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Point count estimation of articulated husk phytoliths of foxtail millet and its prospective use in agricultural archaeology

Xue Shang ^{a, b, *}, Xin Wang ^{a, b}, Pengfei Sheng ^{a, b}, Changsui Wang ^{a, b}

^a Department of Archaeology and Anthropology, University of Chinese Academy of Sciences, Beijing 100049, China

^b Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China

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ABSTRACT

Many researchers have focused on the precise identification of phytoliths extracted from archaeological samples, and on the extraction methods of phytolith from all kinds of samples as well. Additionally, it is important to perfect the scientific method of phytolith quantification. Phytoliths, especially from the husk, are easily broken after being long-buried or during extraction treatment. These can result in uncertainties about quantification of grain numbers and varies under different conditions. Here we introduce a method of point count estimation of articulated millet husk phytoliths, and evaluate its accuracy. As a result, we successfully used the point count method into estimating the area of husk phytolith in unit weight of modern millet samples, which will broaden the available data in archaeobiological research. We suggest precise estimations can therefore be done with this method.

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1. Introduction

Phytolith analysis now plays a significant role in archaeobotanical research for its unique advantages in easier preservation and identification than any other plant macro-/micro-remains (Pearsall et al., 1995; Iriarte, 2003; Crawford, 2005; Harvey and Fuller, 2005; Lu et al., 2009a; Ryan, 2014). Recently, scientists have applied phytoliths to explore the origin and spread of crops (Lu et al., 2009a; Zhang et al., 2012; Ball et al., in press). First domesticated in the Yellow River valley, foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*) are regarded as staple foods in Northern China during the Neolithic period (Zhao, 2005; Crawford, 2005; Hunt et al., 2008; Barton et al., 2009). To date, with the relatively specific anatomical characters (Lu et al., 2009b; Zhang et al., 2011), husk phytoliths of millets extracted from archaeological samples have been frequently used to reconstruct ancient agricultural patterns (Harvey et al., 2005; Lu et al., 2009), especially where macrofossils were not well preserved (Crawford et al., 2005; Harvey et al., 2005; Ryan, 2014).

The now widespread use of phytolith analysis in archaeobotanical studies requires attention to be paid to the accuracy of

the results, and there is little attention paid to this. The husk phytolith of millets with undulated extremities tend to be fragmented after long burial and then during the extraction processes (Wang et al., 2014). The statistical results of millet husk phytolith are thus influenced by many factors, and makes comparison of results difficult compared to proxies of seeds or pollen. A helpful attempt to estimate the total amount of phytolith fragments of millets has been made, which showed that foxtail millet and common millet produce almost the same number of fragments after using the same procedures (Zhang et al., 2010a,b). Nevertheless, it still depends on the characteristics of samples and methods, which make results difficult to compare between different sites or samples, or even different labs. So a scientific-based estimation method needed.

The point count method provides a most efficient way of quantifying the total area of a phase in a plane surface (Inversen, 1964, 1969; Clark, 1982; Whitlock and Larsen, 2001), which involves selecting random points on the microscope slide and determining the percentage of points that overlies charcoal fragments in the eyepieces (Whitlock et al., 2011). It was Robin Clark (1982) who firstly used this method on charcoal analysis, and she also implied this method can be used for other constituents of sediments, such as diatoms, phytoliths, fungal spores or mineral grains. After that, this method became a standardized methodology for estimating the area of charcoal in pollen preparations and was widely used on reconstruction of fire history all over the world

* Corresponding author. Department of Archaeology and Anthropology, University of Chinese Academy of Sciences, 19A, Yuquan Road, Beijing 100049, China.

E-mail address: shangxue@ucas.ac.cn (X. Shang).

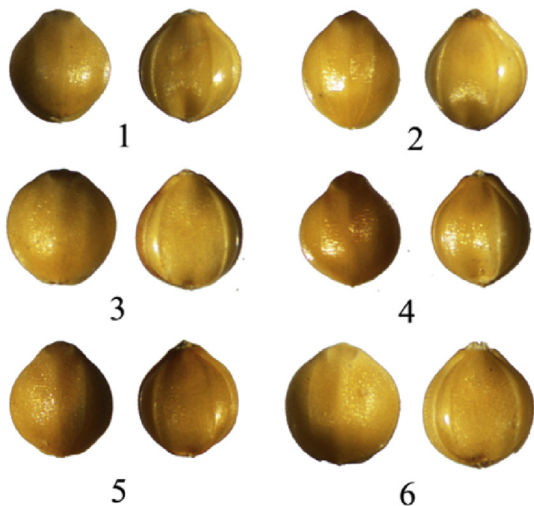


Fig. 1. Images of foxtail millet modern samples. 1. Cishangu; 2. Jigu No.14; 3. Zhuyeqing; 4. Donghuigu; 5. Cixuan No.6; 6. Jinzagu No.3.

(Clark, 1983; Odgaard, 1992; Maenza-Gmelch, 1997; Kershaw et al., 2002; Li et al., 2009; Ballard et al., 2016). Like charcoal particles, articulated husk phytolith of millets are visually recognizable as angular and usually planar fragments, which make it possible to use the similar method to estimate millet husk phytolith abundance.

Here, we introduce the method of point count into quantifying analysis of articulated millet husk phytoliths. In order to test its accuracy, the results of different methods will be compared by using six cultivars of modern samples of foxtail millet (*Setaria italica*) (Fig. 1, Table 1).

Table 1
Information of modern samples

Sample no.	Name of cultivars	Producing area
FM1	Cishangu	Hebei province
FM2	Jigu No.14	Hebei province
FM3	Zhuyeqing	Hebei province
FM4	Donhuigu	Hebei province
FM5	Cixuan No.6	Hebei province
FM6	Jinzagu No.3	Shanxi province

2. Materials and methods

Each sample was weighed to about 1 g. After traditional dry ashing process for modern plant phytolith extraction (Sun et al., 2012), a spike of *Lycopodium* spores was added to each sample in order to estimate the concentration of phytolith abundance

(Stockmarr, 1971). After pretreatment, microscope slides were made for phytolith analysis by the point count method. In the present study, phytolith abundance was estimated by counting the proportion of 5500 points of husk phytoliths that were touching an 11-point ocular scale in 500 different eyepiece views, as described by Clark (1982). Only points falling on particles greater than 5 μm were counted in order to reduce potential error from small particles that are difficult to assign as millet phytolith. By using this method we can get the total area per gram (S_1 , cm^2/g) of articulated husk phytoliths in our samples. Fig. 2.

The area concentration of millet husk phytoliths per gram was then calculated as follows:

$$\text{Formula 1: } S_1 = \frac{PL}{(M \times l \times W)}$$

S_1 : the area in unit of weight of sample (cm^2/g).

P: total point counted that phytolith overlies.

L: the concentration of the *Lycopodium* spores.

l: the counted number of *Lycopodium* spores.

W: the weight of sample (g).

M: the counted number of coordinate points of micrometer in the field of vision (usually $11 \times 500 = 5500$).

In order to test the accuracy of point count method in phytolith analysis, we select 100 grains of foxtail millet at random, and then measured the length, width and thickness of each grain. We made the calculation and statistics on the premise that these tiny grains were approximately oblate spheroids with length, width and thickness (Fig. 1). The following formula is used to calculate the approximate area of 100 millet grains. Then 100 millet grains were weighed. Finally, we can get the approximately total area of the husk phytolith for 1 g (S_2 , cm^2/g).

The approximate area concentration of millet husk per gram was then calculated as follows:

$$\text{Formula 2: } S_2 = \frac{\sum_{i=1}^{100} 4\pi(a_i b_i + b_i c_i + a_i c_i)/3}{w}$$

S_2 : approximately total husk area in unit of weight of sample (cm^2/g).

a: half value of length of one grain.

b: half value of width of one grain.

c: half value of thickness of one grain.

w: the weight of 100 grains (g).

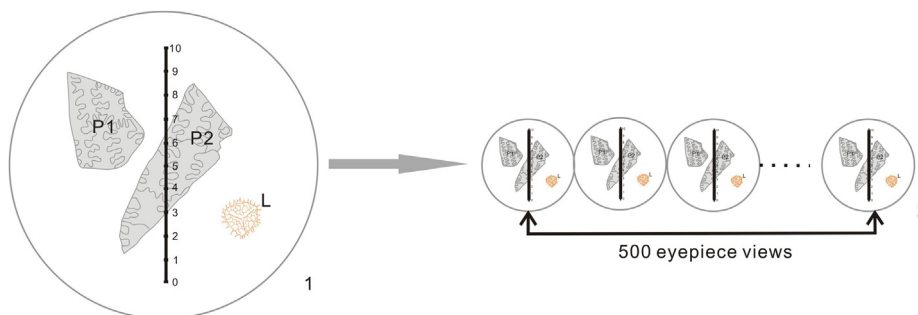


Fig. 2. Diagram of point count method in this paper. 1. A field of view with a standard eyepiece micrometer. In this case, of the eleven points defined by the cross on reticles nearest the numbers, four "touch" phytolith. 2. Then repeating estimation in 500 different eyepiece views.

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