Starch grain analysis is one important avenue of investigation in the reconstruction of prehistoric food processing, however, there has been little research done on quantifying starch deposition and preservation on artifacts. This study documents changes in starch grain deposition and morphology observed from processing of modern plant samples with different characteristics through different types of modern experimental ground stone milling tools. The results have the potential to expand our understanding of subsistence practices and make analysis techniques more comprehensive and quantifiable.

Ground stones have been manufactured and used for thousands of years for a variety of purposes (Adams 2002). One of the most important functions of ground stone tools is the processing of resources for consumption. Archaeologists try to determine the function and role that ground stone milling tools have in resource processing. Ground stone milling tools have many different sizes, shapes and are produced on variety of materials. Some tools are expedient unshaped, while other tools are manufactured and deliberately shaped. Ground stone tools contain microscopic information about resource processing, subsistence practices and environmental constraints. There have been experiments involving resource processing on a variety of ground stone tool sets to describe grinding techniques (Adams 1988, 1989a, 1989b, 1993, 2002, 2010, 2014, Buonasera 2012). Dr. Tammy Buonasera is in the process of conducting an experiment using replicas of prehistoric ground stone milling tools (expedient and manufactured) made from different raw materials to process three different resources to generate data to create models of ground stone processing efficiency. Dr. Buonasera’s research will aid in determining the costs and benefits of manufacturing ground stone milling tools and will help determine processing efficiency of resources by tool form.

Starch grain analysis is one method used to identify plant macrofossils deposited on archaeological artifacts. Starch grains are tiny microscopic plant cell structures that are stored in seeds, stems and underground storage organs (roots, bulb and tubers). Starch grains are important for archaeological research because they are stable and resilient over long periods of time and they stay preserved in archaeological contexts, while other plant structures (pollen, seeds, etc.) may not survive unless optimal conditions are present (Torrence and Barton 2006). While starch grain analysis is common in other countries (Henry and Piperno 2008; Henry et al. 2011; Perry et al. 2007, Piperno and Dillehay 2008; Piperno et al. 2009) it is a fairly new area of research in North America (Herzog 2014; Louderback et al. 2017; Messner 2011; Scholze 2011; Wisely 2016). Most of the studies in North America highlight the importance of analyzing starch grain residue from archaeological artifacts and building regional comparative libraries (Herzog 2014; Scholze 2011; Wisely 2016).

Very often, starch grain analysis research results leave more questions than clear answers. Recent studies have highlighted experimental grinding of a single resource on a single tool to explore the potential of starch grain residue left on artifacts (Hayes et al. 2017; Cnuts and Rots 2017) and the potential contamination of starch grains on artifacts from soil contact (Haslam 2004; Ma et al. 2017). There has been no research that combines experimental grinding of multiple plant resources on multiple sets of different types of ground stone tools made from different raw materials. There is a need to document changes in starch grain morphology and deposition caused by processing samples with different physical characteristics in different types of ground stone milling tools. Buonasera’s replicas provide the opportunity to take a closer look at how starch grains are deposited on ground stone milling tools and the results have the potential to expand our understanding of subsistence patterns and make analysis techniques more comprehensive and quantifiable. The primary objectives of this research include:
1) How and to what degree are the starch grains damaged by prolonged grinding and pounding? Is there a difference in the degree of starch gain alteration in pounding tools versus grinding tools?

2) Does the relative quantity and preservation of grains vary between volcanics’ (e.g., basalt/andesite) with sedimentary rocks (e.g., sandstone)? Do tools made with rugged and porous materials (like sandstone) capture and retain more starch grains than tools made with a fine-grained igneous material?

3) Do manufactured ground stone surfaces retain more starch grains than expedient surfaces?

4) Given that roughly equivalent amounts of time were spent grinding acorns and Indian ricegrass, does one type of starch grain dominate another or is representation fairly even? If they are not equivalent is this due to the presence or greater amounts of starch in one resource versus another or does it have to do with the sequence grinding (e.g., acorns processed after Indian ricegrass)?

METHODS

Comparative Collection

A comparative collection of plant foods traditionally used as food by Native Californians was developed including black oak acorn nuts (*Quercus kelloggii*), valley oak acorn nuts (*Quercus lobata*) and Indian ricegrass seeds (*Achnatherum hymenoides*). This comparative collection was made to obtain data for species-level identifications of starch grains. The nut meats and seeds were ground into a fine powder, and the starch samples were mounted for examination. The mounting medium consisted of a 50/50 combination of distilled water and glycerol. For each species, two slides were prepared and analyzed. Multiple digital photographs were taken at 400x and 1000x magnification under both cross-polarized light and bright field. Multiple measurements (length, width and height) were taken with ImageJ and calibrated with a slide micrometer. Three-dimensional observations and descriptions were recorded for reference.

Sampling Procedures

A total of eight lower stones (four mortars and four metates) and 12 upper stone surfaces were sampled (four manos—five surfaces and five pestles—seven surfaces) were sampled. Experimental tools descriptions and the corresponding plant resource processing times are provided in Table 1. These artifacts represent interactions between different tool shapes (mortars and pestles, grinding slabs and handstones), raw materials of the ground stone tool sets (sandstone and basalt) with different plant resources (acorns and small seeds). Analysis of upper stones will allow comparisons to be made for single plant resource types, while lower stones represent sequential processing of multiple plant resource types.

The equipment used for each sample extraction included a sterile powder-free nitrile gloves, sterile sonic toothbrush heads and base, disposable toothbrushes, disposable pipettes, sterile 15-milliliter centrifuge tubes and sterile 1.5-milliliter centrifuge tubes. For each sample, new sterile equipment (gloves, pipettes, etc.) was used to prevent cross-contamination.

Lower Stone Sample Extraction

The mortars and metates were dry brushed with a new disposable toothbrush to remove any large residue particles left over from processing. Approximately 15 milliliters of distilled water were poured into the deep mortars/metates. For the mortars/metates with shallow depths a sterile clay berm was created to hold the water to facilitate sonication. A sonic toothbrush with a new, sterile head was placed into the water and the mortar/metate was sonicated for 12 minutes. The aqueous solution was collected with a new sterile pipette and placed into a new sterile 15 milliliter centrifuge tube. The centrifuge tube was labeled with the sample information.
Table 1. Experimental Grinding Tools and Resource Processing Times.

<table>
<thead>
<tr>
<th>TOOL SET</th>
<th>STONE TYPE</th>
<th>GROUND STONE FORM</th>
<th>A. HYMENOIDES (INDIAN RICEGRASS) HOURS</th>
<th>Q. KELLOGGII (BLACK OAK) HOURS</th>
<th>Q. LOBATA (VALLEY OAK) HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>sandstone</td>
<td>shallow basin metate</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>sandstone</td>
<td>mano</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>sandstone</td>
<td>shallow mortar</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>sandstone</td>
<td>pestle</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>basalt</td>
<td>shallow basin metate</td>
<td>7</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>basalt</td>
<td>mano – side a</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>basalt</td>
<td>mano – side b</td>
<td>-</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>basalt</td>
<td>shallow mortar</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>basalt</td>
<td>pestle</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>basalt</td>
<td>pestle</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>basalt</td>
<td>conic mortar</td>
<td>6</td>
<td>3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>8</td>
<td>basalt</td>
<td>pestle – side a</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>basalt</td>
<td>pestle – side b</td>
<td>-</td>
<td>3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>9</td>
<td>basalt</td>
<td>bowl mortar</td>
<td>8</td>
<td>3.5</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>basalt</td>
<td>pestle – side a</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>basalt</td>
<td>pestle – side b</td>
<td>-</td>
<td>3.5</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>basalt</td>
<td>unshaped flat metate</td>
<td>4</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>basalt</td>
<td>unshaped mano</td>
<td>4</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>sandstone</td>
<td>unshaped flat metate</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>basalt/andesite</td>
<td>unshaped mano</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Upper Stone Sample Extraction

The manos/pestles were dry brushed with a new disposable toothbrush to remove any large residue particles left over from processing. Each mano/pestle was placed into a new sterile 4-mil bag with 15 milliliters of distilled water. The bag containing the mano/pestle was placed into a Branson sonicator for 12 minutes. The multi-sided manos/pestles were manually submerged in the sonicator to prevent sonication of all sides. The multi-sided tools were air dried before extracting the sample from the other side of the tool. Each mano/pestle was rinsed with distilled water as it was taken out of the 4-mil bag to remove any additional starch grains. The aqueous solution was collected with a new sterile pipette and placed into a new sterile 15 milliliter centrifuge tube. The centrifuge tube was labeled with the sample information.

Starch Grain Processing

Each sample was placed in a centrifuge at 3200 rpm for seven minutes. The sample was distilled by removing the top layer with a new sterile pipette. This process was repeated until each sample was distilled down to 1–2 milliliters. Each sample received 1–2 milliliters of a 2.3 specific gravity solution of Sodium Polytyngstate \((\text{Na}_6[\text{H}_2\text{W}_{12}\text{O}_{40}])\) solution. The sample was centrifuged at 3200 rpm for seven minutes. The top layer (concentrated liquid) was pipetted off and stored in a 1.5 milliliter centrifuge tube. Drops of the concentrated liquid were placed on clean microscope slides; a slide cover was placed over
each sample, and the cover was sealed with Permount to make the slide semi-permanent. These processing methods are adapted from Scholze (2011) and Wisely (2016).

**Starch Identification**

Each sample slide was transected at 100x magnification using cross-polarized light to identify any starch grain. When a starch grain was observed, the microscope was switched to 400x magnification to record and identify each starch grain under cross-polarized light and bright field light. If necessary, magnification was increased to 1000x in order to make identification. Digital photographs were taken to record starch grain presence.

Identifications were based upon a combination of morphological features and measurements (Figures 1 and 2). Morphological features were the primary method of identification, as starch grains can have significant variations in size. If the morphology of the starch grain could not narrow the identification to the genus or species level, a family level identification was made, or the starch grain was listed as “unidentified.”

**RESULTS**

Preliminary results from three of the tools sets are different. Two complete tool sets and one bottom tool have been analyzed (Table 2; Figure 3). The remaining tool sets are currently being analyzed and the data will be added to this report when analysis is complete.

Tool set 3 (shallow basin metate) and 5 (shallow mortar and pestle) are made from sandstone and were used to process one plant resource, Indian ricegrass. Both bottom stones contained a significantly low number of starch grains. The identified starch grains are black acorn (set 5) and unidentified (set 3). Inversely the pestle (set 5) contained the highest amount of starch grains with an estimate of a total more than 39,000 (99% Indian ricegrass). The identified acorn starch grain residues recovered from the tools are likely an environmental contaminant from artifact storage or experiment participant. The mano results from set 3 are predicted to have results similar to the set 5 pestle.

Tool set 6 (shallow basin metate and mano) is made from basalt and processed all three plant resources. The starch grain residue analysis shows that all three plant resources are present on all surfaces. Indian ricegrass was the first resource processed and has the lowest counts for the lower stone. The mano had dedicated specific resource sides (side a – Indian ricegrass; side – b acorn); however, results indicate that users may have deviated from the instructions.

**SUMMARY**

Further analysis is currently underway and results are pending. The preliminary results indicate that the bottom stones manufactured from sandstone material do not retain starch grain residue. This may be due to the abrasive nature of sandstone and when the tool is in use it is consistently exfoliating the top layer of the stone. The reverse seems to be occurring in the sandstone top tool. This may be due to fissures and small cracks forming in the top tool. As plant resources are processed the starch grains fill in the fissures and cracks. If the final results reflect the preliminary results, it could be inferred that bottom sandstone tools do not retain starch grain residue—at least for the type of sandstone used in this experiment—and perhaps resources should not be used to extract starch grains from these types of archaeological artifacts.

Recognizing the challenges of residue analysis will aid in archaeological interpretations and reconstructions. The application of microbotanical research in combination with macrobotanical, faunal and paleoclimatic data sets have the best potential to greatly expand our knowledge of prehistoric lifeways. This study will help guide future starch grain research and will hopefully provide a baseline on how starch grains are retained and affected by different types of ground stone tools.
Table 2. Starch Grain Counts per Tool Set

<table>
<thead>
<tr>
<th>TOOL SET</th>
<th>STONE TYPE</th>
<th>GROUND STONE</th>
<th>A. HYMENOIDES (INDIAN RICEGRASS)</th>
<th>Q. KELLOGGI (BLACK OAK)</th>
<th>Q. LOBATA (VALLEY OAK)</th>
<th>UNIDENTIFIED</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>sandstone</td>
<td>shallow basin metate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>sandstone</td>
<td>shallow mortar</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>basalt</td>
<td>shallow basin metate</td>
<td>39,000 estimated</td>
<td>87</td>
<td>46</td>
<td>152</td>
</tr>
<tr>
<td>6</td>
<td>basalt</td>
<td>mano side a</td>
<td>360</td>
<td>362</td>
<td>313</td>
<td>268</td>
</tr>
<tr>
<td>6</td>
<td>basalt</td>
<td>mano side b</td>
<td>616</td>
<td>1,080</td>
<td>528</td>
<td>440</td>
</tr>
</tbody>
</table>
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