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A novel morphometric method for differentiating wild and domesticated barley through intra-rachis measurements

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The main methods used for determining whether cereal remains from archaeological site were domesticated or not are based on the type of rachis scars and grain size. However, both ways suffer from uncertainties, and more reliable methods are in need. In this work we suggest such a novel differentiating method. We developed two statistical equations, one for unburned and one for burned (charred) wild (*Hordeum spontaneum*) and domesticated (*Hordeum distichum*) barley triplets. These equations are based on clearly measurable characteristics of the upper scars of triplets from modern barley populations. The equations yield the probability (P) that a triplet is from a domesticated variety. Using different batches of barley collected in Israel, it was confirmed that at $P > 0.8$ and $P > 0.7$ for unburned and charred triplets, respectively, domesticated barley was correctly identified at a certainty of >99%.

1. Introduction

Wild wheats and barley disseminate their grains via their brittle ears that disarticulate at maturity into individual dispersal units (called spikelets in the wheats and triplets in barley). In contrast to the wild types, all domesticated forms have non-brittle ears, which remain intact at maturity. Usually, the ears of hulled diploid and tetraploid domesticated wheats (mainly in einkorn and emmer wheats), as well as two-rowed barley, break during threshing at the same point on the rachis where their wild counterparts disarticulate spontaneously. In other words, threshing of the domesticated varieties exhibits the same shattering pattern as their wild progenitors, namely individual spikelets or triplets with the internode at their base (Fig. 1). However, instead of the prevalence of smooth abscission scars that characterize the wild forms, the breakage scars of threshed domestic types tend to be rough. In short: breakage scars in brittle/wild forms are mostly smooth, while those in non-brittle domestic varieties are rough.

In present-day archaeobotany, it is widely accepted that the most effective (and easiest to detect) diagnostic indication for domestication in archaeological cereal remains is finding rough disarticulation scars on the rachis internodes (see Fig. 1). The validity of this diagnostic indicator has been strengthened by molecular research that has shown that non-disarticulation is controlled primarily by a recessive mutated gene in einkorn wheat (Love and Craig, 1924) and by two such mutations in emmer wheat and barley (Takahashi, 1955, 1964, 1972; Zohary, 1969; Komatsuda and Mano, 2002; Nalam et al., 2006). Therefore, in studying the domestication status of wheat and barley from archaeobotanical assemblages, prevalence of distribution units showing rough scars serves as a critical indication of domestication.

Use of the disarticulation scar as a diagnostic tool is not fault-free. In a survey of wild and domesticated barley populations, Kislev (1989) found that about 10% of wild barley triplets do not disarticulate normally and show rough breakage scars (Kislev, 1989); all these triplets originate on the lower parts of the spikes. Additionally, Tanno and Willcox (2012) conducted an extensive survey of archaeological spikelets of hulled emmer and einkorn wheat from eleven Turkish–Syrian sites dated to the Pre-Pottery Neolithic A and B (PPNA-PPNB) periods. They concluded that human threshing/dehusking was the probable cause of the observed damage and fragmentation of the spikelets. Also, only when the archaeological abscission scar is well preserved is it possible to distinguish wild from domestic spikelets. In addition, barley triplets were found to be better preserved, presumably because their processing was less damaging. In most of these sites, there was a

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Fig. 1. Examples of wild barley triplets from Rosh Pina (batch 4). A – smooth scar, B – rough scar. Note that the lower scar in B is torn away.

mixture of wild and domesticated triplets. Regarding the PPNA site at el-Hemmeh, Jordan, White and Makarewics (2011) used the presence of some 22% rough-scarred barley triplets to raise the possibility that the wild barley had been harvested immature. It was already suggested by Kislev et al. (2004) who found that signs of puckering (i.e. wrinkled testa), which is characteristic of younger, unripe grains (Kislev, 1997), were clearly observed in archaeobotanical assemblages. During the spikelets maturity process along wheat and barley ears, there is a gradual decrease in grain moisture. Severity of grains puckering, therefore, hints for the stage of grain maturity. These stages of grain maturity known as "milk-ripe", "yellow-ripe" and "full-ripe" (Percival, 1974), can be used to differentiate between these ripening stages in the assemblage.

Another, but less reliable, diagnostic trait for determining domestication is grain size. Seeds in cultivars are usually plumper and larger than those produced by their wild relatives. However, grain-size increase evolves slowly following the achievement of domestication. As Nesbitt (2002) noted, differences in the size and shapes of wild and cultivated grains are clearly visible in Pottery Neolithic finds, but they are less evident in the Pre-Pottery Neolithic. Likewise, Kislev (1980) already found out that small grained types (e.g. *Triticum pravicoccum*) were selected for millennia after wheat domestication. Recently, Gegas et al. (2010) explored the genetic basis of the variation in wheat grain size and shape. Their work demonstrated that "grain size has progressively increased through alterations both in grain width and length, followed at later stages by modifications in grain shape." Thus, grain size increase in domesticated grain remains is not a fully reliable indication of early stage domestication (see details in Zohary et al., 2012).

Based on the above, it is clear that a novel approach is needed to enforce disarticulation scar types as a fully reliable diagnostic trait for determining whether archaeological, or recent, dispersal units belong to a wild or domesticated type. We attempt here to refine this method for identification. More specifically, we have established, as our first case, a new diagnostic protocol for barley triplets from Israel. The approach is based on clearly measurable parameters of the triplets and their scars, determinations easily carried out on modern samples as well as on ancient remains. Identifications using the new and previously adopted abscission scar approach will be compared.

The following steps were taken (in chronological order):

1) Since wild cereals have been found to contain rough-scarred dispersal units, we examined scar morphology from triplets of several different natural (wild) barley populations in Israel in order to arrive at a more precise evaluation of the percentage of rough-scar triplets in these plants. We followed Kislev (1989), but surveyed >800 ears, in comparison to his 60.
2) As widely acknowledge and recently detailed by Tanno and Willcox (2012), complete spikelets are very rare in archaeobotanical assemblages. In most cases, the lower part of the barley (or wheat) dispersal unit, along with its lower scar, is missing. For this reason, in archaeological findings, one mainly examines the upper scars. We therefore defined the following additional parameters to serve as a basis for distinguishing between wild and domesticated barley: (a) the maximal width of the triplets; and (b) the width; (c) length; and (d) area of the upper scars. Wild and domesticated, two-rowed, modern barley were used in the study to generate correlations between those parameters and the plant of origin. In doing so, we adopted the Probit statistical model. This approach enables devising a probability equation built on a set of input parameters – here, the additional defining parameters chosen for barley – that are associated with a binary variable, namely one with only two possible outcomes – here, domesticated or wild barley. The equation was then used to calculate probabilities of new batches of wild and domesticated barleys in order to test the utility of this approach to distinguish between the two.
3) As charring generally alters the dimensions of archaeobotanical finds, we repeated our previous work using charred barley triplets from contemporary wild and domesticated populations and used their measurements to generate another equation, which was calibrated for heating effects.

2. Materials and methods

2.1. Plant material

Ears (or spikes) of modern wild barley were collected from eight locations across Israel (Table 1). Batches 1–5 were collected in northern Israel, while batches 6–8 were collected in central Israel. When relevant, the degree of maturation of the ears was classified after Kislev (1989) as unripe (green), medium-ripe (yellow-green) and ripe (yellow). The triplets were removed from the ears on the day of collection, and were stored in paper bags for further analysis. Additional batches of domesticated two-rowed barley were used as well; batch 9 (*Hordeum distichum* Noga and Ruth cultivars) was obtained from the Institute for Cereal Crops Improvement, Tel-Aviv University (harvested in 2011), and batch 10 was collected in 2012 from a test field in Havat-Mivhor near Kiryat-Gat (31°36′22″ N, 34°46′18″ E).

Table 1
Locations of collection sites for wild barley (*Hordeum spontaneum*).

Location (batch)	Date of collection
(1) Hoshaya (32°46′02″ N, 35°17′24″ E)	12.5.2011
(2) Ein Kamonim (32°55′09″ N, 35°25′54″ E)	12.5.2011
(3) Elifelet (32°56′59″ N, 35°32′36″ E)	22.5.2011
(4) Rosh Pina (32°58′11″ N, 35°32′18″ E)	22.5.2011
(5) Korazim National Park (32°54′44″ N, 35°33′54″ E)	22.5.2011
(6) Kiryat Ono (32°03′50″ N, 34°50′39″ E)	25.4.2011
(7) Kiryat Ono (32°03′50″ N, 34°50′39″ E)	6.4.2012
(8) Hadassim (32°17′05″ N, 34°53′28″ E)	9.4.2012