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Geometric morphometric analysis of grain shape and the identification of two-rowed barley (*Hordeum vulgare* subsp. *distichum* L.) in southern France

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ABSTRACT

Hulled barley is one of the most frequently recovered cereals in European archaeological sites from Roman and medieval periods. In southern France this cereal is common in carbonized contexts such as cultural layers, ditches, pits, hearths, etc. The distinction between the two subspecies, two-rowed (*Hordeum vulgare* subsp. *distichum* L.) and six-rowed barley (*H. vulgare* subsp. *vulgare* L.) is usually based on morphological characters. The following criteria can be used to discriminate both subspecies from archaeological remains: the number of fertile spikelets per rachis segments, the linear or horseshoe shape depression of the lemma base, the maximum width of the caryopses and the proportion of twisted grains. The recovery of thousands of caryopses, some clearly twisted, and of rachis segments with sterile spikelets from the site of Petit Clos (Perpignan, Pyrénées-Orientales, France) dating to the Roman period suggests that both subspecies were cultivated during this time in southern Gaul. However evidence for two-rowed barley is usually scarce in archaeobotanical reports from Roman and medieval sites. To confirm the presence of two-rowed barley in the carbonized assemblage from Petit Clos and its cultivation, we developed a new method for analysing caryopses shape using geometric morphometrics with landmarks and sliding semi-landmarks. We compared modern reference specimens to the archaeological grains from several excavations from southern France dating from the 1st to the 11th century AD. Several varieties of both subspecies were correctly identified in the modern reference sample using GMM, both before and after carbonization. Archaeological specimens could then be accurately identified. The results confirm that both subspecies of barley were cultivated in southern France during the Roman period.

1. Introduction

Barley (*Hordeum vulgare* L.) has been a staple food source for large portions of the world population since the advent of agriculture. Nowadays hundreds of varieties are known, grouped together as one species, *H. vulgare* (Zohary et al., 2012) comprising of two subspecies, *H. vulgare* susbp. *distichum* L.(two-rowed barley) and *H. vulgare* subsp. *hexastichum* L. (six-rowed barley). The domestication of two-rowed barley (*H. vulgare* subsp. *spontaneum* (C. Koch) Thell.) occurred between 9000 and 8000 cal BC, during the PPNB, in a region between Israel and Syria (Fuller et al., 2012; Tanno and Willcox, 2012; Zohary et al., 2012). Six-rowed forms appeared soon after during the 8th millennia cal BC, a period that shows evidence of increasing intensification of agriculture and domestication (Zohary et al., 2012). Cultivated forms could have been spread from this area by human migration to the Near East, Middle East and Europe (Colledge and Conolly, 2007a). Two-rowed and six-rowed barley are both frequently found on the same sites in Greece from the Neolithic to the Bronze Age (Sarpaki, 1992). Two-rowed barley has also been identified in Neolithic sites in southern Italy (Constantini and Stancanelli, 1994), in three Roman sites in southern France, Le Villard (Puy-de-Dôme, 2nd c. A.D., Bouby, 2001), Bourbousson III (Drôme, 3rd c. A.D., Bouby, 2001) and Petit Clos (Pyrénées-Orientales, 1st c. A.D., Ros, 2010) and is suspected in an early medieval site in the same area of southern France, Manresa (Pyrénées-Orientales, 7th c. A.D., Ruas, 2007 unpublished).

Several criteria are used to discriminate the subspecies in archaeological remains (literature compiled by Jacomet, 2006)

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based on external morphological features. Two-rowed barley is characterized by a single fertile spikelet per rachis segment (the two others being sterile), a horseshoe-shaped depression in the lemma base, and straight shaped grains whose maximum width is below the centre of the grain. By contrast, six-rowed barley develops three fertile spikelets per rachis segment, the depression in lemma base can be either linear or horseshoe shaped and the maximum width of all grains is at the centre.

Another important feature to consider is the straightness of the grain, although this criteria should be used with caution. At each rachis node of six-rowed barley the grains on lateral spikelets are usually twisted while the central grain is straight. The expected ratio of twisted versus straight grains in an assemblage is therefore two to one (literature compiled by Jacomet, 2006). However a small proportion of slightly twisted grains may be present in two-rowed barley, as observed on fresh material (Bouby, 2001). The presence of twisted lateral grains in six-rowed barley is probably due to the presence of three spikelets per rachis segment but a similar reason cannot account for the presence of twisted grains in two-rowed barley and no alternative explanation has been proposed to date.

While these criteria to distinguish the two barley subspecies have been extensively used, most archaeobotanists have expressed reservation towards their use and prefer using "hulled barley" to refer to either subspecies. In the Roman site of Petit Clos, numerous charred hulled barley caryopses were found ($N = 3041$) with only a few chaff remains (lemma bases ($N = 91$) and rachis segment ($N = 6$) (Ros, 2010)). Based on lemma bases and rachis segment criteria, both two- and six-rowed barley were identified. Nonetheless the very small proportion of twisted grains (2%, $N = 61$) favoured the hypothesis of the predominant presence of two-rowed barley. As traditional morphological criteria cannot reliably identify the caryopses, other criteria were explored.

Alternative criteria used to discriminate the caryopses are univariate measurement such as their length, width, and thickness. These were first tested at the Roman site of Bourbousson III (Bouby, 2001) and archaeological charred two- and six-rowed barley grains were both successfully identified and discriminated, confirming potential biometric differences between the two subspecies (Bouby, 2001). In addition the central and lateral grains in six-rowed barley have shape differences, the latter being often narrower than the former. Therefore, six-rowed barley should display greater variation in grain measurements than the two-rowed subspecies that only have central grains. The grains sampled from Bourbousson III and Petit Clos were found to be biometrically similar, indicating the predominant presence of two-rowed barley grains at Petit Clos. Further comparisons with the site of Castlar (Tarn, France, Ruas, 2002) where only six-rowed barley was identified show a large difference in the biometrics of seeds between the two sites (Ros, 2010). The large differences between the six-rowed grains of Castlar and the unidentified caryopses of Petit Clos provide more evidence that the grains of Petit Clos were mainly composed of two-rowed barley (Ros, 2010). Two-rowed barley was therefore most likely present in southern France during the Roman period. Its persistence in this region into the Middle Ages still remains to be investigated.

The comparison of grains measurement is shown to be a useful approach. Nonetheless, this method does not allow identification of individual caryopses. To address this, a precise quantification of the proportion of subspecies in archaeobotanical samples is required. We developed a new method of analysing the shape of caryopses using Geometric morphometrics (GMM). GMM (Adams et al., 2004; Rohlf and Marcus, 1993) allows a more precise quantification of shape than traditional morphometric approaches.

The application of these methods on archaeobotanical remains is increasing, especially on fruits such as olive (Terral et al., 2004), grape (Bouby et al., 2005–2006; Terral et al., 2010), cherry (Burger et al., 2011) and dates (Terral et al., 2012). Nonetheless, these methods have seldom been applied to cereal remains (Apuan et al., 2011) and to our knowledge never to archaeological cereal remains. We chose to apply GMM to archaeological caryopses, as compared to other archaeological cereal remains, grains have the greatest rate of survival (Boardman and Jones, 1990) and are the most common remains in European post-Neolithic archaeological sites (Jacomet and Kreuz, 1999; Colledge and Conolly, 2007b).

We used ten modern cultivated varieties belonging unambiguously to the two subspecies in order to determine if modern caryopses of two-rowed and six-rowed barley can be identified using GMM. This modern reference data was then used to identify archaeological caryopses. Since carbonization can have a strong effect on grain shape (Boardman and Jones, 1990), the fresh referential was analysed before and after experimental carbonization of the dehulled grains (without the lemma and palea).

The archaeological material comes from the sites of Petit Clos and Bourbousson III where the presence of two-rowed barley has been claimed in previous studies (Bouby, 2001; Ros, 2010). To explore the possibility of a continuous presence of two-rowed barley after the Roman period, two Mediterranean medieval sites were also included: Manresa (Pyrénées-Orientales, 7th A.D., Ruas, 2011) and Dassargues (Hérault, 10th A.D., Ros, 2009). The results obtained will provide important information on the role and status of the two barleys during Roman and medieval periods in southern France and show the potential for using GMM in cereal grains identification.

2. Material: archaeological and reference

2.1. Modern reference material

A total of 300 modern dehulled caryopses were analysed. The modern reference sample included five varieties of two-rowed barley (Clarine, Mascara, Mystic, Nectaria, Pastoral) and five varieties of dense-eared six-rowed barley (Actuel, Atenon, Cartel, Esterel, Marcorel) (Table 1). Isolated spikelets were obtained from the Secobra society and grown in Maule's fields (Yvelines, France). For each of the 10 varieties, 30 grains were randomly selected regardless of their central or lateral position on six-rowed barley grains, dehulled and analysed.

The hulls were removed to permit measurement of the grains. After being photographed the 300 caryopses were carbonized in a muffle furnace oven (Nabertherm) to simulate as accurately as possible the effect of carbonization. The grains were charred in reducing conditions, separately wrapped in aluminium and buried in the sand at 250° Celsius for 50 min. This temperature was

Table 1
Hordeum subspecies, varietal denomination and number of modern caryopses analysed as reference material.

Taxa	Cultivated varieties	Number of caryopses
H. vulgare subsp. *distichum* (two-rowed)	Clarine	30
	Mascara	30
	Mystic	30
	Nectaria	30
	Pastoral	30
H. vulgare subsp. *hexastichum* (six-rowed)	Actuel	30
	Atenon	30
	Cartel	30
	Esterel	30
	Marcorel	30