

Beginning of viniculture in France

Patrick E. McGovern^{a,1}, Benjamin P. Luley^b, Nuria Rovira^c, Armen Mirzoian^d, Michael P. Callahan^e, Karen E. Smith^f, Gretchen R. Hall^a, Theodore Davidson^a, and Joshua M. Henkin^a

^aBiomolecular Archaeology Laboratory, University of Pennsylvania Museum of Archaeology and Anthropology, Philadelphia, PA 19104; ^bDepartment of Anthropology, University of Chicago, Chicago, IL 60637; ^cUniversité Paul Valéry-Montpellier 3, Unité Mixte de Recherche 5140, Lattes 34970, France; ^dScientific Services Division, Alcohol, and Tobacco Tax and Trade Bureau (TTB), US Treasury, Beltsville, MD 20705; ^eSolar System Exploration Division, Goddard Space Flight Center, National Aeronautics and Space Administration, Greenbelt, MD 20771; and ^fDepartment of Geosciences, Pennsylvania State University, University Park, PA 16802

Edited by Dolores R. Piperno, Smithsonian National Museum of Natural History and Smithsonian Tropical Research Institute, Fairfax, Washington, DC, and approved May 1, 2013 (received for review September 21, 2012)

Chemical analyses of ancient organic compounds absorbed into the pottery fabrics of imported Etruscan amphoras (ca. 500–475 B.C.) and into a limestone pressing platform (ca. 425–400 B.C.) at the ancient coastal port site of Lattara in southern France provide the earliest biomolecular archaeological evidence for grape wine and viniculture from this country, which is crucial to the later history of wine in Europe and the rest of the world. The data support the hypothesis that export of wine by ship from Etruria in central Italy to southern Mediterranean France fueled an ever-growing market and interest in wine there, which, in turn, as evidenced by the winepress, led to transplantation of the Eurasian grapevine and the beginning of a Celtic industry in France. Herbal and pine resin additives to the Etruscan wine point to the medicinal role of wine in antiquity, as well as a means of preserving it during marine transport.

ancient medicine | biomolecular archaeology | viticulture | Western Mediterranean

Much is already known about the initial domestication of the Eurasian grapevine (*Vitis vinifera* sp. *vinifera*) and the emergence of a “wine culture” in the mountainous Near East, as early as the Neolithic period (1, 2). Less is known about how viniculture moved from east to west across the Mediterranean Sea, eventually reaching Italy and France. Merchant seafarers, including Canaanites and later Phoenicians and Greeks, were the principal conveyors, who progressively established colonies along the coasts and on one island after another.

By at least 800 B.C., the Etruscans of central Italy along the Tyrrhenian Sea had come in contact with the Phoenicians, as shown by their “Orientalizing” industries of metals, pottery, glass, ivory, and preeminently wine. The Phoenician amphora (Fig. 1A) was the prototype for the Etruscan amphora (Fig. 1B), and, where a similarity of form exists, most likely a similar function was intended: primarily to hold grape wine (3), which was supplied by a nascent local industry.

Such wine amphoras eventually filled the holds of Etruscan ships, some of which sank along the Italian and French coasts on their way to southern Mediterranean France, beginning ca. 625–600 B.C. (4–7). On land, the Celts, the native inhabitants of large parts of Western Europe in the first millennium B.C., were lured into the wine culture and eventually saw the advantages of local production to promote their own trading interests. The Gallic wine culture spread inland after the Roman conquest up the Rhone and Rhine rivers to the rest of Europe where, centuries later, primarily monasteries, such as the Cistercian abbey of Vougeot in Burgundy, refined viniculture to such a degree that it became a model for the rest of the world.

Archaeological Samples Chosen for Analysis

The coastal site of Lattara, near the modern town of Lattes south of Montpellier, is key to understanding the transference of the wine culture to Mediterranean France (8). Merchant quarters for the storage, preparation, and transport of imported and exported

goods were newly constructed inside a walled settlement ca. 525 B.C. (Fig. 2). Multiroom buildings along the southwestern wall gave direct access to a lagoon (now partly silted up) connecting to the sea, where boats could have been moored and protected.

Etruscan amphoras, believed to contain wine on archaeological grounds, had already been arriving along the coast of France since the end of the seventh century B.C. Their importation, however, dramatically decreased at many sites after ca. 525 B.C. when the Greek colony of Massalia, founded in 600 B.C. by Phocaean Greeks coming from western Anatolia, began to produce its own wine amphoras. These people began producing a distinctively shaped Massaliote amphora (Fig. 1C) in the second half of the sixth century B.C., thought to have been used to export locally produced wine so as to compete with the Etruscan market. Lattara was the exception to the rule; Etruscan amphoras and other artifacts from Italy, attesting to close commercial contacts, continued to be imported during the heyday of activity in the merchant quarters from about 525–475 B.C.

The critical issue addressed by this study is whether these Etruscan and Massaliote amphoras did indeed contain wine. A biomolecular archaeological argument, as the phrase implies, entails a rigorous assessment of the chemical, archaeological, and, in this instance, archaeobotanical evidence separately and in combination. Absolute certainty is unattainable in a biomolecular archaeological investigation because it is an inherently probabilistic historical field of inquiry. The probability of a solution to an archaeologically relevant problem increases with ever-accumulating data, with the refinement of chemical, archaeological, and archaeobotanical methods, and as more natural products are analyzed and become available for bioinformatics searches.

On this basis, amphora samples were selected for chemical analysis based on whether it (a) was an Etruscan or Massaliote type; (b) was excavated from an undisturbed, sealed context; (c) was part of a whole vessel, with base sherds available for analysis; (d) had remnants of a possible residue on its interior; and (e) was unwashed. Only 13 Etruscan amphoras, lined up in two rows in the southeastern part of the storeroom of a merchants' building in zone 27 (Figs. S1 and S2), met all these criteria. They were clearly in situ and sealed off from later intrusions by a ca. 475 B.C. destruction layer. Another 22 amphoras in this room were more haphazardly arranged and might have been secondarily disturbed.

The 13 Etruscan amphoras belonged to a very specific pottery type (9), amphore étrusque 4 (A-ETR 4), which was likely

Author contributions: P.E.M., B.P.L., A.M., M.P.C., K.E.S., and G.R.H. designed research; N.R., A.M., M.P.C., K.E.S., G.R.H., T.D., and J.M.H. performed research; P.E.M., B.P.L., N.R., A.M., M.P.C., K.E.S., G.R.H., and T.D. analyzed data; and P.E.M., B.P.L., A.M., M.P.C., K.E.S., and G.R.H. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: mcgovern@sas.upenn.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1216126110/-DCSupplemental.

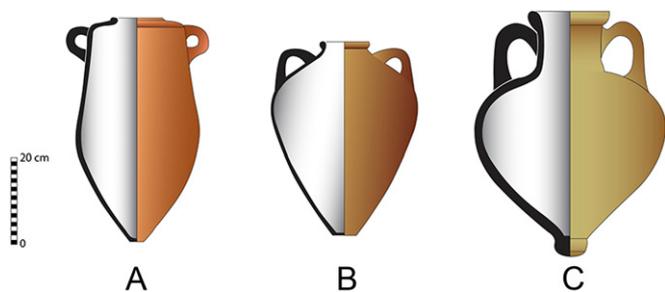


Fig. 1. Representative amphora types from the western Mediterranean: (A) Phoenician amphora (type A-PUN Ch8), ca. 700–600 B.C.; (B) Etruscan amphora (type A-ETR 4), ca. 626–575 B.C.; (C) Massaliote amphora (type A-MA5 1), ca. 550–475 B.C. Drawings by B.P.L., after ref. 10.

manufactured at the Etruscan city of Cisca (modern Cerveteri) ca. 525–475 B.C. (10). The archaeological consensus is that this type was primarily used to transport wine from Etruria to southern France and elsewhere. Three of the 13 amphoras (Dataset S1; nos. 4, 5, and 7) were chosen as representative samples for analysis. These were base sherds because precipitates of liquids settle out and, upon evaporation, concentrate organic compounds there. Two of the sherds (nos. 4 and 5) had small, darkened areas on their interiors, possibly residues of the original contents. Another amphora base (no. 10) of the same Etruscan type from a secure context—the construction level of the building—completed our Etruscan analytical corpus.

To gain a fuller perspective on the possible importation and production of wine at Lattara, two base sherds (nos. 8 and 9) from complete Massaliote amphoras from later (ca. 475–450 B.C.), nearby contexts were also analyzed. No. 9 had a resin-like deposit covering its interior. Archaeologists are in agreement that Massaliote amphoras were almost certainly used for wine.

Additionally, a limestone installation (11) (Fig. 3), dated to ca. 425–400 B.C. and found in situ in a courtyard built over the destroyed merchants' quarters, was analyzed. It has been interpreted as a pressing platform for processing olives or grapes (5–7). Contemporaneous Greek vase paintings (e.g., see Fig. S6) show how such platforms supported baskets of grapes for stomping and collecting the juice. Excavated examples are common throughout the ancient Mediterranean world (1, 7) up until today. Our goal was to determine whether the platform had been used in local production of wine or olive oil.

Archaeobotanical Evidence

The overwhelming predominance of domesticated grape (*V. vinifera* sp. *vinifera*) remains at Lattara, beginning as early as ca. 500 B.C., lends further support to the archaeological interpretation that specific imported amphora types contained wine and that the domesticated grapevine was eventually transplanted to southern France and its grapes pressed to make local wine at the site.

The same merchants' room with the Etruscan amphoras, as well as nearby buildings of the same period, yielded numerous grape seeds, pedicels, and even fruit (skin). For the site as a whole, 15–25% of the cultivated plants and 80% of the fruits were of grape. To date, the only attested fruits other than grape are fig (*Ficus carica*), blackthorn or sloe (*Prunus spinosa*), blackberry (*Rubus fruticosus*), and olive (*Olea europaea* var. *europaea*). The latter occur in very small amounts and with rare exceptions are post-fifth century B.C. Other plants that contain tartaric acid (a principal biomarker for grape—see below and SI Text), such as pomegranate or exotic fruits from distant countries, are totally absent from the site.

A cluster of several thousand carbonized grape seeds, which were found inside a clay container in an earlier phase (ca. 435 B.C.) of the same area in which the pressing platform was

excavated, provides compelling evidence that the latter was used for grapes (12). Masses of grape remains often point to grape pressing and stomping for winemaking (13). By contrast, no olive pits were found near the platform. In general, they are extremely uncommon until Roman times and nearly always occur whole; i.e., they had not been pressed.

Chemical Results

After sample extraction, ancient organic compounds were identified by a combination of chemical techniques: Fourier-transform infrared spectrometry (FT-IR), gas chromatography-mass spectrometry (GC-MS), ultraHPLC tandem mass spectrometry (LC/MS/MS), HPLC with a linear ion trap-Orbitrap mass spectrometry (Orbitrap LC/MS), and headspace solid phase micro-extraction (SPME) coupled to GC-MS (SI Text).

FT-IR showed that nos. 4, 5, 7, 9, and 10 had the characteristic absorptions for a tree resin, according to the results of previous studies (14, 15). Only the spectra for no. 8 and the platform sample were ill-defined. Samples comprised of complex mixtures can be equivocal for FT-IR, and the spectra must be deconvoluted and examined closely for the presence/absence of key absorptions; if a known absorption for a compound is not observed, then that compound is likely not present.

GC-MS (Datasets S1 and S2; Fig. S3) revealed that a tree resin was attested for all of the amphoras, irrespective of whether a possible resin-like residue or resin-like soil inclusions on their interiors were observed. Only the platform lacked resin compounds. The detected compounds, which belong to the abietic acid family (namely, abietic acid and its oxidation products when aged or heat-treated) and the pimaric/sandaracopimaric acid family (pimaric acid, isopimaric acid, and sandaracopimaric acid), are best explained as originating from pine (Pinaceae) resin. The pimaric acid family is lacking for nos. 7, 8, and 10, which might be interpreted as true absence, very low concentration, or differential preservation. Tartaric acid, a principal biomarker for grape wine (see below and SI Text), was weakly detected by this method only in no. 8.

LC/MS/MS demonstrated that tartaric acid/tartrate was unquestionably present in nos. 4 and 8 (Fig. S4), likely present in

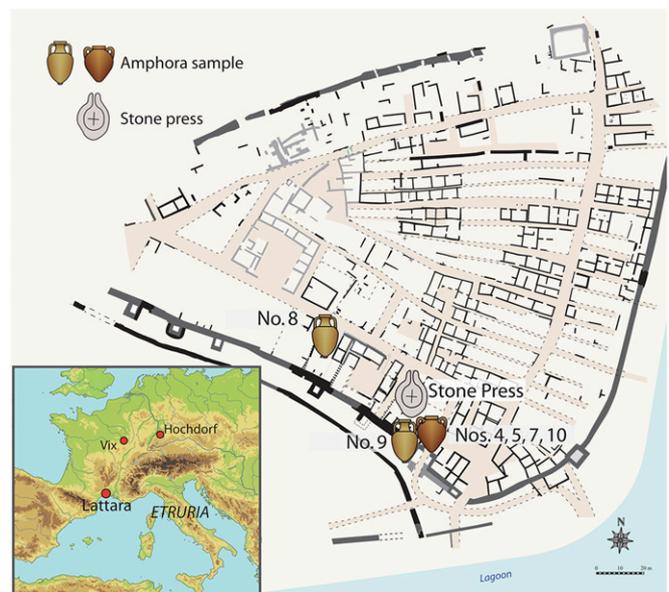


Fig. 2. Map of the ancient settlement of Lattara (modern Lattes), showing the locations of the analyzed samples. Map courtesy of Lattes excavations (redrawn by B.P.L.).



Fig. 3. Ancient pressing platform from Lattara, seen from above. Note the spout for drawing off a liquid. It was raised off the courtyard floor by four stones. Masses of grape remains were found nearby. Photograph courtesy of Michel Py, copyright l'Unité de Fouilles et de Recherches Archéologiques de Lattes.

no. 7, and uncertain for nos. 9 and 10 and the platform sample, based on chromatographic retention time and multiple reaction monitoring (MRM). Our experimental protocol (14) was expanded to include two transitions (149→87 and 149→73) of deprotonated tartaric acid (molecular mass 150.1) instead of only one, providing stronger evidence for the $[M-H]^-$ molecular ion. Tartaric acid was detected at 35 ppb limit, as estimated from the signal-to-noise ratio of the MRM chromatogram of the tartaric acid standard. It was calculated from the tartaric acid peak areas of the standard and archaeological samples that the acid was present at less than 0.5 ppm for all of the positive samples.

Because of uncertainty about the presence/absence of tartaric acid/tartrate in some of the amphoras and especially for the platform, the same prepared extracts for the LC/MS/MS analyses of nos. 4 and 7 were reanalyzed by Orbitrap LC/MS. The advantage of this method is high mass resolution (>27,000 at the tartaric acid mass) and high mass accuracy (<1 ppm error) (16). The platform sample was separately extracted and then purified by solid phase extraction to reduce chromatographic interferences and ion suppression. All these samples were unequivocally positive for tartaric acid/tartrate by Orbitrap LC/MS at the part per billion level (Fig. 4). Other important acids in grape, including succinic, malic, and citric, were also unambiguously identified by chromatographic retention time and accurate mass measurements.

Volatile compounds, which were identified by SPME in what were likely the best-preserved samples (nos. 4 and 5; Fig. S5), shed additional light on the contents of these amphoras (Dataset S3). Pine resin, herbal, and probable grape-derived compounds were the predominant constituents. Detailed information on the extraction methods for the Orbitrap LC/MS and LC/MS/MS analyses and on the experimental conditions for the SPME and liquid-injection GC/MS analyses are provided in *SI Text*.

Discussion and Conclusions

Fermented beverages, especially wine, have long played a crucial role in the transfer of culture from one people to another around

the world (2, 4, 6). The wine trade was one of the principal incentives for the Canaanites and Phoenicians, followed by the Greeks, Etruscans, and Romans, to expand their influence in the Mediterranean Sea. Where wine went, so other cultural elements eventually followed. Technologies of all kinds and new social and religious customs took hold in regions where another fermented beverage made from different natural products had long held sway.

It is not surprising then that the Celts or Gauls along the shore of Mediterranean France between *ca.* 625 and 400 B.C. should have become equally entranced by the cultural and economic possibilities for wine and begun to substitute it for their native beverages, which were likely beers, meads, and mixed fermented beverages (2). This hypothesis, however, has never been tested by biomolecular archaeological methods. Based on our findings, it is now highly probable that (a) the Etruscan amphoras arriving in ports of Mediterranean France, specifically Lattara, contained wine; (b) this wine was pine-resinated; (c) additional botanicals, probably including rosemary, basil and/or thyme, had been added to the wine; and (d) the importation of the Etruscan wine eventually led in a relatively short period to the transplantation of the domesticated Eurasian grapevine and to local wine production in southern France, probably in its initial stages under Etruscan tutelage. These findings bear importantly on the subsequent course of the wine culture in Europe and ultimately the New World.

Our biomolecular archaeological methodology for arriving at these conclusions is very straight-forward: (a) carefully articulate the archaeological problem to be solved; (b) select the best-provenienced, best-dated, and best-preserved archaeological samples for chemical analysis; (c) propose a hypothesis that best explains the interrelated archaeological, archaeobotanical, and chemical data; and (d) subject this hypothesis to ever-more-exacting testing by the same disciplines.

The presence/absence of tartaric acid/tartrate, as a key biomarker of the Eurasian grape, is obviously important to the hypothesis we propose. Based on a thorough bioinformatics search, other compounds, such as malvidin, are less definitive for grape (*SI Text*). One can also legitimately ask whether our detection of this compound necessarily derives from the Eurasian grape and, if it does, whether it is present as grape juice, syrup, or vinegar rather than wine. Archaeological and enological considerations come into play in answering these questions, not just chemical analysis (also see *SI Text*).

A crucial archaeological fact is that the narrow-mouthed, complete amphoras of this study are ideal for preserving tartaric acid/tartrate. Tartaric acid will be absorbed into the pottery, depending on its porosity, and form ionic bonds with the clay, thus helping to preserve the compound. Tartaric acid also readily precipitates out of wine as potassium bitartrate as part of the wine lees. These precipitates collect either as a residue on the bases of the amphoras, which were targeted, or are absorbed into the pottery fabric. In the calcareous geological environment of southern coastal France, tartaric acid also would have been readily converted to insoluble calcium tartrate, further assuring a residue accumulation and/or absorption into the pottery.

Moreover, because the amphoras were likely stoppered (below), any cross-contamination between amphoras would also have been minimized. If tartaric acid escaped from the amphoras into the groundwater, it would have been quickly bound up with calcium and other metallic ions in the calcareous soil, precipitate out, and not have been transported far. It would have been consumed by microorganisms in the soil, especially in relatively anaerobic conditions underground, at a more rapid rate than it was produced by microbes (17). This conclusion was borne out by Orbitrap LC/MS analyses of soil and limestone control samples from the same area and approximate time period as the amphora and pressing platform samples (Dataset S4). The latter had tartaric acid levels that significantly exceeded those of the control samples (*SI Text*).

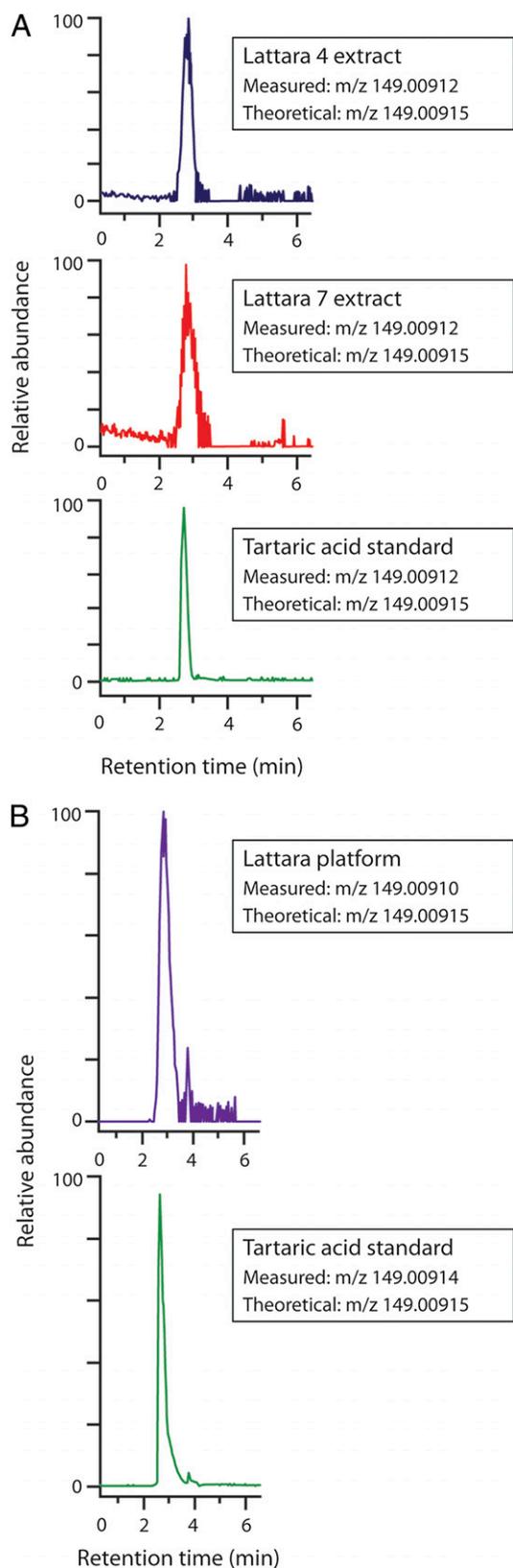


Fig. 4. Extracted ion chromatograms acquired using full-scan Orbitrap LC/MS analysis and a 5-ppm window (at the theoretical mass of deprotonated tartaric acid). (A) Lattara no. 4 extract (Top), no. 7 extract (Middle), and tartaric acid standard (Bottom). (B) Lattara pressing platform (Upper) and tartaric acid standard (Lower). The measured accurate masses, indicated in the boxes, are averages taken across the peaks.

The SPME results for nos. 4 and 5 (Datasets S1 and S3) are also consistent with grape being the source of the tartaric acid. Using standard bioinformatics tools to search the chemical literature (14), constituents of modern grape wine (18) were identified in one or both of the ancient samples tested, including alcohols, esters, aldehydes, and terpenoids. Any ancient ethanol would have been metabolized by microorganisms.

Although benzaldehyde, 2-ethyl-1-hexanol, and nonanal might derive from wine, they could also be contaminants. Other compounds might derive either from ancient and/or modern “background contaminants” due to groundwater percolation or sample handling (e.g., plasticizers and antioxidants from plastic, including compounds in the phthalate family). Possibly, some of the low-boiling compounds up to hexenal were also contaminants, but, more likely, they were preserved within the ionic clay structure.

Botanical additives to the wine in nos. 4 and 5 were also identified. Three natural products account for the greatest number of compounds that are not naturally ubiquitous and are therefore most likely: rosemary, basil, and thyme. These herbs are native to central Italy where the wine was likely made. Rosemary (*Rosmarinus officinalis*) (labeled 3 in Dataset S3), which is widespread throughout the Mediterranean region, accounts for the most number of volatile compounds in Dataset S3, namely, the monoterpenes D-limonene, fenchol (only in no. 4), camphor, borneol and menthol (only in no. 5), the sesquiterpene copaene (only in no. 4), and cuminaldehyde, a benzaldehyde derivative. A previous study of Egyptian wine (14) showed chemically that rosemary had been added to the wine in a Byzantine amphora from Egypt. Basil (*Ocimum basilicum*), a native western Mediterranean plant, can account for the same compounds except copaene and cuminaldehyde; additionally, it contains the sesquiterpene calamanene (only in no. 4) in the naphthalene family, which is rare in the plant world. Although estragole makes up more than half the content of fresh basil, its allylic and benzylic structure makes it highly unstable to bio- and photodegradation, and it would not be expected to survive for thousands of years. Thyme (*Thymus vulgaris*), which grows widely around the Mediterranean, is another possibility, but it lacks calamanene and copaene.

All of the Lattara amphoras contained compounds (labeled 4) from pine resin. Natural untreated pine resin also contains the monoterpenes fenchol, camphor, and borneol (19). This resin is still used today to make Greek *retsina*, the only modern carry-over of ancient tradition.

Resinated wines with many of the same compounds as those attested for the Lattara amphoras are reported for a bronze cauldron (*situla*), part of the drinking equipment in a wealthy Etruscan tomb, dated to ca. 450–400 B.C., at the Adriatic Sea port of Spina at the mouth of the Po River in Italy (20). DNA analyses (20) of amphoras, which were recovered from shipwrecks found in the Aegean Sea and off the coasts of western Anatolia and Corfu (fifth–third centuries B.C.), further substantiate the presence of similar botanicals to those in the Lattara amphoras—namely, rosemary, thyme, and pine resin. An SPME study of a Greco-Roman amphora from Campania in Italy, dated ca. 200 B.C.–A.D. 200, from a shipwreck in the Adriatic Sea off the coast of Croatia, yielded a suite of compounds (21) that is consistent with a pine-resinated herbal wine like those in the Lattara Etruscan amphoras. The compounds include alcohols, esters, ketones, and aldehydes characteristic of wine, the monoterpenes fenchone, camphor, and borneol, the sesquiterpene calamanene, and members of the abietic and pimaric/isopimaric acid families, together with possible naphthalene and phenanthrene-related derivatives originating from heat processing and/or oxidative aging of pine resin.

The relative prominence of retene in the Lattara amphoras might imply that a heated tree tar or pitch was applied to their interiors or to a now-disintegrated stopper (22). Only one amphora body sherd (no. 9), however, appeared to have a tar or

resin lining on its entire interior surface. An accumulation of resin at the bottom of the base with none continuing up the side wall (no. 7), isolated small darkened areas (nos. 5 and 9), and resin-like particles dispersed in soil on the inside of nos. 4 and 8 are better interpreted as resulting from the precipitation of a resin or tar added as a preservative or flavorant to the wine, with subsequent degradation to the oxidized diterpene acid forms. Wine transported by ship also kept better when it was resinated.

Perhaps the most important finding of this study, with obvious implications for the beginning of winemaking in France and Europe as a whole, is that the pressing platform at Lattara was already being used to stomp grapes and to produce local wine *ca.* 425–400 B.C. To date, nothing comparable has been reported from the region, especially at Massalia, which is believed to have begun exporting native wine in its distinctive amphoras as much as a half century earlier. The pressing platform is remarkably like the grape-stomping platform that is shown on a black-figured vase (Fig. S6) by the Amasis Painter of sixth century B.C. Athens, recovered from the Etruscan site of Vulci. This ceramic masterpiece is the earliest depiction in the Greek world that shows a sequence of vinicultural activities (picking, treading, and fermentation) and uniquely illustrates the intimate association of wine with the arts.

The question remains whether similar archaeological, chemical, and botanical evidence for local wine production as that from Lattara will be forthcoming from Massalia or another site in the region. It is reported that large quantities of presumably domesticated grape seeds have been recovered from sixth century B.C. levels at Massalia, and by the end of the century, the production of Massaliote amphoras, probably for transporting local wine, had sky-rocketed (9, 23). Could it be that the Phocaeans brought a tradition of winemaking with them from Anatolia when they founded Massalia or adopted it early on from the Etruscans? Large numbers of grape remains, including seeds, pedicels, and grape skins, are also reported from fifth century B.C. Coudounèu (24), a site within the economic sphere of Massalia, 75 km to the northwest. At the same time at Roquepertuse (25), even closer to Massalia, pips of the domesticated Eurasian grape have been reported.

The real issue, however, is not whether Lattara, Massalia, or another French site proves to have the earliest evidence for local wine production. According to the Lattara evidence presented here, we can now state that local winemaking was in place in Mediterranean France by at least the fifth century B.C., and that the groundwork for this crucial development was preceded by a trade in wine amphoras coming from Etruria where local winemaking was already well-established.

Similarly to the transfer of winemaking by the Canaanites to the Egyptian Nile Delta millennia earlier (1, 2), the native Celts at Lattara would have needed the expertise and knowledge of the Etruscans to plant their own vineyards and begin making wine. They might have had general knowledge of the Eurasian grape, which grew wild along the northern Mediterranean shore and which they might have used to make a native fermented beverage. However, such exploitation and the morphological transition between wild and domestic grapes is not attested until at least the third century B.C., particularly at Port Ariane, about a half

kilometer distant from Lattara (26). Moreover, much more horticultural knowledge and technological proficiency would have been needed to transplant the domesticated grapevine, successfully tend it, vinify the grapes into wine using specialized equipment, and preserve the wine in sealed vessels with tree resins.

Plantings of the domesticated Eurasian grapevine in Mediterranean France were probably transported on Etruscan ships. A fourth century B.C. Punic shipwreck off the coast of Mallorca at El Sec (27, 28) illustrates how it might have been accomplished: grapevines on this ship were embedded in soil in the cool hull of the ship, which would have enabled them to travel well and be replanted. This ship also carried numerous amphoras from throughout the Mediterranean and Black Sea, specialized drinking vessels, and cauldrons and buckets of types well-documented elsewhere in Europe for making and serving a mixed fermented beverage.

The Etruscan shipwreck of Grand Ribaud F (27, 29), found off the coast of the Hyères Islands, east of Marseilles, and dated to *ca.* 515–475 B.C., is especially pertinent to the transfer of winemaking to Mediterranean France. Its hold was filled with grapevines, which the excavator argues were for cushioning the shipment (dunnage) of some 700–800 amphoras rather than for transplantation. Significantly, all of the Etruscan amphoras on board this ship, which had been carefully stoppered with cork (among the earliest evidence for this technology, which is also attested by two examples from Lattara, dated *ca.* 475 B.C.) and stacked at least five layers deep in the hull, are of the same pottery type (A-ETR 4) and contemporaneous with the Etruscan amphoras analyzed and reported on here. The ship's final destination was quite possibly Lattara.

Finally, it should be stressed that ancient wine, such as that imported into Lattara and later made there, served as more than a social lubricant or aromatic beverage, as is customary today. In addition to its eventual role as a powerful religious symbol, grape wine and other alcoholic beverages were the medicines of antiquity, as evidenced by the pharmacopeias of Egypt, China, Greece, and Rome (30) (*SI Text*). Alcoholic beverages were an excellent means to dissolve and administer botanical concoctions externally and internally.

Much more remains to be discovered about the progress of viticulture, winemaking, and the cultural impact of grape wine in France and Europe beginning with the Celts of Mediterranean France. Future biomolecular archaeologists will increasingly be called upon not only to identify biomarker compounds by ever more sensitive techniques, but also to correlate and assess their findings in light of ever more precise archaeological and archaeobotanical data.

ACKNOWLEDGMENTS. Thierry Janin, Michel Py, and Denis Lebeaupin kindly provided the samples for analysis. Jean-Pierre Brun, Denis Lebeaupin, Anne-Marie Curé, William Meyer, and Jean MacIntosh Turfa consulted on archaeological interpretation; Ariane Vacheret and Gaël Piquès assisted in sampling; and W. Christian Petersen (Winterthur Museum) and Edith C. Stout advised on chemical matters. Abdul Mabud and Jeffrey Ammann (Scientific Services Division of the Alcohol and Tobacco Tax and Trade Bureau) have provided significant and ongoing support of the study. Additional support was provided by the Centre National de la Recherche Scientifique, the Université Paul Valéry-Montpellier 3, the University of Chicago, and the regional government of Languedoc-Roussillon. This research was initiated under and funded by National Science Foundation Grant BCS-0935847.

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