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Vincent Bonhomme, Clémence Pagnoux, Laurent Bouby, Sarah Ivorra, Susan E. Allen, Maria Valamoti Sultana

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COOKING WITH PLANTS IN ANCIENT EUROPE AND BEYOND

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COOKING WITH PLANTS IN ANCIENT EUROPE AND BEYOND

*Interdisciplinary approaches to
the archaeology of plant foods*

SOULTANA MARIA VALAMOTI, ANASTASIA DIMOULA AND MARIA NTINOU (EDS)

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Early viticulture in Neolithic and Bronze Age Greece: looking for the best traditional morphometric method to distinguish wild and domestic grape pips

Vincent Bonhomme, Clémence Pagnoux,
Laurent Bouby, Sarah Ivorra, Susan E. Allen,
Soultana Maria Valamoti

Abstract

The origins of viticulture and the domestication of the grapevine have been the subject of much research, notably on the basis of archaeobotanical remains. To identify wild and domesticated grapevines, analyses using various measurements have been performed on archaeological grape pips: length and breadth of the pip, length of the stalk, placement of the chalaza and indices/formulas involving these measurements. The performances of traditional methods based on size parameters, however, can vary largely when applied to modern cultivars. In addition, recent studies on the morphological distinction between wild and domesticated grape pips using size measurements and ratios highlighted the importance of the comparisons between the archaeological material to a representative reference collection of grape pips from diverse modern cultivars and wild populations. Given that different methods have been proposed for the distinction between wild and domesticated grape pips, some based on measurements and others on shape, this paper tests for the first time these methods based on a standardized collection of modern pips and compares their performances. The analyses were performed on a set of modern grape pips consisting of 34 cultivated varieties and 13 wild genotypes. Then, archaeological grape pips were compared to this set of modern reference material using the same measurements and methods. We used grape pips from three sites located in Northern and Southern Greece and dated from the Late Neolithic (ca 4500-4000 BC) to the Late Middle Bronze Age (ca 1700-1620 BC): Dikili Tash, Tzoungiza and P.O.T.A. Romanou. Our results show that shape analysis is the most efficient method to identify wild and domesticated grapevines, while traditional measurements are also highly reliable. The Stummer index represents the least accurate method. According to our analyses the archaeological pips from Late Neolithic and Early Bronze Age Greece belong to the wild morphotype. The Late Middle Bronze Age assemblage from Tzoungiza offers the first reliable evidence of morphologically domesticated grape pips, but the relative proportions of each type vary according to the methods used. This clearly shows how narratives about the origins of early viticulture and grapevine domestication may be influenced by the choice of identification method applied to archaeological grape pips.

Keywords: *grape domestication, traditional and modern morphometrics, archaeobotanical identification, form, shape and distance measurements*

3.1 Introduction

Grapevine is one of the emblematic plants of the Mediterranean. It is considered to have been first domesticated in South-West Asia between 6000 and 3000 BC, but its domestication history is still debated. Indeed, evidence of early domestication from South-West Asia is limited, and new data question this hypothesis. The earliest finds of grapevine in archaeological contexts come from the region of the Dead Sea and date to the Pleistocene (Kislev et al 2004), and grape pips are found at several sites in the Near East during the Neolithic (Zohary et al 2012; Fuller and Stevens 2019), although large concentrations are not recorded before the 3rd mill BC (Miller 2008). In addition, the results from chemical analyses have been put forward to advocate the production of wine in the South-Caucasus as early as 6000 BC (McGovern 2003; McGovern et al 2017). But no evidence of grapevine domestication is currently available at such an early date and the onset of domestication in the area is still uncertain (Bouby et al 2021). Molecular studies confirm the existence of a main domestication centre in South-West Asia (Arroyo-Garcia et al 2006; Myles et al 2011; Bacilieri et al 2013; Riaz et al 2018) although the results of genetic research have been advanced to support the hypothesis of other centres of domestication (Grassi et al 2003; Arroyo-Garcia et al 2006). Parallel or secondary events (involving both local wild grapevine and varieties introduced from the putative primary centre) of domestication may have occurred anywhere in the area of natural distribution of the wild grapevine, from the Himalayas to Western Europe and around the Mediterranean basin (Levadoux 1956; Arnold et al 1998), including Greece (Logothetis 1970). Wild (*Vitis vinifera* subsp. *sylvestris*) and domesticated grapevine (*V. vinifera* subsp. *vinifera*) differ regarding their reproductive biology, and several phenotypic changes, including the morphology of the pips.

The question of grapevine domestication in Greece has recently been addressed (Pagnoux et al 2021). According to the dominant hypothesis, viticulture and wine making are related to the emergence of palatial civilizations in Crete and in the Peloponnese during the Bronze Age (Renfrew C 1972). However, evidence of wine making and grapevine management as early as the 5th mill BC (i.e. much earlier than the Late Bronze Age) in Northern Greece, far from Mycenaean Greece and Minoan Crete (Renfrew JM 1995; Valamoti 2009, 2015; Valamoti et al 2007, 2015, 2020; Pagnoux 2019; Pagnoux et al 2021) calls into question the origin of viticulture and its relation with the emergence of hierarchical societies in Prehistoric Greece (Valamoti 2017, 178). More recently, evidence from morphometric analyses, together with other indications offered by archaeobotanical and archaeological data, raises the hypothesis of a local domestication in the Aegean. A continuous use of local wild grapevines since the Late

Neolithic would have led to the emergence of domesticated types during the Middle Bronze Age, and to the cultivation of various fully domesticated types from the Late Bronze Age onwards. Introduced cultivars from more Eastern regions may have played a role in the emergence of variability in the domesticated types in Greece (Valamoti et al 2020; Pagnoux et al 2021).

Archaeobotanical inference of this domestication process is a longstanding challenge that has traditionally been based on the morphology of the grape pips. Measurements of specific features have long been used to distinguish wild and domesticated grapevine. The first index, proposed by Stummer was based on the size of the pips (Stummer 1911) and widely used by archaeobotanists. However, it was argued that this method presented a wide overlapping zone between the measurements of wild and domesticated pips and that several archaeological pips fall into this zone (e.g. Renfrew JM 1995). Furthermore, several works have pointed out the distortion induced by carbonisation and the discrepancy when comparing carbonised archaeological materials with non-carbonised modern ones (Logothetis 1970, 1974; Smith and Jones 1990). Both of these limitations led to a renewed search for measurements and indices that would both more accurately distinguish between domesticated and wild types and be less subject to the effects of deformation and size change caused by charring.

Morphometric analyses based on combinations of traditional measurements (size measurements including length, breadth, length of the stalk, position of the chalaza, thickness of the pips, etc) have been developed with the aim to better distinguish wild and domesticated grape pips (e.g. Kislev 1988; Di Vora and Castelletti 1995; Mangafa and Kotsakis 1996). Based on the analysis of modern pips from two Greek varieties and three wild populations, Mangafa and Kotsakis (1996) proposed four formulas allowing the distinction of wild and domesticated grapevine. Their work takes into account the influence of carbonisation on the measurements and the accuracy of the measurements including the difficulties in taking them. Their proposed formulas are designed to be applicable to any *Vitis vinifera* archaeological seed, which is a big advantage. However, some analyses of modern grape pips from France based on these formulas call into question their accuracy, especially regarding the identification of the domesticated grape pips, and suggest that the performance of the formulas in correctly identifying wild and domesticated pips should be tested on modern material from more numerous varieties, including specimens from other origins before their application to archaeobotanical material (Bouby and Marinval 2001). The same remark could be addressed to the Stummer index, which was based on Austrian grapevines only.

Another attempt to focus on shape variability was the use of discrete measurements (as referred to above: length, breadth, length of the stalk and position of the chalaza) converted into log shape-ratios: each variable is divided by the geometric mean of all 4 variables and then log-transformed (Bouby et al 2013; Bouby 2014). This method would also be more directly applicable to distorted charred remains.

More recently, a method based on the outline analysis of the grape pips has been developed (Terral et al 2010). Geometric morphometrics allow the researcher to analyse the shape of the objects separately from their size thereby limiting the influence of environment and, more particularly, reducing biases related to the effect of charring. Moreover, geometric morphometrics are regarded to allow a better discrimination performance of wild and domesticated grapevine than methods based on the measurement of distances (Bouby et al 2006; Bouby 2014). It also offers information beyond the wild/domesticated distinction, through the identification of domesticated grape types (varieties, or groups of varieties) (Terral et al 2010; Pagnoux et al 2015, 2021; Bouby et al 2021).

In this paper, we benchmarked the different morphometric methods to compare their accuracy at discriminating between wild and domesticated types on a modern reference collection, more representative than the ones used by Mangafa and Kotsakis (1996) and Stummer (1911). This extended collection was used to train new linear discriminant analyses, based on shape and size descriptors separately, that were compared to Stummer index and to Mangafa and Kotsakis formulas. All these methods were then applied to grape pips from three archaeological sites dated to the period corresponding to the shift from morphologically wild to morphologically domesticated grapevine and to the beginning of the varietal diversification of grapevine in Greece, i.e. from Late/Final Neolithic (4500-4000 BC) to Late Middle Bronze Age (1700-1620 BC). Our chief aim was to test if the results of these different methods for determining wild versus domesticated pips were congruent or if their discrepancies may lead to different readings of the history of grapevine domestication in Greece.

3.2 Material and methods

We used a modern reference collection with 47 accessions, including 34 *Vitis vinifera* subsp. *vinifera* (domesticated grapevine) varieties and 13 *V. vinifera* subsp. *sylvestris* (wild grapevine) individuals (Table 3.1; Bonhomme et al 2020a). For each accession, 30 normally developed berries have been randomly collected from a single, fully ripe bunch, and a single pip was extracted from each berry. Domesticated material was sampled in the French central ampelographic collection (INRAE, Vassal-Montpellier

Domesticated varieties	L	LS	PCH	B
Ain el Bouma	5.58 ± 0.41	1.94 ± 0.2	3.45 ± 0.25	3.52 ± 0.26
Alvarinho	5.2 ± 0.3	1.35 ± 0.21	2.68 ± 0.24	3.52 ± 0.2
Babeasca neagra	5.5 ± 0.27	1.44 ± 0.14	2.85 ± 0.21	3.72 ± 0.17
Barbera	6.77 ± 0.37	1.96 ± 0.16	3.62 ± 0.26	3.68 ± 0.22
Cabernet-Sauvignon	5.95 ± 0.24	1.8 ± 0.16	3.11 ± 0.18	3.6 ± 0.14
Carignan	6.93 ± 0.26	2.16 ± 0.2	3.9 ± 0.25	3.52 ± 0.21
Chaouch blanc	6.91 ± 0.37	1.75 ± 0.17	3.83 ± 0.34	4.12 ± 0.31
Chardonnay	5.35 ± 0.44	1.44 ± 0.24	3.1 ± 0.29	3.77 ± 0.3
Chasselas	5.93 ± 0.31	1.63 ± 0.22	3.06 ± 0.23	3.8 ± 0.26
Chevka	6.01 ± 0.32	1.49 ± 0.16	3.03 ± 0.18	3.72 ± 0.19
Cinsaut	5.99 ± 0.33	1.74 ± 0.18	3.38 ± 0.21	3.7 ± 0.22
Debina	7.02 ± 0.49	1.73 ± 0.2	3.7 ± 0.32	4.16 ± 0.27
Ferral tamara	6.75 ± 0.24	1.94 ± 0.17	3.5 ± 0.19	4.16 ± 0.18
Feteasca alba	5.23 ± 0.24	1.43 ± 0.13	2.7 ± 0.2	3.48 ± 0.19
Gaidouria	5.67 ± 0.36	1.64 ± 0.19	2.99 ± 0.24	3.81 ± 0.2
Grenache	5.29 ± 0.27	1.47 ± 0.1	2.83 ± 0.15	3.37 ± 0.21
Hunisa	8.28 ± 0.35	2.28 ± 0.19	4.43 ± 0.25	4.95 ± 0.26
Pehlivan kara = Papigi kara	6.61 ± 0.36	1.91 ± 0.22	3.21 ± 0.31	3.68 ± 0.19
Kravi tztzi	7.02 ± 0.32	1.92 ± 0.23	3.5 ± 0.21	3.94 ± 0.17
Local black	5.52 ± 0.36	1.46 ± 0.22	3.31 ± 0.28	3.81 ± 0.2
Mavrud	6.86 ± 0.27	2.33 ± 0.18	3.93 ± 0.2	4.29 ± 0.19
Merlot	6.07 ± 0.33	1.51 ± 0.16	3.01 ± 0.22	3.85 ± 0.25
Meslier Saint-François	5.6 ± 0.63	1.54 ± 0.25	3.09 ± 0.42	3.3 ± 0.29
Mourvèdre	6.21 ± 0.42	1.52 ± 0.17	3.09 ± 0.24	3.73 ± 0.29
Muscat à petits grains noirs	7.26 ± 0.26	2.24 ± 0.17	4.14 ± 0.25	4.14 ± 0.23
Muscat à petits grains roses	5.31 ± 0.29	1.37 ± 0.2	3 ± 0.26	3.13 ± 0.19
Pembe Gemre	6.94 ± 0.35	2.11 ± 0.24	3.92 ± 0.3	3.76 ± 0.16
Pinot noir	6.37 ± 0.33	1.73 ± 0.21	3.47 ± 0.19	3.9 ± 0.28
Roussanne	6.52 ± 0.26	1.43 ± 0.17	3.33 ± 0.26	4.15 ± 0.23
Sauvignon	6.51 ± 0.32	1.91 ± 0.18	3.49 ± 0.24	3.69 ± 0.22
Sliva	6.91 ± 0.43	1.9 ± 0.25	3.63 ± 0.29	3.98 ± 0.21
Syrah	5.42 ± 0.37	1.66 ± 0.2	3.16 ± 0.29	3.22 ± 0.19
Tachtas	5.98 ± 0.24	1.64 ± 0.15	2.99 ± 0.21	3.79 ± 0.21
Vermentino	6.52 ± 0.39	1.98 ± 0.22	4.16 ± 0.3	3.59 ± 0.15

Wild individuals	L	LS	PCH	B
La Calmette (Fr)	5.43 ± 0.29	0.97 ± 0.18	2.69 ± 0.24	3.7 ± 0.24
La Calmette 10 (Fr)	5.07 ± 0.36	1.23 ± 0.21	2.78 ± 0.25	3.34 ± 0.24
Camp Saure 11 (Fr)	5.3 ± 0.18	1.07 ± 0.14	2.51 ± 0.14	3.37 ± 0.17
Chalabre 4 (Fr)	4.91 ± 0.23	0.95 ± 0.11	2.32 ± 0.15	3.62 ± 0.2
Col de la Babourade 7 (Fr)	5.13 ± 0.38	1.02 ± 0.19	2.52 ± 0.21	3.29 ± 0.29
L'Escale 13 (Fr)	5.62 ± 0.37	1.13 ± 0.18	2.88 ± 0.23	3.6 ± 0.18
L'Escale 14B (Fr)	4.82 ± 0.33	0.81 ± 0.12	2.22 ± 0.17	3.31 ± 0.16
L'Escale 16 (Fr)	5.34 ± 0.3	1.19 ± 0.14	2.73 ± 0.2	3.61 ± 0.25
L'Escale 17 (Fr)	4.91 ± 0.27	1.01 ± 0.16	2.36 ± 0.18	3.58 ± 0.2
L'Escale 18 (Fr)	5.16 ± 0.47	0.71 ± 0.16	2.31 ± 0.22	3.94 ± 0.39
L'Escale 20 (Fr)	5.17 ± 0.3	0.91 ± 0.14	2.44 ± 0.18	3.47 ± 0.19
Pic Saint-Loup H (Fr)	5.19 ± 0.4	0.79 ± 0.15	2.27 ± 0.21	3.77 ± 0.29
Rivel 1 (Fr)	5.92 ± 0.6	1.01 ± 0.3	2.79 ± 0.32	4.08 ± 0.3

Table 3.1 Accessions used in this study. Dimensions are reported with mean±sd and given in mm.



Fig. 3.1 Localisation of the investigated sites.

Grapevine Biological Resources Center; <https://www6.montpellier.inra.fr/vassal>); it consists of varieties from seven countries from continental Europe and the Near East, where viticulture has a long history. Wild individuals were sampled *in natura* from seven populations in France. Our reference modern dataset thus gathered 1410 pips.

Archaeological material comes from three sites, as summarized in Table 3.2: Dikili Tash in the region of Macedonia, northern Greece (Late Neolithic, 4500-4000 BC) and two sites in the Peloponnese, southern Greece, P.O.T.A. Romanou (Early Bronze Age, ca 2700-2200 BC) and Tsoungiza (Late Middle Bronze Age, ca 1700-1620 BC) (Fig. 3.1). All these sites have yielded large concentrations

of carbonised grape pips. All grape pips analysed consist of subsamples of at least 100 pips from larger assemblages, in order to first test the morphometric methods. Grape pips from P.O.T.A. Romanou have already been studied using geometric morphometrics (Valamoti et al 2020, Pagnoux et al 2021) and those from Tsoungiza have been studied (Allen and Forste 2020) using the metrical indices from Stummer (1911) and Mangafa and Kotsakis (1996).

All pips, modern and archaeological, were photographed in dorsal and lateral views using a stereomicroscope coupled with a digital camera. On each pip, four length measurements were manually recorded using ImageJ (<http://imagej.nih.gov/ij>): total length (L), length of

Table 3.2 Archaeological samples used in this study.

Site	Region	Chronology	Number of samples	Number of pips	Reference
Dikili Tash	Macedonia	Late Neolithic	4	200	Pagnoux et al 2021; Valamoti unpublished
Romanou	Peloponnese	Early Bronze Age	2	210	Valamoti et al 2020; Pagnoux et al 2021
Tsougiza	Peloponnese	Late Middle Bronze Age	1	100	Allen and Forste 2020

the stalk (LS), position of the chalaza (PCH) and maximum breadth (B). Nomenclature followed and measurements replicated the methods of Mangafa and Kotsakis (1996). To focus on relative changes, log-shape ratios (lsr) were calculated as follows:

We also derived the synthetic index proposed by

$$lsr_{\{L,LS,PCH,B\}} = \log \frac{\{L,LS,PCH,B\}}{\sqrt[4]{L * LS * PCH * B}}$$

Stummer (1911), and the four proposed by Mangafa and Kotsakis (1996) (hereafter cited as $MK_{1,2,3}$ and 4) as follows: Here, we calculated two new indices, lsr and lsr_trad,

$$Stummer = \frac{B}{L}$$

$$MK_1 = -0.3801 - \frac{30.2 * LS}{L} + 0.4564 * PCH - 1.386 * L + \frac{2.88 * PCH}{L} + 9.4239 * LS$$

$$MK_2 = 0.2951 - \frac{12.64 * PCH}{L} - 1.6416 * L + 4.5131 * PCH + \frac{9.63 * LS}{L}$$

$$MK_3 = -7.491 + 1.7715 * PCH + \frac{0.49 * PCH}{L} + \frac{9.56 * LS}{L}$$

$$MK_4 = 0.7509 - 1.5748 * L + 5.297 * PCH - \frac{14.47 * PCH}{L}$$

using linear discriminant analyses (further abbreviated LDA, Fisher 1936), based on natural measurements and log-shape ratios, respectively. Looking for such first-order linear combination has the merits of simplicity (only measurements and not ratios/products are used) and consistency (each measurement is used once and weighted by its own coefficient).

Traditional morphometrics were compared to geometric morphometrics analysis as the current gold standard for wild/domesticated identification. We used outline coordinates (x; y) extracted from the images, centered, scaled using the centroid size, aligned and normalized for the position of their first points. For each view, elliptical Fourier transforms were used to convert the contour geometry into “Fourier coefficients” (Bonhomme et al 2014). The number of harmonics retained assembled 95% of the total harmonic power (5 for both views). With four coefficients per harmonic, 40 Fourier coefficients were used as quantitative variables describing the shape (which is the form minus the size) for all subsequent analyses.

For all the LDAs, a permutational approach allowed us both to address the imbalance between wild and domesticated sample sizes in our dataset (Evin et al

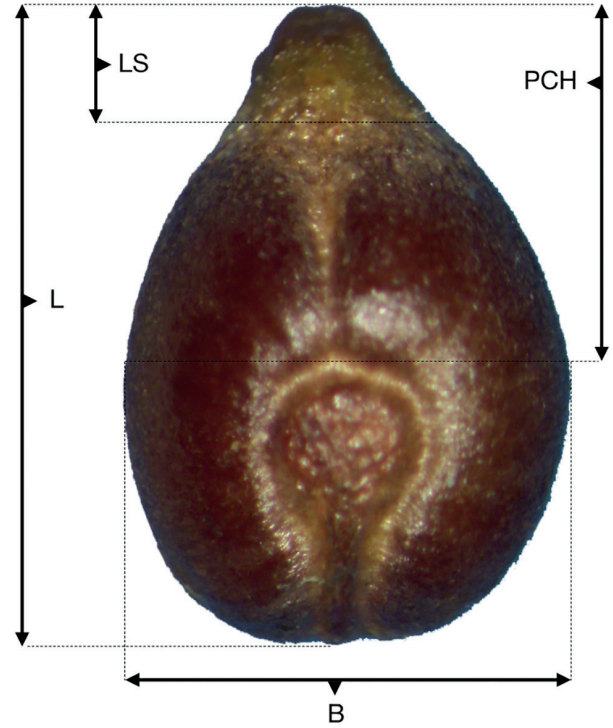


Fig. 3.2 Dorsal view of a grapevine pip, here from the *Vitis vinifera* subsp. *sylvestris* (wild) individual “Pic Saint-Loup 13”, with indications of morphometric measurements: L (total length), LS (length of the stalk), PCH (position of the chalaza), B (pip breadth).

2013; Bonhomme et al 2020b), and to increase sampling robustness. We sampled, 10^3 times, 390 pips in each class (the smallest sample size between wild and domesticated classes). Fig. 3.3 presents the density distributions for both natural measurements, log-shape ratios, and all the indices derived from them, for wild and domesticated accessions.

We then benchmarked the performance at distinguishing modern wild and domesticated pip forms based on these seven indices (*Stummer*, MK_1 , MK_2 , MK_3 , MK_4 , *trad* and *lsr_trad*) and on shape descriptors (*EFT*) using “leave-one-out” cross-validation. Accuracies (the proportion of correctly classified pips) were reported both globally and by class (Fig. 3.4). A good model should both maximize global accuracy and minimize (absolute) difference accuracies between classes, i.e. not over/underestimate a certain class (wild or domesticated).

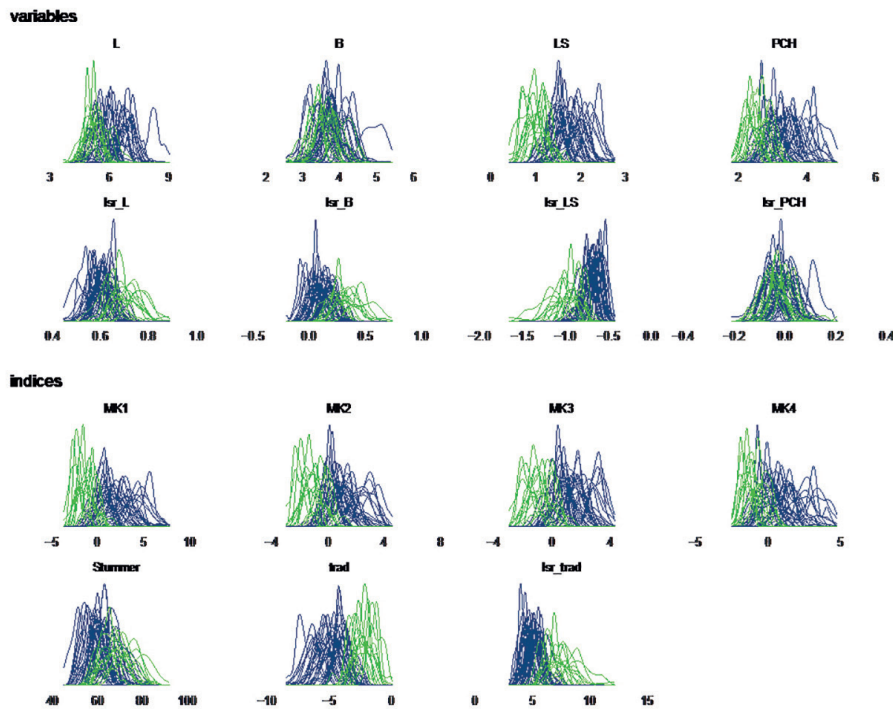


Fig. 3.3 Distributions of the length measurements and their corresponding log-shape ratios (variable panel), and of the former synthetic indices and those introduced here (indices panel). Mean density distribution (thick trait and filled are) are displayed for wild (green) and domesticated (blue) classes. Individual accessions are also represented with thin coloured traits.

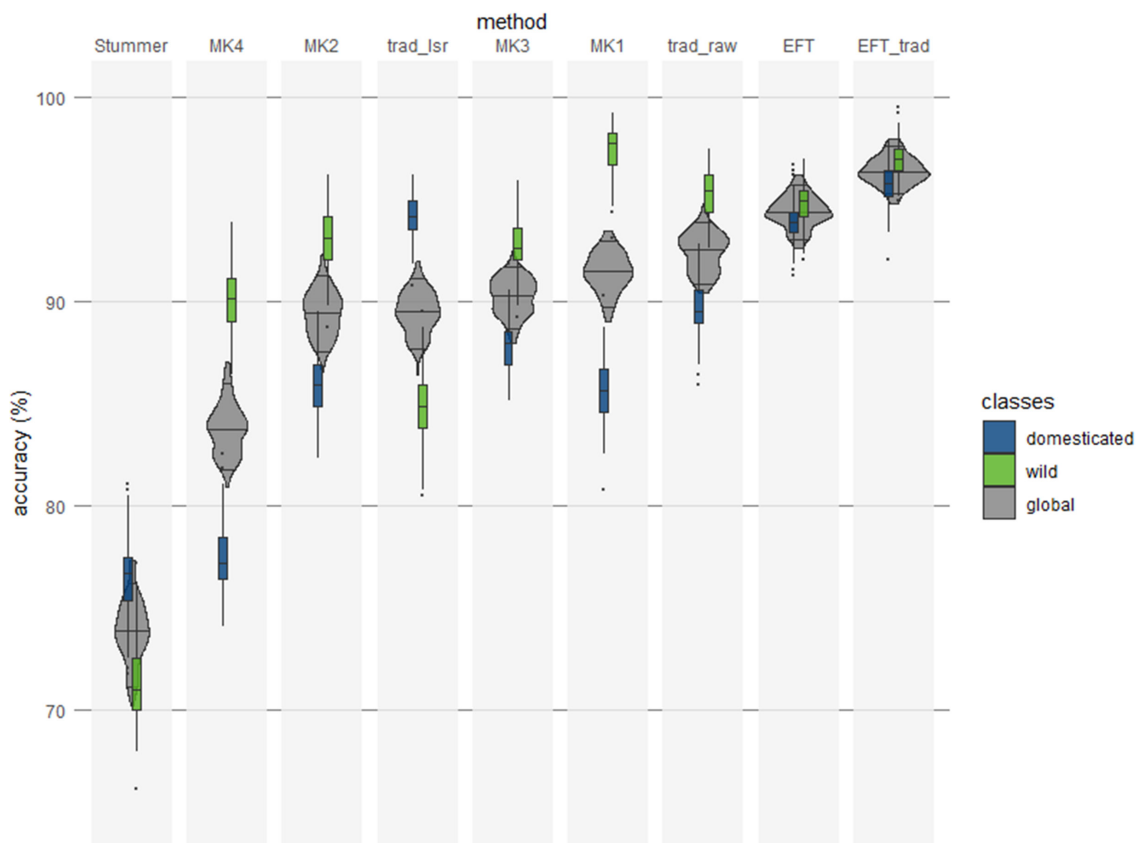


Fig. 3.4 Benchmarking identification performances for the seven indices for the reference collection. Violin distributions indicate global accuracies; boxplot classes accuracies. Distributions were obtained from 1000 permutations of balanced dataset (N=420).

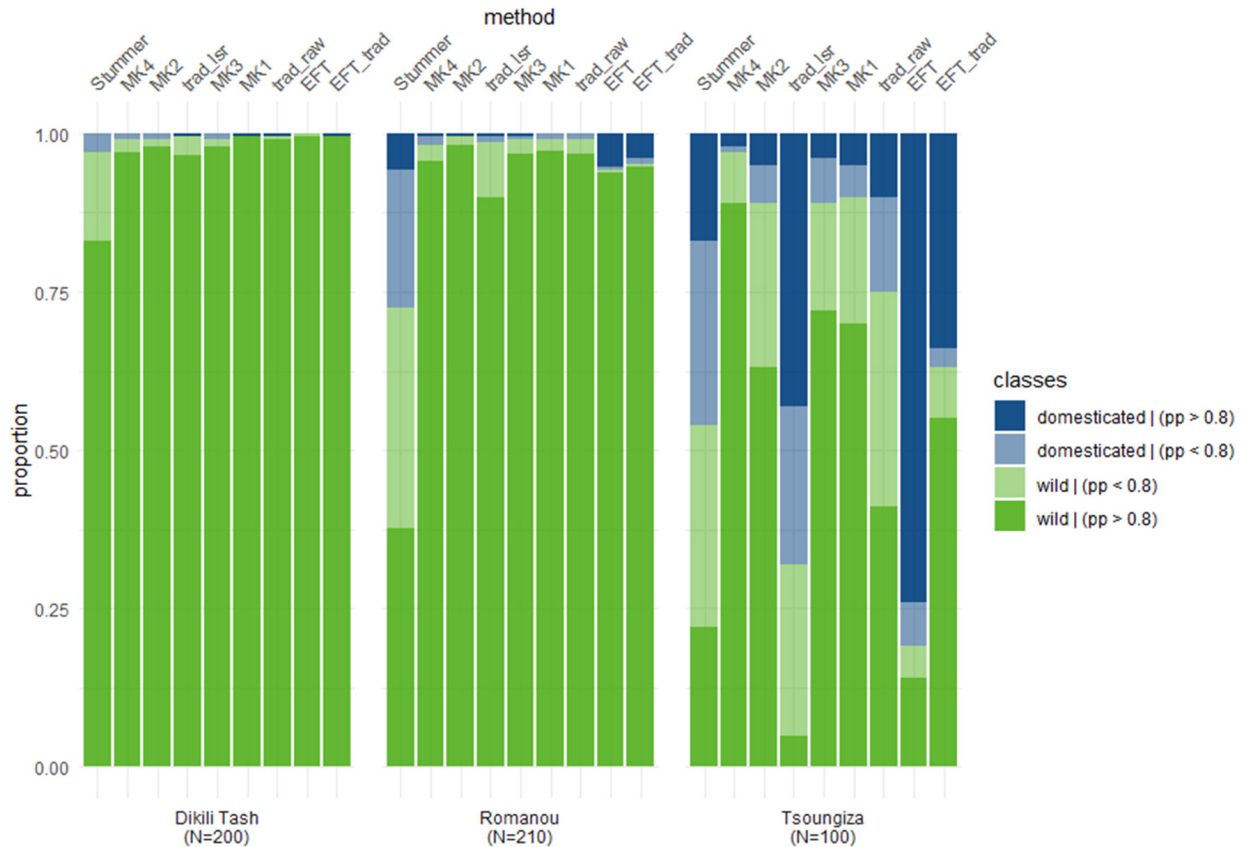


Fig. 3.5 Archaeological inference. Proportion of wild and domesticated grapevine are displayed for each site (panels) and each index (columns). Assignations are based on majority rule over 1000 permutations. Transparent areas indicate pips for which posterior probabilities were, on average, below 0.8.

Finally, the (10^3 permutations \times 8 methods) LDAs obtained on the modern material were used to infer the domestication status (wild/domesticated) of the archaeological materials. For each of the eight methods, each pip was classified 10^3 times as either wild or domesticated: we used a majority rule for the definitive assignation and also calculated the average posterior probability associated with these winning assignations. Eventual ties (exactly 500 votes for “wild” and for “domesticated”) were resolved using the highest mean posterior probability. For each site, we calculated the proportion of pips assigned to the domesticated/wild type according to each model, and distinguished those with an average posterior probability > 0.8 (Fig. 3.5).

3.3 Results

The four measurements on the 1410 pips allowed us to assess the resulting distributions to identify wild and domesticated grapevines (Fig. 3.3). The density distribution for LS, the “beak length” was the single measurement that shows the best differentiation between wild and domesticated grapevine, on average, but also at

the accession scale. By contrast, B (the breadth of the pip) is the worst descriptor in these respects. Log-shape ratios seems to improve differentiation (e.g. lsrB compared to B) compared to single measurements, except for lsrPCH that shows an increase in distribution overlapping, compared to PCH.

We benchmarked the performances of the five synthetic indices already published, those of the two indices we calculated from our measurements (trad and lsr_trad) and of outline analysis (EFT – Fig. 3.4). First, the results confirm that the best discrimination between pips of wild and domesticated grapevines is obtained using geometric morphometrics. EFT gives the best global accuracy (94.4%) and the minimal difference between performances for wild and domesticated grape pips. One can see that results obtained from traditional measurements, although inferior, are very good; trad provide correct identification for 92.4% of the pips, on average. Moreover, the formulas from Mangafa and Kostakis also give good results, especially MK1 and MK3 which allow the correct identification of respectively more than 91.4% and 90.2% of the pips. The trad index outperforms the MK1 index only

by 1.9%. The worst discrimination accuracy was obtained with the Stummer index (73.9%). MK4 was quite better (83.7%) but less accurate than the three other indices proposed by Mangafa and Kotsakis and the *lsr_trad* index.

The absolute difference between class accuracies (i.e. the difference between correct identification of the wild and domesticated compartments) is clearly higher for all the indices based on traditional measurements than for EFT. Most of the methods (MK formulas and *trad*) allow the discrimination of wild grape pips with a greater accuracy than the domesticated ones. This means that, when these methods are applied to archaeological pips, the relative proportion of wild grapevine to domesticated will likely be overestimated. In contrast, the Stummer and *lsr_trad* indices identify with greater class accuracy the pips from domesticated grapevines and are therefore expected to overestimate the proportion of domesticated grapevine in archaeological assemblages. Because these last two methods (Stummer and *lsr_trad*) use ratios and logged measurements for *lsr_trad* rather than natural measurements, they put less emphasis on pip size. This difference may reduce confusion about the identification of smaller domesticated seeds, which overlap in size with wild seeds.

Once the differences in the performance of the various methods on fresh material have been established, it is also necessary to consider possible interactions with the deformations induced by the carbonisation of the archaeological material. Various experimental studies have indeed shown that carbonisation leads to a reduction in the size and certain deformations in the shape of the seeds (Smith and Jones 1990; Mangafa and Kotsakis 1996; Uccesu et al 2016; Bouby et al 2018). Generally, the domesticated seeds take on a more roundish appearance more characteristic of the wild grapevine. It should be stressed, however, that the long beak, which is very characteristic of the domestic vine, is weakly affected by these deformations. The consequence is that experimentally charred wild and domesticated *Vitis* seeds can still be reliably discriminated using both *lsr_trad* and EFT methods (Bouby et al 2018). However, the performance of other methods will necessarily be more affected by charring, those that take into account size, which is strongly modified by charring, and those that do not take into account beak size or shape.

If we now consider the archaeological material, in Dikili Tash, the earliest site (Late Neolithic), 0 to 1% of pips were classified as of the domesticated form with a posterior probability > 0.8, irrespective of the method applied. The same results were observed for Romanou (Early Bronze), setting aside the results obtained with the Stummer index, which classifies 30% of the assemblage as domesticated. The first solid evidence of domesticated forms appears in the Tsoungiza assemblage (Late Middle

Bronze / Early Mycenaean) where more than 75% of the pips are identified as domesticated using EFT. The *trad* index, the best performing index on modern material, only reports 25% of pips of the domesticated form, including 11% with a posterior probability > 0.8. Both the Stummer and the *lsr_trad* indices report a majority of pips of the domesticated form.

Among all the indices based on length measurements *lsr_trad* is the one giving the best results on modern domesticated pips. Considering the changes in size and shape caused by carbonization it is likely that this index performs, comparatively, even better on charred pips, being less affected by size changes.

3.4 Discussion

3.4.1 Assessing the performances of traditional morphometrics on modern reference material

The modern reference material used in our study has proven crucial towards choosing a method that reliably allows the distinction between wild and domesticated grapevine. Testing measurements, indices, formulas or any method first on modern material is crucial to evaluate the accuracy and the suitability of the chosen descriptors. Recent works confirm that a wide reference collection allows to build more robust models, based on more representative modern groups (wild and domesticated compartments), and therefore to identify archaeological material with higher confidence (Terral et al 2010; Bouby et al 2013, 2021; Pagnoux et al 2015; Bonhomme et al 2020b). Here we have used an extended modern collection, which is used to systematically compare the performances of various methods based on traditional morphometric descriptors (Stummer index, MK1, MK2, MK3, MK4, *trad* and *lsr_trad*) to outline analysis (EFT).

The first point to be made is that traditional measurements of grape pips allow a distinction between wild and domesticated grapevines with a high accuracy, except for the Stummer index, which allows the correct identification of a smaller proportion of modern pips. The limited distinctive potential of the Stummer index lies in the fact that it considers only two descriptors of the grape pip, the distances B and L. The index of these measurements does not take into account a main distinctive feature, that of the length of the stalk, and thus insufficiently reflects the pips morphological characteristics between the two subspecies of grape, wild and domesticated.

As a matter of fact, to distinguish between the two subspecies, the length of the stalk is of key importance. This descriptor has long been considered as a characteristic of the domesticated grapevine (Levadoux 1956). Previous studies aiming to identify wild and domesticated grapevine among archaeological grape pips have revealed the value

of this measurement (Smith and Jones 1990; Mangafa and Kotsakis 1996; Bouby et al 2013). Additionally, a more recent experimental approach of the effect of charring on the identification of grape pips also demonstrated the robustness of the length of the stalk in distinguishing wild/domesticated grape even after charring (Bouby et al 2018). The stalk dimension is also a reliable criterion as regards its independence related to the number of pips per berry: it is not affected by the number of pips per berry compared to other size and shape characters (Bonhomme et al 2020a). Along with the length of the stalk, the length of the pip and the position of the chalaza appear to be relevant to the distinction between wild and domesticated pips, even when carbonized (Mangafa and Kotsakis 1996). Our study confirms that by contrast, the breadth of the pip is the less powerful descriptor to distinguish between wild and domesticated grapevine (Mangafa and Kotsakis 1996; Bonhomme et al 2020a).

However, the use of shape-based rather than size-based criteria allow an even better identification of domestication status, because shape is less affected by environmental parameters than size. Shape is also less affected by carbonization. For all these reasons, already underlined by previous studies (Mangafa and Kotsakis 1996; Bouby et al 2018), the use of criteria based on shape allow a better identification of grape pips and especially of carbonized pips. Geometric morphometrics give the best results, but formulas MK1 and MK3, based on composite indices and not only on size parameters, give good classification rates, superior to 90%. In their paper, Mangafa and Kotsakis recommend the use of MK2 and MK3, as more adapted to the analysis of carbonized material because they use fewer simple size variables than ratio variables (Mangafa and Kotsakis 1996). Our benchmark confirms that MK3 allows a better identification of domesticated grape pips compared to the other formulas: this formula may therefore be considered as a very good method to identify archaeological grape pips. On the basis of our results, MK2 is less powerful to distinguish between wild and domesticated, but more efficient than MK4.

Log-shape ratios allow a good distinction between wild and domesticated grapevine when applied to charred remains, as it has already been demonstrated (Bouby et al 2013; Bouby et al 2018). EFT offer even more accurate criteria but data acquisition is less straightforward.

3.4.2 Selecting the appropriate method for exploring charred archaeological pips

In light of our results, different histories of grapevine domestication could be written by picking one method rather than another. The use of the Stummer index, whose performance is limited on modern material as we have shown, may have dated the identification of the first domesticated grapevines in Greece and elsewhere

to an earlier period. Indeed, this method is as powerful as the other ones in identifying “typical” wild type, but less powerful when dealing with “less typical” shapes, as shown by our results on the archaeological material. In this study, up to 25% of the sample from Early Bronze Age P.O.T.A. Romanou can be classified as domesticated using the Stummer index, while the other methods classify less than 10% as domesticated. Previous studies based on the Stummer index support the interpretation that the first domesticated morphotypes occurred as early as the Late Neolithic at Dimitra (Renfrew, JM 1997) and Sitagroi (Renfrew, JM 2003) in Macedonia, while the grape pips from Dikili Tash are considered as wild or “intermediate” (Logothetis 1970). Our results suggest that these conclusions should be considerably tempered.

Conversely, while the Stummer index overestimates the presence of domesticated forms, the MK formulas overestimate the presence of the wild grapevine. In our study, more or less 10% of the archaeological pips dated to the Late Middle Bronze Age (Tsoungiza, Peloponnese) are classified as domesticated using any of the four formulas or direct traditional measurements, while this rate is close to 75% using geometric morphometrics and log-shape ratios. Therefore, the use of MK formulas could have led to underestimate the presence of domesticated grapevine in Greece during the Bronze Age, as shown by a recent research based on geometric morphometrics (Pagnoux et al 2021).

Two sources of bias are combined when applying models trained on modern material to archaeological material. First, a purely statistical by-product arises from contrasted class accuracies: here the probability of being classified as “wild” is overestimated. Since it is obtained on modern material, this bias is likely carried, but also overwhelmed by an opposite effect, when inferring domestication status on archaeological material. As a matter of fact, when charred, pips tend to become rounder, which is not a statistical but a taphonomic effect which is expected to unduly classify as wild, “true” domesticated pips.

As mentioned above, charring has a strong impact on seed size and also affects seed shape, leaving the beak relatively unaffected. Charring therefore has a variable impact on the performance of the methods aiming to discriminate wild/domesticated grape pips, depending on whether they take into account the size of the seeds and the shape of the stalk. As a result, the EFT and *lsr_trad* methods are relatively unaffected by charring, because they do not use size while they take into account the stalk. The methods MK1, MK2, MK3, MK4 and *trad* will necessarily be more affected by charring, because they directly rely on distance measurements that express size. The performance of the Stummer index will be even more impacted as it does not directly take into account

the size and shape of the beak. Further deciphering the interrelated effects of the statistical method and of the deformations due to charring will require more dedicated experimental work.

3.4.3 Archaeological application and the domestication of grapevine in Greece

In this study, all methods give the same result for the Late Neolithic Dikili Tash, and this result is consistent with previous studies on other samples from the same site, analyzed using MK formulas (Mangafa and Kotsakis 1996) or geometric morphometrics (Pagnoux et al 2021): grape pips are all morphologically wild. However, indications of grapevine cultivation during the Late Neolithic are numerous (for a review, see Pagnoux et al 2021), and the pips from Dikili Tash may represent grapevine under cultivation, wild or at an early stage of domestication, whose seeds do not bear any morphological sign of domestication (Valamoti 2015).

Describing the situation of the grapevine in Greece during the Early Bronze Age is more challenging. Few grape pips are available, contrary to the Late Neolithic finds. Grape pips from Early Bronze Age Lerna (Peloponnese) have been analyzed using the Stummer index: most of them fall into the overlapping zone, while few are considered as typical of wild grapevine and even fewer as typical of domesticated grapevine (Hopf 1962). This pattern led Hopf to suggest that grapevine is at a transitional stage at Lerna during the Early Bronze Age. However, Smith and Jones (1990) and Mangafa and Kotsakis (1996) argued that this overlapping zone corresponds not to an admixture or intermediate forms, but results from the use of inappropriate parameters.

Smith and Jones compared the results obtained by Hopf to their experimental work and suggested that these “transitional” pips could correspond to domesticated ones, since pips from cultivars experimentally charred fall into this overlapping zone of the Stummer index (Smith and Jones 1990). However, because neither we nor Smith and Jones had access to the Lerna material, it was not possible to test this hypothesis. Similarly, Early Bronze Age pips from Dikili Tash analyzed by Logothetis are not available for reanalysis. Grape pips and even more morphometric data on grape pips dated to the Early Bronze Age are hence still lacking, such that only minimal comparison material can be found. The material from P.O.T.A. Romanou, analyzed here, is therefore highly significant, as it is one of the few Early Bronze Age assemblages that can help to better understand the changes undergone by grapevine during this period. More than 90% of the pips are, on the basis of all traditional approaches, Stummer and EFT set aside, closer to the wild grapevine. However, as shown in a recent study, grape pips from Romanou are slightly different from those dated to the Neolithic: they are more

elongated and/or with a longer stalk, and they tend to be less typical of the modern true wild grapevine; this change in pip shape may reflect an early stage of selective pressure towards domestication (Valamoti et al 2020). Combined to other evidence of grapevine management and intensive use since the Late Neolithic (for a recent review see Pagnoux et al 2021), the results presented here are further clues to a possible autochthonous domestication of the grapevine in Greece.

The grape pips from Tsoungiza, analysed in this study, and previously by Allen and Forste (2020) using Stummer, MK1 and MK2, provide the first morphometric results on Late Middle Bronze Age material from Peloponnese. On the basis of geometric morphometrics and log-shape ratios, they are mostly close to the domesticated grapevine. Allen and Forste (2020) reach the same conclusion, not only on the basis of morphometric results, but also due to the presence of many immature pips, that are regarded as typical of domesticated grapevine (Kroll 1999).

This result is also consistent with previous studies on Late Bronze Age sites from Central and Northern Greece (Pagnoux et al 2021) that indicate the cultivation of domesticated varieties at the time. Material from earlier periods and from other Middle and Late Bronze Age sites is nonetheless still needed to better understand the development of domesticated grapevine in Peloponnese. Indeed, wine making and wine consumption has long been thought to have started with the Mycenaean civilization (1650-1100 BC) in this region, but current archaeobotanical and morphometric data seems to challenge this assumption. At Neolithic Dikili Tash, evidence of wine making has been found, consisting of grape pressings (Valamoti et al 2007, 2015) and chemical evidence in vessels (Garnier and Valamoti 2016). These elements are associated with large quantities of morphologically wild grape pips, showing that the first wine in the Aegean was probably made using wild grapevine or grapevines in an incipient form of cultivation, before the emergence of fully domesticated types; at Dikili Tash the latter seems more likely because of the large quantity of the harvest. Previous studies in the Near-East suggested that the first wine was made using grapes from wild grapevine (Miller 2008), and experimental microvinifications indeed reveal that wild grapevines are suitable to make wine (Arroyo-Garcia et al 2016), although the small size of the berries and the limited and irregular production of fruits pose challenges for wine-production (Bouby et al 2021). Evidence of wine predating the presence of the domesticated type has also been suggested in the Caucasus (Bouby et al 2021).

3.5 Conclusions

The efficiency and relevance of the methods used to distinguish wild and domesticated archaeological pips has been and still is the cause of debate, as well as the question

of grapevine domestication. In this article we compare for the first time, based on a unique and diversified modern reference collection, the performance of geometric morphometrics (EFT) to traditional length measurements, and for the latter we compare the different known indices and methods mentioned in the literature. First, our results underline the necessary use of a diversified reference collection to reliably assess the different methods used for the distinction of wild vs domesticated grapevine. Then, except for the Stummer index, this study confirms the good overall performance of the methods based on length measurements compared to EFT, which, however, is still more accurate.

A good method would allow distinguishing modern wild and domesticated grapevine with a good global accuracy, would minimize differences in accuracy between wild and domesticated types and would be efficient in identifying archaeological grape pips despite the distortion induced by charring. Consequently, we show that picking one method rather than another may lead to write a different history of the grapevine domestication.

The use of wild grapevine during the Late/Final Neolithic (4500-4000 BC), perhaps at an early stage of cultivation, is confirmed by this study, as well as the use of fully domesticated type during the Late Bronze Age. The situation is still unclear concerning the Early Bronze Age (2700-2200 BC): grape pips from one site, P.O.T.A. Romanou in south Peloponnese, show a morphology close to the wild type but different from the shape of the Late Neolithic grape pips. On the basis of these results, a local domestication of the grapevine may be suggested for Greece. Notably in Late Middle Bronze Age Tzoungiza (1700-1620 BC) domesticated grapevine is clearly identified. If we take into account the temporal relationship between the P.O.T.A. Romanou and Tzoungiza archaeological assemblages, it appears that from the 3rd mill BC to the first half of the 2nd mill BC there is a considerable increase of domesticated grape pips that may reflect a regional change due to local cultivation leading to the development of morphologically domesticated pips.

Many questions remain open: from a methodological point of view, the importance of the operator in taking length measurements need to be investigated, while more archaeological material is necessary to better understand the history of grapevine domestication in the Aegean.

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