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EXAMINING THE USE OF STARCH GRAIN RESIDUES TO IDENTIFY PLANTS PROCESSED IN STONE AGE CONTEXTS*

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ABSTRACT

Residues present on the surface of stone tools have the potential to identify the kinds of substances certain tools were used on and possibly elucidate tool function. In particular, it has been suggested that starch grains present from plant residues may be used to identify the genus or species of the plant. A study was conducted to determine the feasibility of extracting starch residues from grindstones and using starch grain size and morphology to determine the type of plant processed. A reference collection was established with various indigenous southern African plants. Starch grains extracted from the grind stones were compared with reference collection samples. Results indicate that using starch grain morphology and size as a means of plant identification in a Stone Age context is difficult, if not impossible.

INTRODUCTION

Stone tools of every kind are probably the most abundant archaeological material found on Earth. While their size and morphology have been used to place them into broad categories, there is very little about these two features that can tell us how they were used, and what kinds of materials they were used on. In recent years, progress has been made in use-wear analysis and the study of organic residues on the surface of stone tools. These two fields can now provide some clues as to tool use in the past. Most analyses of organic residues begin with the microscopic examination of the stone tools. A study was undertaken to examine the feasibility of extracting starch grains from tools such as grind stones that are too large to place under a microscope. The second part of the work was to examine the possibility of identifying plants worked on with these and other southern African tools based on the size and morphology of the starch grains.

Starch grains have been found to be present on the surfaces of stone tools from all around the world (Loy *et al.* 1992). These are usually present in large amounts on tools that can be examined under the microscope. Their abundance, as well as distinctive properties, has lead some researchers to claim that identifying these grains to the genus or even species level is possible (Loy *et al.* 1992; Barton & White 1993).

A number of techniques exist that have been used to identify starch grains according to their species of origin. These include both chemical and morphological methods. Chemical methods include the temperature at which the grains loose their structure, the rate at which they are affected by iodine and the rate at which they loose their structure when exposed to caustic chemicals. These techniques however are limited for use in an archaeological contexts due to the typically small number of starch grains preserved on tool surfaces as well as their destructive nature (Loy *et al.* 1992). Morphological properties such as size and shape have been used in several studies and appear to be more promising (Loy *et al.* 1992; Fullagar 1994; Barton 1994). In most studies, the tools were examined using light microscopy and tentative identification was based on the comparison of starch residues with a reference collection in terms of size and shape.

Starch grains are composed of two related polymers, amylose and amylopectin, and are formed within subcellular structures known as plastids. Plastids are defined primarily by their ability to produce starch and are divided into several types, of which chloroplasts and amyloplasts are the most important for archaeological purposes. Chloroplasts are mainly associated with leaves, contain chlorophyll and are involved in photosynthesis. Amyloplasts are found in the storage organs of plants such as roots, tubers and corms. Starch grains are formed from a nucleation point and grow by the accumulation of layers of amylose and amylopectin around this point which is commonly known as the hilum. When viewed under cross-polarized light, these layers, together with the quasi-crystalline nature of the amylose and amylopectin, produce a birefringent effect in the form of an "extinction cross" or "Maltese cross". This pattern

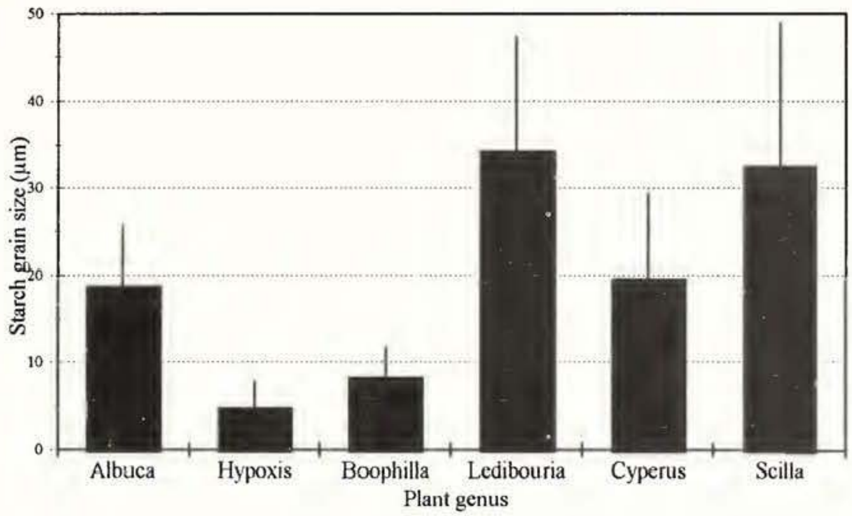

Fig. 1: Average size and standard deviation of reference collection.

consists of two dark bars crossing at the hilum with bright areas of light in between them.This characteristic of starch grains is very useful for distinguishing them from other residues.

OBTAINING THE SAMPLES

Twenty grindstones of varying size and shape were taken from two different levels from Rose Cottage Cave in the eastern Free State. All except three of the stones were too large to be examined using a microscope. Samples were taken at random from the entire surface of the tool, with particular attention being paid to cracks where microscopic residues would be expected to occur. Samples were obtained by applying $\sim 100\mu l$ of sterilised distilled water to the tool and scraping the surface to slightly loosen residues. The solutions were removed using a micro-pipette and placed into an eppendorf tube. Approximately $50\mu l$ of each sample was then applied to a glass slide and allowed to dry. Finally they were covered with a coverslip to protect the sample.

Each slide was examined using a transmitted light microscope. The samples were rehydrated using $40\mu l$ of ultra-pure water in order that the grains may regain their natural shape. All slides were examined within an hour of rehydration. The samples were initially scanned under polarized light at a magnification of 160x. If the characteristic "Maltese cross" was observed, the starch grain was photographed under a higher magnification under normal transmitted light and its sized recorded. The three smaller tools were examined directly under incident light for starch grains and other plant residues.

ESTABLISHING A REFERENCE COLLECTION

The plants used to establish the reference collection included the following genuses: *Gynandiris, Cyperus, Gladiolus, Hypoxis, Ledibouria, Albuca* and *Scilla*. All of the above plants have species that are edible or are known to have been used in a medicinal context in traditional societies. Only the bulbs, corms or their underground storage organs were used in establishing the reference collection. At least three samples of each genus were obtained in order to observe any variation within genuses. Reference collection samples were prepared by macerating tissue from each plant and applying a small amount to a glass slide. Two slides were prepared for each specimen.The first was stained with iodine and fixed semi-permanently using glycerol. The second was allowed to air-dry and protected with a glass coverslip. This was done in order to determine whether the effect of dehydration had any significant effect on the average size and morphology of the grains. As with the samples collected from the tools, these specimens were rehydrated using ultra-pure water before being examined under the microscope. Specimens from each genus were photographed at various magnifications. Average starch grain size was also determined. This was done by selecting five sites at random on each slide and measuring the minimum and maximum length of 20 starch grains at each site. Mean size and standard deviation are displayed in Figure 1. The reference collection samples are classified according to shape in Table 1.

RESULTS

The method described above proved effective at removing starch grains from grind stones. Of the twenty grind stones examined, thirteen of them had significant numbers of starch grains. In particular, Mn5 and Mn7 had samples with well over 100 grains. Where only a few grains were observed, futher sampling would hopefully provide a more significant number. It should be noted that the absence of starch grains in the samples from the

Table 1. Shape categories of the reference collection based on the classification by Fullagar (1994).

Genus	Shape category	Comments
Hypoxis	Round	Tend to be small
Albuca	Elliptical	Characteristic notch, smaller grains tend to be round
Gynandiris	Elliptical	Fairly irregular in shape
Ledibouria	Elliptical	Larger grains tend to be irregular
Scilla	Round / Elliptical	Smaller to medium grains tend to be round
Gladiolus	Sub-angular	Sometimes two or more facets

Table 2. Size and shape category of starch grains found on grindstones.

	Mn1	Mn3	Mn5	Mn7	Mn9	A1	A2	A4	A6	A7
Round	8μm - 10μm		5μm - 15μm	4μm - 23μm		4 8μm -		6μm	1.6μm - 5μm	12μm - 27μm
Sub-round	10μm - 13μm		5μm - 8μm		7μm - 10μm	9μm		4μm - 12μm	8μm - 13μm	10μm - 19μm
Eliptical	15μm - 23μm						4.5μm - 4.8μm		11μm - 19μm	11μm - 21μm
Angular	12.5μm - 25μm				12μm		14μm	26μm	14μm - 24μm	
Sub-angular		9.5μm							14μm - 16μm	
Undefined				12μm	14μm			12μm		

other seven tools may be due to sampling error and is not necessarily an indication that they were not used to work plant resources.

Starch grains were classified according to their basic shape as described by Fullagar (1994) (Table 2). It was noted that dehydration had little or no effect on the size or shape of the starch grains, although these reference samples had only been dehydrated for a few weeks, not the years that would be expected from archaeological samples. Several tools in the collection had grains of a very distinctive shape. Many had round grains of varying size, while others had angular grains with five sides. None of these angular grains matched any of the plants in the reference collection. The round grains pose a problem in that many of the plants in the reference collection have varying proportions of round grains. All three of the tools examined directly under the microscope had a substantial number of starch grains as well as other plant fibres. These grains tended to be very small, in the region of 2-4μm.

DISCUSSION AND CONCLUSIONS

While this proved an effective method for starch grains from grind stones in significant numbers, the identification of these grains to genus or species was difficult. The reference collections in the case proved to be too small to allow the positive identification of any plants based on their starch grains found on the surface of the tools. However, even when large reference collections are available, identification of the plant of origin based on the size and shape of starch grains in any archaeological context is problematic (Therin 1994). This is due to the nature of the archaeological record as well as the nature of starch grains. Archaeological samples are usually small in nature. This problem is compounded by the fact that more than one species may be present on any particular tool. Variation in size and morphology can also occur not only within each species, but within any particular plant, a fact that many researchers have not taken into account. Variation in starch grains size depends on several factors (Therin 1994). The location and function of starch grains within the plant are important in determining size and morphology. For instance, starch grains produced in the chloroplasts by photosynthesis are usually very small. They grow in available spaces and therefore have no defined shape. On the other hand, the morphology of starch grains found in the amyloplasts is thought to be controlled genetically and tends to be more defined. The degree to which the grains are packed seems to play an important role in their morphology, with loose packing resulting in rounder grains and close packing producing more angular grains. The age of the plant organ in which growth occurs effects the size and shape of the grains. In general, the longer the period of reserve starch growth, the larger the granules become (Badenhuizen 1964). These larger granules tend to have a more regular shape than the smaller and younger granules. Factors such as the availability of water and nutrients as well as soil composition constrain plant growth and can affect size and shape of starch grains.

Thus on any particular tool, variation in starch grain size and shape could be the result of several factors; the presence of more than one species, the part of the plant processed with the tool, the age of the plant processed and environmental conditions governing the growth of the plant. These factors are complicated by the fact that at present, shape is defined in terms of a small number of categories. With the number of plant species available to hunter-gatherers living in the eastern Orange Free State it is likely that several species or genuses will be of the same shape category and have size ranges that overlap. It is clear that even where an extensive reference collection is available, the use of starch grain size and morphology as a means of identifying the plant origin is problematic. There are however, some cases where identification may be possible, as long as the limitations are kept in mind. For instance, where it is likely that grindstones were used to process large amounts of produce from the same part of plants that were harvested at the same time. Such an example would be Iron Age contexts where it may be possible to determine whether the staple crop was maize as opposed to sorghum or millet in areas where this is disputed. Given a large enough reference collection as well as large, well-preserved samples it may be possible to resolve such disputes based on the techniques described above. It would seem however, in stone age contexts, where so many factors may affect the size and shape of starch grains present on the tool, the identification of plant species will continue to remain problematic, if not impossible.

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