Supplemental Material, Text 3, Lab Procedures, Contamination Protocols, and Comparative Collections

University of Missouri - Columbia and University of Central Florida: Neil Duncan and Danielle Young

Samples processed for the pilot project were sent to the University of Missouri-Columbia under the guidance of Deborah Pearsall and Neil Duncan. At the University of Central Florida, samples were process by Neil Duncan and Danielle Young at the Paleoethnobotany and Environmental Archaeology Laboratory.

Clean lab protocols were followed in the recovery of microremains at both locations. These include using sterile tools, beakers, test tubes, and powder-free nitrile gloves, slides, and coverslips each designated for single, one-time use per sample. To prevent cross contamination, lab surfaces were periodically sprayed with alcohol-based cleaner and wiped clean. Blank microscope slides are prepared periodically to control for contamination caused by airborne starches. All comparative microremain samples are prepared in a separate room not attached to the wet lab – a room separate from where the archaeological microremain samples are processed.

Extraction of microremains followed published methods (Henry and Piperno 2008; Pearsall 2015; Zarrillo and Kooyman 2006,). For scraped ceramic residue and dental calculus, starches and phytoliths were isolated with a three phase process involving a gentle chemical dispersion using a 10% solution of sodium hexametaphosphate (NaEDTA), disaggregation in a vortex machine, rinsing and centrifuging, and mounting of the starch and phytolith residue solution on a standard microscope slide with glycerin under a coverslip sealed with lacquer.

Because there are C4 grasses native to the project area, there was a concern that some of these could mask as maize. Comparative studies of grasses have consistently distinguished maize from other grasses based on size, shape, fissure, and overall morphology (Holst et al. 2007; Messner 2011; Musaubach et al. 2013; Pagán-Jiménez 2007; Piperno et al. 2009). Messner’s (2011: see supplemental material) analysis of starches from eastern woodland grasses includes several genera that are also native in the Midwest, in particular Panicoideae grasses such as *Setaria spp., Panicum spp., Schizachyium scoparium, Tridens flavus, Sorghastum nutans,* and *Andropogon gerardi.* Clear distinctions can be made that allow for the identification of maize starch grains in comparison to other grasses.

Specific to differentiating maize from *Tripsacum sp.* (gammagrass, which is local to the study area), the UCF observations of *Tripsacum dactyloides* starch grains are similar to Piperno and Holst (1998) who first characterized starch of this genus. The grains are simple, sometimes compound, and very spherical with one or more slight depressions that we observe to be linear to y-shaped. Hila are large relative to grain size. The starch grains also have characteristically prominent fissures that radiate from the hilum. In addition, size of the grains does not overlap with maize, in our comparative samples from local Florida grown *T. dactyloides,* starch grains ranged from 2 to 14 microns in length.

Starches from *Zea mays* can be distinguished from starches of tripsacum. Maize starches are spherical, however most often grains are slightly irregular to multi-faceted. Central fissures in maize starch grains tend to be deep, sometimes stellate-shaped, unlike tripsacum. Also, maize grains are distinguished by their continuous double border. Some races of maize produce starches with radiating fissures similar to tripsacum (Piperno and Holst 1998) but retain a double border, deep fissure, and slightly irregular shape with pronounced striations radiating from the depressed central hilum to the edges of the grain (Holst et al. 2007:17611). In our comparatives, metate-ground maize flour also produced grains with radiating fissures. Archaeological starches identified to maize in this study are consistent with maize starch comparatives, not tripsacum.

Maize was confidently identified when the starch grain exhibited all the diagnostic attributes including (1) double outline, (2) faceted, (3) transverse or y-shaped fissure over central hila, and (4) roughened/bumpy surface. If a starch grain suspected to be maize fit only 3 of these attributes, it was identified as cf. maize.

Direct sampling of ceramic interior surfaces for microremains utilized a new sterile brush-head attached to a sonicating toothbrush for each artifact. Each artifact was placed in a sterile plastic bag large enough to allow the artifact to be brushed with the sonicating toothbrush and rinsed with distilled water. The water containing the microemains was then transferred to 50ml test tubes and centrifuged to concentrate the sample at the bottom of the tube. The supernatant was then pipetted off. A 10% solution of NaEDTA was added to each sample. Each tube was then vortexed and centrifuged again to concentrate the sample and the supernatant was pipetted off. Samples that were visibly silty were then subjected to density separation using lithium metatungstate (LMT). The samples were rinsed and centrifuged to remove remaining LMT and centrifuged again to concentrate the sample prior to transferring the residue to a microscope slide, coverslip sealed with lacquer. Samples which were visibly clear were centrifuged then transferred to microscope slides with glycerin.

Starch and phytoliths recovery from the mano and groundstone artifacts followed a procedure that produces three sediments from each artifact. Artifacts were first brushed with a new, unused sterile toothbrush for each artifact. The dislodged material is collected as Sediment 1. Sediment 2 is produced by rinsing the artifact with distilled water and brushing. The final sediment comes from sealing the artifact in a sterile plastic bag filled with distilled water and placed in a sonicated bath to dislodge remaining material adhering to the surface or in crevices and pores of the artifact. The three sediments are processed further to isolate starch and phytoliths through a gentle chemical dispersion using NaEDTA and disaggregation in a reciprocal shaker. Remaining organic material are exposed to a 6% solution of hydrogen peroxide for about 10 minutes, rinsed and centrifuged, rinsed again, with supernatant solution decanted each time. Starch granules were then separated from the remaining soil using LMT or sodium polytungstate (SPT), centrifuged and rinsed several times. Samples were then transferred to microscope slides with glycerin.

Slides were scanned under a Zeiss Axio-Imager A.2 transmitted light microscope under polarized light and non-polarized light at 400x.

University of Kansas: Steve Bozarth

Samples submitted for this project were processed in the Department of Geography, Palynology and Phytolith Laboratory. Alconox (a laboratory grade detergent) is used on a regular basis to clean all of the beakers, bottles, and centrifuge tubes as well as the counter tops and fume hood.

The calculus and residue samples were processed as follows; 1) removal of carbonates with dilute (10%) HCL; 2) oxidation with Schulze’s solution in a hot-boiling water bath; 3) dehydration of isolate with butanol; 4) dry storage in 1-dram vial. Once the samples are transferred from the original containers into the beakers to start the processing, the beakers are always covered with watch glasses.  The centrifuge bottles are covered with screw on tops and the centrifuge tubes are always covered with rubber stoppers.

The entire isolates were analyzed with a research grade Zeiss microscope at a magnification of 625X.

University of Minnesota: Robert Lusteck (affiliation at the time of analysis)

Samples were processed in the Limnological Research Center's LacCore labs, a facility funded in part by the National Science Foundation. The facility is part of the University of Minnesota campus and is located six floors below ground, thus providing some protection from contamination. However, blanks were run periodically, based on the volume of slides prepared. Pollen samples were also prepared on a regular basis in the lab and air traps were used to control for contamination.

Extraction of microremains from sherd scrapings and artifacts followed Pearsall (Pearsall 2015). All samples were prepared in fume hoods. Sherds were sampled with clean dental tools (all samples), then with a sonic toothbrush. The sample was transferred to a 50 ml test tube using high purity distilled water. A few drops of TBA (tertiary butyl alcohol) were added to prevent bubbles that might allow bits of the sample to float. The sample was centrifuged to concentrate the residues in the bottom of the tube and the supernatant decanted. The sample was then treated with a series of warm chemical baths (@90° C) to remove the organics. Standard laboratory grade nitric acid (65%) and hydrogen peroxide (30%) was used. The samples were bathed twice in nitric acid for approximately 4 hours each time, and twice in hydrogen peroxide for 2 hours each. The sample was rinsed with distilled water and placed in 15 ml test tubes. LST (lithium heteropolytungstate) at a specific gravity of ~2.3 was added to the sample to further separate the silica. The sample was vortexed with LST and centrifuged to float the biogenic silica. The resulting material was rinsed 5 times with distilled water. Finally, the samples were placed in 1 dram vials with 90% ethyl alcohol and slides were prepared using permount.

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