a naturally sheltered harbor–Bay of Saint-Roch)–Antipolis occupied a strategic position along the maritime trade routes linking Marseille to the Italian coast. This advantageous location, combined with the exploitation of maritime resources such as fish salting and the manufacture of garum (fermented fish sauce), contributed to the prosperity of the Greek, and later Roman, settlement.

The excavation documented the complete sequenced of infilling of the ancient bay, allowing researchers to reconstruct the major phases of silting. Archaeological deposits spanning from the 4th century BCE to the 6th century CE attest to the site's sustained role as an active harbor. The harbor floor functioned as a dumping ground, accumulating thousands of objects discarded from anchored vessels, or left behind during cargo transfers. These materials offer valuable insights into the daily lives of sailors and the dynamics of maritime trade. The spatial distribution of debris varies chronologically, likely reflecting changes in harbor activities and modifications of the riverbank⁴³.

Waterlogged conditions at the site enabled exceptional preservation of abundant organic remains, including a Roman boat without its cargo. The preserved dimensions of the vessel are approximately ~15 meters in length, and 6 meters in width. It was constructed from a variety of timbers, including coniferous species such as *Pinus halepensis*, *Pinus pinea*, and *Abies alba*, as well as angiosperms such as *Quercus ilex*, *Corylus avellana* and *Olea europaea*⁴⁴. Further plant remains were recovered through the sieving of several 1-liter sediment samples. These included fragments of wood and charcoal, as well as a diverse assemblage of fruits and seeds. The fruit remains consist largely of commonly cultivated and locally available species, such as *Ficus carica*, *Juglans regia*, *Morus nigra*, *Prunus avium/cerasus*, *Olea europaea*, *Prunus domestica*, *Prunus persica*, and *Vitis vinifera*. The assemblage also includes more exotic species, such as *Cordia myxa* and *Ziziphus cf. ziziphus*⁴⁵.

- **15 : Terrasses de Montfau, Magalas**

The Roman-period site of Magalas (southern France), occupied from the 1st to the 4th century CE, comprises a sanctuary complex and an adjacent winemaking establishment. Ancient DNA samples were recovered from the lowermost layers of well PT 4000, located within the sanctuary area. The well contained abundant archaeological material, including ceramic vessels, metal objects, wood remains, and plant macroremains such as *Vitis vinifera*, *Juglans regia*, *Corylus avellana*, and *Cyperus longus*. (for additional details, see Ramos-Madrigual et al⁴⁶).

- **L16 Mas de Vignoles XIV, Nîmes (Gard)**

The Mas de Vignoles XIV site, located in the alluvial plain of the Vistre River (southern France), was occupied from the Neolithic to the Middle Ages and excavated during rescue operations by Inrap in 2010–2011. The most substantial occupation corresponds to a large Iron Age (Roman Republican) farm dated to the 2nd–1st century BCE, later reorganized with orthogonal ditches delimiting agricultural areas. Grape pips analyzed in this study were recovered from medieval wells radiocarbon-dated to 417–515 CE and 731–851 CE, corresponding to the transition between Late Antiquity and the Early Middle Ages. (for additional details, see Ramos-Madrigual et al⁴⁶).

- **L17 Îlot Saint-Géraud, Aurillac (Cantal)^{47–49}**

Rescue excavations were conducted by Mosaiques Archéologie (directed by N. Clément) in 2013–2014 at the site known as Îlot Saint-Géraud in Aurillac (Cantal), situated to the south of the medieval abbey church of the Monastery of Saint-Géraud. Covering an area of 3 000 m², the excavation revealed evidence of nearly continuous occupation from the Early Middle Ages to the present day.

The earliest phases of occupation, dating from the 7th to the 10th century CE, included masonry buildings associated with refuse pits, as well as a structure interpreted as having a cultic and/or funerary function, indicated by the presence of several wooden sarcophagi. During the 12th to 16th century CE, the site was further developed with the construction of an abbey and a range of service buildings, including a stable that was later destroyed by fire. A cemetery, extending over at least 1,000 m², also formed part the monastic complex during this period. In the 17th and 18th centuries CE, the southern part of the former monastic complex was transformed into a garden, creating a large agricultural space within the very heart of the town.

The waterlogged pips analyzed in the present study originate from a fill layer of Early Medieval pit 3027, identified as an open-air refuse pit. Numerous uncharred seeds and fruit pits were uncovered during the excavation from several stratigraphic units (US3028, 3159, and 3162). Carpological analysis of a sample from the upper fill layer revealed a highly diverse botanical assemblage, including rye, naked wheat, vetch, melon or cucumber, fig, wild strawberry, walnut, several *Prunus* species, blackberry, black elderberry, and grapevine. Additionally, the assemblage contained fish vertebrae, charcoal fragments, and dipteran puparia. The uncharred plant remains (seeds and pips) likely derive from food waste or excrement.

Accelerator Mass Spectrometry (AMS) dating of the seeds yielded a broad calibrated chronological range of 777–986 CE (857–986 CE at 83% probability, Ly-13922). Ceramic material from the same context correspond to the 7th–9th centuries, which combined with radiocarbon dating, supports the attribution of this feature to the 9th century CE. Evidence of grapevine use on site was also identified in the form of a charcoal fragment from a fireplace associated with the Modern period.

- **18 Cine Serra, Ibiza (Espagne)⁵⁰**

Rescue excavations were carried out in 2017 by Antiquarium (directed by J. Torres Costa and E. Marlière) at the site of the former "Cine Serra" cinema in the city of Ibiza (Ibiza Island, Spain). The excavation uncovered the remains of a 12th-century CE irrigation system

associated with an agricultural zone. This system, locally known as *Ses feixes*, was based on the reclamation and drainage of coastal wetlands to create long, narrow fields (*feixes*) separated by irrigation channels (*séquies*). Designed to optimize agricultural productivity, the *Ses Feixes* system remained in use until the late 1950s and enabled the cultivation of both marshy and arid soils.

The waterlogged grape pips analyzed in the present originate from a sediment layer at the base of one of the 12th-century CE irrigation channels. This layer contained rich organic deposits, including abundant plant remains, faunal material, seeds, charcoal, and wood fragments. The carpological remains identified in this context include species likely cultivated on-site—such as grapevine, celery, almond tree, cypress, and fig—as well as wild plants, possibly representing agricultural weeds or elements of the surrounding natural environment. An AMS radiocarbon date obtained from a seed sample (10-CINESERRA118) places this layer firmly within the period of Muslim occupation of the island, yielding a result of 955±30 BP (1027-1160 CE, 95.4% probability).

- **L19 Ancien Couvent des Ursulines, Auvillar (Tarn et Garonne)**^{51,52}

Rescue excavations were conducted by Hades (directed by L. Leroux) between 2017 and 2018 in Auvillar (Tarn-et-Garonne), on the site of the former Ursuline convent. The 370 m² excavation area lies to the north of the town, on the edge of a plateau overlooking the Garonne river by approximately 50 meters⁵¹. The investigation aimed to document the historical development of this area during the medieval and modern periods.

The earliest activity identified on the site dates to the 9th or early 10th century CE and is marked by the excavation of a large defensive ditch (feature Fs24). In the 11th and early 12th centuries CE, a funerary area was established, followed by the development of an urban settlement that gradually grew in density through the end of the Middle Ages. During the 12th-13th centuries CE, the ditch—subsequently replaced by an earthen rampart—was backfilled with substantial quantities of domestic refuse. In the early modern period, occupation became less dense, prior to the establishment of the Ursuline convent in the 17th century CE.

The waterlogged grape pip analyzed in this study derives from layer 24.5, a fill deposit within ditch Fs24 composed primarily of clay and well-preserved plant macroremains. This layer also contained a notable proportion of animal bones and ceramic fragments, suggesting that the ditch had been repurposed as a dumping ground for the household waste from the adjacent settlement. The ceramic material, along with a radiocarbon dating obtained from a grape pip (Lyon-14778: 815± 30 BP; 1168-1266 cal CE), places the deposit in the second half of the 12th or 13th century CE.

Archaeobotanical analysis confirms the abundance of seeds and fruit remains in the deposit, primarily comprising food waste, particularly waterlogged fruits⁵². Grape remains are especially prevalent. While the presence of fecal material is evident, the co-occurrence of grape pips with pedicels, stalk fragments and grape skins may also point to the disposal of grape-pressing residues.

- **L20 Rue des Hospices, Valenciennes (Nord)**^{53,54}

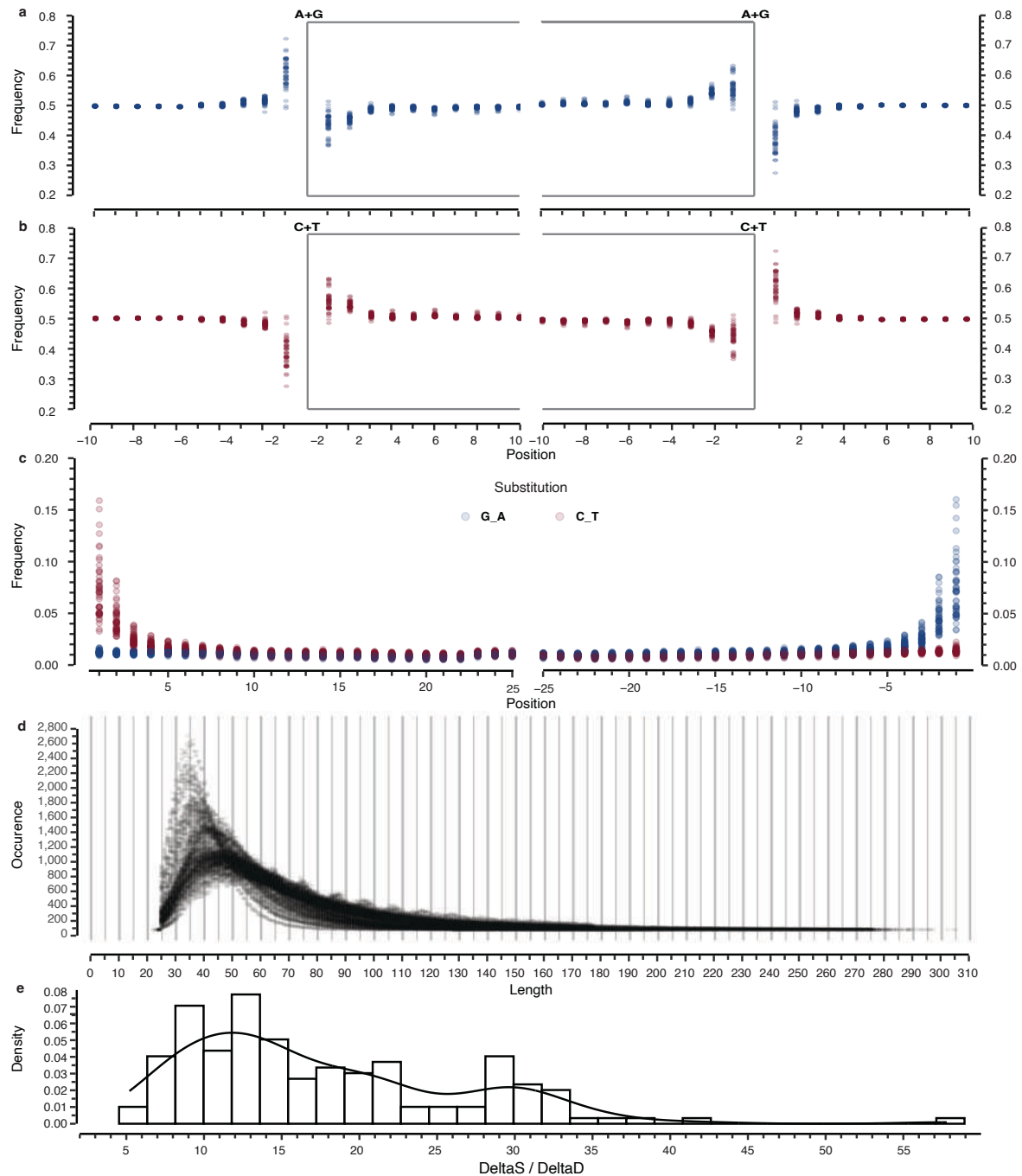
Excavation carried out in 2016 by the Service archéologique de Valenciennes (directed by D. Delassus) in the center of the modern—corresponding to the core of the medieval town—uncovered an area of 1,100 m², close to the St Catherine river. The site, characterized by fluvial deposits and high humidity, provided excellent conditions for the preservation of organic remains⁵³. The earliest human occupation dates to the 10th–11th centuries CE and is represented by pits, ditches, and wooden post-built structures, in line with what is known from historical records as the “old town” of Valenciennes.

One of the two waterlogged grape pips analyzed in this study comes from cesspit St 3739, attributed to this first phase of settlement. Although not directly associated with any standing structure, its fill is dated by ceramics to 12th century CE.

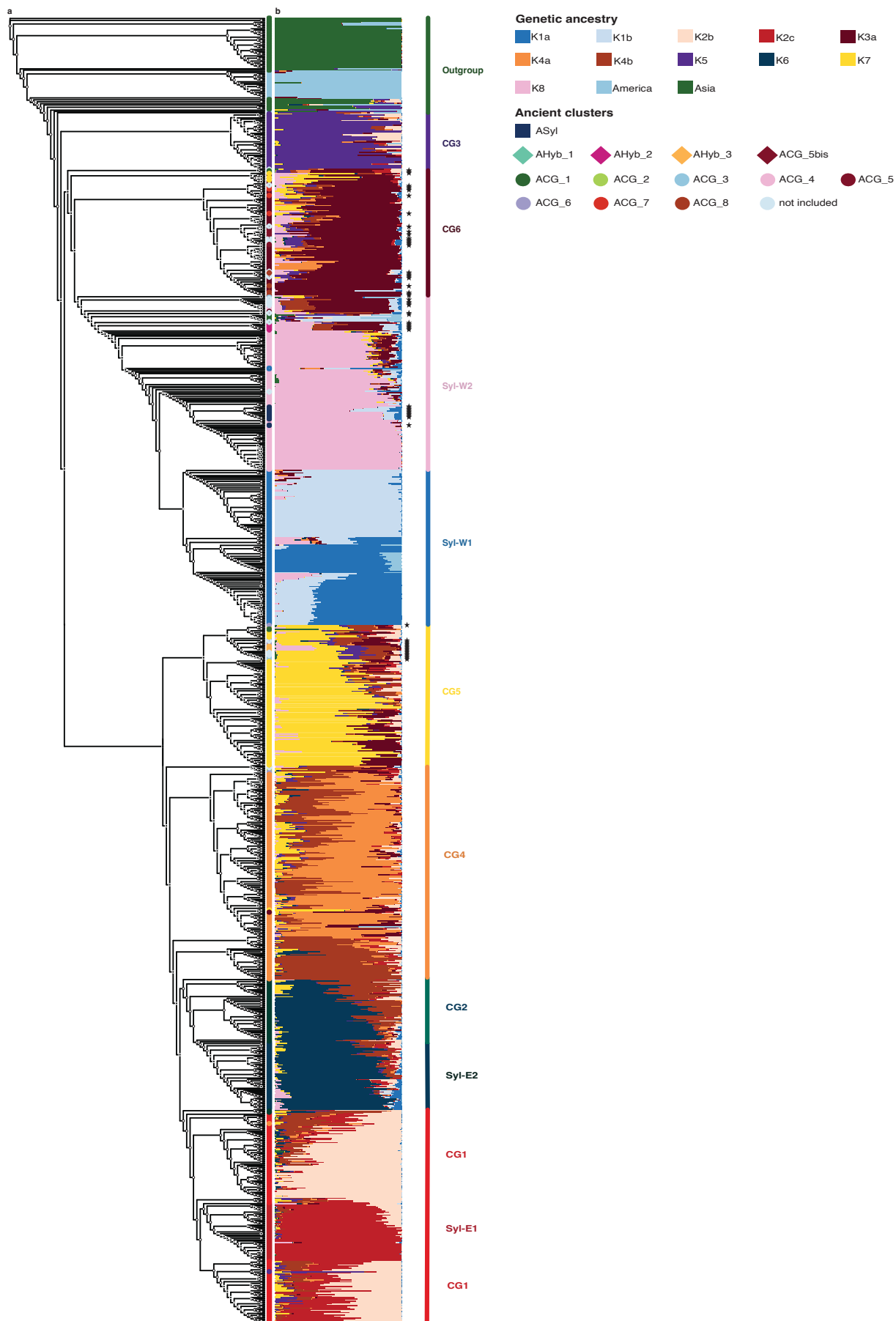
From the late 13th century CE onward, stone residential buildings were constructed at the site. Around 1430, the city’s first hospital, known as “Hôtel-Dieu”, was established here.

Excavations revealed the western part of the main hospital building, including the chapel, the large patient ward, a cellar, and a cistern. The second grape pip analyzed in the present study comes from cesspit 1029, associated with the large patient ward. The filling of the cesspit, especially US 9 which yielded the grape pip, is dated by ceramic material to the 15th century CE.

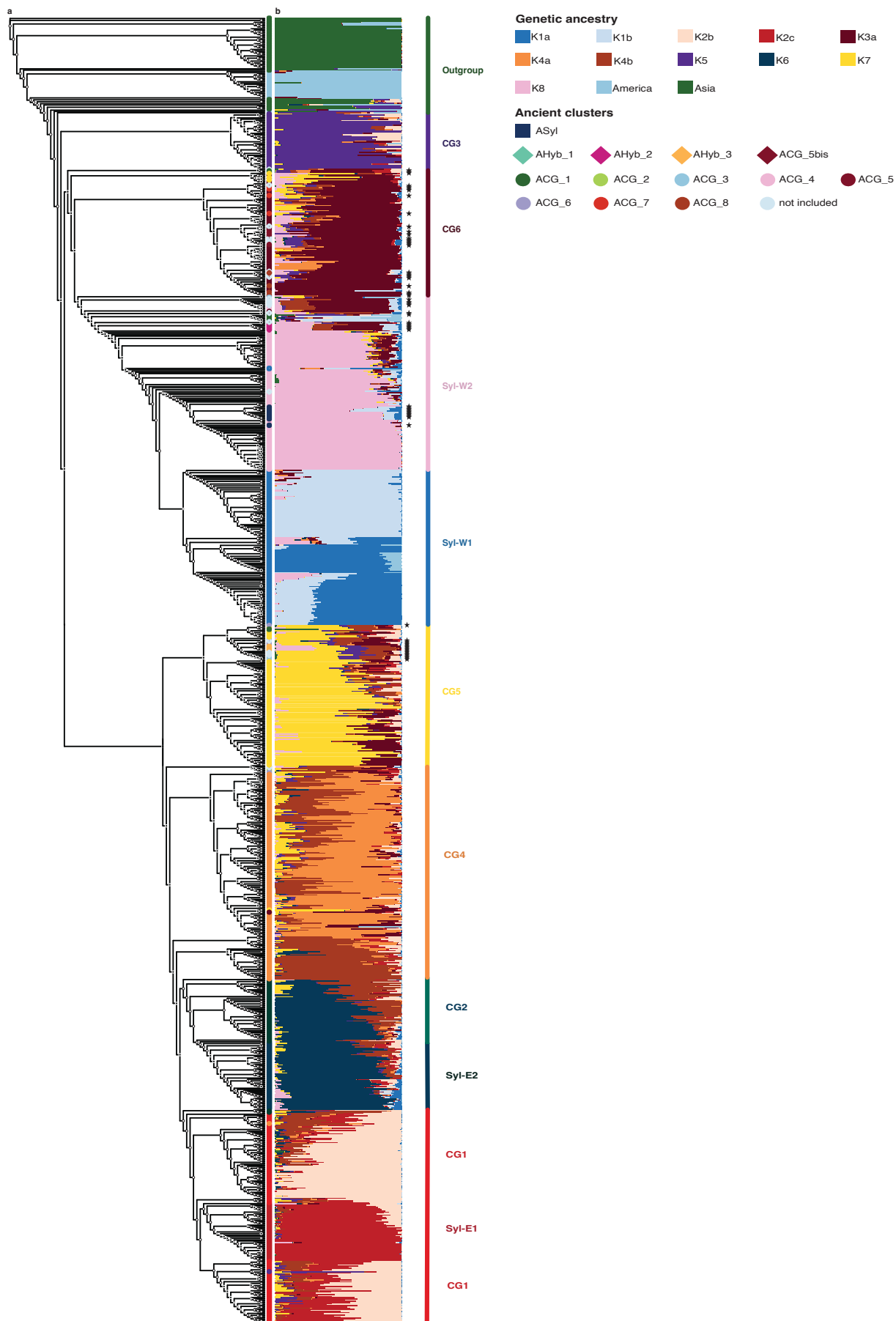
As is typical of latrine contexts, the archaeobotanical assemblage from the cesspits reflects dietary waste, with an abundance of fruit remains. In addition to grape pips, seeds of fig and strawberry were common, alongside those of blackberry, mulberry, medlar, cherry, plum, and peach⁵⁴ and stones.



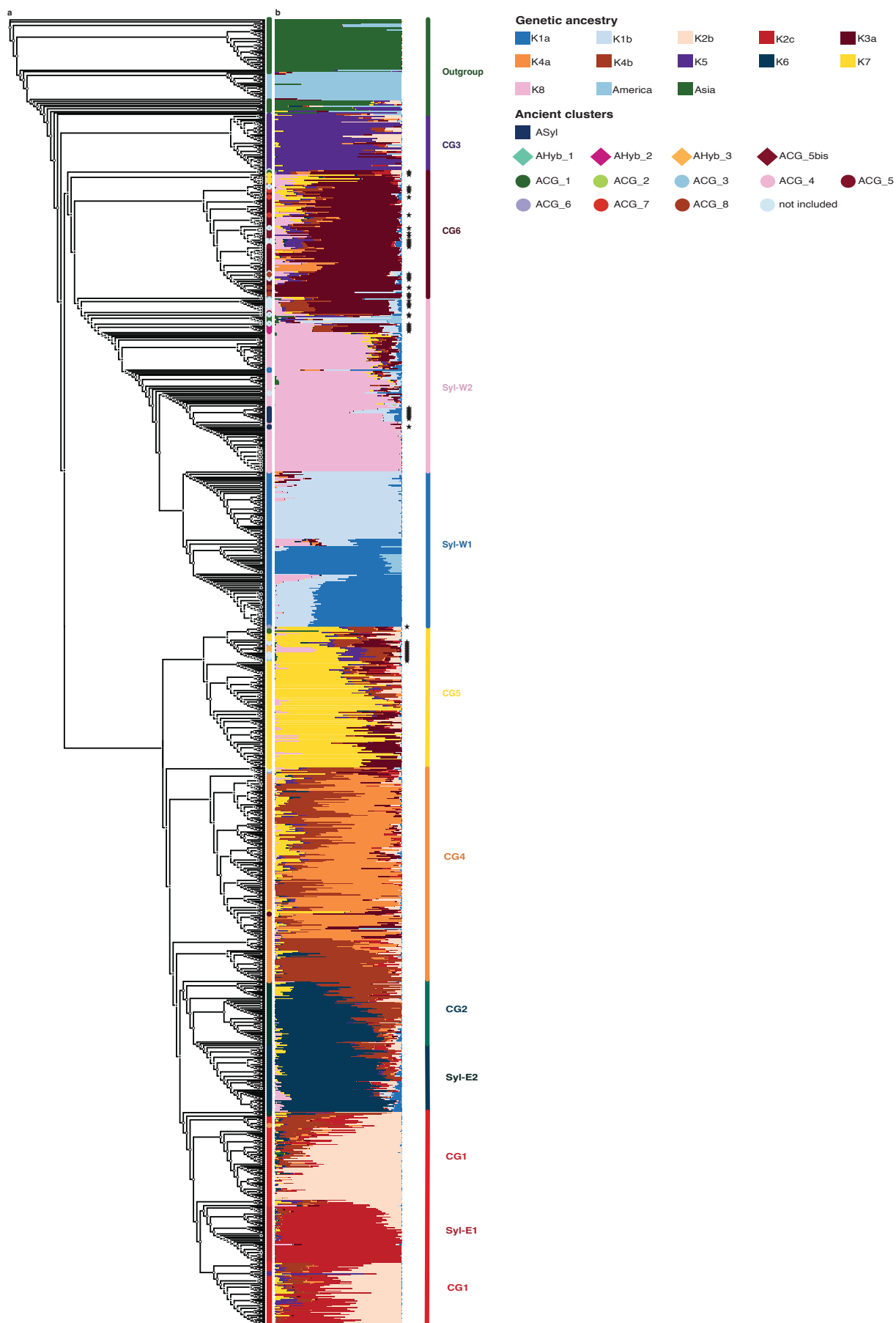
Supplementary Fig. 1. Post-mortem DNA damage patterns in newly sequenced ancient grape pips ($n = 49$) a) Base composition profiles. Purine (G+A) frequencies at the ten nucleotide positions flanking read-to-reference genome alignments (left, positions -1 to -5, and; right, positions +1 to +5), and the ten first (1 to 10) and last (-10 to -1) alignment positions. b). Same as a) for pyrimidines (C+T). c) Nucleotide mis-incorporation profiles. C>T substitution rates within the first (left) and last (right) 25 positions in read-to-reference genome alignments. d) Single-end read length distribution e) Distribution of the ratio between deamination rate in single-stranded overhangs (ΔS) and double-stranded regions (ΔD). Each point represents the average measurement in a given sample.



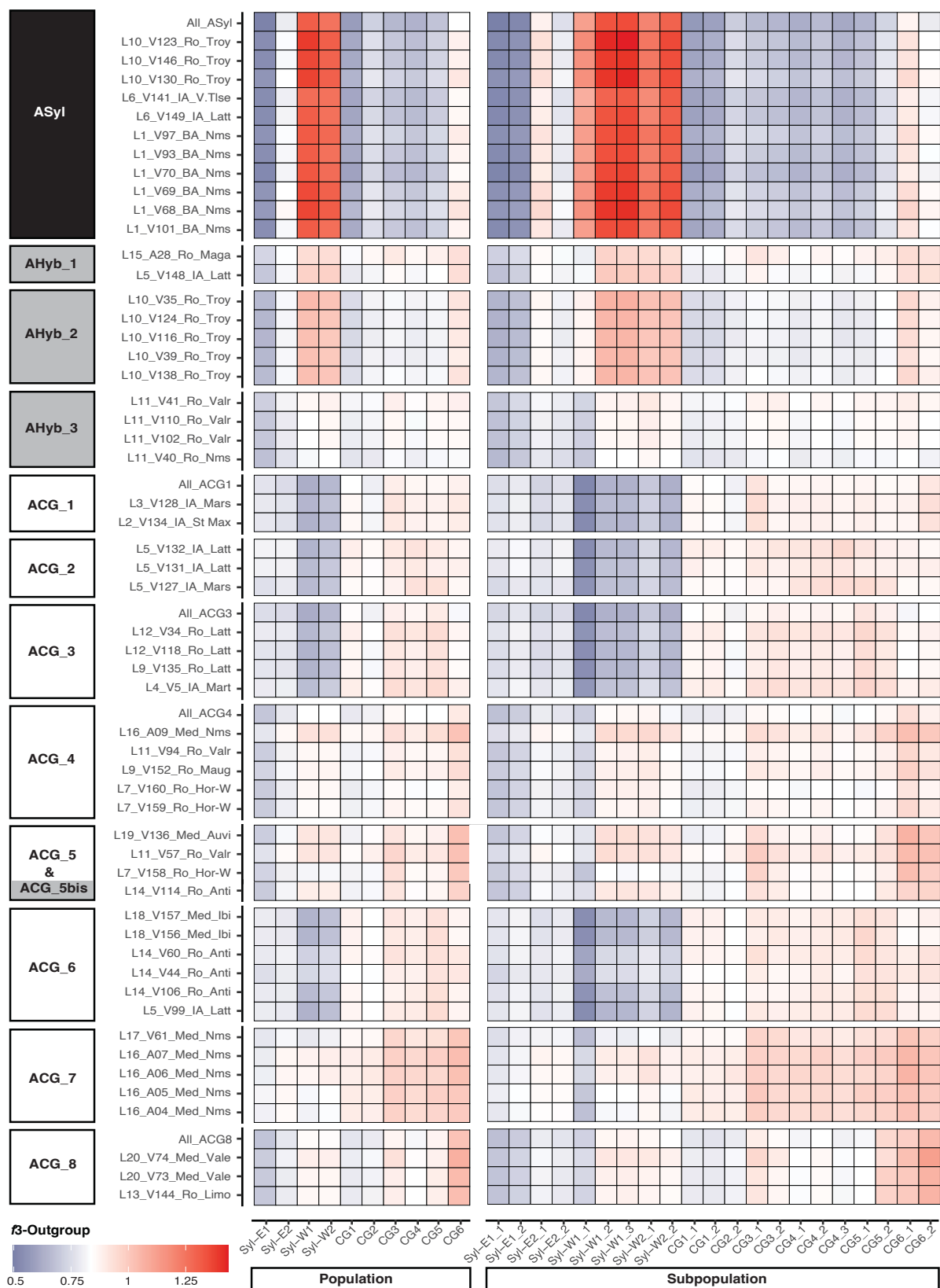
Supplementary Fig. 2. Neighbor joining phylogenetic tree and unsupervised ADMIXTURE⁵⁵ profiles of the 1,071 accessions from the core datasets. a) Neighbor-joining phylogenetic tree with node colors representing support, as assessed from 100 bootstrap pseudo-replicates (BV): white >90%, grey >70% and ≤90%, and black <70%. Tips are colored according to population, following Dong et al⁵⁶. b) Unsupervised ADMIXTURE⁵⁵ clustering at K = 13 aligned to tree phylogenetic placement. Colors represent the main genetic ancestry components originally defined by Dong et al with minor modifications. K1 ancestry is subdivided into K1a and K1b, both detected within the Syl-W1 population. Similarly, K2 is divided into K2b and K2c, which are primarily associated with the CG1 and Syl-E1 populations, respectively. Finally, K4 ancestry is also subdivided into K4a and K4b, both characterizing the CG4 population. The main population clusters considered are indicated on the right. Ancient samples are marked by black stars.



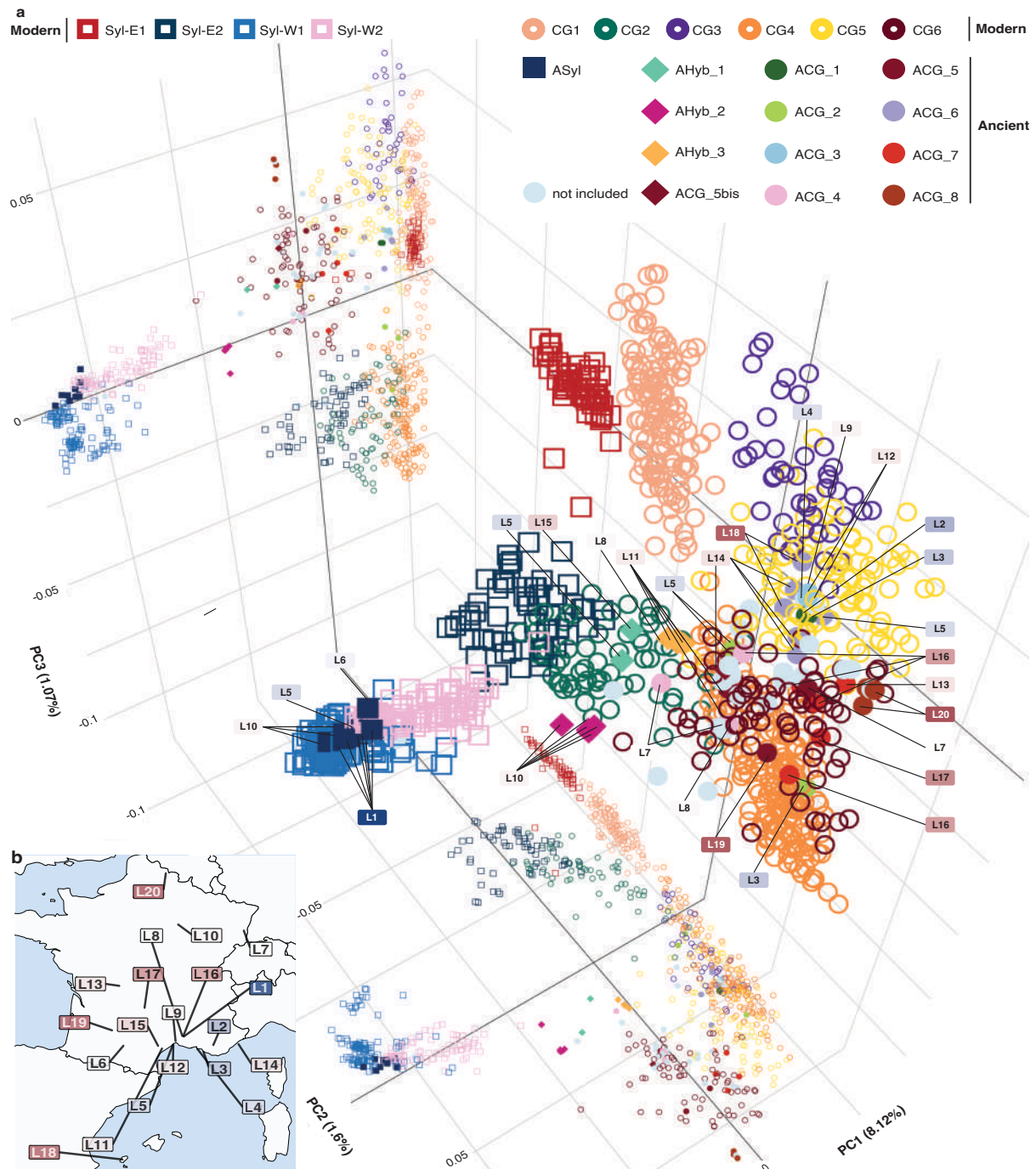
Supplementary Fig. 3. Neighbor joining phylogenetic tree and unsupervised ADMIXTURE⁵⁵ profiles of the 850 accessions from the core datasets. a) Neighbor-joining phylogenetic tree with node colors representing support, as assessed from 100 bootstrap pseudo-replicates (BV): white >90%, grey >70% and ≤90%, and black <70%. Tips are colored according to population, following Dong et al⁵⁶. b) Unsupervised ADMIXTURE⁵⁵ clustering at K = 13 aligned to tree phylogenetic placement. Colors represent the main genetic ancestry components originally defined by Dong et al⁵⁶ with minor modifications. K1 ancestry is subdivided into K1a and K1b, both detected within the Syl-W1 population. Similarly, K2 is divided into K2a and K2b, associated with the CG1 population, and K2c, found in the Syl-E1 population. Finally, K6 ancestry is also subdivided into K6a and K6b, characterizing the CG2 and Syl-E2 populations respectively. The main population clusters considered are indicated on the right. Ancient samples are marked by black stars



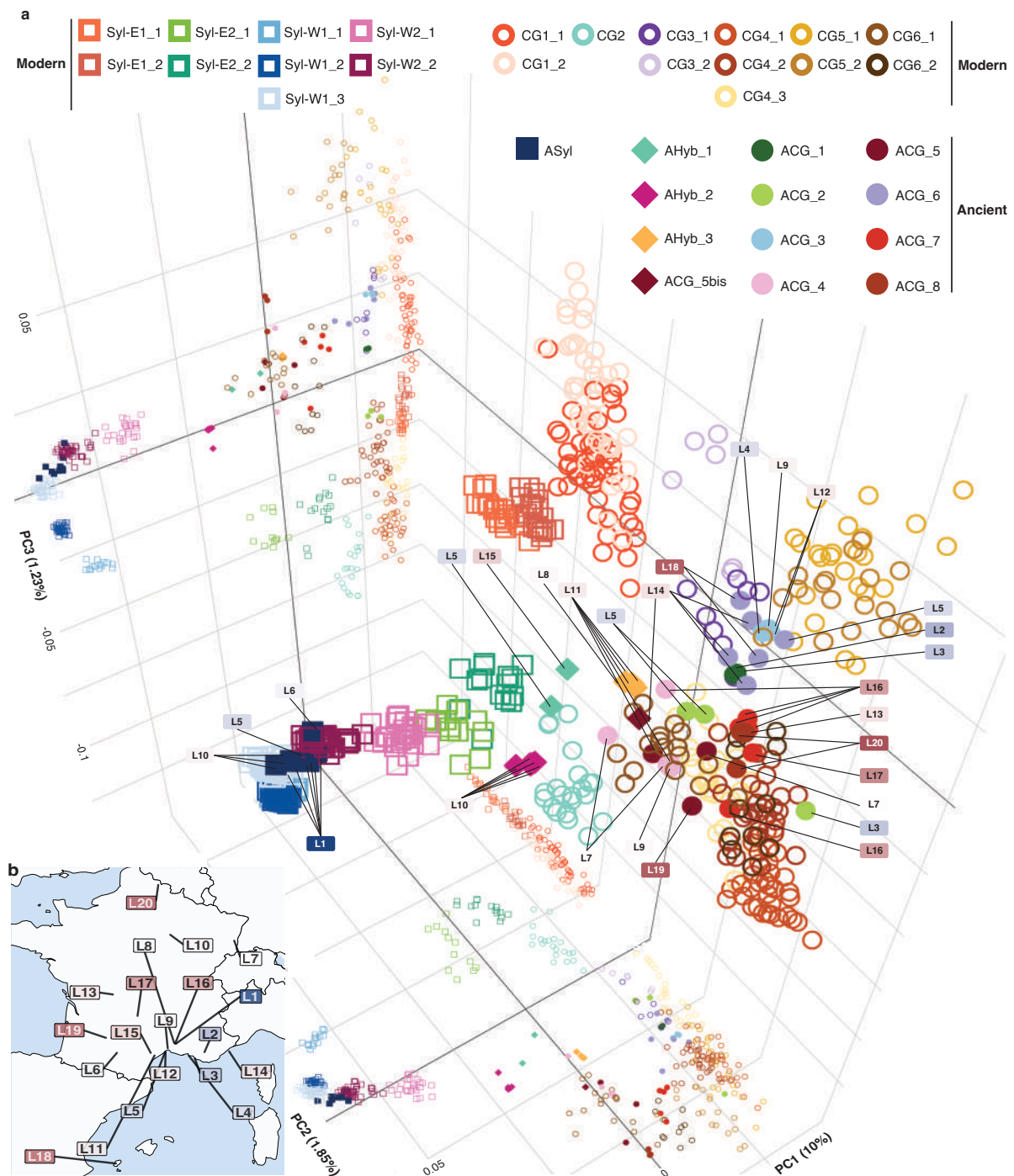
Supplementary Fig. 4. Neighbor joining phylogenetic tree and unsupervised ADMIXTURE⁵⁵ profiles of the 613 accessions from the core datasets. a) Neighbor-joining phylogenetic tree with node colors representing support, as assessed from 100 bootstrap pseudo-replicates (BV): white >90%, grey >70% and ≤90%, and black <70%. Tips are colored according to population, following Dong et al⁵⁶. b) Unsupervised ADMIXTURE⁵⁵ clustering at K = 11 aligned to tree phylogenetic placement. Colors represent the main genetic ancestry components originally defined by Dong et al⁵⁶ with minor modifications. K1 ancestry is subdivided into K1a and K1b, both detected within the Syl-W1 population. Similarly, K2 is divided into K2b and K2c, which are primarily associated with the CG1 and Syl-E1 populations, respectively. The main population clusters considered are indicated on the right. Ancient samples are marked by black stars.



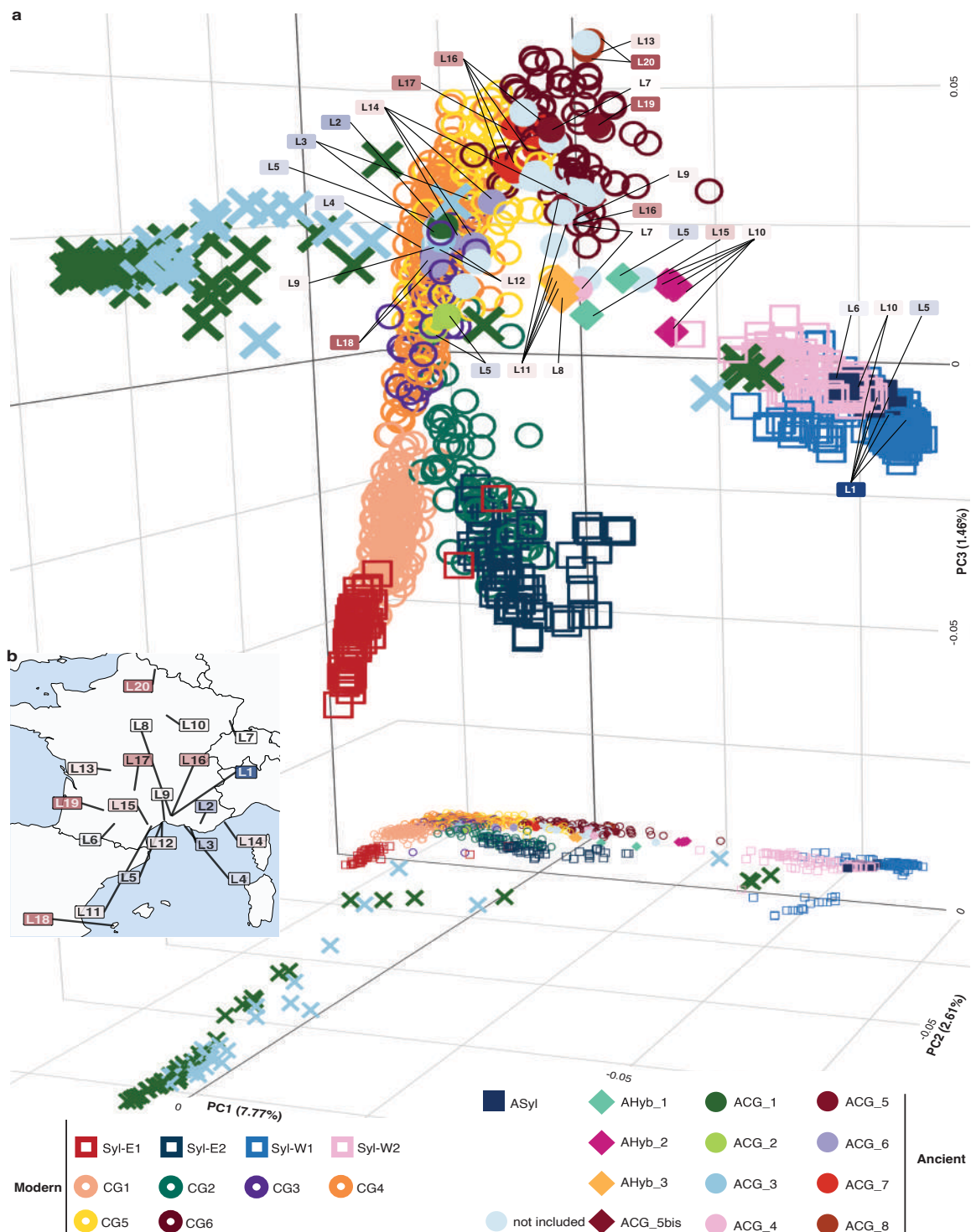
Supplementary Fig. 5. Matrix of f_3 -outgroup statistics between ancient samples (n=54) and modern grapevine populations (left) and sub-populations (right) of the extended dataset. f_3 -outgroup statistics, using non-*Vitis vinifera* species as outgroups, measure the extent of shared genetic drift between pairs of samples. The color gradient represents lower (blue) to higher (red) levels of shared genetic affinity. Sample names include original labels, age categories (BA, Bronze Age; IA, Iron Age; Ro, Roman Period; and Med, Medieval), and archaeological sites. Labels framed in black, grey, and white rectangles on the left panel correspond to ancient genetic clusters identified among wild, hybrid, and domesticated accessions, respectively. Groups identified with a “hyb_” prefix correspond to the data merger several samples identified as genetically identical.



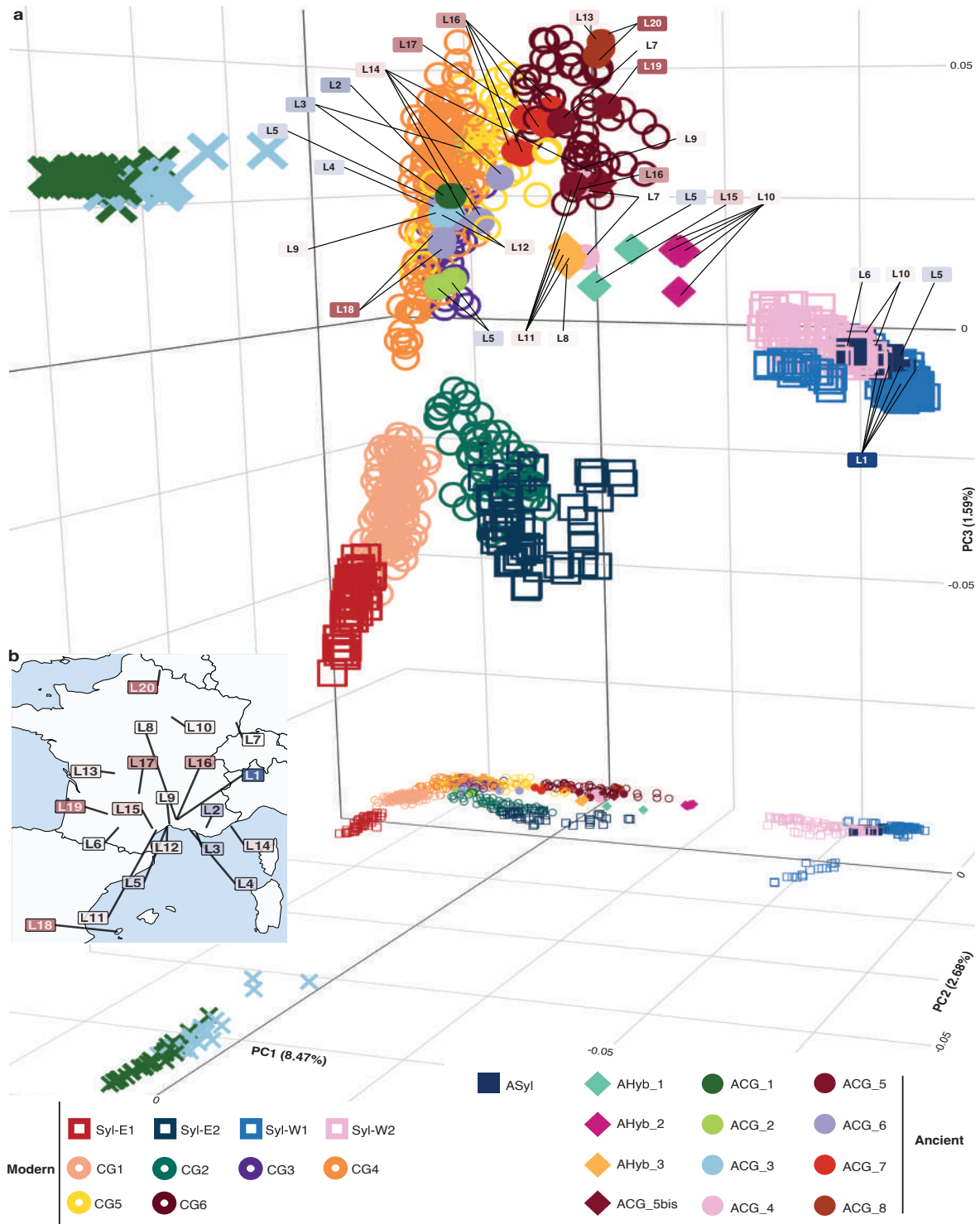
Supplementary Fig. 6. 3D-PCA projection of ancient samples ($n = 80$) in the space defined by all modern grapevine accessions of the core dataset ($n = 908$). a) PCA showing the first three principal components, with their respective fraction of variance explained indicated in parentheses. 2D-PCAs are shown on the 2D-plans formed by each pair of axes. Squares, circles, and diamonds represent *Vitis sylvestris*, *V. vinifera*, and their hybrids, respectively. Open symbols indicate modern accessions while filled symbols indicate ancient samples. Symbols are colored by sub-population. Archaeological sites of ancient samples are labeled with simplified codes and colored by age, following the same gradient as in Figs. 1ef. b) Summary map of the archaeological sites and their labels.



Supplementary Fig. 7. 3D-PCA projection of ancient samples ($n = 54$) in the space defined by all modern grapevine accessions of the core dataset at the sub-population level ($n = 506$). a) PCA showing the first three principal components, with their respective fraction of variance explained indicated in parentheses. 2D-PCAs are shown on the 2D-plans formed by each pair of axes. Squares, circles, and diamonds represent *Vitis sylvestris*, *V. vinifera*, and their hybrids, respectively. Open symbols indicate modern accessions while filled symbols indicate ancient samples. Symbols are colored by sub-population. Archaeological sites of ancient samples are labeled with simplified codes and colored by age, following the same gradient as in Figs.1ef. b) Summary map of the archaeological sites and their labels.

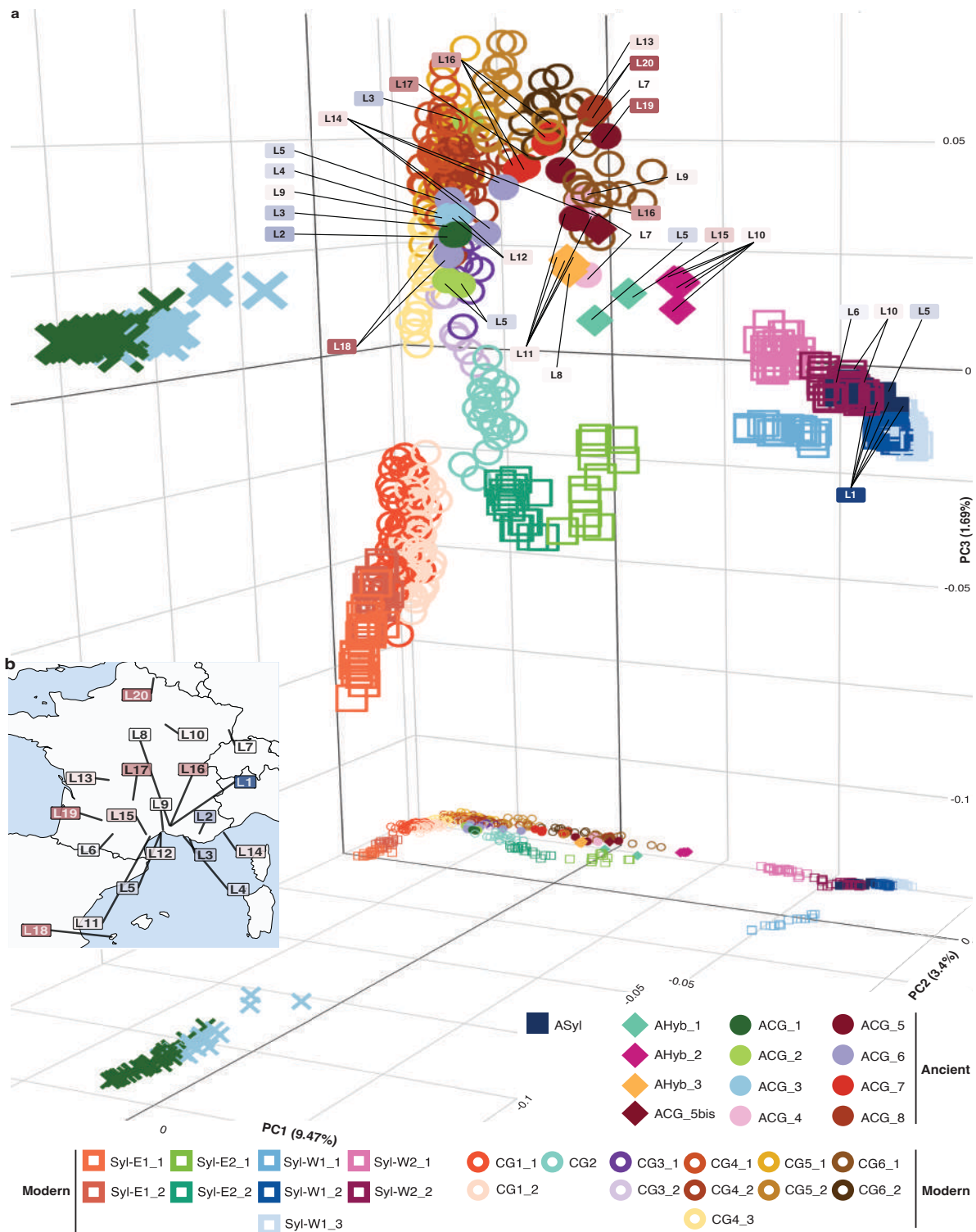


Supplementary Fig. 8. 3D-PCA projection of ancient samples ($n = 80$) in the space defined by all modern accessions of the core dataset ($n = 991$). a) PCA showing the first three components, with their respective fraction of variance explained indicated in parentheses. 2D-PCA is shown on the PC1-PC2 plane. Squares, circles, diamonds, and crosses represent *Vitis sylvestris*, *V. vinifera*, their hybrids, and non-*V. vinifera* species, respectively. Open symbols indicate modern accessions; filled symbols indicate ancient samples. Symbols are colored by population. Archaeological sites of ancient samples are labeled and colored by age, following the same gradient as in Figs. 1ef. b) Summary map of the archaeological sites and their labels.

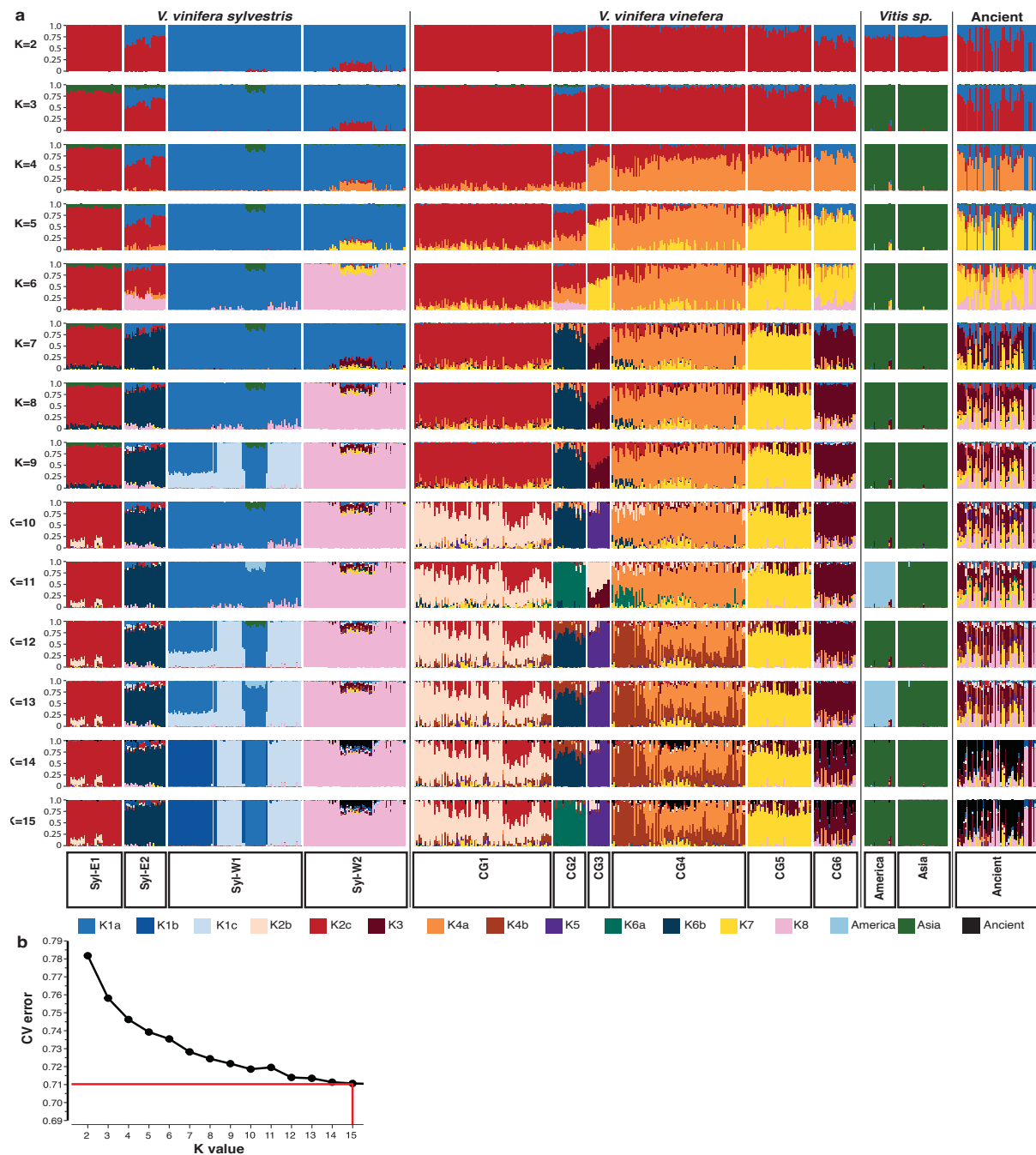


Supplementary Fig. 9. 3D-PCA projection of ancient samples ($n = 54$) in the space defined by all modern accessions of the core dataset at the population level ($n = 796$).

a) The first three PCA axes are displayed, with their respective fraction of variance explained indicated in parentheses. 2D-PCAs are shown on the PC1-PC2 plane. Shapes represent taxonomic groups: squares for *Vitis sylvestris*, circles for *V. vinifera*, diamonds for hybrids, and crosses for non-*V. vinifera* species (crosses). Open symbols denote modern accessions, filled symbols denote ancient samples. Symbols are colored by population. Archaeological sites of ancient samples are labeled and colored by age, following the same gradient as in Figs. 1ef. b) Summary map of the archaeological sites and their labels.

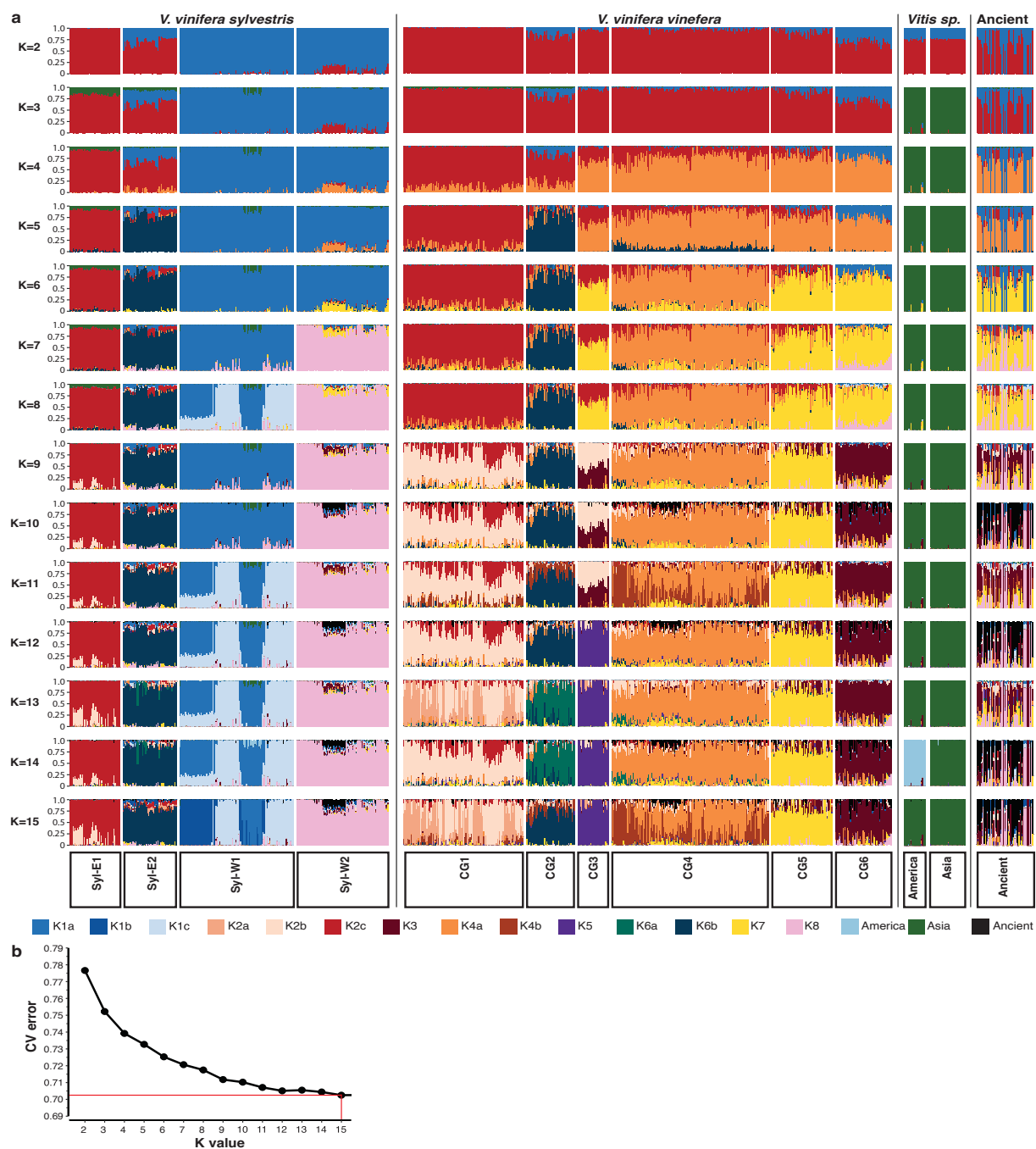


Supplementary Fig. 10. 3D-PCA projection of ancient samples ($n = 54$) in the space defined by all modern accessions of the core dataset at the subpopulation level ($n = 559$). a) The three principal components are shown, with their respective fraction of variance explained indicated in parentheses. 2D-PCAs are shown on the PC1-PC2 plane. Symbols represent *Vitis sylvestris* (squares), *V. vinifera* (circles), hybrids (diamonds), and non-*V. vinifera* species (crosses). Open symbols represent modern accessions, filled symbols represent ancient samples. Colors indicate subpopulation. Archaeological sites of ancient samples are labeled and colored by age, following the same gradient as in Figs. 1ef. b) Summary map of the archaeological sites and their labels.



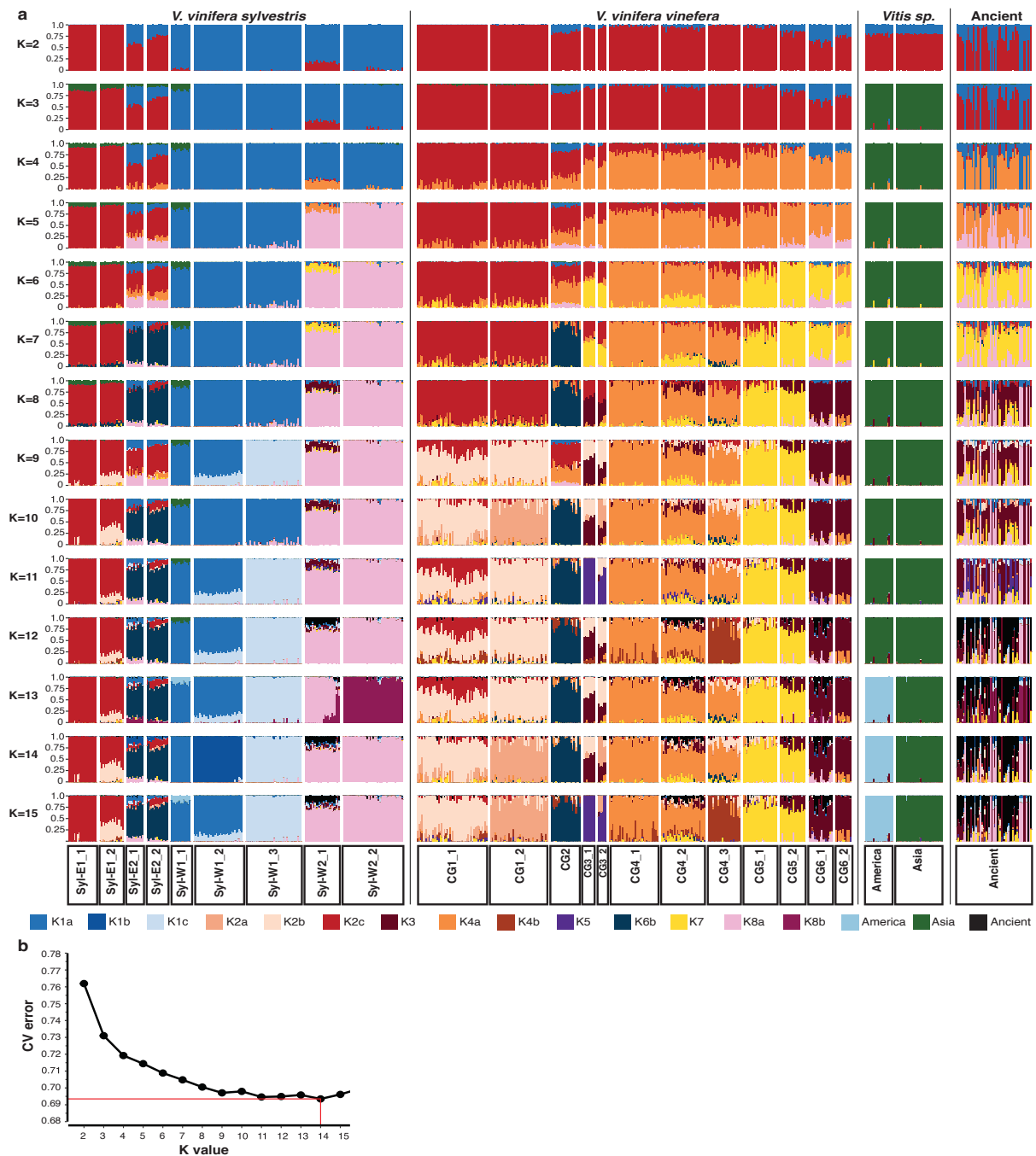
Supplementary Fig. 11. Unsupervised ADMIXTURE⁵⁵ analysis on all the accessions of the core dataset ($n = 1071$). a) Genetic ancestry proportions from $K = 2$ to 15, with K representing the number of independent genetic ancestries. Accessions are ordered from left to right according to taxonomic status: *Vitis sylvestris*, *V. vinifera*, outgroups, followed by ancient samples. Colors represent the main genetic ancestry components originally defined by Dong et al.⁵⁶, with minor modifications. K1 ancestry is subdivided into K1a, K1b, and K1c, all observed within the Syl-W1 population. Likewise, K2 is divided into K2b, associated with the CG1 population, and K2c, found in the Syl-E1 population. K4 ancestry is further divided into K4a and K4b, both present in the CG4 population. Finally, K6 ancestry is subdivided into K6a and K6b, characterizing the CG2 and Syl-E2 populations, respectively. Outgroups and ancient samples are shown in green and black, respectively. In cases where substructure is observed within outgroup populations, species from Asia are depicted in green, while those from the Americas are shown in blue. Cluster labels are indicated at the bottom, inside white

rectangles. A distinct ancestry component appears at $K = 14$, mainly found in ancient samples. b) Cross-validation error plot for the unsupervised ADMIXTURE⁵⁵ analysis (--cv=10), from $K = 2$ to 15 CV errors decrease continuously with increasing K , indicating improving model fit. Model fit improvement is marginal from $K = 14$ to 15.



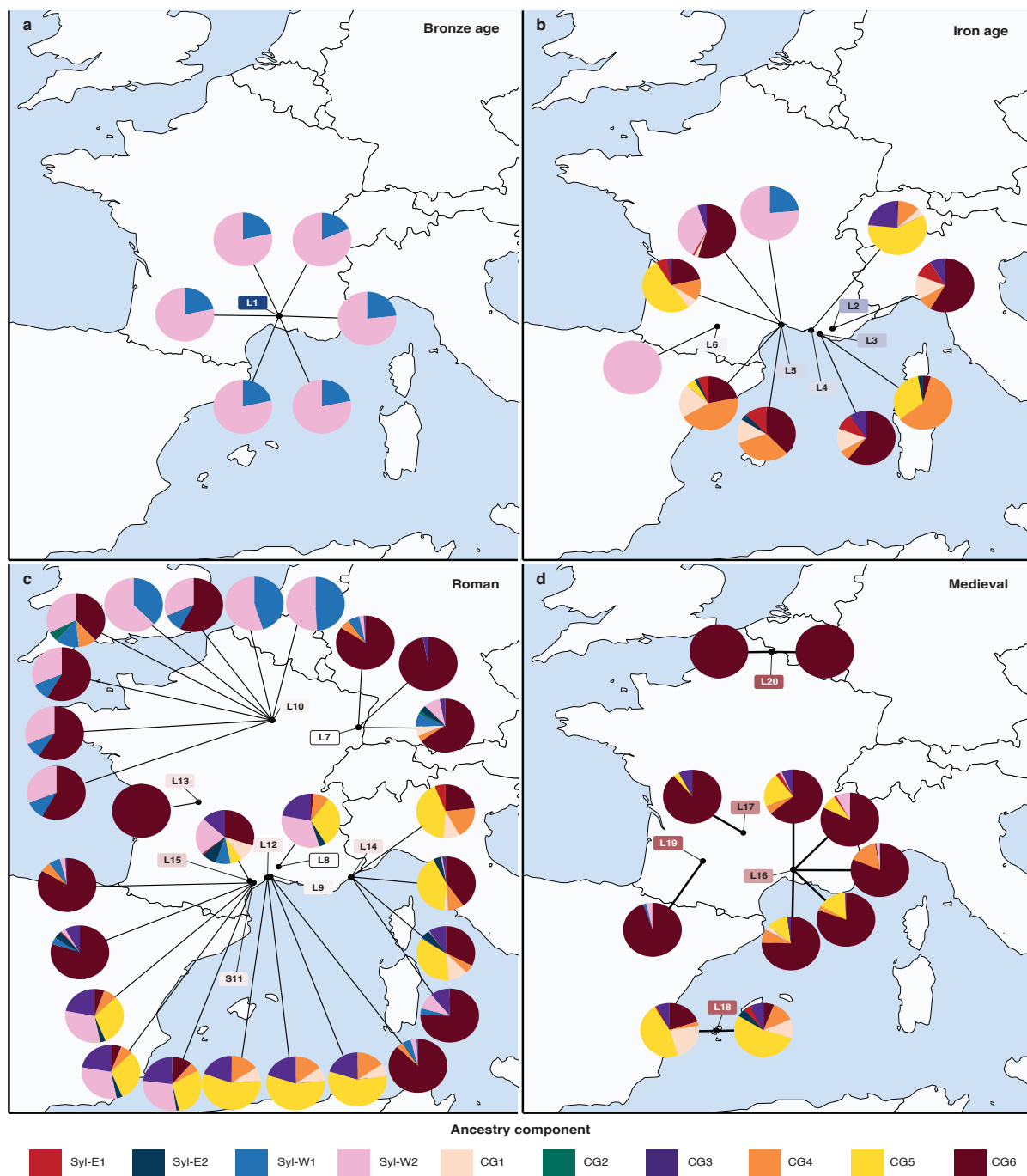
Supplementary Fig. 12. Unsupervised ADMIXTURE⁵⁵ results on the core dataset at the population level ($n = 850$). a) Genetic ancestry proportions from $K = 2$ to 15, with K representing the number of independent genetic ancestries. Accessions are ordered from left to right according to taxonomic status: *Vitis sylvestris*, *V. vinifera*, outgroups, followed by ancient samples. Colors represent the main genetic ancestry components originally defined by Dong et al.⁵⁶, with minor modifications. K1 ancestry is subdivided into K1a, K1b, and K1c, all observed within the Syl-W1 population. Likewise, K2 is divided into K2a and K2b, both associated with the CG1 population, and K2c, found in the Syl-E1 population. K4 ancestry is further divided into K4a and K4b, both present in the CG4 population. Finally, K6 ancestry is subdivided into K6a and K6b, characterising the CG2 and Syl-E2 populations, respectively. Outgroups and ancient samples are shown in green and black, respectively. In cases where substructure is observed within outgroup populations, species from Asia are

depicted in green, while those from the Americas are shown in blue. Cluster labels are indicated at the bottom, inside white rectangles. A distinct ancestry component appears at $K = 14$, mainly found in ancient samples. b) Cross-validation (CV) error plot from unsupervised ADMIXTURE⁵⁵ analysis ($--cv=10$) for $K = 2$ to 15. The CV error decreases steadily, suggesting increasing model fit with increasing K values.

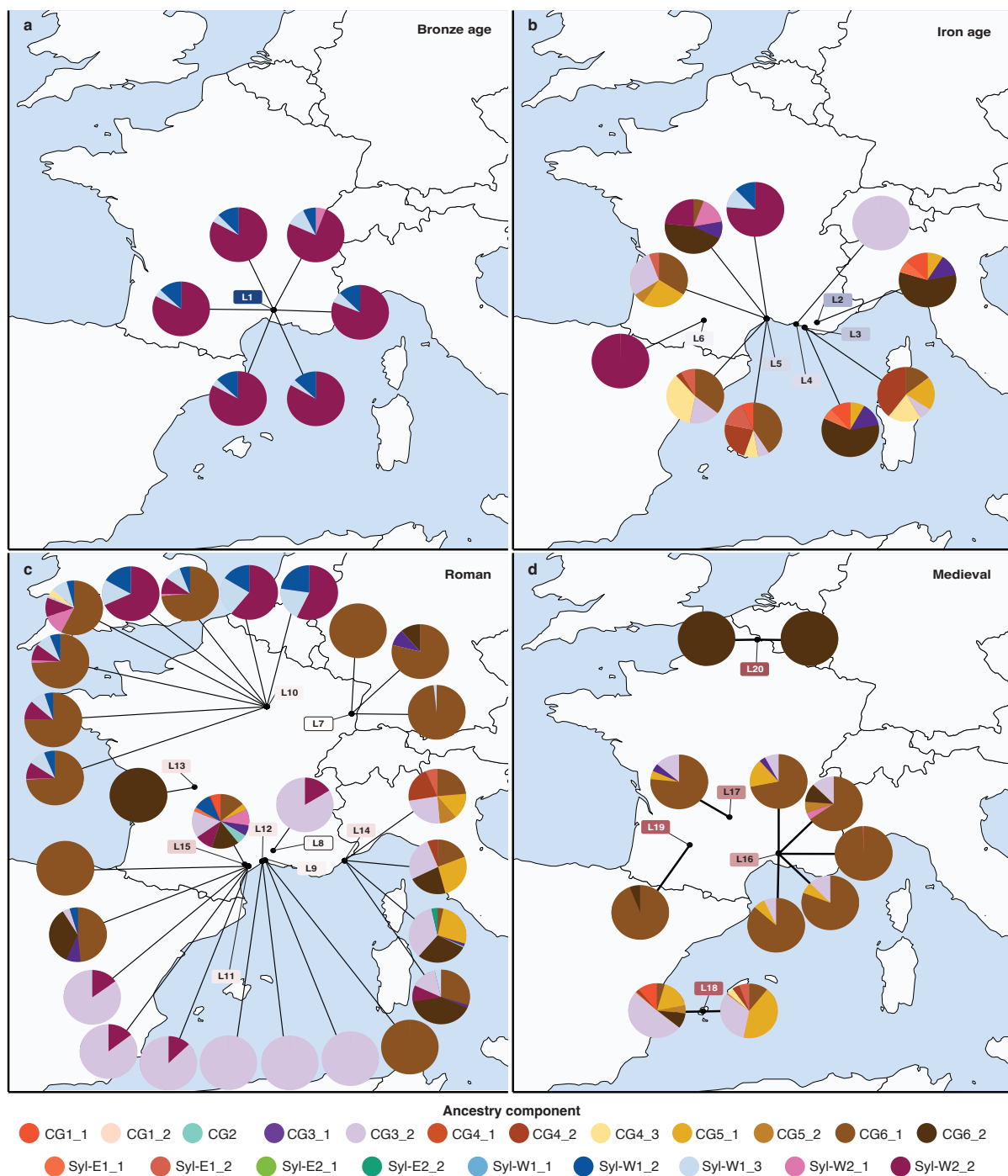


Supplementary Fig. 13. Unsupervised ADMIXTURE⁵⁵ results on the core dataset at the subpopulation level ($n = 613$). a) Genetic ancestry proportions from $K = 2$ to 15, with K representing the number of independent genetic ancestries. Accessions are ordered from left to right according to taxonomic status: *Vitis sylvestris*, *V. vinifera*, outgroups, followed by ancient samples. Colors represent the main genetic ancestry components originally defined by Dong et al.⁵⁶, with minor modifications. K1 ancestry is subdivided into K1a, K1b, and K1c, all observed within the Syl-W1 population. Likewise, K2 is divided into K2a and K2b, both associated with the CG1 population, and K2c, found in the Syl-E1 population. K4 ancestry is further divided into K4a and K4b, both present in the CG4 population. Finally, K6 ancestry is subdivided into K8a and K8b, characterizing the Syl-W2 population. Outgroups and ancient samples are shown in green and black, respectively. In cases where substructure is observed within outgroup populations, species from Asia are depicted in green, while those

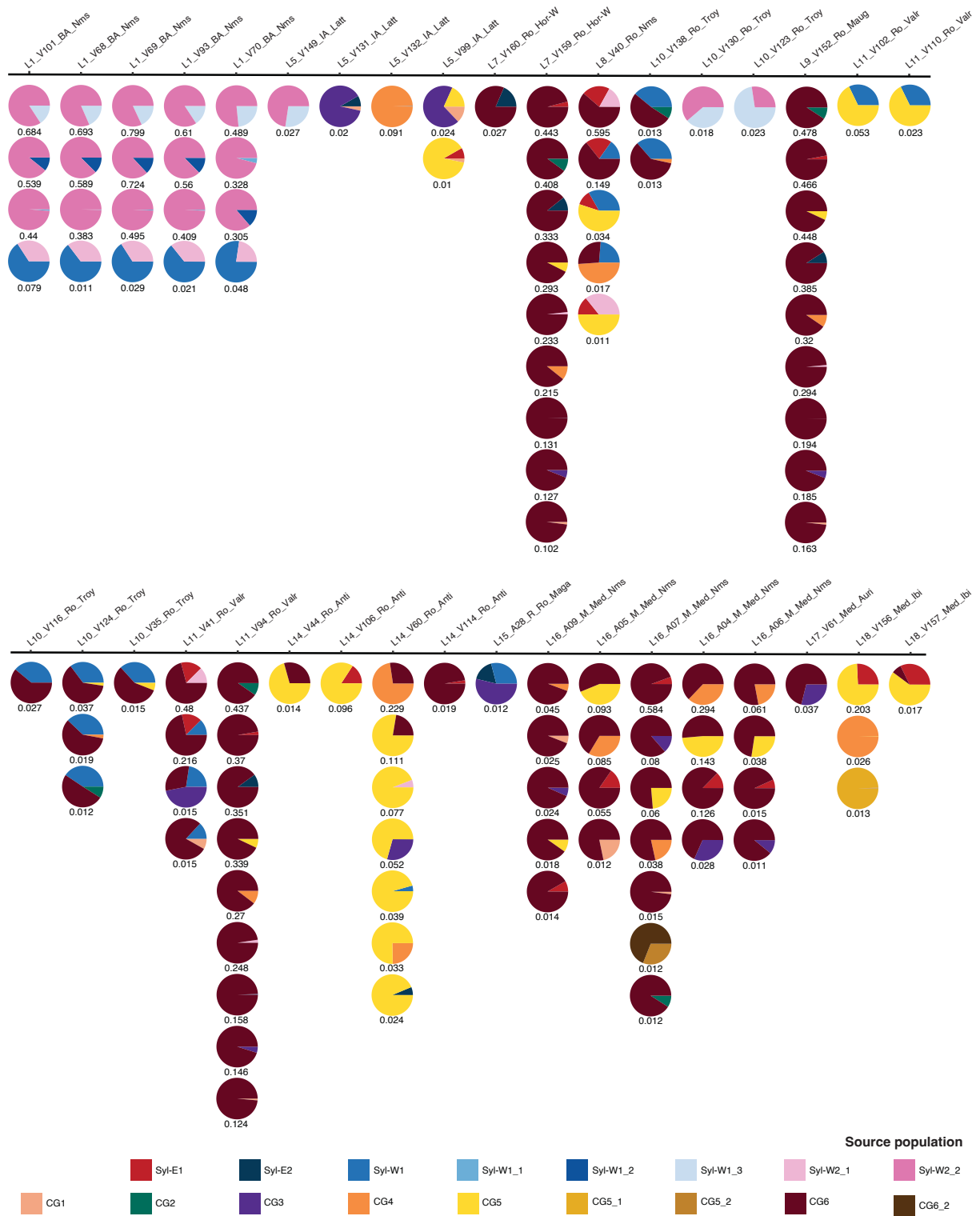
from the Americas are shown in blue. Cluster labels are indicated at the bottom, inside white rectangles. A distinct ancestry component appears at $K = 14$, mainly found in ancient samples. b) Cross-validation (CV) error plot from unsupervised ADMIXTURE⁵⁵ analysis (--cv=10) for $K = 2$ to 15. The CV error decreases steadily until $K = 9$ and reaches its minimum at $K = 14$.



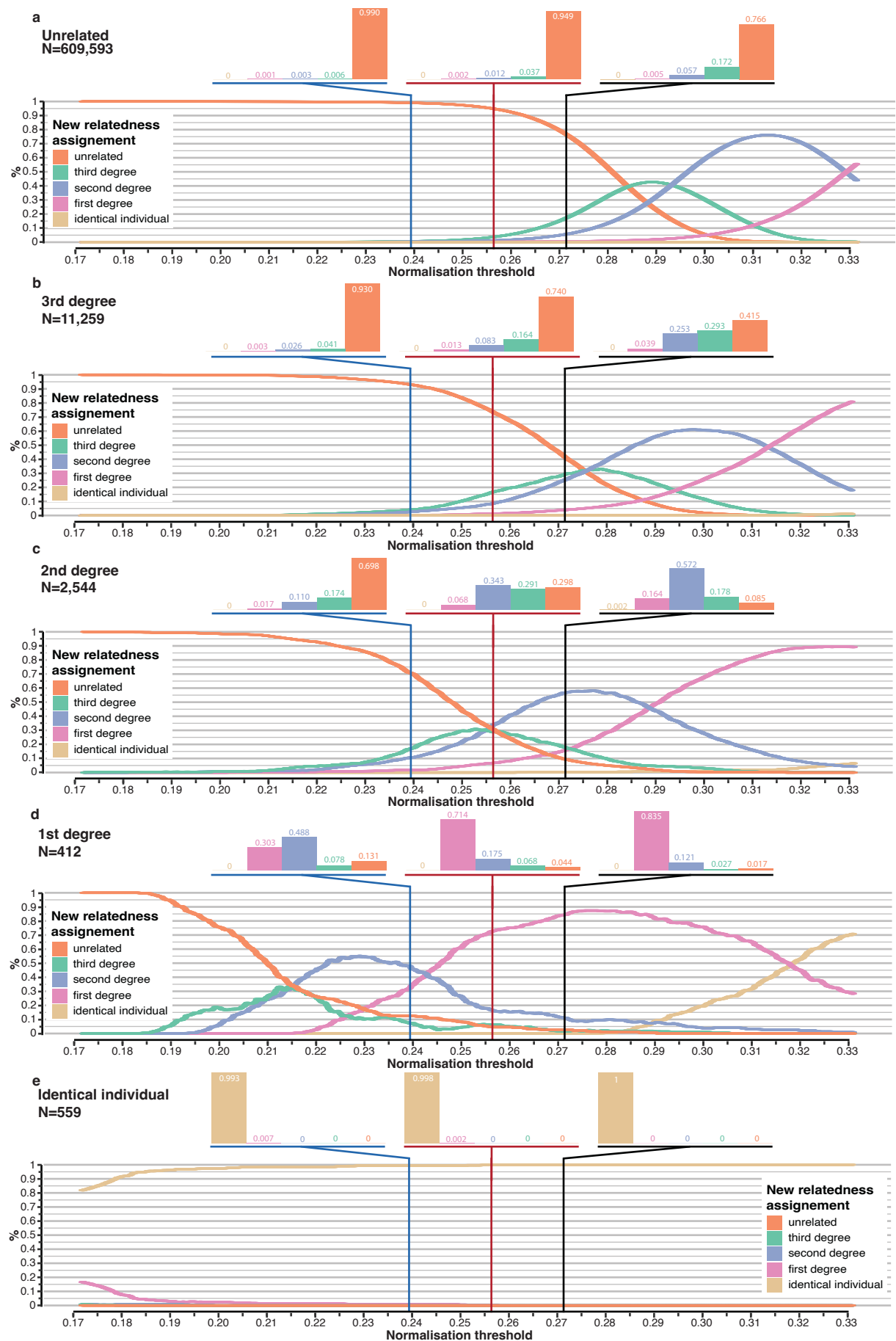
Supplementary Fig. 14. Supervised ADMIXTURE⁵⁵ analysis on the core dataset at the population level by period. a) Genetic ancestry profiles of Bronze Age samples ($n = 6$). b) Same as a), for Iron Age samples ($n = 10$). c) Same as a), for Roman Period samples ($n = 27$). d) Same as a), for Medieval samples ($n = 11$). Colors correspond to major ancestry components. Site numbers correspond to those indicated on Fig. 2d.



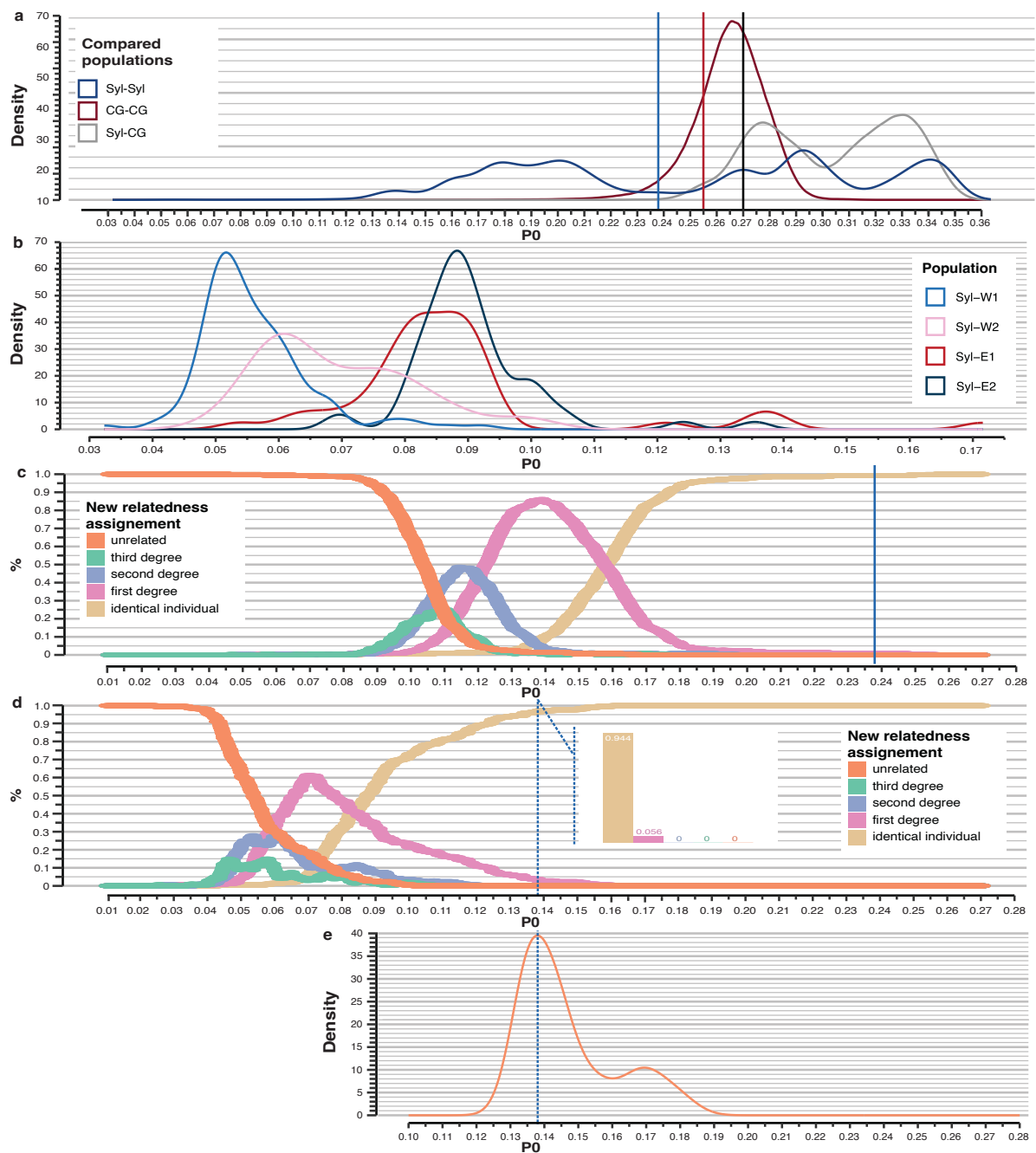
Supplementary Fig. 15. Supervised ADMIXTURE⁵⁵ analysis on the core dataset at the subpopulation level by period. a) Genetic ancestry profiles of Bronze Age samples ($n = 6$). b) Same as a), for Iron Age samples ($n = 10$). c) Same as a), for Roman Period samples ($n = 27$). d) Same as a), for Medieval samples ($n = 11$). Colors correspond to major ancestry components. Site numbers correspond to those indicated on Fig. 2d.



Supplementary Fig. 16. qpADM⁵⁷ modeling results for each ancient sample ($n = 36$) on the extended dataset. Only statistically significant models (i.e., associated with $p \geq 0.01$) models are shown. Only those most parsimonious models (i.e., associated with minimal numbers of independent sources) are considered. Ancient samples are ordered from oldest (top-left) to most recent (bottom-right). P values are displayed below each plot, with models ranked from most significant (left) to least (right). Sample names include their original label followed by age category (BA = Bronze Age, IA = Iron Age, Ro = Roman, Med = Medieval) and archaeological site.

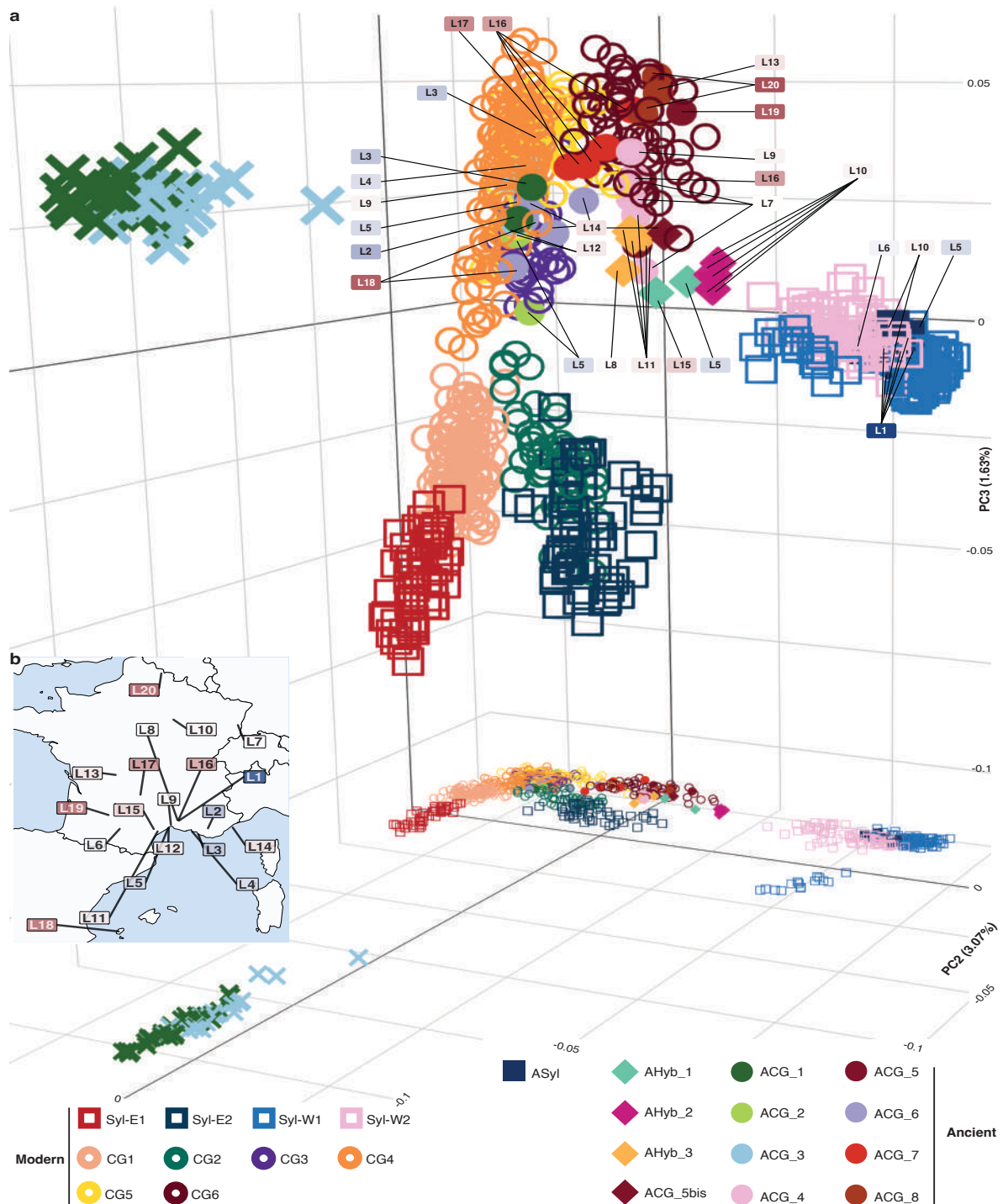


Supplementary Fig. 17. Normalisation threshold selection for relatedness analyses amongst cultivated ancient samples of the extended dataset. a) Simulation of the impact of the normalisation threshold for P0 in READv2⁵⁸ analysis on relatedness assignment among modern cultivars known to be unrelated. The vertical axis provides the fraction of individual pairs assigned to a given relatedness level given the normalisation threshold provided on the horizontal axis, with colors reflecting the relatedness level (orange, unrelated; green, 3rd-degree; blue, 2nd-degree; pink, 1st-degree, and; beige, identical). b) Same as a) for 3rd-degree relatives. c) Same as a) for 2nd-degree relatives. d) Same as a) for 1st-degree relatives. Cultivar relatedness was obtained from the Vitis International Variety Catalogue (VIVC)⁵⁹. e) Same as a) for cultivars known as identical. Identical cultivars were obtained by repeating twice the pseudo-haploidisation procedure. Vertical lines decompose the proportion of pairs assigned to each relatedness category at three specific thresholds, the ones leading to 99% (blue) and to 95% (red) of unrelated pairs correctly identified, versus the by-default one (black) proposed by READv2⁵⁸. The number of relationships considered at each relatedness level is indicated at the top left of each panel.

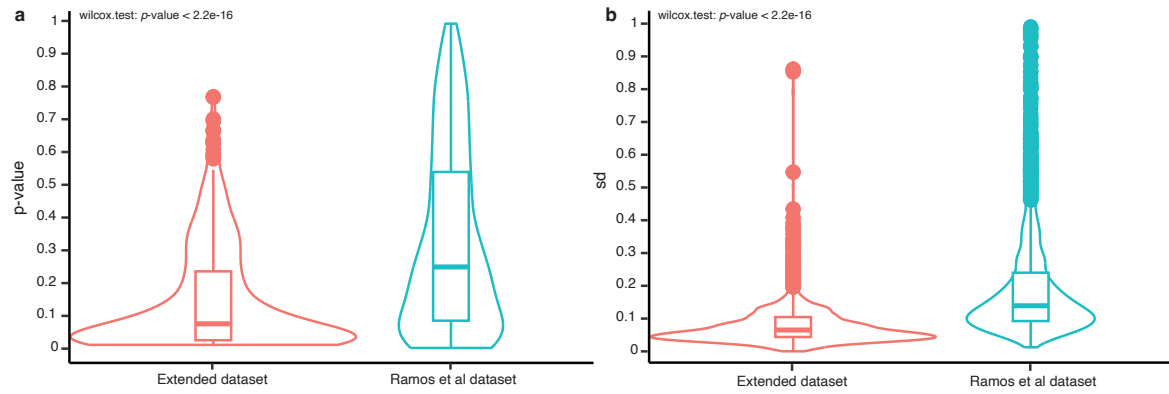


Supplementary Fig. 18. Selection of the normalisation threshold used for relatedness analysis in wild and hybrid ancient grapevine samples of the extended dataset. a) Distribution of non-normalized P values between pairs of *Vitis sylvestris* individuals, colored according to the populations from which each pair originates. The three candidate normalisation thresholds presented in Supplementary Fig. 17 are shown here. B) Distribution of non-normalized P values between pairs of identical *V. sylvestris* individuals, also colored by population of origin. Given that the ancestry of wild and hybrid ancient samples is primarily explained by Syl-W components, only Syl-W populations are considered. c) Simulation of the effect of varying the normalisation threshold (from 0.17 to 0.33) on relatedness assignment in READV2⁵⁸ for *V. vinifera* cultivars known to be genetically identical. The curves show the proportion of pairs assigned to each relatedness category across the threshold gradient: orange (unrelated), green (3rd-degree), blue (2nd-degree), pink (1st-degree) and beige (identical). The vertical blue line indicates the threshold used for

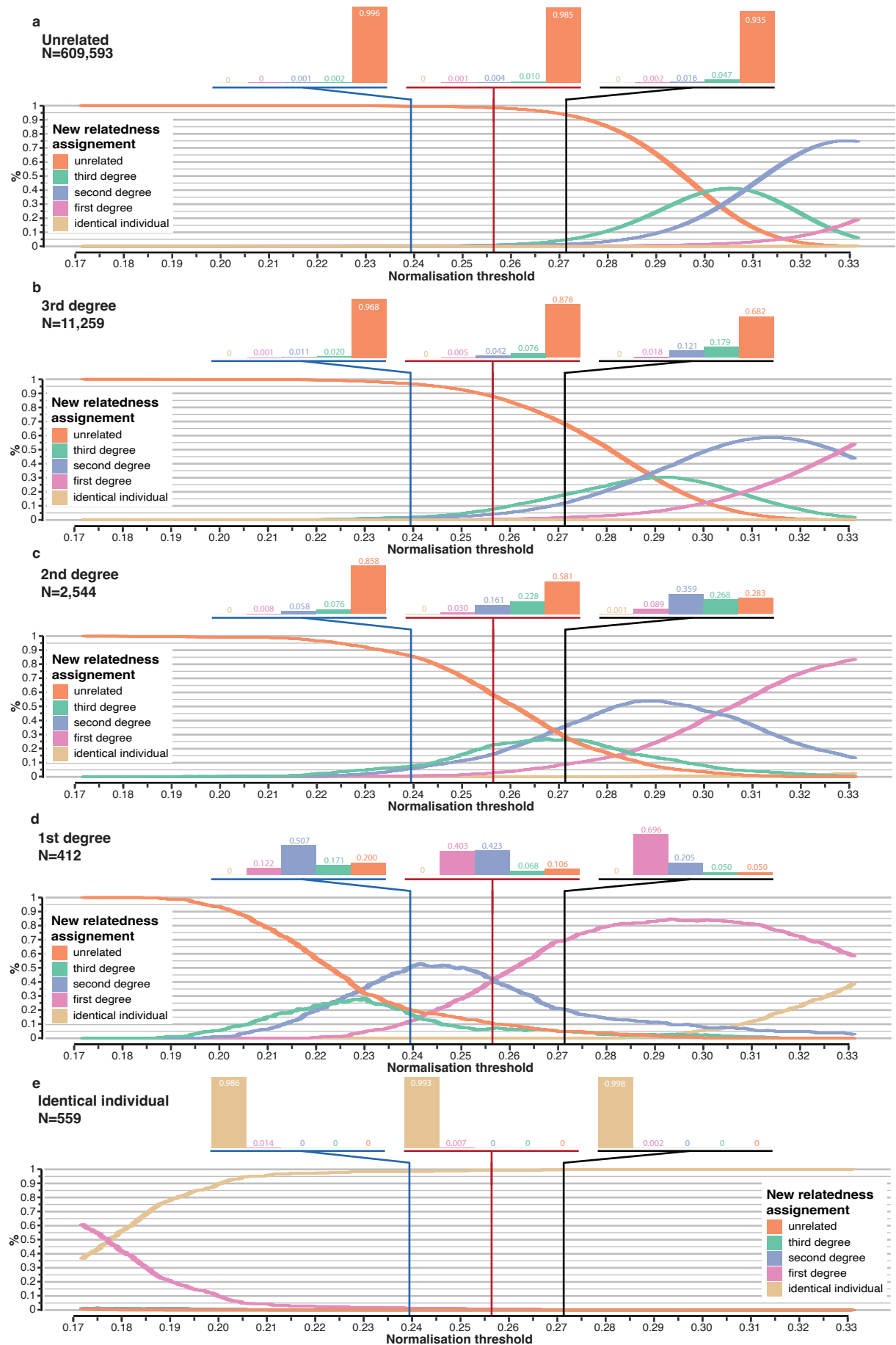
domesticated ancient samples. d) Same analysis as in c), but for identical Syl-W individuals. The dashed blue line represents the normalisation threshold selected for wild and/or hybrid ancient samples. e) Proportions of relatedness categories among identical Syl-W individuals according to READv2⁵⁸ using the newly defined normalisation threshold for wild/hybrid ancient samples. d) Distribution of inferred candidate thresholds for wild/hybrid ancient samples, based on the relative positioning of the threshold used for domesticated samples to the unrelated and identical curves in c), and its transposition onto the unrelated and identical curves in d). The dashed blue line indicates the mode of this distribution, which was chosen as the normalisation threshold for wild and hybrid ancient samples.



Supplementary Fig. 19. 3D-PCA projecting ancient samples onto the genetic space defined by modern *Vitis* accessions at the population level using genomic positions from Ramos-Madrigal et al.⁴⁶ found in the core dataset ($n = 4,363$). a) The three axes represent the first three principal components with the percentage of variance explained indicated in parentheses. A 2D-PCA projection on the PC1-PC2 plane is also shown. Symbols represent taxa: squares (*Vitis sylvestris*), circles (*V. vinifera*), diamonds (hybrids), and crosses (non-*V. vinifera* subsp.). Open symbols correspond to modern accessions, filled symbols to ancient samples. Colors reflect population origin. Simplified labels indicate archaeological sites where samples were excavated; their colors correspond to sample ages using the gradient shown in Figs. 1ef. b) Summary map of archaeological sites with corresponding labels.



Supplementary Fig. 20. Distribution of p -values and standard deviations (sd) for statistically significant models identified with qpADM⁵⁷ using whole-genome data versus sites from Ramos-Madrigual et al.⁴⁶ found in the extended dataset ($n = 4,363$). a) Violin and boxplots of p -values from all significant qpADM models performed with whole-genome data (orange), or restricting the analyses to those genomic positions from Ramos-Madrigual et al.⁴⁶(blue). Wilcoxon test p are shown at the top left, indicating that whole-genome data significantly decrease qpADM p . b) Same as a), but for the standard deviation of the modeled ancestry proportions across all significant qpADM models. Use of whole-genome data significantly reduces the sd of ancestry proportion estimates.



Supplementary Fig. 21. Normalisation threshold selection for relatedness analyses amongst cultivated ancient samples, using sites from Ramos-Madrigal et al.⁴⁶ found in the extended dataset ($n = 4,363$). a) Simulation of the impact of the normalisation threshold for P0 in READv2⁵⁸ analysis restricted to sites from Ramos-Madrigal on relatedness assignment among modern cultivars known to be unrelated. The vertical axis provides the fraction of individual pairs assigned to a given relatedness level given the normalisation threshold provided on the horizontal axis, with colors reflecting the relatedness level (orange, unrelated; green, 3rd-degree; blue, 2nd-degree; pink, 1st-degree, and; beige, identical). b) Same as a) for 3rd-degree relatives. c) Same as a) for 2nd-degree relatives. d) Same as a) for 1st-degree relatives. Cultivar relatedness was obtained from the Vitis International Variety Catalogue (VIVC)⁵⁹. e) Same as a) for cultivars known as identical. Identical cultivars were obtained by repeating twice the pseudo-haploidisation procedure. Vertical lines decompose the proportion of pairs assigned to each relatedness category at the specific thresholds from Supplementary Fig. 17, the ones leading to 99% (blue) and to 95% (red) of unrelated pairs correctly identified, versus the by-default one (black) proposed by READv2⁵⁸. The number of relationships considered at each relatedness level is indicated at the top left of each panel.

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