Journal of Archaeological Science 86 (2017) 60–67

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Identification of inter- and intra-species variation in cereal grains through geometric morphometric analysis, and its resilience under experimental charring

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ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form
1 November 2016
Accepted 7 September 2017
Available online 14 September 2017

Keywords:
Elliptical Fourier transforms
Archaeobotany
Cereal grain
Experimental charring
Einkorn
Emmer
Barley

ABSTRACT

The application of morphometric analysis in archaeobotany has the potential to refine quantitatively identifications of ancient plant material recovered from archaeological sites, most commonly preserved through charring due to exposure to heat. This paper uses geometric morphometrics, first, to explore variation in grain shape between three domesticated cereal species, einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and barley (*Hordeum vulgare*), both before and after experimental charring at 230 and 260 °C. Results demonstrate that outline analysis reliably reflects known variations in grain shape between species and differences due to charring observed in previous experimental work, and is capable of distinguishing the species, with near-perfect results, both before and after charring. Having established this, the same method was applied to different accessions of the same species, which indicated that three different grain morphotypes of einkorn and two, possibly three, of emmer could be identified in the uncharred material, and that at least two different morphotypes for each species could be distinguished even after charring at temperatures up to 260 °C. This opens up the possibility of tracking evolutionary change in crops, both chronologically and geographically, through morphometric analysis.

1. Introduction

The taxonomic identification of ancient plant material found on archaeological sites is fundamental to its interpretation in terms of past plant use, agricultural practices etc., and these archaeobotanical remains are most commonly preserved through charring due to exposure to heat. Problems of taxonomic identification of charred plant remains are widely recognised (e.g. Jones, 1997; Hillman, 2000; Van der Veen, 1992). As well as natural overlap in morphology within and between species, distortion due to the charring process presents further difficulties for taxonomic identification. Cereal grains, which are largely composed of starch, are particularly susceptible to distortion through charring (Charles et al., 2015).

Although it is possible to identify well preserved cereal grains to species even after the distorting effects of charring, more subtle variations within species have not commonly been explored, due to the lack of reliable methods for distinguishing between different sub-species or varieties. Morphometric methods have been used to address intra-species variation for other archaeobotanical remains such as grape pips (Bouby et al., 2013; Terral et al., 2010), and Ros et al. have recently investigated grain shape variation between sub-species and varieties of barley (Ros et al., 2014). This type of investigation is best achieved through the analysis of variation in modern material where the species and source of the grain is already known, before attempts are made to apply the method to archaeologically preserved material where taxonomic identity must be inferred from the remains themselves. This paper explores

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http://dx.doi.org/10.1016/j.jas.2017.09.010
0305-4403/© 2017 Published by Elsevier Ltd.

the potential to refine quantitatively taxonomic identification though the geometric morphometric analysis of grain shape to determine the extent to which inter- and intra-species differences can be identified, both before and after charring.

Morphometrics, the description of shape and its (co)variation, encompasses three different approaches: "classic" identification, "traditional" morphometrics and geometric (also called "modern") morphometrics (Bookstein, 1991; Rohlf and Bookstein, 1990). Archaeobotanical identification is classically based on a series of diagnostic traits, including descriptions of shapes, that are assessed by eye, and that can be recognised consistently by trained specialists. Identification by eye, however, leaves limited capacity for quantifying variation within or between archaeobotanical assemblages. In contrast, a morphometrics-based approach allows shape variation to be directly quantified and, further, plant remains can be classified probabilistically. The ability to quantify grain shape variation also holds great potential for tracing past phenotypic variation in cereal populations both temporally and spatially, thus documenting diversity, chronological change and geographic movements of cereal crops.

"Traditional" morphometrics, the measurement of linear dimensions (typically length, breadth and thickness for grains) and calculation of ratios of these dimensions, is occasionally used to aid identification of archaeobotanical remains, for instance between wild and domesticated varieties (Colledge, 2001). Measurements are not, however, routinely taken in archaeobotanical studies.

Geometric morphometrics represent shapes by quantitative variables using a mathematical framework defined by the nature of the shapes studied. The manner in which this is achieved depends on whether there are many features present that can be landmarked, or whether curves, outlines and surfaces are the shapes' main homologous features. Recently, application of geometric morphometrics to archaeobotanical material has proven helpful to aid species identification (García-Granero et al., 2016) and, beyond this, to examine variation within species (Burger et al., 2011; Newton et al., 2006; Orrù et al., 2013; Pagnoux et al., 2014; Ros et al., 2014; Terral et al., 2012, 2010, 2004; Ucchesu et al., 2016). Studies to date have, however, focused on fruit stones such as grape and olive, while the application of geometric morphometrics to cereal grains has been treated with caution due to the known shape distortion caused by charring of starch-rich grain compared with the relative shape stability of woody fruit stones. As well as distortion depending on the type of material charred, the conditions under which charring occurred (e.g. temperature, oxygen availability, and, to a lesser extent, duration of heating) are also important (Bouby et al., *in press*; Charles et al., 2015; Ucchesu et al., 2016).

Previous work on cereals has demonstrated that grain distortion increases with charring temperature, with a noticeable difference between wheat grains charred at 230 and 260 °C (Charles et al., 2015). Grains charred at these temperatures are comparable to well-preserved grains recovered from archaeological sites, both in terms of appearance and internal structure as seen through scanning electron microscopy (Charles et al., 2015). At higher temperatures, grain shape changes more dramatically, making it difficult to distinguish species and even genera; bubbles may appear on the grain surface and, in extreme cases, the endosperm is exuded from the grain (Braadbaart, 2008; Charles et al., 2015). As intra-species differences are unlikely to be preserved where species or genus is indeterminable, this paper focuses only on grains charred under conditions that generate well-preserved remains. Charring also causes an overall reduction in size but, as size is not a useful characteristic for distinguishing between grains of domesticated wheat and barley, we have restricted our analyses to shape differences.

For morphometric analysis of charred archaeological cereal grains to be considered meaningful, it must be established that, for well-preserved grains (charred at relatively low temperatures), the effects of charring do not obscure or distort grain shape to the point where variation due to charring is greater than the inherent differences between species or between different populations within species. The ability of morphometric analysis to distinguish between grains of known cereal species is also an essential prerequisite for attempting to use the technique for exploring more subtle within-species variations. Having established this, an analysis of grain shape variation within species can follow.

Two key questions are therefore addressed: i) whether geometric morphometrics, specifically outline analysis using elliptical Fourier transforms, can satisfactorily distinguish modern grains of three domesticated cereal species commonly found archaeologically: einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and barley (*Hordeum vulgare*), in uncharred material and in material charred at 230 and 260 °C; and ii) whether any of the accessions of grains from different populations of the same species exhibit characteristic shape differences and, if so, whether these are still distinguishable after charring. An ability to identify plant populations using geometric morphometrics, despite morphological changes due to charring, would indicate that this approach would be applicable to the archaeobotanical record and could then be used to seek out distinct cereal populations in antiquity.

2. Materials and methods

2.1. Materials

Three accessions each of three cereal species, einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and barley (*Hordeum vulgare*), were included in this study. The accessions originated from various locations in Turkey, Jordan, Iran and Syria, and were provided by the John Innes Centre (UK), GRIN (USA) and IPK Gatersleben (Germany) (see supplementary material, Table A). At least 18 grains were sampled for each accession. For none of the accessions was the shape of the grains obviously distinctive, though grains of the einkorn accession Tm3 were unusually large compared to those of accessions Tm1 and Tm2. There was therefore no certainty, prior to analysis, that it would be possible to distinguish any of the accessions from others of the same species on the basis of their grain shape.

Grains were taken from spikelets throughout the ear, except for the very basal and terminal spikelets, where the grains are sometimes underdeveloped. Einkorn grains were taken only from one-grained spikelets and, for emmer, only two-grained spikelets were sampled (those containing one grain being, in any case, primarily from the bottom and terminal spikelets), and both grains in the spikelet were used. For barley, only two-row varieties were sampled. Wheat grains (einkorn and emmer) were dehusked by hand to remove the surrounding glumes. For barley grains (which were all of the hulled type), the paleas and lemmas were partially peeled off to expose the grain shape at the ends, and to better replicate archaeobotanical remains, where the complete hulls are rarely preserved.

Each grain was photographed in dorsal, lateral, and polar views, the latter capturing the cross-sectional shape of the grain (Fig. 1; see also Jacomet, 2008) using a Leica Z6 apochromatic microscope, Retiga 2000R camera and Media Cybernetics® Image Pro Premier 9 software®.

2.2. Controlled charring

Each grain was given a unique identification code to facilitate