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Identification of archaeological barley grains using geometric morphometrics and experimental charring

Angèle Jeanty^{a*}, Jérôme Ros^a, Cyprien Mureau^a, Camille Dham^a, Célia Lecomte^a, Vincent Bonhomme^{a,b}, Sarah Ivorra^a, Isabel Figueiral^{a,c}, Laurent Bouby^a, Allowen Evin^a

^a ISEM, CNRS, EPHE, IRD, Université de Montpellier, Montpellier, France

^b Athéna, Lacamp, Roquedur, France

^c Inrap DST Paris, Rue de l'Acropole, 34420, Villeneuve-les-Béziers, France

Abstract

Barley is one of the main cereals found in archaeological sites in the north-western Mediterranean basin, over the last 8 millennia. Grains are preserved in archaeological sediments by charring after or before dehusking. Morphological criteria for distinguishing 2-row from 6-row barley, but also hulled barley from naked barley, can be affected by charring and this can complicate identification of barley subgroups. In the last decade, geometric morphometrics applied to uncharred barley grains has shown the possibility of identifying barley subgroups, but its applicability to archaeological remains still needs to be ascertained. We used 3985 raw and charred grains of 113 current varieties to (1) assess charring effects on the shape of barley caryopses, depending on their subgroup and whether they were charred husked or dehusked and (2) select the best dataset for identifying barley subgroups. We also used 700 archaeological grains, from the Neolithic period to the end of the Middle Ages, to (3) compare the morphometric taxonomic assignment of the grains with their carpological identification based on discrete anatomical features in a series of 7 archaeological samples. Our results indicate size and shape differences between barley grains when charred husked or dehusked. Although the charring process results in greater morphometric homogeneity, it allows subgroups of barley to be identified. For the 2-row vs. 6-row identification, more than 71% of the charred modern grains can be correctly identified. For the hulled vs. naked identification, the correct cross-validation percentages range from 70 to 73%. Finally, the good correlation between carpological and morphometric identifications of archaeological grains suggests that it is possible to identify archaeobotanical samples in the future.

Keywords

Archaeobotany; *Hordeum vulgare*; Evolution; Domestication; Elliptical Fourier Transforms; Discriminant analysis; GMM

Introduction

In archaeobotany, taxonomic identification is essential for documenting the processes of domestication and geographical diffusion of plants over time. Based on the observation of anatomical and morphological characteristics, the identification of archaeological remains is possible at the family, genus, species, or even subspecies level. However, several factors can limit the identification of ancient remains, such as taphonomy (i.e. the preservation processes). Charring, waterlogging or mineralisation can affect the characteristics needed to make a taxonomical identification. There may also be intrinsic morphological limitations, as some species or varieties produce organs that are too similar to allow their specific or intraspecific identification. To refine the identification of certain taxa, morphometrics, and more recently geometric morphometrics, have been used in archaeobotanical research (Evin et al., 2022). This research has been particularly developed on fruit macroremains, such as grapevine (e.g Terral et al., 2010; Pagnoux et al., 2021; Bouby et al., 2021; Bonhomme et al., 2021), olives (Newton et al., 2014; Bourgeon et al., 2018; Margaritis et al., 2021; Terral et al., 2021; Tsirtsis et al., 2023) and dates (Terral et al., 2012; Gros-Balthazard et al., 2017; Sallon et al., 2020), but also on cereals (Ros et al., 2014; Bonhomme et al., 2016; Roushannafas et al., 2022) and legumes (Tarongi et al., 2021). Cereal preservation is mostly dependent on charring (e.g. Leroyer et al., 2018), which is detrimental to ancient DNA preservation and subsequent palaeogenetic studies (Lundstrom et al., 2018). Consequently, morphometrics appears to be an ideal tool for the study of archaeological cereal remains, which may include experimental charring of modern cereals of known taxonomy for performing the identifications.

Barley (*Hordeum vulgare* L.) is one of the main cereals found in European archaeological sites. Today, this species is one of the most important cereal crops worldwide (Carena 2009; Zohary et al., 2012). It is primarily used for human and animal food, for the chemical and agricultural industries and for the production of alcoholic beverages (Bothmer 2003; Newman and Newman 2006; Carena 2009; Riehl 2019). These different uses are associated with a very large diversity of varieties (Knüpffer et al., 2003).

Modern varieties are divided into two subspecies: two-row barley (*Hordeum vulgare* subsp. *distichum*) and six-row barley (*Hordeum vulgare* subsp. *vulgare* also called *Hordeum hexastichum*). They differ in spike morphology and number of fertile spikelets at each node of the rachis (Bothmer 2003; Jacomet, 2006; Zohary et al., 2012). Each subspecies comprises hulled and naked types, depending on how strongly the inner envelopes (lemma and palea) are attached to the grain at maturity. Therefore, 4 subgroups can be distinguished based on general morphology: 2-row hulled, 2-row naked, 6-row hulled and 6-row naked barley.

Archaeologically, the identification of these subgroups depends on the preservation of different anatomical parts of the ear. A simple observation of the caryopsis morphology may be sufficient to distinguish naked and hulled types (Jacomet, 2006). The number of fertile spikelets per rachis node is the best indicator of the number of rows, but these fragile elements are generally eliminated during crop processing (Hillman 1981,

1984) or during charring. Charred caryopses are frequently the only observable organ in archaeobotanical samples; ascertaining the exact number of rows solely from these elements can be difficult through a macroscopic observation (Bouby 2001). Identification of archaeological grain assemblages is often based on the proportion of twisted (lateral) grains in the sample: a fraction of 2/3 twisted grains corresponds to 6-row barley; in 2-row barley, all the grains are straight. In the north-western part of the Mediterranean basin, both 2-row and 6-row barley were present from the Early Neolithic onwards (Antolín 2016; Bouby et al., 2020). During the Neolithic, the cultivation of naked barley was dominant (Antolín 2016; Bouby et al., 2020). It then declined in favour of hulled barley from the Bronze Age onwards (Stika and Heiss 2013; Lister and Jones 2013; Bouby et al., 2017). Later on, its presence is still mentioned in written sources (e.g. Ferdière 1988) but is rare in the archaeological record.

In order to go beyond classical morphological observations, several authors have attempted to identify barley subgroups using linear measurements such as grain length, width and thickness (Bouby 2001). More recently, geometric morphometrics, in particular seed outline analyses, has opened up new perspectives for identifying ancient barley characteristics (Ros et al., 2014; Bonhomme et al., 2017; Wallace et al., 2019; Jeanty et al., 2023). Results from morphometric analysis of grain outlines suggested that 2-row and 6-row barley caryopses could be distinguished, when experimentally charred (Ros et al., 2014). This first result, initially based on a small number of varieties, was later confirmed by two other publications (Bonhomme et al., 2017; Wallace et al., 2019). More recently, the discrimination between 2-row and 6-row barley and between hulled and naked forms has been further explored, using a larger comparative collection of 58 modern varieties and a larger number of caryopses (Jeanty et al., 2023). While all of these studies paved the way for an understanding of grain morphometric variation, many questions remained, particularly regarding the possibility of identifying charred archaeological grains. On one hand, the effect of charring on the grain, whether burnt husked or dehusked, had never been tested experimentally. While archaeobotanists often assume that ancient barley grains were burnt husked, the morphometric references used to identify archaeological barley are often based on charred dehusked caryopses. However, previous experiments on glume wheats have shown that the presence or absence of the husks during charring has an effect on grain preservation and distortion (Boardman and Jones 1990; Braadbaart 2004).

As in barley spikelets grains are only protected by the palea and lemma, while those of hulled wheat also have outer husks, it became clear that the effect of husk protection on grain shape needed to be investigated on barley. On the other hand, the impact of charring on the ability to identify ancient grains is not yet fully understood and previous studies have used reference collections consisting of either charred (Wallace et al., 2019) or uncharred grains (Bonhomme et al., 2017; Jeanty et al., 2023), or a mixture of both (Ros et al., 2014).

This paper aims to ensure a more accurate identification of archaeological barley using geometric morphometrics applied to grain shape variation between 2-row/6-row and hulled/naked barley before and after experimental charring. Our aims are threefold: (1) to assess the effect of charring on the shape of barley caryopses depending on their subgroup and on whether they were charred husked or dehusked; (2) to select the best dataset for identifying charred barley grains; and (3) to identify a series of 7 archaeological samples, used as case studies, to contrast the morphometric identification with carpological identification.

Material and methods

Collection of modern varieties

A total of 113 accessions belonging to 105 known varieties were sampled in two different conservatories: the Small grain cereals Biological Resources Centre (INRAE, Clermont, France) and the Secobra Recherches society (Maule, France) (SI Table I). For each variety, a total amount of 50 spikelets was selected: 20 were kept husked, while 30 were randomly selected and manually dehusked to extract the grain.

Experimental charring

Husked and dehusked grains were experimentally charred to make them comparable to archaeological grains. The charring protocol was adapted from the literature (Boardman and Jones 1990; Braadbaart 2004; Ferrio et al., 2004; Ros et al., 2014; Charles et al., 2015; Berihuete-Azorín et al., 2019) and allows for both homogeneous charring of the grains and minimal number of damaged grains. Each husked and dehusked grain was wrapped in aluminium foil, buried in sand to obtain reducing conditions, and charred in a Thermolyne type 48000 oven at 240°C for 210 min. After charring, the spikelets were dehusked and the grains extracted. A total of 2412 uncharred and 3985 charred grains, including 2412 charred dehusked and 1573 charred husked caryopses, were analysed (Table 1). From now on we will mention ‘dehusked caryopses’ when referring to grains already without glume, while ‘husked caryopses’ will correspond to those still with glume after charring.

	2-row hulled	2-row naked	6-row hulled	6-row naked
Uncharred caryopses	872	248	1048	244
Charred dehusked caryopses	872	248	1048	244
Charred husked caryopses	617	140	690	126
Total number of charred caryopses	1489	388	1738	370

Table 1. Number of caryopses analysed by charring condition and subgroups.

Archaeological samples

A total of 700 charred archaeological caryopses were analysed (Table 2). They were recovered from 7 ancient settlements dating from the Late Neolithic to the High Middle Ages, located in the north-western part of the Mediterranean basin (Fig. 1). All the archaeobotanical assemblages studied were well preserved and correspond to closed finds (grains were deposited all at once). In this case, only grains which present no morphological damage and whose outer limit can be clearly delineated without showing any obvious deformation were taken into account. They were all carefully examined under a stereomicroscope by expert carpologists who identified them as 2-row or 6-row, and as naked or hulled, according to macroscopic criteria (Jacomet, 2006). However, at Zac du Mas des Abeilles, identification remained restricted to ‘naked barley’ due to the variable morphological characteristics of the grains. A total of 100 complete caryopses were selected from each sample, with one sample per site.

Site name	Cadenet et Perrieres	ZAC du Mas des Abeilles	Dassargues	La Cougourlude	Château Royal	Bourbousson III	Baume Layrou
Town	Sernhac	Nîmes	Lunel	Lattes	Collioure	Crest	Trèves
Latitude	43,91122	43,81367	43,68477	43,56386	42,52563	44,72817	44,08861
Longitude	4,555323	4,3684129	4,156787	3,902262	3,084791	5,022559	3,403056
Sample identification	US 1753	US 2014	US 3027	US 31007	US 1016	US 616a inf.	sector 15
Chronoculture	Roman Empire	Late Neolithic	High Middle Age	Iron Age	High Middle Age	Roman Empire	Late Bronze Age
TPQ	1 CE	3500 BCE	900 CE	510BCE	1135 CE	275CE	1200BCE
TAQ	200 CE	3100BCE	1100CE	475 BCE	1200CE	300 CE	900 BCE
Mean TPQ/TAQ	100,5 CE	3300BCE	1000CE	492,5 BCE	1167,5 CE	287,5 CE	1050BCE
Reference	Bouby 2014	Bel 2009	Ros et al. 2019	Daveau 2014	Ros 2020	Bouby 2001	Fages and Bouby 2006
Carpologist	Bouby	Figueiral	Ros	Figueiral & Bouby	Ros	Bouby	Bouby
2-row/6-row carpologic identification	6-row	undetermined	6-row	6-row	6-row	2-row	6-row
Hulled/naked carpologic identification	hulled	naked	hulled	hulled	hulled	naked	hulled
Archaeological context	Fireplace	Pit	Silo	Well	Silo	Destruction of an antic building	Cave

Table 2. The 7 archaeological samples studied. Code = sample code; site name = name of the archaeological site; town = location of site; latitude; longitude; sample identification = name of the archaeological sample available in carpological publication; Chronoculture; TPQ (Terminus post quem in years); TAQ (Terminus ante quem in years); Mean TPQ/TAQ (in years BCE (Before Common Era) or CE (Common Era)); Reference; Carpologist; Carpological identifications found in publication and archaeological context where ancient material was found.

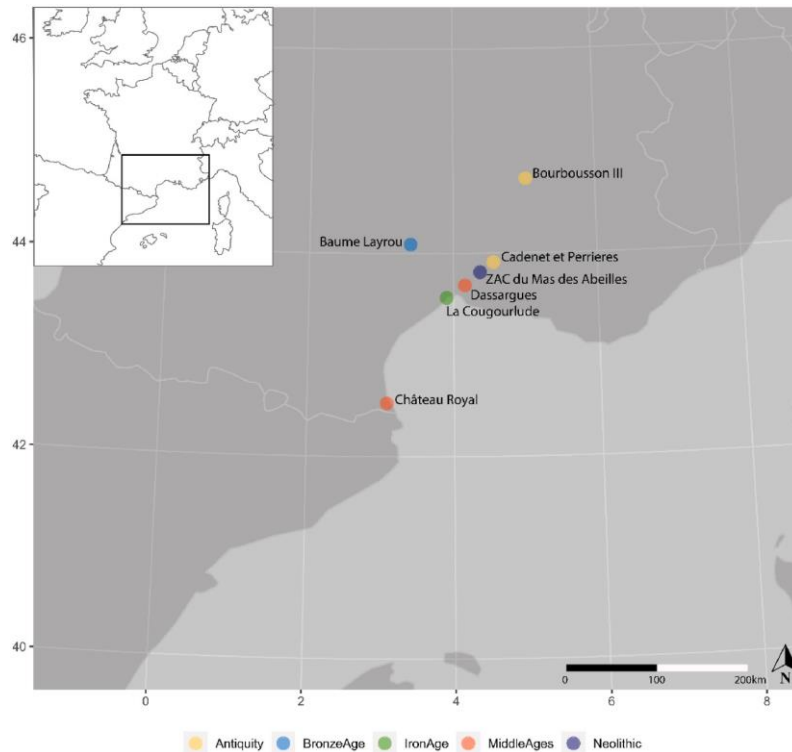


Fig. 1. Geographical location of the 7 archaeological sites: Baume Layrou, Bourbousson III, Cadenet et Perrieres, Château Royal, Dassargues, La Cougourlude, ZAC du Mas des Abeilles. Colors correspond to chronology: dark blue for Late Neolithic, light blue for Late Bronze Age, green for Iron Age, yellow for Antiquity and dark orange for High Middle Ages. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Acquisition of 2D images

All caryopses were photographed with modern samples photographed twice, before and after charring. Each grain was identified by an identification code, to allow a one-to-one comparison before and after charring (SI Table I).

Each grain was placed on plasticine with a scale bar and its lateral and ventral views photographed using an Olympus SZ-ET microscope coupled with an Olympus DP26 Camera. In the case of charred and archaeological grains, each photograph was carefully examined to eliminate caryopses whose morphology had been altered during charring. These changes include the presence of protrusion (i.e. albumen protruding from the seed) and partially split or blown grains (like popcorn). An explanatory diagram of seed checking is given in SI Fig. 1.

As the number of grains damaged during charring differed among varieties, the final number of charred grains (husked + dehusked caryopses) per variety varied between 18 and 105. Husked and dehusked caryopses were examined for each variety except one (Table I, SI Table I). On each photograph, the blue background corresponding to the plasticine used to position the grain was removed, and the resulting image was converted to a black mask using Adobe Photoshop CS6 software.

Morphometrics

On each mask, for both lateral and ventral views, 360 outline coordinates were automatically extracted using R (v. 4.1.3) and the Momocs package (v. 1.4.0; Bonhomme et al., 2014). Coordinates were scaled and

normalised before applying an Elliptic Fourier transform (EFT) approach (Giardina and Kuhl 1977; Kuhl and Giardina 1982). Outlines were decomposed into successive harmonics whose coefficients were statistically analysed. The number of harmonics retained to adequately capture the grain geometry was determined to be 5 for the lateral view and 7 for the ventral view (following Bonhomme 2012).

Statistics

Quantification of barley diversity

Principal component analysis (PCA) on the raw EFT coefficients was used to reduce the dimensionality of the data, assess the overall variation in grain shape and detect potential outliers. Analyses of variance (ANOVA) and multivariate analyses of variance (MANOVA) were then used to test for size and shape differences between the groups, respectively, while linear discriminant analyses (LDA) were applied to quantify the differences between them. Leave-one-out cross-validation accuracies (CVP) and their 90% associated confidence intervals were used as performance indices of the discriminant analyses.

MANOVA and LDA were first used to assess the differences in grain shape between those charred husked and dehusked. A 2-way MANOVA with subgroup and charring condition (husked vs. dehusked) as factors was used to assess the subgroup homogeneity of the charring effect on grain shape.

The same approach was then used to compare the 2-row vs 6-row and naked vs hulled subgroups using different data sets: uncharred grains, - dehusked charred grains, - husked charred grains, - the combination of these last two charred data sets, and – the combination of all grains including the charred and uncharred data sets. The uncharred data set, although not corresponding to an archaeobotanical state, was used to obtain the best possible discrimination values, unaltered by charring.

The ability to discriminate charred barley grains from uncharred reference samples and uncharred barley grains from charred reference samples was estimated, using 100 predictive discriminant analyses calculated on reference groups. The number of grains per group was constrained, by resampling to the size of the smallest group (pdam function in Evin et al., 2013, 2015). For each grain, the resulting percentage corresponds to the number of times it was identified as belonging to a particular group. The dominant identifications of the grains from one sample were then confronted with the carpological identifications, resulting in a percentage of grains correctly identified by the analysis. If it is possible to successfully identify charred grains from uncharred grains, it may not be necessary to char a modern reference collection before using it to establish the identification of the archaeological grains.

The structure of the morphometric variation was also captured using a phenotypic dissimilarity network, based on the Mahalanobis distances calculated in the discriminant space.

Shape differences were visualised using thin plate splines on mean shapes.

Identification of archaeological specimens

Once the dataset with the highest correct cross-validation was identified, it was used to assign archaeological grain to a subgroup.

Linear predictive discriminant analyses were performed on shape data to identify archaeological grains using the resampling approach previously used (Evin et al., 2015). In this study, only classifications with

posterior probabilities above 70% were retained. In order to move from seed by seed to entire sample identification, the majority within the sample is retained for the whole sample. For example, for a sample containing 73 seeds identified as 2-row and 27 identified as 6-row, the whole sample will be identified as 2-row barley. The morphometric identifications were then compared with the carpologist initial identifications, using Jacomet, 2006 criteria.

All morphometric and statistical analyses were performed using the Momocs, stats and MASS R packages to calculate CVP. The ggplot2 (v. 3.4.1) and patchwork (v. 1.1.2) R packages were used to visualise the results. Finally, all p-values were adjusted according to the Benjamini-Hochberg method (Benjamini and Hochberg 1995) using the p.adjust function (stats R package).

Results

Impact of charring conditions: husked vs. dehusked grains

Grains charred after dehusking or husked, differ in all size and shape comparisons (Table 3) and these differences are not homogeneous between subgroups (interaction terms of the MANOVAs are significant Table 3). Grains charred husked tend to be longer, wider and thicker than those charred dehusked (Table 3).

	Length		Width		Thickness		Lateral shape		Ventral shape	
	χ^2	p	χ^2	p	χ^2	p	F	p	F	p
Grains charred dehusked vs grains charred husked	20.315	6.568e-6	12.04	0.0005206	81.169	<10-15	28.37	<10-15	3.0959	<10-7
2-row vs 6-row	7.4063	0.0065	232.47	<10-15	88.083	<10-15	43.184	<10-15	22.749	<10-15
Hulled vs naked	22.4	<10-15	16.402	5.123e-5	35.546	<10-7	44.037	<10-15	8.6941	<10-15
2/6 rows * charring	3.552	0.0595	0.773	0.37936	9.044	0.00265	2.442	0.0003439	2.1984	0.0002784
Hulled/naked * charring	5.21	0.022511	1.7	0.1924	80.66	<10-15	8.623	<10-15	1.924	0.002431

Table 3. Effect of charring on grain size and shape variation for 2-row/6-row and hulled/naked barley. Results of groups differences using Kruskal Wallis tests for size (χ^2 and p-value) and MANOVA for shape (F and p-values). Results of interactions between factors using MANOVA for shape (F and p-values) and ANCOVA for size (F and p-value). (P-values are still significant after adjustment for multiple comparisons.)

Mean shapes of barley grains charred husked (blue outline in Fig. 2) differ from those charred dehusked (purple outline). Barley grains charred husked are wider and thicker than the others (Table 3, Fig. 2). The main differences, highlighted in red (Fig. 2) are located on the dorsal part of the lateral shape and on the outer edges of the ventral view.

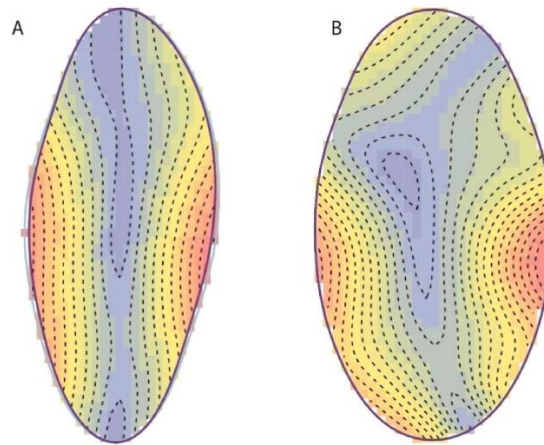


Fig. 2. Lateral (A) and Ventral (B) mean shapes representing the mean outlines of charred dehusked (purple outline) and husked (blue outlines) grains. Colors represent outline shape differences in both groups with warmer colors highlighting the areas that differ the most. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

However, despite these differences, charring conditions have relatively little effect on differences between groups (Fig. 3), considering different charring conditions cluster by subgroup.

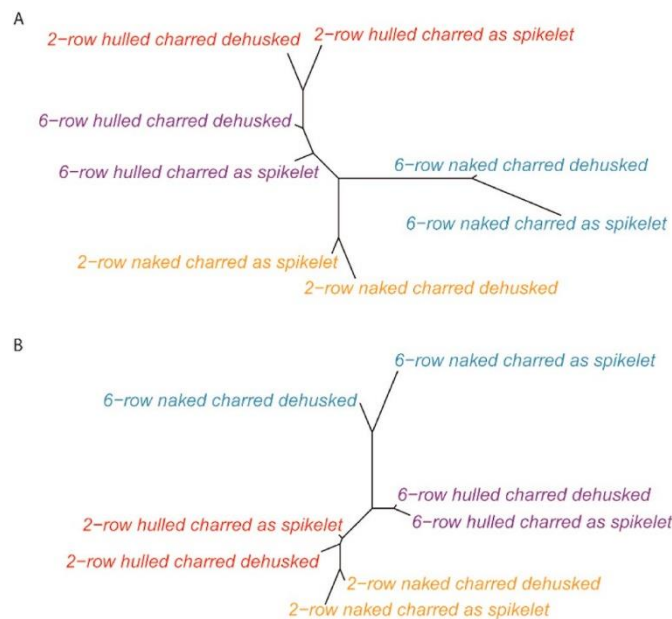


Fig. 3. Dissimilarity networks between barley subgroups charred dehusked and husked for the lateral (A) and ventral (B) views of the grain. Colors indicate barley subgroups: 2-row hulled in red, 2-row naked in orange, 6-row hulled in purple and 6-row naked in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Differences between barley subgroups

Differences between 2 row vs 6 row, and between naked vs hulled barley subgroups were assessed using 4 different data sets, depending on whether the grains were charred or not and how they were charred (i.e dehusked or husked). The highest cross-validated correct classification between 2-row vs 6-row barley was obtained for uncharred grains reaching 88.05% (CI = 87.92–88.19). By comparison, the various data sets of charred grains only reached ~71% (Table 4). Grains charred dehusked or husked, and the combination of

both gave similar percentages (Table 4). In all cases, the combination of the lateral and ventral views discriminated better between the two subgroups than each view separately.

	2-row vs 6-row			Hulled vs naked		
	VL	VV	VVVL	VL	VV	VVVL
Uncharred caryopses	77.64 [77.47–77.85]	80.54 [80.42–80.69]	88.05 [87.92–88.19]	82.57 [81.52–83.7]	70.37 [69.13–72.04]	82.52 [81.67–83.41]
Charred dehusked caryopses	67.68 [67.03–68.22]	67.71 [67.25–68.26]	71.25 [70.71–71.76]	73.48 [72.05–74.81]	59.64 [57.41–61.9]	70.73 [69.21–72.57]
Caryopses charred husked	68.94 [68.53–69.5]	65.68 [65.12–66.22]	71.87 [71.43–72.27]	72.65 [70.46–74.83]	58.56 [54.31–61.28]	72.26 [69.74–74.63]
All charred caryopses	68.14 [67.74–68.6]	66.33 [66.08–66.6]	71.41 [71.1–71.71]	71.8 [70.71–72.89]	59.77 [58.3–61.21]	69.72 [68.33–70.98]
All caryopses	71.62 [71.42–71.82]	70.81 [70.61–71.06]	75.79 [75.6–75.99]	75.96 [75.14–76.83]	61.64 [60.6–62.53]	74.34 [73.48–75.04]

Table 4. Percentage classification accuracy (cross-validated) of 2-row vs 6-row and hulled vs naked barley grains. Values were obtained from uncharred grains, charred dehusked grains, grains charred husked, the combination of the two charred categories, and the combination of all available charred and uncharred grains. Values were calculated for the lateral (VL), ventral (VV) views and their combination (VVVL); 90% confidence intervals are given in brackets.

When comparing hulled and naked barley, the highest discrimination was again obtained for uncharred grains reaching 82.57 % (CI = 81.67–83.41). Charred dehusked grains, grains charred husked and their combination allowed the two subgroups to be discriminated with a similar percentage of 71/72% (Table 4). In all hulled vs naked comparisons, the lateral view performed better than the ventral view and the combination of the two views did not perform better than the lateral view alone (Table 4). In the corpus of modern varieties, for all identifications, the combination of uncharred and charred grains allowed a better discrimination of the groups than charred grains alone, though lower than the uncharred grains alone. For the identification of 2-row vs. 6-row group, charred caryopses identified using uncharred barley grains gave similar values (75.7%) as uncharred identified using charred caryopses (73.7%). The identification of naked vs hulled subgroups also gave similar result for the lateral view, but the opposite for the ventral view and the combined lateral and ventral views (Table 5).

	2-row vs 6-row			Hulled vs naked		
	VL	VV	VVVL	VL	VV	VVVL
Charred caryopsis identifications using uncharred caryopsis	74.8	75.7	73.6	62.6	56.9	60.9
Uncharred caryopsis identifications using charred caryopsis	71.8	73.7	64.9	60.4	60.2	62.0

Table 5. Percentages of caryopses correctly identified between 2-row vs 6-row and between hulled barley vs naked barley, obtained after identification of charred caryopses by uncharred caryopses and identification of uncharred caryopses by charred caryopses. Percentages are given for lateral (VL), ventral (VV) and both combined (VVVL) views.

Identification of archaeological barley samples

To identify the charred archaeological barley grains (Table 6), we used the reference collection comprising all available grains, charred and uncharred, in order to capture as much diversity as possible along the gradient from strongly to slightly charred archaeological grains. Identification of 6-row barley predominated in six of the archaeological assemblages, with 60%–76% of the grains identified as such. The 2-row morphology was predominant in only one assemblage (Bourbousson III), where 37% of the grains were identified as 6-row, 62% as 2-row barley, 1 grain remaining unidentified. In the last assemblage,

almost half (47%) of the grains were identified as 2-row and the other half (52%) as 6-row barley, with 1 grain remaining unidentified.

Site name	2-row	6-row	Undetermined-rows	hulled	naked	Undetermined-husks	2/6 rows identification	Hulled/naked rows identification
Baume Layrou	40	60		73	25	2	6-row	hulled
Bourbousson III	62	37	1	12	88		2-row	hulled
Château Royal	37	62	1	29	70	1	6-row	hulled
La Cougourlude	24	76		70	29	1	6-row	hulled
Dassargues	25	75		54	45	1	6-row	hulled
ZAC du Mas des Abeilles	47	52	1	35	64	1	6-row	naked
Cadenet et Perrieres	33	67		75	25		6-row	hulled

Table 6. Identification of the 7 archaeological samples. Each sample is composed of 100 archaeological barley grains that were identified as 2-row or 6-row and as hulled or naked barley. The grains with all posterior probabilities below 70% remained unidentified. The two last columns provide the majority identification of the assemblage.

In terms of hulled vs naked identifications, the hulled form was predominant in six of the assemblages with 54%–88% of the grains identified as such. In one assemblage (ZAC du Mas des Abeilles), the majority (64%) of the grains were identified as naked.

These morphometric identifications were then compared with the carpological identification of the assemblages (Fig. 4). Five of the seven carpological assemblages (Baume Layrou, Château Royal, La Cougourlude, Dassargues, Cadenet and Perrières) were identified as 6-row barley by both morphometrics and carpology. The sample from Bourbousson III was identified as 2-row barley by both approaches. The last assemblage from the site of Zac du Mas des Abeilles for which the carpologist did not propose an identification regarding the 2–6 row type, was identified morphometrically as a mixed assemblage (Table 6).

Morphometric identifications as hulled or naked were all congruent with the carpological identifications with six assemblages being identified as predominantly hulled and only one, ZAC du Mas des Abeilles, identified as naked.

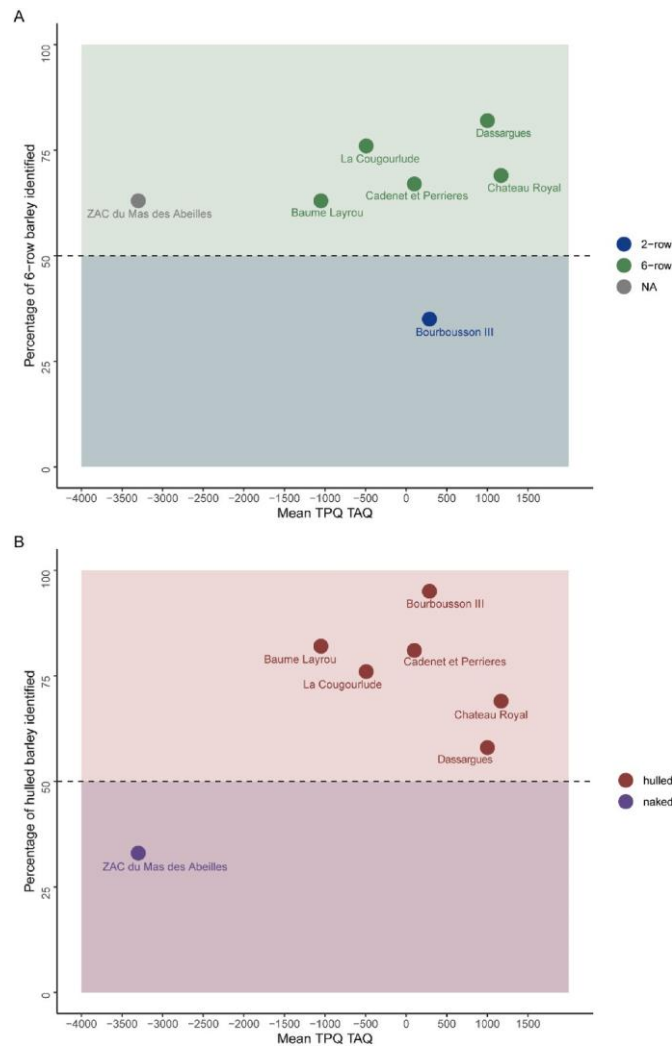


Fig. 4. Comparison of the carpological and morphometric identifications. The percentage of grains morphometrically identified as 2-row/6-row and hulled/ naked was compared with the carpological identifications. Each point corresponds to a sample. The X axis represents time while the Y-axis represents the percentage of grains morphometrically identified as 6-row (A) or hulled (B). The colors of the dots represent the carpological identification: 2-row (blue), 6-row (green), hulled (red) and naked (purple) barley. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Discussion

Differential impact of charring on husked and dehusked grains

In this study, we demonstrate the role of husks during charring on grain morphology, and how the differences highlighted did not prevent the discrimination of 2-row vs 6-row and naked vs hulled subgroups. The overwhelming majority of archaeobotanical assemblages are preserved by charring, which has a strong influence on grain preservation and morphology (Boardman and Jones 1990; Gustafsson 2000; Charles et al., 2015; Stroud et al., 2023). Previous morphometric studies concluded that charred grains can still be identified, albeit with less accuracy than uncharred modern grains (Ros et al., 2014; Bonhomme et al., 2017; Wallace et al., 2019).

However, these studies were carried out exclusively on modern caryopses that were dehusked prior to charring; a condition imposed by the fact that the morphology of non dehusked grains cannot be assessed, due to the presence of the husk. Charring, with or without the husk, can have a marked effect on grain preservation (Boardman and Jones 1990; Braadbaart 2004). In our archaeological corpus, the presence of husk fragments on barley grains among the best-preserved archaeobotanical assemblages suggests that they were commonly charred husked (Ros and Ruas 2017; Ros 2020). This is why our work aimed to understand how the presence of the husk on the grain during charring influenced the resulting morphology of the grain.

We have shown that, depending on whether they are charred husked or dehusked, grains differ morphologically after charring. Grains that were charred husked are longer, wider and thicker than those dehusked before charring. Charring results in a decrease in grain length, and an increase in width and thickness, leading to a homogenization of grain shapes (Charles et al., 2015). Hence, the presence of husks does not constrain the grain, which would have resulted in its smaller size compared to dehusked grains. An explanation may be that gaseous components that cause the grain to increase in size remain trapped in the husks during the charring process, which may result in the larger size of the caryopses charred husked.

Charring and taxonomical identification

However, these morphometric variations do not prevent the identification of the 2/6-row and naked/hulled barley subgroups. Our modern reference collection, consisting of 105 varieties and 2412 caryopses, allows to discriminate the 2/6-row subgroups with a probability of 88.05%, and 82.57% as naked or hulled when the grains are uncharred. As expected, these probabilities decrease with charring. Using the same reference collection, only 71.87% of the grains are correctly identified as 2-row or 6-row, and 73.48% as hulled or naked. Interestingly, equivalent performance is obtained when modern grains are charred husked or after dehusking. Our results therefore confirm previous geometric morphometric studies, which demonstrated that modern caryopses reference collections are suitable for identifying 2-row/6-row and hulled/naked barley subgroups, even when applied to charred barley grains (Ros et al., 2014; Bonhomme et al., 2017; Wallace et al., 2019).

For the 2-row/6-row identification, the percentages of correct identifications of charred barley grains from an uncharred barley reference sample are similar to the CVP obtained from charred reference samples. However, concerning the distinction hulled/naked, the identification of charred caryopses from uncharred barley grains is less efficient (62.7%) than the identification from charred barley grains (71.8%). In addition, the correct cross-validation percentage for identifying 2-row/6-row and hulled/naked is higher when combining uncharred and charred caryopses than using charred caryopses only. The combination of charred and uncharred caryopses therefore appeared to be the best approach for identifying ancient grains. It can potentially accommodate a wide variety of charring conditions, while at the same time providing good enough percentages for the identification of archaeological assemblages. Thus, if applied to large numbers of archaeological samples, GMM could be a key technique for tracing long-term and large-scale changes in the cultivation of barley types.

Identification of ancient barley grain samples

Overall, our results demonstrate the ability to identify barley subgroups with probabilities good enough to be applied to archaeological charred grains. This is an important result as, in many cases, with no traces of lemma or palea on the surface of the grain, it is difficult to estimate whether they have been charred before or after dehusking.

The distortion of cereal caryopses is highly sensitive to charring conditions, particularly temperature and oxygen availability (Boardman and Jones 1990; Braadbaart 2004). Well-preserved archaeological grains are generally comparable to caryopses that were experimentally charred at relatively low temperatures (220–240°C) (Charles et al., 2015). Indeed, archaeological grains charred at high temperatures are unlikely to be included in morphometric analyses, as they are often too distorted and poorly preserved. However, the archaeological grain samples we studied are the result of likely variable and unknown charring conditions. It is therefore important to be able to identify charred barley grains with high CVP values. In order to maximise the variability taken into account for the identification of archaeological grains, we decided to use a reference collection combining uncharred and charred grains, including grains charred dehusked and husked. Such a combination gives an accuracy of 77.5% for 2-row/6-row discrimination and 78.1% for hulled/naked.

The applicability of outline morphometric analysis to identify archaeological material is supported by 7 case studies. These sites were selected because they yielded closed assemblages of well-preserved barley grains, dating to different periods and confidently identified by several archaeobotanists as belonging to the different barley subgroups. Barley grain assemblages were characterised by retaining the dominant identification for the whole sample. All the samples used as case studies show congruent carpological and morphometric identifications. It is worth noting that concerning the sample from Mas des Abeilles not assigned to either 2-row or 6 row types by the traditional archaeobotanical study, the morphometric identification gives a mixed signal, with only a majority of 52% assigned to the 2-row type. It is possible that the sample is a mixture of 2-row and 6-row naked forms. However, it should be noted that none of the 7 assemblages have 100% of their grains identified to one single group, at best, 88% of the grains show the same identification. This could mean that all assemblages are mixtures in varying proportions or alternatively, and more likely, that not all grains are morphologically typical of the group they were attributed to. This is also suggested by the modern reference material where only 88% of the grains can be re-assigned to the correct subgroup. The methodology we have developed and the modern and archaeological samples we have studied offer new perspectives for the study of the bioarchaeological history of barley. These approaches are not free from the problems inherent in archaeobotanical remains, such as the effect of carbonisation on grain preservation, or the difficulty of drawing firm conclusions when few remains have survived. Nevertheless, the framework we have used will improve our knowledge of barley and could be applied to other species such as wheat, which also has a complicated taxonomy, or other regions such as the Near East, where domestic barley was first domesticated in the Neolithic.

Conclusion

Whilst the identification of archaeological barley caryopses using traditional macroscopic criteria remains challenging, we demonstrate here the ability of grain shape analysis using the Elliptic Fourier Transforms to distinguish the four main subgroups traditionally recognized for barley. Although it cannot account for the full diversity of shape changes caused by charring events, the proposed method provides a quantitative tool for identifying charred grains with satisfactory success. Our results demonstrate that geometric morphometrics can be applied on a large scale to archaeological samples to identify the main barley subgroups. Morphometrics provides a quantitative, probabilistic approach that is applied to individual grains. However, we advise caution when looking at the identification of individual grains, and instead consider the results at the scale of archaeobotanical samples composed of several grains, as we have done in the present paper. Morphometrics remains a very useful tool to complement carpological identifications using a subsample of an assemblage, or to identify assemblages of grains that are not readily identifiable, e.g. due to surface abrasion. This non-destructive method can be coupled with biomolecular approaches, such as isotope analysis or radiocarbon dating. The next step should be to compare the central and lateral grains of 6-row barley with 2-row barley. This could reduce the percentage of error associated with the identification of charred caryopses, as some grains were initially recognized as 2-row, but they could actually correspond to 6-row central grains. Other lines of research will investigate the diachronic evolution of the different barley types, their past distribution in time and space and their relative abundances, all of which will shed light on human selection and preferences in terms of agriculture and economy.

Data reporting

Morphometric data of current charred and archaeological barley are available here: <https://github.com/angelejeanty/Identification-of-a-rchaeological-barley-grains.git>.

Supporting information

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jas.2023.105924>

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Credit authorship contribution statement

Angèle Jeanty: Writing original draft, Methodology, Formal analysis, Conceptualization.

Jérôme Ros: Writing review & editing, Validation, Supervision.

Cyprien Mureau: Writing review & editing, Resources.

Camille Dham: Resources, Data curation.

Célia Lecomte: Resources, Data curation.

Vincent Bonhomme: Writing review & editing, Visualization, Validation, Methodology, Formal analysis, Conceptualization.

Sarah Ivorra: Writing review & editing, Visualization, Validation, Methodology.

Isabel Figueiral: Writing review & editing, Resources.

Laurent Bouby: Writing review & editing, Validation, Supervision, Conceptualization.

Allouen Evin: Writing review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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