

An exploration of image recognition in archaeological starch analysis

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Abstract

The goal of this thesis was to produce a statistical method of assigning species to starch granules recovered from eastern North American archaeological assemblages. Starch grain analysis is an archaeobotanical tool that can be used to answer important questions about prehistoric diet and medicine use. In eastern North America this technique can be useful in tracking the spread and adoption of Peruvian and Mexican cultivars maize and beans, as well as identify the use of locally occurring starch rich plants which would have been used as food and medicines.

This thesis is divided into two chapters. The first is a literature review describing some of the more recent developments in starch grain analysis. Six major research streams are reviewed: (1) assigning species to starch granules recovered from archaeological residues; (2) the earliest evidence of the use of domesticates and plant processing techniques; (3) the recovery of ancient starch from human dental calculus; (4) the taphonomy of starch granules; (5) methodologies to control for starch grain contamination in the field and lab; and (6) the use of starch grain analysis in paleoenvironmental reconstruction. It is argued that assigning species to starch granules recovered from archaeological material is fundamental to investigating all other streams of ancient starch research, and that statistical methods should be developed to assign species.

The second chapter describes a method of assigning species to starch granules using image recognition software. It is designed to classify the starches of 17 plant taxa expected to be recovered in eastern North American archaeological assemblages, including the cultigens maize and beans. This technique is shown to be cost-effective and relatively quick while producing

accurate results. Directions for further research are provided, most importantly the application of this technique to archaeological materials.

Ancient starch analysis is a burgeoning aspect of archaeobotany and holds promise in answering important questions about diet and other plant uses. After reading this thesis, the reader can expect to have a thorough understanding of the various streams of ancient starch analysis and be capable of reproducing the classification technique described herein which can be applied to their own research questions.

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Chapter One: Ancient starch research literature review

Abstract

This chapter was written to review the developments in ancient starch research from 2016 to 2020. Bibliometrics, a statistical technique for literature review was used to analyze articles within this time frame. This method reduces researcher bias and allows for the extrapolation of broader inferences about ancient starch research as-a-whole. Six lines of research are identified: (1) the earliest evidence of the use of cultigens or plant-processing techniques; (2) the use of morphometrics to assign species to starch granules of unknown origin; (3) the recovery of ancient starch from dental calculus; (4) the study of ancient starch taphonomy; (5) the creation of methods to control for contamination of starch samples; and (6) the use of starch as a proxy to reconstruct paleoenvironments. These research areas are briefly summarized and discussed.

1.0 Introduction

This chapter reviews the development of starch grain analysis within archaeology. Starch grains, like pollen and phytoliths, are plant microfossils that can be recovered from archaeological residues. Unlike these other microfossils, starch grains are the carbohydrate final product of photosynthesis, and they can be found in large numbers within storage parts of plants such as edible seeds and tubers (Gott et al. 2006). As such, starches recovered from archaeological material are typically interpreted as evidence of plant processing like cooking

(e.g., Duncan et al. 2009, Larby et al. 2019), milling (e.g., Aranguren et al. 2007, Li et al. 2019) or fermentation (e.g., Logan et al. 2012, McGovern et al. 2017).

Starch research in archaeology is a relatively new field (Dozier 2016) though the number of published articles has increased substantially since 2006. Reliable documents written on starch analysis prior to 2006 are scant. The most rigorous is likely “The differentiation and specificity of starches in relation to genera, species, etc.” (Reichert 1912) which was not written for an archaeological audience. That source was a precursor to the International Code of Starch Nomenclature (ICSN 2011) and as such it provides thorough descriptions of the starch grains of many plant species. A review by Barton and Torrence (2015) describes the developments in ancient starch research between years 2006-2015. Their study demonstrated a spike in articles written on ancient starch research following the publication of the book “Ancient Starch Research” (Torrence and Barton 2006) which was published in 2006. That book contains chapters on topics critical to the analysis of starch grains recovered from archaeological residues such as microscopy, cell biology, recovery methods, taphonomy and classification.

Barton and Torrence (2015) found that prior to the publication of the book “Ancient Starch Research,” archaeological starch analysis was conducted primarily within Australia and Oceania. Following its publication ancient starch research became more widespread worldwide and a substantial amount occurred within the People’s Republic of China. Barton and Torrence’s (2015) article identified sources for the recovery of ancient starch, the most prevalent being grinding stones, followed by sediments, flaked stone, ceramic, and dental calculus. Two emerging lines of research were highlighted in their article, the spread of maize into North America and the recovery of starch from Pleistocene-aged materials.

This chapter provides a summary of trends, challenges, and prospects of ancient starch research in the five years following Barton and Torrence's (2015) publication (i.e., 2016-2020). This was achieved with the aid of bibliometric analysis. This technique is a statistical method that can be used to analyze the citation histories of articles. The approach used in this chapter was modeled after a method used by Maditati et al. (2018) in their review of green supply chain management. Using techniques like bibliometrics has some advantages over traditional literature review. For the research described in this chapter, the advantages are: (1) articles were collected in a way that removed my own personal bias on which articles to include, and (2) since the sample size of articles is arguably representative, the data within this chapter can be used to extrapolate trends within the literature written on ancient starch research as-a-whole. The use of statistics in literature review has its own unique limitations, however. For example, articles published in prestigious journals may be overrepresented, and otherwise valuable research may be missed due to it being published in relatively obscure journals. Importantly, there is no statistical replacement for sound reasoning and a critical understanding of a series of articles' subject matters.

In general, recent starch research addresses many of the same themes first described by Barton and Torrence (2015), although new streams of research are also identified within this chapter. Topics familiar to archaeologists such as taphonomy and contamination (as they relate to ancient starch research) were also reviewed in Barton and Torrence's (2015) review article, and developments in these topics are also discussed here.

2.0 Creation of co-citation maps and content matrices

A sample of journal articles on ancient starch research published between January 2016 and December 2020 were analyzed bibliometrically. This was done to identify streams of research that may have developed following the publication of Barton and Torrence (2015) in a controlled way. The sampled articles (n=45) were recovered from Clarivate™ Web of Science™ (WoS™) from the following website <http://webofscience.com/wos/woscc/basic-search> by searching the terms “starch AND archaeology.” Although a broader search may have increased the number of sampled articles, the search used produced a similar number of articles to what was analyzed in Barton and Torrence’s (2015) review; these authors sampled 110 articles over a ten-year period, roughly 11 per year. This is comparable to 45 articles published over five years, or nine per year used for the review in this chapter. Barton and Torrence (2015) reported that a “significant” number of articles (i.e., n=12 or ~9.2%) within their study were published in the Proceedings of the National Academy of Sciences of the United States of America (PNAS). They highlighted this to show the importance of ancient starch research in making discoveries about crop dispersal or the use of plants prior to the adoption of agriculture. Of the 45 articles sampled for the review written for this chapter, three (~6.7%) were published in PNAS.

WoS™ was chosen as a source for articles because it is compatible with HistCite™ software (Garfield 2009) and has elsewhere been identified as the most reputable academic database (Maditati et al. 2018). This database only includes peer-reviewed journal articles which may somewhat limit the ability of the results from the bibliometric analysis to be extrapolated to ancient starch research as-a-whole. It has been suggested that archaeological data is often cited from monographs and unpublished grey literature such as dissertations, book chapters and site reports (Börjesson 2015). Nevertheless, peer-reviewed journal articles are the standard for the

most rigorous level of academic literature and should be expected to include much of the most salient information on ancient starch research.

While analyzing literature published about ancient starch research between January 2016 and December 2020, two bibliometric analysis tools were used. These were the creation of co-citation maps and content matrices. These techniques were modeled after methods described by Maditati et al. (2018) to help identify trends, themes and other patterns in the content and citation histories of the articles sampled for the review in this chapter. By and large, these techniques are heuristic devices. Co-citation maps help identify trends specifically by displaying articles on a chart so that researchers can visualize differences in the references cited by each article. The main reason why articles were collected from WoS™ for this analysis is that only WoS™ (as opposed to other databases like Scopus, Google Scholar, etc.) database output files include metadata that lists each individual cited reference, something necessary for HistCite™ to generate co-citation maps. Content matrices on the other hand are charts that cannot be created automatically using software. They are used to list information from each article, some of which can only be gathered by a human analyst. In the case of the research provided in this chapter, I first read each article and then asked myself two questions about each: (1) What are the research questions, and (2) what are the key results? Beyond that, what is included in the content matrices was copied such as the titles, authors, and key words.

Further details about the methods and mechanisms used to create the co-citation maps and content matrices are explained in the appendices (A.1 and A.2). The completed co-citation maps and content matrices used in the literature analysis within this chapter can also be viewed in those appendices (A.1 and A.2).

Through the creation of co-citation maps (Appendix A.1) and content matrices (Appendix A.2), it was possible for the articles sampled by bibliometric analysis to be grouped together into six groups. These were: earliest evidence, paleoenvironment, dental calculus, morphometrics, taphonomy and contamination. Although not exhaustive of the body of literature written on ancient starch research, all the articles included in the bibliometric analysis were published in prolific journals. The articles therefore include many of the most salient topics in ancient starch research between the years they were published (2016-2020). After reviewing the sampled articles, it was possible to write about each of the six groupings of ancient starch research. The information gathered from these sampled articles were further embellished upon by citing other pertinent articles published prior to, afterward or contemporaneously to the sampled articles.

3.0 Content analysis

3.1 Assigning species to unknown ancient starch granules using morphometrics

Morphometrics are the analytical techniques used by archaeobotanists to assign species to starch granules of unknown origin. The ability to assign species to archaeological starch grains of unknown origin is critical to many forms of ancient starch analysis. For example, to trace the use and spread of cultigens it is necessary for researchers to be able to differentiate the starch grains of cultigens from those of other species. Typically, this means first studying and classifying the starch grains of modern plants, then comparing those to starches recovered from archaeological materials. The identification of unknown starch species has received little study outside of archaeology and so it is nearly exclusively an archaeological science. One exception is the research conducted by Tong et al. (2008) which describes a method that can be used to verify the identities of starchy plants sold as traditional Chinese medicines. Tong et al.'s (2008) method

can not be applied to archaeological materials because it requires many starch grains known to be of the same species to calculate a size distribution. Archaeological starch assemblages are therefore incompatible with Tong et al.'s (2008) methodology because archaeological assemblages typically include more than one species, some of which may only be represented by one or two granules.

The most prevalent method of starch granule identification is done by visual comparison of archaeological starch granules with modern reference collections (Field 2006, Lentfer 2009) and comparison with published descriptions of starch granules (e.g., Brown and Louderback 2020, Reichert 1912, Musaubach et al. 2013, Gismondi et al. 2019, Mercader et al. 2018a, Holst et al. 2007). Starch granules identified by visual comparison can be further verified by using dichotomous keys (e.g., Messner 2011, Lints 2012, Yang and Perry 2013, Louderback et al. 2022) or statistical techniques (e.g., Torrence et al. 2004, Coster and Field 2015, Arráiz et al. 2016, Wilson et al. 2010). Statistical techniques used for starch grain analysis are especially useful because they can provide number values such as identification accuracies given as percentages (e.g., Torrence et al. 2004, Arráiz et al. 2016, Coster and Field 2015, Wilson et al. 2010). Researchers can then use these data to compare and evaluate each statistical method. Examples include comparing the accuracies of starch identification methods to one another, demonstrating that certain species are more readily identifiable than others (e.g., Torrence et al. 2004, Mercader et al. 2018a, Coster and Field 2015, Arráiz et al. 2016, Wilson et al. 2010), and comparing identification accuracies against human analysts (e.g., Arráiz et al. 2016).

Several (n=4) articles used a morphometric technique called the geometric morphometric approach (GMA) (defined in Coster and Field 2015 and Coster and Field 2018). (Appendix A.1; Figs. F.A.1.1 and F.A.1.2; articles 3, 11, 36 and 41). This makes it the one of the most popular

statistical techniques identified within the bibliometric analysis. For GMA, first a reference collection is created by measuring values such as area, perimeter, and location of center mass (aka the “hilum”) of between 80 and a hundred or more starch grains per target species. The Fourier transform, a measurable deconstruction of a starch grain’s shape can also be recorded; however, it can not be recorded for starches with convexities. These measurements are used to train classification algorithms that can then be used to assign species to starches of an unknown source. This method was developed specifically to be used with Oceanian assemblages such as in Australia (Field et al. 2016), Indonesia (Lape et al. 2018) and New Guinea (Shaw et al. 2020, Field et al. 2020), and therefore mainly features plants like yam (*Dioscorea spp.*), taro (*Colocasia spp.*), banana and plantain (*Musa spp.*).

3.2 Observing the earliest evidence of cultigen exploitation and plant processing techniques using ancient starch analysis

A preoccupation with uncovering the most ancient evidence of human behaviour is a hallmark of archaeology, though lines of evidence used to demonstrate these behaviours tend to attract controversy. Some examples include the early dates of between approximately 18,000 to 14,500 cal. BP for the New World occupation at Monte Verde, Chile (Dillehay et al. 2015), and 24,000 cal BP for another New World occupation at Bluefish Caves, Canada (Bourgeon et al. 2017). Another example is the early reported age of New World footprints located at White Sands National Park, USA. These were reported to be between approximately 23,000 to 21,000 years old by radiocarbon dating seeds in layers that bracketed the prints (Bennett et al. 2021). Recently, it has been argued that the reservoir effect exaggerated the age of the spiral-ditchgrass (*Ruppia cirrhosa*) seeds that were dated and that the footprints may be approximately 13,000

years old and of Clovis age (Haynes 2022), which would fit accepted paradigms of the peopling of the Americas. The preoccupation with uncovering the most ancient evidence of human behaviour is shared by ancient starch researchers, such as when investigating the use of cultigens (Boyd et al. 2008, Boyd and Surette 2010, Lints 2012, Zarillo and Kooyman 2006, Albert et al. 2018, Rumold and Aldenderfer 2016, Louderback and Pavlik 2017) and in some cases these studies attract similar controversies.

As explained by Barton and Torrence (2015) in their review article, starch analysis can allow researchers to track the spread of cultigens such as maize (*Zea mays*) into the Canadian prairies (e.g., Boyd et al. 2008, Boyd and Surette 2010, Lints 2012, Zarillo and Kooyman 2006). Not all archaeologists agree with the timeline for the introduction of maize based on starch assemblages preserved in food residues in this region, however. This is because of the potential for maize starch cross-contamination of archaeological materials which may occur in the field (Mercader et al. 2017) or the lab (Crowther et al. 2014), and because maize macroremains (e.g., kernels and cob fragments) generally have more recent dates. In one study, maize starch and phytoliths were recovered from carbonized food residues dated to 200 cal BC; this is 800 years older than any maize macroremains that have been recovered in that same region (Albert et al. 2018). The history of maize in North America is an ongoing discussion with multiple perspectives (Boyd et al. 2008, Boyd and Surette 2010, Lints 2012, Zarillo and Kooyman 2006, Albert et al. 2018) and starch analysis will likely continue to play an important role in future research written on this topic.

Research into the earliest evidence of the spread of cultigens has also been conducted while investigating the oldest use of potatoes in the New World. Researchers recovered the starch granules from domesticated potato (*Solanum tuberosum*) from Late Archaic-Early

Formative ground stone tools collected from the Jiskairumoko site in the Titicaca Basin region of Southern Peru. This is evidence of some of the earliest domesticated potato consumption in the region, dating between 3,400 and 1,600 cal BC (Rumold and Aldenderfer 2016). Starch from wild potato (*Solanum maglia*), a possible ancestor to the modern domesticate (*Solanum tuberosum*) has been reported in Chile at the site of Monte Verde, dating approximately 13,000 years old (Ugent et al. 1987). This is the oldest reported wild potato in the New World, though there is some controversy over the dating of the Monte Verde site. There is no evidence that potatoes were domesticated outside of South America; however, wild potato (*Solanum jamesii*) was reported in Utah from residues recovered from ground stone tools dating between 8,950 and 8,150 cal. B. P. This is the oldest date published for potato use in North America. The antiquity of potato use in North America raises questions of whether independent domestication may have occurred north of the Andes (Louderback and Pavlik 2017).

Archaeologists can use ancient starch research to observe the earliest evidence of plant processing techniques. For example, the earliest evidence of wine making was reported from the analysis of archaeological materials, including grape starch granules and seeds recovered from early Neolithic Georgia (ca. 6,000 to 5,000 BC) in the South Caucasus region (McGovern et al. 2017). The taphonomic effects of fermentation on starch granules has received study and so this process can be recognized archaeologically. Typically, fermentation will result in a combination of the enzymatic degradation and gelatinization of starches. Enzymatic degradation of cereal starches will result in the formation of pits, while for some tubers it will give starches a rough surface texture overall. Gelatinization of starches at temperatures below 70° C results in swelling of starch grains that more-or-less retain their original shape. The preparation of “mash,” the main

ingredient of beer must be done below boiling temperature, which is fortunate since starches gelatinized above 70° C may become unrecognizable (Wang et al. 2017).

The most widely cited article in this study reported the earliest evidence of wine making in Georgia (McGovern et al. 2017). That article was cited 125 times (Web of Science, accessed December 19, 2022). This could be in part due to the interdisciplinary as well as multiproxy (pollen, starch, chemical, etc.) approaches taken by the researchers. Wine and alcohol consumption in general is a topic that is familiar and of interest to many people, academic or otherwise, and that may have contributed to the article's popularity. It may be possible to apply the analysis of fermented starch grains outside of Eurasia, such as in the Andes where the fermented maize beverage *chicha* was consumed. Archaeologists have already tracked the production of *chicha* using phytolith analysis (Logan et al. 2012) and this type of research would likely benefit from the recovery and analysis of starch grains.

3.3 Recovery of ancient starch from dental calculus

The recovery of starch granules from dental calculus was cited by Barton and Torrence (2015) as a useful application of ancient starch analysis to Pleistocene-aged archaeological material (i.e., Henry et al. 2011, Henry et al. 2014). This is because starches can at times out survive other plant parts, which is important when dealing with especially old remains. However, only one article out of the 45 sampled for bibliometric analysis were written about the recovery of starches from dental calculus of Pleistocene age (i.e., Cristiani et al. 2018) (Appendix; Figs. F.A.1.1 and F.A.1.2; article 15). It is likely that a greater number of articles were not written about dental calculus from Pleistocene skeletons only because skeletons of this age are relatively rare when compared to more modern skeletons. The remaining three articles were written to

describe the recovery of starches from the dental calculus of Neolithic (Goude et al. 2020), Pheonecian (D'Agostino et al. 2020) and Roman (D'Agostino et al. 2019) remains (Appendix; Figs. F.A.1.1 and F.A.1.2; articles 30, 42 and 44).

Sample sizes of starches recovered from dental calculus are typically minute (Copeland and Hardy 2018). For example, D'Agostino et al. (2019) sampled the dental calculus of 40 skeletons and recovered only 571 starch grains total, an average of just under 15 per individual. This could be in part why there weren't a greater number of articles written on the recovery of starches from dental calculus within the bibliometric sample. Although the yield of starch grains recovered from dental calculus may be low, it is not a reason to discontinue this type of research because starches recovered from dental calculus demonstrate the direct evidence of diet. The data collected from multiproxy analyses such as the inclusion of isotopic or phytolith analyses can be used to strengthen the interpretive value of starch granules recovered from dental calculus (e.g., Cristiani et al. 2018, Goude et al. 2020, Wesolowski et al. 2010, Mickleburgh and Pagán-Jiménez 2012, Mickleburgh et al. 2018, Tromp and Dudgeon 2015, Henry et al. 2011). Similar examples of sources of direct evidence of human diet from the recovery of starch granules are available and being explored. These sources include coprolites (e.g., Vinton et al. 2009) and mummy gut contents (e.g., Chen et al. 2020).

Research has been conducted to develop and enhance methods of analyzing starch granules recovered from dental calculus. Tavarone et al. (2018) developed a method to remove modern contaminants from archaeological starch recovered from dental calculus using distilled water and centrifugation. Efforts have also been made to assess the applicability of scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDX) to observe starch granules adhered to teeth in situ (Power et al. 2014). SEM-EDX has elsewhere been shown to be

useful in observing starch granules adhered to the surfaces of stone tools in situ (Hayes et al. 2019).

3.4 Taphonomy of ancient starch

The study of taphonomy is fundamental to archaeology and so it is natural that the study of taphonomy has existed as a stream of research within ancient starch research since its inception. It is remarkable that starch granules, essentially parcels of high-caloric food can persist in the archaeological record in environments where other plant materials (e.g., kernels, cobs, tubers, etc.) disintegrate. Early research suggests that the quality of their substrate or the matrix in which starch granules are deposited influences their survivability. For example, Freitas and Martins (2000) suggested that starch granules adhering to calcite crystals may persist favourably. Barton (2009) and Langejans (2010) each ran experiments testing how starch granules survive deposition after being adhered to stone tools and buried for either one (Langejans 2010) or two (Barton 2009) years. Barton (2009) found that starches adhered to lithics and left on the ground's surface survived better than those that were buried. Langejans (2010) concluded that pH (either high or low), abundance or lack of moisture, and presence of heavy metals in soil may be produce the most favourable matrices for the survival of starch residues. Starch grains are recoverable regardless of soil pH; however, soils with a neutral pH promote biological degradation from organisms such as fungi. Since biological degradation is responsible for the most damage to ancient starch (Langejans 2010), this means that excessively acidic soils such as those found in the Boreal forests of Northwestern Ontario may be an ideal source of intact and therefore identifiable starch granules.

Experimental archaeology is critical for continued research into the taphonomy of starch granules. Cooking experiments have been conducted in various ways, such as to track the movement and morphological changes of starch granules baked in earth ovens (Thoms et al. 2015); the transformation of maize (*Zea mays*) starch granules into spherulites during the nixtamalization process (Johnson and Marston 2020), a process which enhances the nutritional value of maize (Katz et al. 1974); or the changes in starch grain morphology which occur from grinding (Ma et al. 2019). It was demonstrated that earth ovens can cause starches to gelatinize, at times to the point of becoming unidentifiable (Thoms et al. 2015). Nixtamalization can cause starch to transform into spherulites possessing a highly conspicuous “rainbow” morphology (Johnson and Marston 2020). Grinding has the effect of creating pressure facets or rupturing starch grains (Ma et al. 2019).

The results obtained through experimental archaeology should not be considered definitive, however. Experience has shown us that interpretations based on experimental archaeology can lead to controversy and at worst, to incorrect claims. For example, the debate that occurred when paleontologists published their findings on the 130,000-year-old Cerutti Mastodon site in California (Holen et al. 2017). They reported the recovery of mastodon bone fragments in direct context with cobbles interpreted as hammerstones and anvils. These conclusions were reached by conducting use-wear analysis on the cobbles and experimenting with cow and elephant bones to recreate the bone breakage patterns. The authenticity of the Cerutti Mastodon site has not been widely accepted within the archaeological community, and some researchers use it as an example of how the results from experimental archaeology can be misleading or even abused (e.g., Eren and Bebbler 2019).

Archaeobotanists should be wary of interpreting taphonomic changes to starch grain morphology as evidence of the use of specific processing techniques by humans in antiquity. For example, some taphonomic changes seen in starch granules can also occur in native (i.e., unmodified, or undamaged) grains (García-Granero 2020). Furthermore, experiments to track the effects of long-term deposition on starch grain morphology are constrained by time. Many masters and doctoral programs are between one and four years in length so these types of research programs are incapable of experimentally studying the effects of a decade of deposition, and certainly can not capture the effects of deposition for centuries or millennia. It has been proposed that starch grains undergo permineralization (Barton and Torrence 2015, Mercader et al. 2018b), and essentially become “fossils” through the absorption of aqueous minerals. The process of starch grain fossilization has not been investigated experimentally. However, the silicification (a form of permineralization, aka petrification) of alder (*Alnus pendula*) wood has been investigated experimentally. It was found that under suitable conditions, in this case submergence in a hot spring possessing a high silica content, wood can become permineralized in as short as tens of years (Akahane et al. 2004).

3.5 Methodologies to control for contamination of starch samples

Contamination has been a central concern of starch analysts and their critics since the introduction of ancient starch research. It is known that starch can become airborne (Dozier 2016, Balme and Beck 2002) so it is reasonable to have concern for accidental contamination. Research has shown that wind is a significant factor in the dispersal of airborne starches. For example, when grinding maize with a mano outdoors starches can travel as far as 10m from the source (Dozier 2016). Although airborne movement of starch is limited when indoors (such as

when a researcher is in a laboratory), it is still especially a concern for ancient starch researchers reporting maize (*Zea mays*) starch. Beyond being present in many foods, cornstarch is a widely used industrial product that can be found in many common items, for example powdered gloves (Crowther et al. 2014). As such, starch researchers have developed strategies to mitigate the risk of potential contamination, like having dedicated starch laboratories; using blank slides as starch “traps” in the laboratory to track airborne contaminants; and using power-free gloves before handling starch samples (e.g., Kooiman et al. 2021, Boyd et al. 2014). The ability for starch to become airborne may also be useful for archaeologists when interpreting sites. It was noted that starches will aggregate along the interior walls of rock shelters due to wind (Balme and Beck 2002), and so it is possible that starches may also aggregate along the interior walls of structures, such as for example wigwams and longhouses.

Potential contamination in the field must also be accounted for. Samples collected for starch analysis should be immediately bagged in the field (e.g., Field et al. 2020) to mitigate risks of contamination during and following excavation. It may also be necessary to investigate the land-use history of an archaeological site to rule out the possibility of contamination. For example, since maize agriculture is a widespread modern practice throughout the New World, some researchers make efforts to specify that archaeological materials were not recovered from a plough zone (e.g., Albert et al. 2018) ruling out the possibility of starch contamination from modern maize farming. This level of assurance could be unnecessary since it has been demonstrated that starches do not preserve well in active maize and wheat fields in northern China. Researchers Ma et al. (2017) attempted to recover maize and wheat starch from the surface soil of a maize and wheat farm, as well as surrounding environs. Even though these plants seasonally produce starch rich seeds, no starch grains were recovered from any of the

surface samples. Ma et al. (2017) attributed these observations to a combination of human and natural influences. The modern techniques used in maize and wheat farming in northern China leave few grains behind and in some cases involve the burning of fields post-harvest.

Furthermore, grazing animals, microbes and fungi are likely to scavenge whatever grains remain upon the surface after harvesting. It is therefore unclear whether this pattern would be seen elsewhere in the world, such as in modern or prehistoric maize farms located in the New World.

Very few (n=2) of the (n=45) articles reviewed here were written exclusively on methods for mitigating the risk of starch contamination (Appendix; Figs F.A.1.1 and F.A.1.2; articles 7 and 17). This could indicate that the controls for starch contamination developed prior to 2016 have been adequate during the following years, but I would be skeptical to make this claim. One article was written about the removal of starch contaminants from dental calculus (Tavarone et al. 2018), something that has received further publication by other scholars (e.g., Soto et al. 2019). Starches within dental calculus become encrusted and shielded from outside contaminants, so researchers can remove contaminants from the exterior of the encrustations non-destructively using water. The second article that was selected for bibliometric analysis was written about the creation of an identification key for possible contaminant species that occur naturally in Tanzania (Mercader et al. 2017). I did not review any articles that used this method to control for contamination, nor did I review any articles that recreated this method for other geographical regions so Mercader et al.'s (2017) method may be unique. Lentfer et al. (2002) used a similar concept to Mercader et al.'s (2017) to create a starch grain reference collection for paleoenvironmental recreation in Papua New Guinea, however. This gave me the impression that it could be possible to develop methods that investigate both ancient contamination and paleoenvironmental reconstruction simultaneously using starch as a proxy.

3.6 Reconstruction of paleoenvironments using ancient starch

Articles written about the application of ancient starch research to paleoenvironmental reconstruction were not common ($n=3$) within the sample of ($n=45$) articles presented here (Appendix, Figs. F.A.1.1 and F.A.1.2, articles 3, 14 and 20). This could be in part due to the small numbers of starch granules that are typically recovered from sediment samples (Ciofalo et al. 2018), an important source of starch useful for environmental reconstruction (e.g., Boyd et al. 2013, Lentfer et al. 2002, Field et al. 2016, Farley et al. 2018). Paleoenvironmental reconstruction is not limited to the analysis of sediment samples, however. Researchers have shown that starch grains recovered from artifacts can be used to supplement (Field et al. 2016), and in lieu of (Yang et al. 2018) starch data collected from sediments while reconstructing paleoenvironments.

The analysis of starch granules can be used to reconstruct long-term or short-term changes in environment. Longer-term research includes the work done by Yang et al. (2018) in northern China. In their work, a period from 25,000 to 5,500 cal. BP (from the Last Glacial Maximum to the mid-Holocene) was assessed. This was a diachronic study that followed the history of the domestication of millets. In northern China during the LGM, both wild wheats (tribe Triticeae) and wild millets were exploited. There, a gradual change in climate during the Holocene began favouring the growth of millet over wheat. Yang et al. (2018) argue that this is a significant reason why early farmers in the region chose to domesticate millets rather than wheats.

Starch analysis to recreate paleoenvironments can also be used to study relatively short time frames. For example, Field et al. (2016) reconstructed the paleoenvironment for a period ranging from about 2kya to the present to better understand how people adapted to the expansion

of tropical rainforests in northern Queensland, Australia, through niche construction of “eucalyptus pockets.” Eucalyptus pockets are areas adjacent to rainforests which would have been routinely cleared and managed in prehistory to grow specific cultigens. In their study, researchers collected starch granules and phytoliths from sediment samples and grinding stones associated with two archaeological sites, one within the rainforest and another in a eucalyptus pocket. The recovery of starches from economic plants walnut (*Endiandra spp.*, *Belschmiedia bancroftii*) and macadamia (*Lesjia whelani*) from within the eucalyptus pocket allowed Field et al. (2016) to determine it was maintained for around two thousand years. Another shorter-term study was conducted by Farley et al. (2018) on Ulong Island, Palau, which spanned from 3000 years ago until present. They recovered starches from a core taken from a sinkhole that would have been used for cultivation. Starches from banana (*Musa spp.*), yams (*Dioscorea spp.*), arrowroot (*Tacca leontopetaloides*), chestnut (*Inocarpus fagifer*) and breadfruit (*Artocarpus sp.*) were recovered in the oldest sediments dated to 3-2 kya. During the thousand years that follow, bananas disappear as land-use patterns transition from intensive clearance and gardening to reduced, stabilized gardening. Ulong is known to have been abandoned between 500-300 BP, and this portion of the sequence is characterized by a reduced variety of starches and a dearth of charcoal. A key advantage to relatively short-term studies that follow sequences extending into the present is that ethnographic data can be used to strengthen environmental reconstructions (e.g., Field et al. 2016, Farley et al. 2018).

Paleoenvironmental reconstruction can be reinforced by using proxies other than starch, for example phytoliths (e.g., Field et al. 2016), geochemical evidence, and charcoal (Farley et al. 2018). Some studies have used starches alone to reconstruct paleoenvironments. For example, Yang et al. (2018) reconstructed a nearly 20,000-year sequence of farming in northern China

using only the starch analysis of grinding stones. Their study included many starch granules (n = 1834) recovered over nine different archaeological sites. The richness in starch assemblages in this case therefore may have precluded the need for additional proxies.

4.0 Future directions for ancient starch research

It goes without saying that ancient starch researchers, like all archaeologists, should be informed of the most recent developments in their field. The Journal of Archaeological Science was identified as a valuable source of publications on ancient starch research between 2006-2015 (Barton and Torrence 2015), and the results from this chapter indicated the same was true for the years 2016-2020. However, journals may change their focus through time, as new editors take charge, new ‘hot topics’ develop, and new journals are created with similar mandates. Although it has been a leader in starch analysis for two decades, the Journal of Archaeological Science may not remain the richest source of articles written about ancient starch.

Many articles written about ancient starch that have been published in the prestigious journals *American Antiquity* and *PNAS* were written about the earliest evidence of the use of various cultigens (ie. Boyd and Surette 2010; Albert et al. 2018; Rumold and Aldenderfer 2016; Louderback and Pavlik 2017). This is consistent with observations made by Barton and Torrence (2015) where they specifically mentioned the importance of ancient starch research to investigate the spread of maize into North America.

Maize is an excellent candidate for the study of ancient starch because its seeds contain a relatively large number of relatively large starch granules when compared to many other species. Because of the prevalence of modern maize in food and other products like powdered gloves, controls for contamination in ancient maize labs have become quite sophisticated (e.g., Albert et

al. 2018; Boyd and Surette 2010; Kooiman et al. 2021). It is unclear whether these controls remove all risk of modern maize starch contamination, although it's not impossible that they do. It is essential that new methodologies created to control for maize contamination be useful in addressing other lines of research (e.g., paleoenvironmental reconstruction) since there is a risk that further controls for contamination may be unnecessary.

No ancient starch researcher has yet studied whether modern maize starches representing contamination (whether industrial or from food products) are distinguishable from heirloom species, the most likely cultigens to be recovered from archaeological materials. Messner (2011) published the description of the starches from three subspecies of an heirloom 'flint' maize that would have been cultivated in eastern North America. These three closely related species of starch appear to be distinguishable based on Messner's (2011) descriptions. In a different study, researchers demonstrated that the starches of four species of Mexican heirloom maize could be distinguished from teosinte based on physical characteristics (Holst et al. 2007). Taxonomically speaking, domesticated maize (*Zea mays ssp. mays*) shares a species name with the teosinte thought to be its wild ancestor (*Zea mays ssp. parviglumis*) and are distinguished in literature at the subspecies level. There are also some teosintes that do not share a species name with maize (e.g., *Zea luxurians*, *Z. perennis*) which are not thought to be the direct ancestors of maize. Holst et al. (2007) demonstrated that the starches of domesticated maize; the teosinte that is directly ancestral to maize; and teosintes of species other than *mays* are distinguishable, notably at the species and subspecies levels. Neither of the two aforementioned studies (i.e., Messner 2011 and Holst et al. 2007) included the description of modern cultivars of maize. Because the appearance of maize starch is thought to be genetically controlled (Holst et al. 2007) and the domestication process is known to exert a strong selective pressure on a plant's genes, it is reasonable to

speculate that modern maize (such as the kind that would be found in modern foods and industrial items and would represent modern contaminants) could be visually distinguishable from starches originating from the heirloom species used in prehistory. Indeed, it is already known that in crops such as maize, manioc, and *Capsicum* pepper that a marked increase in size is associated with domestication (Rumold and Aldenderfer 2016). Before the advent of ancient starch research in archaeology, Reichert (1912) analysed the starch of nine species of maize including modern and heirloom species. Here he reported that the histological characteristics of all species of maize were “essentially the same” (Reichert 1912: 354). There have been significant developments in ancient starch research since Reichert’s (1912) publication, however. His work does not preclude the potential differentiation of species of maize starches using modern statistical techniques though, such as the geometric morphometric approach (Coster and Field 2015).

Whether or not it is possible to distinguish starch originating from modern contaminant maize from heirloom species using statistical techniques, objective approaches to the identification of unknown starch grains are of utmost importance to the development of ancient starch research. It is critical for ancient starch researchers to provide robust identifications along with realistic and measurable estimates of confidence. The use of statistical techniques has been shown to strengthen paleoenvironmental recreations (e.g., Field et al. 2016, Farley et al. 2018), and should be applied to other streams of ancient starch research like the spread of cultigens into North America.

Current statistical methodologies used to classify ancient starch grains research rely heavily on continuous metrics like length, area, and hilum offset. These techniques include the geometric morphometric approach (GMA) (Coster and Field 2015) and linear discriminant (LD)

analysis (Torrence et al. 2004, Brown and Louderback 2020). These techniques present challenges distinguishing between grains that overlap in size and may only differ in their appearance, such as differences in their surface textures or overall shape. Presently, there is no objective method of recording these types of differences. Recent developments in the training of artificial intelligences (AI) to identify microbotanicals such as pollen (Sevillano et al. 2020, Dunker et al. 2020) and phytoplankton (Dunker et al. 2018) may be of use to ancient starch researchers wanting to assign species to archaeological starches since AI can objectively record subtle differences in traditionally subjectively recorded characteristics like texture and shape. It is possible that AI trained to perform image recognition on starches to assign species could perform at the same level as existing techniques like LD and GMA. It is also possible that image recognition could be used in conjunction with such techniques to improve the results of species identification. Preliminary research into the efficacy of training image recognition software to assign species to starch granules is described in the following chapter of this thesis.

5.0 Conclusion

Starch grain analysis is at an intriguing place in its development. Although relatively new, it has become an increased focus of study since the 2006 publication of “Ancient Starch Research” (Torrence and Barton 2006). Since 2015, cutting-edge research has been conducted in the investigation of the earliest use of cultigens (e.g., Boyd and Surette 2010, Albert et al. 2018, Rumold and Aldenderfer 2016, Louderback and Pavlik 2017), as well as the earliest evidence of fermentation (e.g., McGovern et al. 2017). Statistical techniques, most notably the geometric morphometric approach (GMA) (Coster and Field 2015) have been developed to provide ancient starch researchers with numerical measures of confidence in their identification of starch species

and to also demonstrate that identifications made using the traditional method of visual comparison are valid. The applicability of GMA has been demonstrated through paleoenvironmental reconstruction (e.g., Field et al. 2016). Dental calculus remains an important source of direct evidence of plant use (e.g., Cristiani et al. 2018, Goude et al. 2020, D'Agostino et al. 2020, D'Agostino et al. 2019) and methodological improvements are actively being developed (e.g., Tavarone et al. 2018, Power et al. 2014). Taphonomy remains a central area of research in ancient starch analysis. Since studying the effects of long-term degradation is in many cases unfeasibly time-consuming, researchers may benefit from applying field-based or case study approaches to understanding the long-term effects of taphonomy. Experimental archaeology will likely continue to play a role in understanding taphonomic effects of short-term processes, such as cooking, grinding, nixtamalization, or fermentation (e.g., Thoms et al. 2015, Ma et al. 2019, Johnson and Marston 2020, Wang et al. 2017). Contamination remains a concern for ancient starch researchers, though few new methods to control for it have been published between 2016 and 2020. Creative new methods should be developed that can be used both to control for contamination while being applied to other lines of ancient starch research.

The period in which ancient starch research finds itself is a sort of adolescence where there is still much room for development. However, there has also been enough time for a solid foundation of methodologies to have already been created, tested, and applied to archaeological materials. It is up to researchers to build upon this foundation in creative ways to allow ancient starch research to develop further.

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Chapter Two: Application of deep learning to the classification of some key plant taxa in eastern North America

Abstract

The classification of archaeological starch granules can be useful in studying various important archaeological themes such as the spread of agriculture and identifying the early cultivation of plants. These types of analyses typically require measurements of large numbers of starch granules which can pose technical difficulties in addition to being time consuming. This chapter describes a novel application of high-throughput microscopy and image-recognition software to address these challenges in the development of a starch grain classification tool. Seventeen species present in eastern North America, most of which are known to have been used as foods or medicines, were included in this analysis. Results were comparable to other published starch classification methods. Maize, and potato were highly recognizable using this method of image recognition, while some other species were shown to be less reliably identified, or not identifiable at all. Further research should include experimentation using archaeological materials.

6.0 Introduction

The purpose of this chapter is to discuss the potential of using computer AI (i.e., deep learning) to develop identification keys for the analysis of archaeological starch residues likely to be recovered from eastern North American assemblages. The recognition, observation, and identification of “types” is a critical part of archaeology, whether it is to identify archaeological

sites from remote sensing, or to classify artifacts and ecofacts to their respective periods or species. Specifically, this chapter describes a reproducible and affordable methodology which exploits image recognition software to classify species of starch granules. Recently, artificial intelligences (AIs) have been trained to identify microbotanicals such as pollen (Sevillano et al. 2020, Dunker et al. 2020) and phytoplankton (Dunker et al. 2018) using image recognition algorithms. Modern image recognition AIs use convolutional neural networks (CNNs) to emulate the way in which eyes function, and recently have been shown to outperform humans at some image recognition tasks (Dunker et al. 2020). CNNs may also be useful in detection of archaeological sites and have been demonstrated to be capable of classifying Maya sites from LiDAR data (Bundzel et al. 2020).

Image recognition can help researchers create identification schemes that are almost completely devoid of jargon. For example, in the classification scheme described in this chapter there is no need to understand the terms “longitudinal cleft” (i.e., lengthwise splits), “hila” (i.e., the core of the granules from where the growth rings arise), “fissures” (i.e., surface cracks), or other obscure, inconsistent language that pervades reports on ancient starch research (Brown and Louderback 2020). Analysts using this type of scheme would instead first allow the algorithm to suggest a species, and then verify or refute that classification by visually comparing the image to hundreds, or potentially thousands of reference photos.

As in many other fields of archaeology (i.e., zooarchaeology, ceramic analysis, etc.), the traditional method of identifying archaeological starches is done by visual comparison with modern reference material. Since visual comparison introduces a considerable amount of subjectivity, many researchers have developed statistical techniques to add validity to their identifications (e.g., Torrence et al. 2004, Coster and Field 2015, Wilson et al. 2010, Arráiz et al.

2016). The method described in this chapter is akin to those statistical techniques in that the output data is given as numbers which can be then used to make comparisons between other numerical datasets. The method described in this chapter however differs in a major way. To date, all statistical techniques used to classify starches rely on collecting continuous measurements (i.e., length, circularity, area, hilum offset, etc.) whereas image recognition does not. Although the sizes of starch grains do affect their appearance, and so in a sense continuous measurements do play a factor in distinguishing one species from another, image recognition also captures discrete differences automatically such as texture; presence and appearance of ornamentation (for example fissures; lamellae; and stellate, Y-shaped, or hollow hila); as well as subtle variations in the overall shape of granules in an objective way. Discrete qualities of starches are at times included in statistical classification schemes, and some researchers have suggested discrete characteristics are the most powerful distinguishers when compared with continuous measurements (i.e., Torrence et al. 2004). The use of image recognition is an objective way to capture discrete characteristics since these are captured as pixel data. To date there has been no objective way to record and compare the discrete characteristics of starch granules except for with the methodology described in this chapter.

The methodology for classifying starch granules described in this chapter was targeted at identifying three Indigenous food crops: maize (*Zea mays ssp. mays* L., *Zea mays ssp. everta* L.), common-bean¹ (*Phaseolus vulgaris* L.), and potato (*Solanum tuberosum* L.). This is due to the importance of these plants in the archaeological record of Ontario, Canada, and elsewhere in eastern North America. This key is also designed to identify wild plants known to have been Indigenous foods and/or medicines in this region. Some domesticated species, particularly maize,

¹ Note: common names of plants are hyphenated throughout this chapter to help readers recognize strings of words as being representative of single plants.

tend to produce greater amounts of starch compared to wild species. In the case of agricultural sites in eastern North America, domesticates would have also played a more major role as food staples. This makes the recovery of starches from domesticated species more likely from sites where agriculture was present. However, the classification method described in this chapter can also be applied to pre-agricultural eastern North American sites since starches have always been an essential part of human diets. Furthermore, the recovery and classification of starches from plants used for medicine can be applied to any archaeological assemblage where those plant medicines were used regardless of time-period.

7.0 Background

Starch granules are microscopic particles produced largely in the seeds and underground storage organs (USOs) of many edible plants. They are the carbohydrate rich final product of photosynthesis and are the plant's own, or their offspring's source for calories necessary for growth and other biological functions. Starch granules can be observed under a compound microscope, and their recognition can be aided by using cross polarized (XP) light. Under these lighting conditions, many starch granules will display a conspicuous "extinction" or "Maltese" cross (Gott et al. 2006).

Starch granules from different species can be distinguishable, in some cases allowing researchers to assign species to starches recovered from archaeological material. Identifications are frequently made by comparing archaeological starches to modern reference material, although these identifications can be further strengthened by using dichotomous keys (e.g., Messner 2011, Lints 2012, Yang and Perry 2013, Louderback et al. 2022, Perry and Quigg 2011b) and/or statistical techniques (e.g., Torrence et al. 2004, Coster and Field 2015, Arráiz et

al. 2016, Brown and Louderback 2020, Wilson et al. 2010). These methods rely on comparing morphological traits such as the presence/absence and quality of lamellae (i.e., growth rings), the position and shape of hila (i.e., the point of attachment), length (varying from near 0 μm to 30 μm or more) and two-dimensional shape (e.g., round, bell, kidney, irregular, etc.). In some cases, taxonomical attribution is only reasonable at levels higher than species (e.g., genus, family, etc.), and in others no reasonable taxonomical attribution can be given at all.

Artifacts like pottery and lithics, as well as the archaeological matrix can be productive sources of starch granules. Pottery may possess encrustation of carbonized food that can contain identifiable starch granules (e.g., Boyd et al. 2008, Kooiman et al. 2021) and grinding stones are popular reported sources for starches recovered from cereals, nuts, and tubers (e.g., Li et al. 2019, Owen et al. 2019, Hayes et al. 2021, Field et al. 2016, Field et al. 2020, etc.). Some other potential sources, such as fire-cracked-rocks (FCRs), have received relatively little study (e.g., Perry and Quigg 2011a). FCRs are plentiful in many archaeological sites around Ontario, Canada, and elsewhere in eastern North America. Identification of starches recovered from FCRs might make it possible to identify the FCRs as cooking elements. This may be useful in tracking the earliest use of cultigens in the Lake Superior region such as maize and northern-wild-rice (*Zizania palustris*). Perry and Quigg (2011a) argued that FCRs were used as cooking elements based on the recovery of starches from features containing large numbers of these artifacts in Texas, USA. Ethnographic literature indicates northern-wild-rice (*Zizania palustris* L.) was parched before consumption by Ojibway peoples in the Lake Superior region (Smith 1932). If residues recovered from FCRs in that region produced starch granules consistent with northern-wild-rice, an argument could be made the FCRs were similarly used for food-processing. As with all starch analyses of artifacts, researchers should be cautious of interpreting the presence of

starch in an FCR residue as representing the use of a specific plant with that artifact. This is because there is always a risk that an artifact could have been contaminated with starches from the surrounding archaeological matrix.

Maize is widely reported in starch granule analyses (e.g., Boyd et al. 2006, Boyd et al. 2008, Albert et al. 2018, Bérubé et al. 2020, Kooiman et al. 2021, etc.). The earliest domestication of maize likely began in Mexico around 9,000 years ago, and it was established in South America by 6,500 years ago (Kistler et al. 2018). By no later than 500 AD this crop had spread as far north as the Canadian Subarctic (Boyd and Surette 2010). Identifiable starch granules have been recovered from 28,000-year-old deposits (Loy et al. 1992) meaning all archaeological sites where maize agriculture existed have potential for maize starch granule recovery. However, not every archaeological site where maize agriculture existed will necessarily produce starches since there are post-depositional taphonomic processes that can destroy starches; these include (though are not limited to) biological or enzymatic degradation (Langejans 2010), and exposure to high (ca. $>70^{\circ}$ C) heat (Wang et al. 2017).

Some identification methods for maize starch already exist (e.g., Musaubach et al. 2013, Messner 2011, Holst et al. 2007), although none to date have incorporated image recognition technology. Furthermore, these methods use the traditional method of visual comparison and do not employ statistical techniques. The most similar method to the classification scheme described in this chapter used a combination of multispectral imaging flow-cytometry (MIFC) and a deep learning algorithm (Inception v.3) to automatically classify 35 species of pollen granules for environmental analysis (Dunker et al. 2020). Accuracy was measured at an average of 96%. There are two major obstacles to training image recognition software that Dunker et al.'s (2020) methodology overcomes. These are: (1) deep learning algorithms typically require extremely

large numbers of photographs for training, and (2) deep learning algorithms benefit from a uniform, consistent background to help differentiate objects from the background. The MIFC used in their experiment solves both these issues because it is capable of capturing photographs at a rate of approximately 5,000 particles per second on a neutral, consistent background.

MIFCs can photograph any particle that is miscible in phosphate buffered solution (PBS), which includes pollen (Dunker et al. 2020), phytoplankton (Dunker et al. 2018), red blood cells (Doan et al. 2020), and as this chapter demonstrates, starch grains. PBS has a refractive index (1.33 at 25° C) nearly identical to water, which is considered a suitable mounting medium for observing starch grain morphology (Barton and Fullagar 2006).

At the outset of the research described here, it was unclear whether a deep learning algorithm could be trained to identify species of unknown starch granules. There was also a suspicion that some species of granules would be more readily identifiable since this has been frequently reported (e.g., Messner 2011, Mercader et al. 2018, Torrence et al. 2004, Coster and Field 2015). The research presented in this chapter addresses these uncertainties and furthermore lays the groundwork for the application of this type of identification key to other archaeological materials.

8.0 Materials and methods

8.1 Species selection

A total of 17 species within nine plant families were selected for analysis. These included the eastern North American crops maize and common-bean. Although squash was an important staple in eastern North American diet, this plant was not included in this study because its starch grains fall below the size cutoff used in the IFC experiments (15 μ m; see section 8.3). Wild plants

known to have been used as food and/or medicine in eastern North America were also included (Table T.2.1). Any plant whose native range includes North or South America is categorized in that table (T.2.1) as “New World” plants. “Ethnobotanical” plants are only listed as such if they have been reported as plant food or medicines in the ethnobotanical record of eastern North America (specifically Densmore 1928, Tantaquidgeon 1942, Curtin 1949, and Smith 1932). In the case of each species only one tissue was sampled. This was either the underground storage organ (USO) of the plant (i.e., tuber, corm, rhizome, etc.) or the seed (i.e., grains, or in the case of common-bean cotyledons). See section 8.2 for more information why these tissues were selected.

One Old World domesticate, wheat (*Triticum* spp.), was included to determine whether the classification scheme described in this chapter could be used to detect modern contamination that may have occurred in the field or lab. Two wild species, Michigan-lily (*Lilium michganense*) and one-flower-broomrape (*Orobanche uniflora*) whose ranges include eastern North America and have no known recorded use by humans were included because their identification could be used for paleoenvironmental reconstruction and/or to track contamination that occurred in the field. These two plants share a genus level relation to one or more food/medicinal plant included in this analysis, specifically Canada-lily (*Lilium canadense*), Philadelphia-lily (*Lilium philadelphicum*) and Louisiana-broomrape (*Orobanche ludoviciana*). It was unclear whether or how accurately the classification scheme described in this chapter would discriminate between species of the same genus.

Table T.2.1: List of plants whose starch was analyzed for descriptive statistics and algorithm training.

Family	Genus	species	name	New/Old World	domesticate/wild	ethnobotanical?	tissue sampled
Alismataceae	<i>Sagittaria</i>	<i>latifolia</i>	wapato	New	wild	yes	USO
Araceae	<i>Arisaema</i>	<i>triphyllum</i>	jack-in-the-pulpit	New	wild	yes	USO
Araceae	<i>Peltandra</i>	<i>virginica</i>	green-arrow-arum	New	wild	yes	USO
Boraginaceae	<i>Hydrophyllum</i>	<i>canadense</i>	Canada-waterleaf	New	wild	yes	USO
Boraginaceae	<i>Hydrophyllum</i>	<i>virginianum</i>	Virginia-waterleaf	New	wild	yes	USO
Colchicaceae	<i>Uvularia</i>	<i>perfoliata</i>	perfoliate-bellwort	New	wild	yes	USO
Colchicaceae	<i>Uvularia</i>	<i>sessilifolia</i>	wild-oat	New	wild	yes	USO
Fabaceae	<i>Phaseolus</i>	<i>vulgaris</i>	common-bean	New	domesticate	yes	seed
Liliaceae	<i>Lilium</i>	<i>canadense</i>	Canada-lily	New	wild	yes	USO
Liliaceae	<i>Lilium</i>	<i>michiganense</i>	Michigan-lily	New	wild	no	USO
Liliaceae	<i>Lilium</i>	<i>philadelphicum</i>	Philadelphia-lily	New	wild	yes	USO
Orobanchaceae	<i>Orobanche</i>	<i>ludoviciana</i>	Louisiana-broomrape	New	wild	yes	USO
Orobanchaceae	<i>Orobanche</i>	<i>uniflora</i>	one-flower-broomrape	New	wild	no	USO
Poaceae	<i>Triticum</i>	<i>spp.</i>	wheat	Old	domesticate	no	seed
Poaceae	<i>Zea</i>	<i>mays ssp. mays</i>	maize	New	domesticate	yes	seed
Poaceae	<i>Zea</i>	<i>mays ssp. everta</i>	popping-corn	New	domesticate	yes	seed
Solanaceae	<i>Solanum</i>	<i>tuberosum</i>	potato	New	domesticate	yes	USO

8.2 Reference material

All plants sampled for this study were collected from the Department of Anthropology (Lakehead University) starch grain reference collection. This reference collection was created by an employee of the university (Clarence Surette) under the supervision of a faculty member (Matthew Boyd). This collection contains locally available and exotic species of economically important and wild plants including several domesticates. These specimens were originally either collected from the wild, obtained from Lakehead University's herbarium, or purchased from a grocery store. In most cases, more than one reference sample was available per species, so starches from different organs within the same plant could be compared. Each sample has an associated slide fixed in Entellan (an anhydrous mounting medium with a refractive index of 1.490-1.5 manufactured by Sigma-Aldrich consisting of a polymer of acrylates dissolved in toluene used for the permanent mounting of samples) for study using conventional microscopy and are curated as starch pellets contained in microcentrifuge tubes for further sampling. It was from these pellets that the starches analyzed in this study were collected.

Starch pellets were produced according to the following procedure: (1) plant material was cut into separate sections (i.e., seed, rhizome, shoot, etc.), (2) if the plant was desiccated (i.e., collected from the herbarium) it was allowed to soak in water for several minutes, (3) plant materials had their skin, membranes or other outer tissues removed with a clean knife to decrease the risk of capturing contaminants, (4) materials were pulverized in distilled water using a ground stone (granite) mortar and pestle, (5) suspended plant materials were recovered using a fresh pipette then filtered through a 100 μm or smaller fabric sieve, (6) sieved materials were recovered with a fresh pipette, deposited into microcentrifuge tubes, then centrifuged until a pellet formed, (7) the supernatant was removed using a fresh pipette and the remaining pellet was

allowed to air dry for at least 24 to 48 hours, and (8) the pellet was soaked in an excess of 70% ethanol and vortexed to resuspend the pellet. Ethanol was used to reduce the risk of biological degradation during storage. Each time this procedure was repeated on a new plant, or a different organ of the same plant the knife, mortar and pestle were thoroughly cleaned with water to avoid cross contamination. Over the years these samples remained undisturbed in the lab so the starches and other materials mostly precipitated to the bottoms of their tubes as pellets.

In most cases, starches sampled for the research described in this chapter were collected from the prepared pellets of underground storage organs (USOs) such as tubers, corms, and rhizomes (see Table T.2.1). The exceptions were maize, common-bean, wheat, and popping-corn whose pellets were prepared from their seeds. In all cases, the pellets sampled for the research described in this chapter were chosen because their associated slides contained any combination of large, abundant, and/or remarkable grains relative to other tissues from the same plant.

Sampling of starches for the research described in this chapter was conducted using the following procedure: (1) using a micropipette and vortexing, starch pellets were resuspended in their 70% ethanol solution, (2) subsamples were collected using a clean micropipette, filtered through a 100 μm fabric sieve, and deposited into fresh microcentrifuge tubes, (3) subsamples were centrifuged back into pellets, (4) supernatants were removed using fresh pipettes, and (5) pellets were suspended in distilled water.

8.3 Image acquisition

Starch samples were shipped to Lunenfeld-Tanenbaum Research Institute, Toronto, Canada, suspended in water. Although I was present in the lab during photography, samples were shipped ahead to save time. Samples were resuspended upon receipt in phosphate buffered

solution (PBS) and photographed using a multispectral imaging flow cytometer (MIFC) (Amnis ImageStream™ MKII) (Basiji 2016). This instrument is a microscope capable of capturing thousands of images per second. Particles are forced through a fine nozzle where they are photographed up to 12 times into two brightfield and 10 fluorescent channels. This is achieved by reflecting the image of the particle through the objective lens into a network of mirrors ending at each of the 12 photo receptors. Images from fluorescent channels were not collected since starch granules, unlike some other plant structures like chloroplasts are not naturally fluorescent.

The MIFC can take photographs at 40x and 60x magnification. An attempt was made to collect images at 60x however the relatively large granules of majority of target species proved to be better captured at 40x, so that magnification was used. This resulted in a relatively lower level of resolution and made granules of smaller sizes less clearly visible.

Images were saved as raw image files (*.rif) on an external hard drive, then downloaded to an Acer Spin™ laptop. Raw image files (*.rifs) are a proprietary format designed to be used with the software IDEAS (v6.2) (Luminex 2021) which was installed on the laptop. The software was developed specifically to be used with images collected from an Amnis ImageStream™. Opening *.rif files in IDEAS triggers the creation of *.caf, and *.daf files, which are smaller files used for data analysis. These formats allow the user to create various populations, either by hand, or by excluding images according to measurements such as length. Images can be plotted on graphs as points, which when selected will bring the user to the selected image. The x and y axes of these graphs can be customized, for example width on the x-axis and length on the y. In this example, if a researcher was interested in elongated grains, they could be accessed by selecting the various plotted points in the top-left, or bottom-right quadrants of the graph.

The raw images were “gated” to exclude any particles with lengths between 15 and 30 μm . Gating in IDEAS is analogous to “screening” in archaeology, where archaeologists will use sieves of various sizes to separate sediments (artifacts, ecofacts, etc.) by size. Gating, though, can exclude particles for other morphological differences besides size (e.g., aspect ratio, circularity, symmetry, lobe count, etc.), and for non-morphological differences between photographs, such as flow-speed and time. The range 15 and 30 μm was chosen for four reasons. Firstly, smaller grains in some cases were seen to be less easily identifiable by the algorithm (see section 8.4). Secondly, to simplify identification of starches that would be recovered from archaeological residues by removal of smaller, harder-to-see, granules. The images collected by MIFC of archaeological residues would include many images of debris. MIFC is incapable of cross polarized (XP) light microscopy, so researchers cannot rely on the extinction cross to identify starches. They would instead rely on common traits of starch granules such as the presence of a hilum (fissures, lamellae, etc.). Due to the low resolution of the MIFC photos, it could be challenging for researchers to distinguish these features on smaller granules. It is important to note that species with smaller granules, like taro and yam whose granules measure 2-10 μm are identifiable using conventional microscopy (e.g., Farley et al. 2018). Thirdly, a limited size range of starches used was to facilitate applying this classification scheme to archaeological materials. By choosing 30 μm as a maximum length, archaeological residues may be sieved to this extremely fine fraction before photographing the particles by MIFC. This would reduce the number of images of debris that an analyst would have to parse through to find starch granules. From experience I have noted species producing starches larger than 30 μm typically (maybe always) also include granules that are between 15 and 30 microns in length. I have observed that these relatively “small” granules in larger-grain species tend to be produced in greater numbers

than those longer than 30 μm . Finally, should this experiment be repeated, excluding starches $>30 \mu\text{m}$ may allow for the use of the higher (60x) magnification setting of the MIFC.

To accurately remove particles smaller than 15 μm and larger than 30 μm , a custom “mask” needed to be created. A mask in the IDEAS program is a polygon that is automatically superimposed over every single particle within the image set of a *.caf or *.daf file (Fig. F.2.1). The program possesses a default mask which can be modified in several different ways (e.g., “erosion,” “dilation,” etc.). Due to a “haloing” effect common to images of starch granules, the default mask over-exaggerated the size of starch granules (Fig. F.2.1). To correct for this discrepancy, the default mask was modified by using “erosion,” a function that removes a “selected number of pixels from all edges of the starting mask” (Luminex 2021: 222). The number of pixels to remove was set to seven pixels, as it appeared to best approximate the true shape of granules (Fig. F.2.1). This effect was not perfect, and it was noted anecdotally that it was more accurate for larger granules and would under-exaggerate the lengths of smaller ones. The accuracy of this custom mask was tested by comparing the range of length measurements it collected from maize granules of all sizes (i.e., not only between 15 to 30 μm) to maize lengths published in another identification key (i.e., Messner 2011) (Appendices, A.3.1).

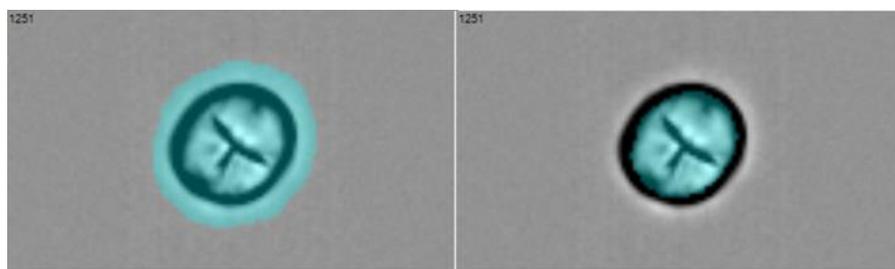


Fig. F.2.1.: The effect of applying the “erosion” effect to the “mask” of a maize starch granule. The left image is the default mask, and the right image is the mask after the application of erosion effect.

Following gating, I carefully selected images to include those which were free of debris; that were of unblurred, undamaged, individual grains; and were without overexposure, lens flares, or other optical effects but this was not always possible. Samples included variable amounts of non-starch material. Less pure samples had smaller numbers of photographed starch, which reduced the overall number of suitable images. Therefore, to allow for the inclusion of the greatest number of species in this study, some image sets are relatively overexposed, blurry, or are otherwise of inferior quality. Images of compound, and semi-compound grains were included, although aggregates, or clusters were not.

Of the 500 starch granule images per species, each grouping was divided into training and validation sets containing 300 and 200 images, respectively. These images were then exported into *.tif format, and organized into folders appropriate for the image recognition software. This was organized in the following way: “Experiment_title” > “Training_set” and “Validation_set” > “Species_name” > *.tif images. The number 300 was selected for the training sets because this is considered a robust number of grains to measure in recent publications describing starch grain identification (e.g., Brown and Louderback 2020; Mercader et al. 2018). Since the analysis described in this chapter includes 17 species, a total of 8,500 images were collected for analysis. All the images measured for this chapter are available online from my personal Google DriveTM account and can be access at the following web address:

https://drive.google.com/drive/folders/1LGVRaEUltZUioVuCvtg36m0_Cj4wJmlz?usp=sharing.

The total size of the reference image set is 1.03GB.

8.4 Algorithm training

To train the image recognition algorithm, the widely available scripting program Matlab (v R2021b) was utilized. This program has been used by other ancient starch researchers (e.g., Coster and Field 2015). Matlab has modules that allow researchers to download “pre-trained” image recognition algorithms, which are particularly useful because they take advantage of “transfer learning.” Transfer learning is when an algorithm has been previously trained in a similar task, in this case the algorithm would have been already trained in the classification of images such as animals and vehicles. Just as a human would, the algorithm applies what it has already learned to a new task, in this case the classification of images of starch grains. Two benefits of transfer learning are that smaller image sets can be used to train algorithms, and overall computing time is reduced considerably. Although there are a fair number of pre-trained algorithms that could have been accessed through Matlab, ‘resnet-18’ was selected. For resnet-18 to work in Matlab the modules “Computer Vision Toolbox,” and “Deep Learning Toolbox” needed to be downloaded. The measurable results from the training of deep learning, image recognition software is given as a percentage value, called the “validation accuracy.” In any given training regiment, the algorithm needs to be provided with a certain number of “training” images, and a separate number of “validation” images. The test for validation accuracy is like an open book exam where the computer compares the “known” training image set to the validation images of “unknown” species. The closer the validation accuracy is to 100%, the more reliable the algorithm’s ability is to successfully classify images.

Experiments were carried out either on a desktop computer equipped with a GeForce GTX 960 graphical processing unit (GPU), or an Acer Nitro™ 5 possessing a GeForce GTX1650 GPU. The scripts (Fig. F.2.2, Fig. F.2.3) were run 60 times using various numbers of

training images, validation images and training epochs. The number of training images used per species was either 300 or 150. The number of validation images were either 200 or 100. Epochs, which are the total number of times a machine learning algorithm passes through an entire dataset were set at either 5, 10, or 20. The final (60th) iteration of the experiment, whose results are cited in in this chapter (see section 9.1), used 300 training images, 200 validation images, and 10 epochs. All image recognition experiments were conducted concurrently to the image sets being processed. This is because image processing in IDEAS takes considerably longer than running the image recognition algorithm making results more-or-less available “in real time.” Initially image recognition experiments were conducted using all sizes of grains (i.e., not just those 15 μm to 30 μm in length), though this strategy was abandoned after the 27th iteration to improve the average classification accuracy. Perhaps due to the low resolution of the images collected by MIFC, smaller grains seemed more difficult for the algorithm to identify, and their inclusion reduced the overall classification accuracy in certain cases by about 5%. For example, when the algorithm was trained with 4 species (maize, jack-in-the-pulpit, wapato, and eastern-skunk-cabbage) using grains of all sizes, the classification accuracy was on average 91.9%. When smaller grains of those species were excluded, the classification accuracy increased to 97% on average. The number of species included in experiments varied from between four to 22. The additional species that did not end up being included in this analysis were squash, indian-cucumber (*Medeola virginiana* L.), eastern-skunk-cabbage (*Symplocarpus foetidus*), northern- (*Zizania palustris* L.), and southern-wild-rice (*Zizania aquatica* L.), which were ultimately excluded from the final classification methodology due to their small size.

```

clear

rng('shuffle')      %% creates a different seed (starting number from a series of numbers from a random
                    %% number generator) each time the script is run. This stops the script from producing the same results each time
                    %% it is run

outputFolder = fullfile('D:\Maizeolus_v2\Training_set');      %% folder where training images are located
trainDigitData = imageDatastore(outputFolder,'IncludeSubfolders',true,'LabelSource','foldernames');

outputFolder = fullfile(['D:\Maizeolus_v2\Validation_set']);      %% folder where validation images are located
testDigitData = imageDatastore(outputFolder,'IncludeSubfolders',true,'LabelSource','foldernames');

inputSize = [224 224 3];  %% sets the image size (resnet-18 can only use images of this size)
augimdsTrain = augmentedImageDatastore(inputSize,trainDigitData,'ColorPreprocessing','gray2rgb');  %%
augimdsValidation = augmentedImageDatastore(inputSize,testDigitData,'ColorPreprocessing','gray2rgb');  %%
                    %% changes greyscale training images into three separate images representing red, green and blue channels
                    %% changes greyscale validation images into three separate images representing red, green and blue channels

numClasses = 17;  %% total number of species. In this experiment, there were 17 species, so this is set to 17

problem2;
miniBatchSize = 32;      %% changes batch size. Can be changed based on the power of your computer
validationFrequency = floor(numel(trainDigitData.Labels)/miniBatchSize);

options = trainingOptions('sgdm',...
    'LearnRateSchedule','piecewise',...
    'LearnRateDropFactor',0.1,...
    'LearnRateDropPeriod',2,...
    'MaxEpochs',10,...
    'InitialLearnRate',0.001,...
    'MiniBatchSize',miniBatchSize,...
    'ValidationData',augimdsValidation, ...
    'ValidationFrequency',validationFrequency);

convnet = trainNetwork(augimdsTrain,lgraph,options);      %% trains image recognition algorithm

[YPred] = classify(convnet,augimdsValidation);  %% creates array of all guesses

plotconfusion(testDigitData.Labels,YPred);  %% uses array to create a confusion matrix and display error
YPred

```

Fig. F.2.2: First Matlab script (“main”) used in classification experiment.

```

net=resnet18('Weights', 'ImageNet'); % Using Transfer Learning method, a resnet-18 network with ImageNet
dataset pretrained
lgraph = layerGraph(net); %% this creates a graph that allows the user to track variables for each
experiment, in order to track the performance of the algorithm

layersToRemove = {
'fc1000' %% resnet-18 usually has 1000 classes. Here the script needs to change that number to the
number of possible species (ie. 17)
'prob'
'ClassificationLayer_predictions'
};

lgraph = removeLayers(lgraph, layersToRemove); %% "lgraph" creates a chart to track the performance of
the convolutional neural network (the classification algorithm) through each epoch (ie iteration)

newLayers = [
fullyConnectedLayer(numClasses, 'Name', 'FC1')
softmaxLayer('Name', 'Softmax')
classificationLayer('Name', 'classoutput')
];

lgraph = addLayers(lgraph, newLayers);
lgraph = connectLayers(lgraph, 'pool5', 'FC1');

```

Fig. F.2.3: Second script “problem2” referenced in the main code (Fig. 2.2).

8.5 Descriptive statistics

After the images used for algorithm training were prepared, lengths of all those images were recorded because length is one of the most valuable measurements that can be used to distinguish starch granules (Torrence et al. 2004). Collecting length measurements was very simple using the IDEAS software and extremely quick. Measuring the lengths of the 500 starch grains selected from each species took the program only a matter of seconds, whereas doing the same by hand would have taken substantially longer. Length distributions were recorded as histograms (see section 9.2; Figs. F.2.5-2.21). Because the images used for algorithm training were the ones that were measured, all measured grains were between 15 to 30 μm in length. Although all plant species studied in this report produce starch granules between 15 to 30 μm , each has its own frequency distribution within this range (which is not the full range for many, and quite likely all plant species). By reviewing this distribution, researchers can gather an

understanding of which lengths (within the 15 to 30 μm range) occur most frequently in any given species, which could then be used to strengthen identifications.

9.0 Results

9.1 Species classification of image recognition algorithm training

Although the overall validation accuracy of the algorithm was 85.8%, it performed differently depending on which species was being classified. This can be visualized through the creation of a confusion matrix (Fig. F.2.4). In the confusion matrix, the values along the diagonal from top left to bottom right corners represent the validation accuracy for each individual species. In addition to displaying how the algorithm performed for each given species, this chart shows what the misclassified images were classified as.

		Target class																	
		Alismataceae		Araceae		Boraginaceae		Colchicaceae		Fabaceae	Liliaceae			Orobanchaceae		Poaceae			Solanaceae
		<i>S. latifolia</i>	<i>A. triphyllum</i>	<i>P. virginica</i>	<i>H. canadense</i>	<i>H. virginianum</i>	<i>U. perfoliata</i>	<i>U. sessilifolia</i>	<i>P. vulgaris</i>	<i>L. canadense</i>	<i>L. michiganense</i>	<i>L. philadelphicum</i>	<i>O. ludoviciana</i>	<i>O. uniflora</i>	<i>Z. mays</i> spp.	<i>Z. mays</i> spp. everta	<i>T. spp.</i>	<i>S. tuberosum</i>	
Output class	<i>S. latifolia</i>	95.5					1.5	1					1	0.5	2	6	1		
	<i>A. triphyllum</i>		97																
	<i>P. virginica</i>	1		86				0.5			0.5				1	0.5			
	<i>H. canadense</i>				96		6	0.5		0.5							2		
	<i>H. virginianum</i>					98.5													
	<i>U. perfoliata</i>						73	4									11.5		
	<i>U. sessilifolia</i>				3.5	1	14.5	92.5			0.5						0.5		
	<i>P. vulgaris</i>		0.5	3					81.5						1	0.5			
	<i>L. canadense</i>		0.5			0.5				99.5									
	<i>L. michiganense</i>	1.5		1			1				76	23.5	4.5	8.5					
	<i>L. philadelphicum</i>			9.5			0.5	0.5	0.5			19		0.5	0.5				
	<i>O. ludoviciana</i>	0.5		0.5					14		21.5	56.5	94.5	1.5		1			
	<i>O. uniflora</i>	1.5			0.5		1	1.5	3.5		2.5		1	88			3	0.5	
	<i>Z. mays</i> ssp. <i>mays</i>														96.5	27.5			
	<i>Z. mays</i> ssp. <i>everta</i>		2											0.5	1.5	67.5	0.5		
	<i>T. spp.</i>						2							0.5			62		
	<i>S. tuberosum</i>						0.5										14	98.5	

Fig. 2.4: Confusion matrix describing the validation accuracy of the identification key reported. The darker gray diagonal represents the individual validation accuracies for each given species. All values are given as percentages (%). Species with >90% validation accuracy are highlighted with a black background and green text. Grey cells not in the diagonal represent misclassified species, where the ‘target’ was the known species and the ‘output’ was the classification made by the algorithm.

Seeing the validation accuracies displayed differently for each species can be useful in comparing how well an algorithm identifies different species of the same plant. For example, if we were interested in the algorithm's ability to identify maize specifically. The confusion matrix (Fig. F.2.4) shows that the algorithm correctly classified the images of popping-corn (*Zea mays ssp. everta*) 67.5% of the time. It also shows that 27.5% of the time images of popping-corn (*Zea mays ssp. everta*) were misclassified as the other cultivar of corn in the experiment, maize (*Z. mays ssp. mays*). If these misclassifications were instead treated as correct because they are the same species, *Z. mays ssp. everta* should have received a 95% validation accuracy. Similarly, images of *Z. mays ssp. mays* were misclassified as *Z. mays ssp. everta* 1.5% of the time. If those were treated as correct, *Z. mays ssp. mays* would have a validation accuracy of 98%. If an average is then taken for *Z. mays ssp. everta* and *Z. mays ssp. mays*, it could be said the algorithm correctly identified maize/corn to the species level only, with a 96.5% validation accuracy. This is coincidentally the same validation accuracy that was provided for *Z. mays ssp. mays* alone.

The confusion matrix (Fig. F.2.4) shows that Canada-lily, wapato, jack-in-the-pulpit, Canada-waterleaf, Virginia-waterleaf, wild-oat, Louisiana-broomrape and potato were all reliably identified (with >90% validation accuracies). Of these, Canada-lily stands out as the plant with the highest validation accuracy attained (99.5%). Furthermore, Canada-lily was the only plant within the sample that ever attained a 100% validation accuracy throughout all 60 of the training experiments, and it did so more than once. The starch from this plant was analyzed by Messner (2011) and no specific mention was made of any unique qualities besides the presence of fissures and lamellae. These features are present in far fewer than 99.5% of the starch granules included in this study (see section 9.2.6; Fig. F.2.13) so it unlikely these features

alone are responsible for explaining how this species was able to achieve 100% validation accuracy. After reviewing the images used to train the algorithm and comparing them with others, I cannot make sense what the algorithm is “seeing” that makes Canada-lily so conspicuous.

Although closely related to Canada-lily which received an exceedingly high level of correct identification throughout experimentation, the plant misclassified most often was Philadelphia-lily. This plant was more often misclassified as Michigan-lily (23.5%) than correctly identified (19%). Philadelphia-lily was never classified as Canada-lily and was most frequently classified as the completely unrelated, parasitic plant Louisiana-broomrape (56.5%). Incidentally, the plant that was most frequently (i.e., 191/556 or 34.4%) classified incorrectly by the algorithm was also Louisiana-broomrape. It is unclear why this pattern occurred, although it was noted that this species morphology appeared highly variable upon review. The overall shapes ranged from somewhat round to drastically lobed. Faceting was noted as well as a range of hilum forms. The size of the grains is not overly large so the overlap in size with other species may have also played a role in the algorithm’s misclassifications. The second most frequent misattribution of species given by the algorithm was Michigan-lily (14.4%). However, if misclassification of lilies at a genus level were considered correct (i.e., misattributions of Philadelphia-lily as Michigan-lily), the percentage falls considerably to 6%.

It has been suggested that using a larger number of granules to train classification schemes may produce better results (Coster and Field 2015). To test whether there was a statistically significant difference in validation accuracy when using a larger image set, an experiment was run using only 100 training images as opposed to the 300 used to produce the confusion matrix (section 8.4; Fig. F.2.4). The number 100 was chosen because it is common for

reports describing the classification of starch granules to measure between 100 (e.g., Torrence et al. 2004) and 300 granules (e.g., Brown and Louderback 2020). This iteration of the experiment received a validation accuracy of 76.94%, representing an approximately 10% reduction. These results were tested using a McNemar's test (Dietterich 1998). This test is like a chi-square test that has only one degree of freedom. Unlike a regular chi-square test however, McNemar's tests can only compare two dichotomous variables. In this case, the dichotomous variables were: (1) the total number of instances that one experiment correctly classified a validation image, while the other did not; and (2) vice versa. The reasoning why other variables are ignored in a McNemar's test (i.e., when both experiments classify the same image correctly or vice versa) is that in these cases both experiments are equally accurate (or inaccurate). It can be preferable to perform five iterations of a twofold cross-validation test since it is slightly more powerful than a McNemar's test (Dietterich 1998). Five iterations of a twofold cross-validation test were not conducted because the McNemar's test produced an acceptable p-value (chi-square = 170.73, df = 1, $p < 0.00001$). These results suggest that identification keys created following the one in this chapter should include at least 300, and not 100 images. It is unclear whether this rule should be applied to identification keys created by other means.

9.2 Descriptive statistics

Below is a series of figures (Figs. F.2.5 to 2.21) illustrating the descriptive statistics of all 17 species analyzed in this study. Each figure includes the count, mean, median, standard deviation and maximums (within the 15 to 30 μm pre-defined range, rationale given in section 8.3) of the lengths of the starch granules selected for the image recognition experiments. It is important to note that these statistics are constrained by the 15 to 30 μm size range that was

measured; if a different range was set, the means, medians, standard deviations, and maximum lengths for each taxon might be different. However, it is not necessarily true that the measurements would be different, especially if the grains outside this range are outliers. Since the application of these experiments as a classification scheme was only developed to be used on starch granules measuring 15 and 30 μm , and all species recorded possessed minimum lengths of 15 μm , no minimum lengths are provided. The distribution of lengths of each species are displayed as frequency histograms to provide the shape of each species' length distribution.

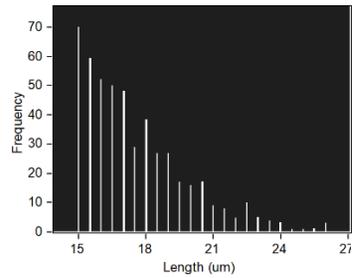
Included beside each histogram for length is a gallery of 45 randomly selected starch granules images from the corresponding species. Since 500 granules from each species were chosen, these galleries represent 9% of the entire population of photographs for each analyzed species. These are presented to give the analyst a snapshot of each species appearance, and relative size overall at-a-glance. These images could be used as reference images when attempting to identify the species of unknown starch granules. These galleries should not be considered exhaustive for starch granule types for any of the species.

It is worth emphasizing that since the measurements taken for starch granules were only from those selected for image recognition, all measurements are between 15 to 30 microns. Because of this pre-determined range, some (possibly all) descriptive statistics provided in this section may not be accurate representations of the full variability within each target species. Typically starch grain lengths follow a bimodal or normal distribution, though they are sometimes negatively skewed. Species included here with length histograms that appear positively skewed (e.g., green-arrow-arum, wild-oat) may have normal distributions with modes near the lower limit of the size range measured in this study; thus, the true mean or median lengths for these species would be smaller than the means or medians provided in this section.

9.2.1.0 Alismataceae (Water-plantains)

9.2.1.1 *Sagittaria virginica* (wapato)

wapato



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
wapato	500	17.54	17	2.312	26

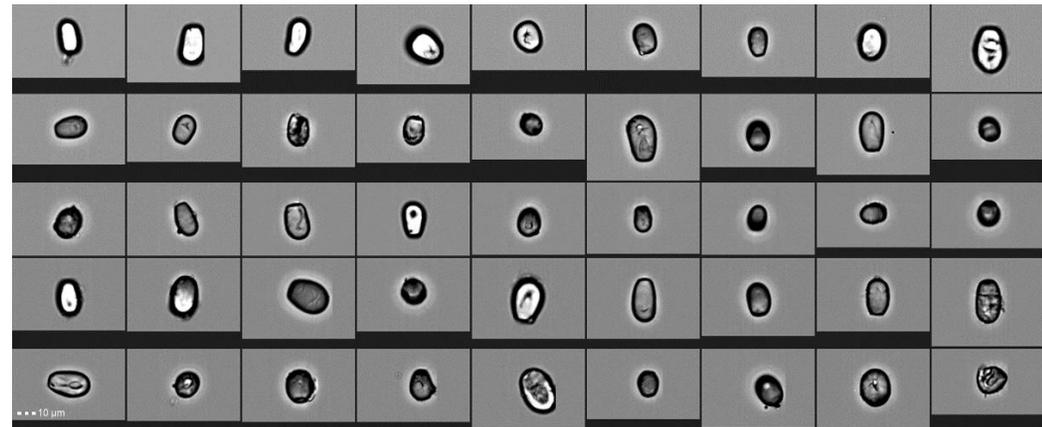
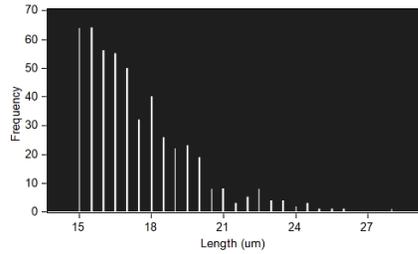


Fig. F.2.5: Descriptive statistics of length, and sample gallery for *Sagittaria latifolia* (wapato).

9.2.2.0 Araceae (Arums)

9.2.2.1 *Arisaema triphyllum* (jack-in-the-pulpit)

jack-in-the-pulpit



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
jack-in-the-pulpit	500	17.42	17	2.221	28

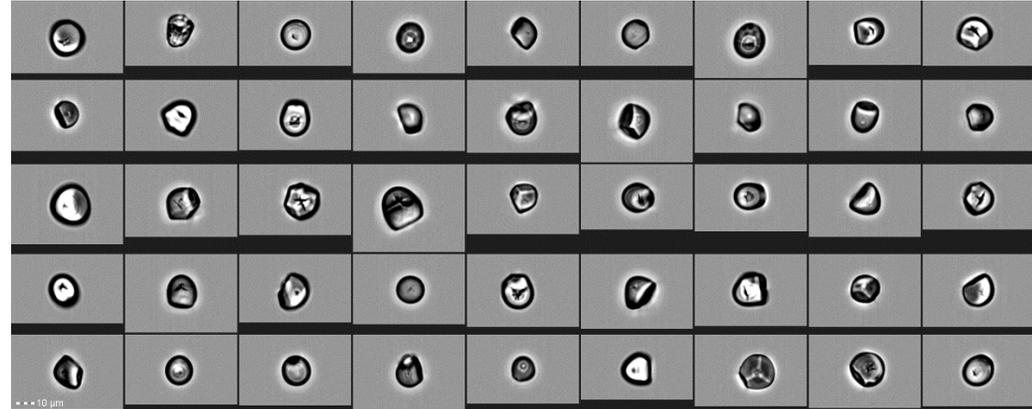
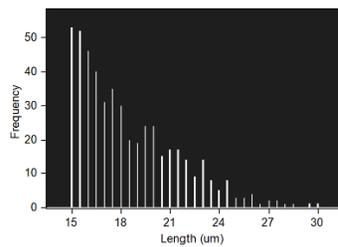


Fig. F.2.6: Descriptive statistics of length, and sample gallery for *Arisaema triphyllum* (jack-in-the-pulpit).

9.2.2.2 *Peltandra virginica* (green-arrow-arum)

green-arrow-arum



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
green-arrow-arum	500	18.46	17.5	3.046	30

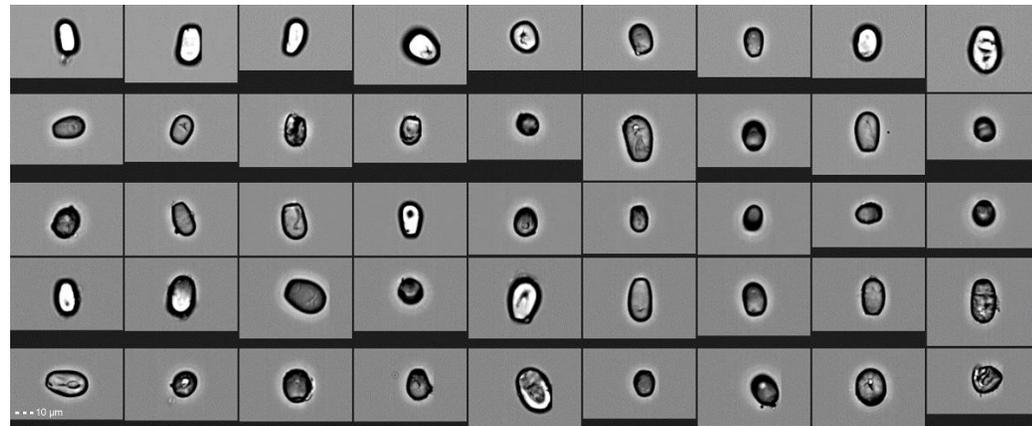
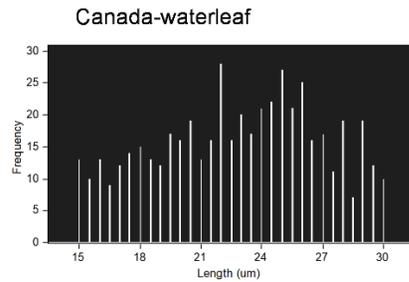


Fig. F.2.7: Descriptive statistics of length, and sample gallery for *Arisaema triphyllum* (jack-in-the-pulpit).

9.2.3.0 Boraginaceae (Borages)

9.2.3.1 *Hydrophyllum canadense* (Canada-waterleaf)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
Canada-waterleaf	500	22.83	23	4.071	30

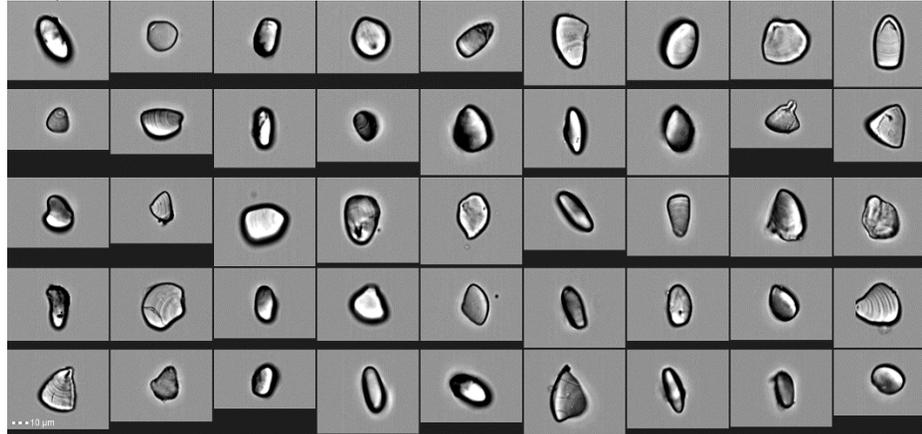
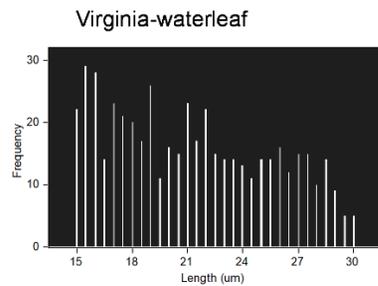


Fig. F.2.8: Descriptive statistics of length, and sample gallery for *Hydrophyllum canadense* (Canada-waterleaf).

9.2.3.2 *Hydrophyllum virginianum* (Virginia-waterleaf)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
Virginia-waterleaf	500	21.25	21	4.294	30

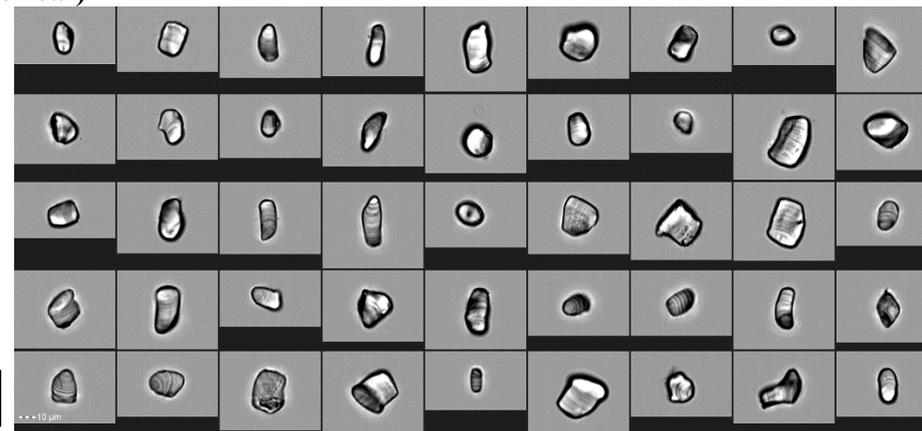
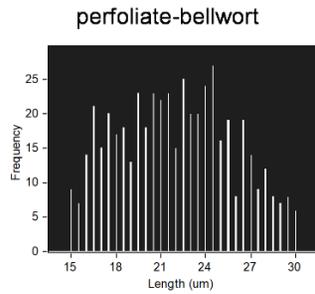


Fig. F.2.9: Descriptive statistics of length, and sample gallery for *Hydrophyllum virginianum* (Virginia-waterleaf).

9.2.4.0 Colchicaceae (Colchicums)

9.2.4.1 *Uvularia perforliata* (perfoliate-bellwort)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
perfoliate-bellwort	500	22.01	22	3.839	30

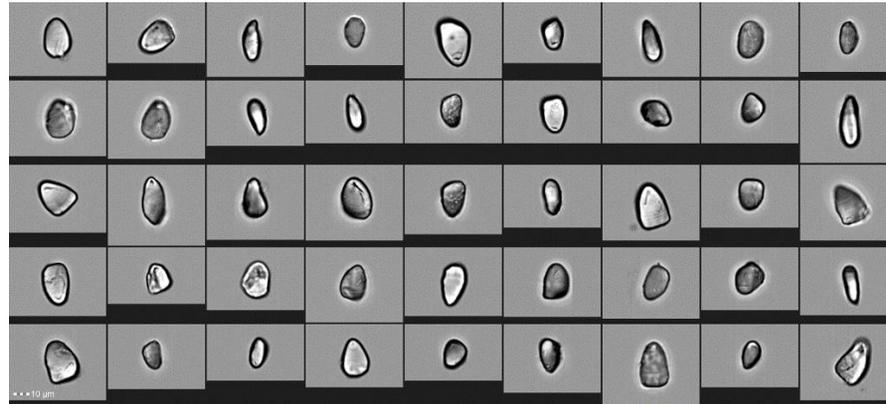
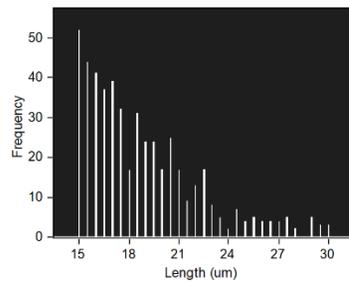


Fig. F.2.10: Descriptive statistics of length, and sample gallery for *Uvularia perforliata* (perfoliate-bellwort).

9.2.4.2 *Uvularia sessilifolia* (wild-oat)

wild-oat



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
wild-oat	500	18.82	18	3.461	30

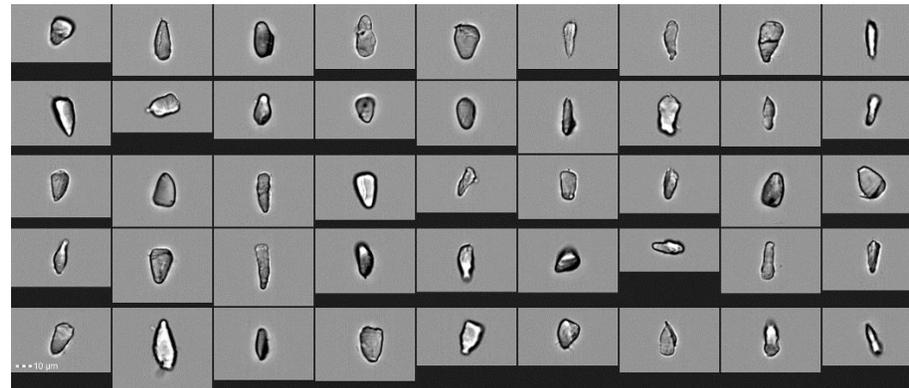


Fig. F.2.11: Descriptive statistics of length, and sample gallery for *Uvularia sessilifolia* (wild-oat).

9.2.5.0 Fabaceae (legumes)

9.2.5.1 *Phaseolus vulgaris* (common-bean)

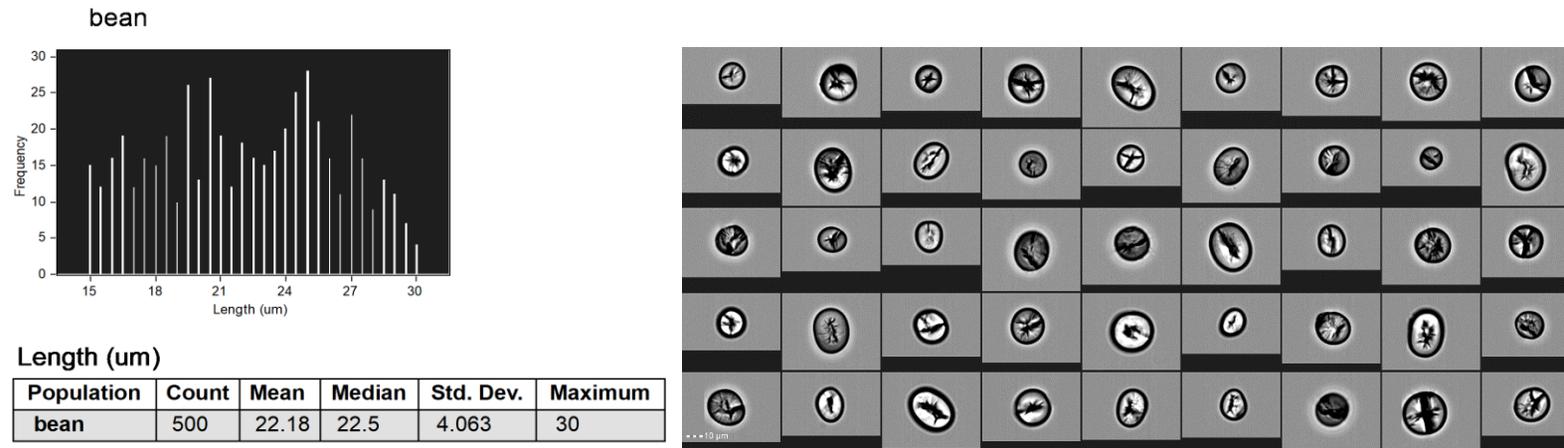
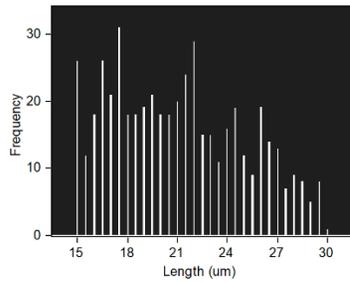


Fig. F.2.12: Descriptive statistics of length, and sample gallery for *Phaseolus vulgaris* (common-bean).

9.2.6.0 Liliaceae (Lilies)

9.2.6.1 *Lilium canadense* (Canada-lily)

Canada-lily



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
Canada-lily	500	21.08	21	3.976	30

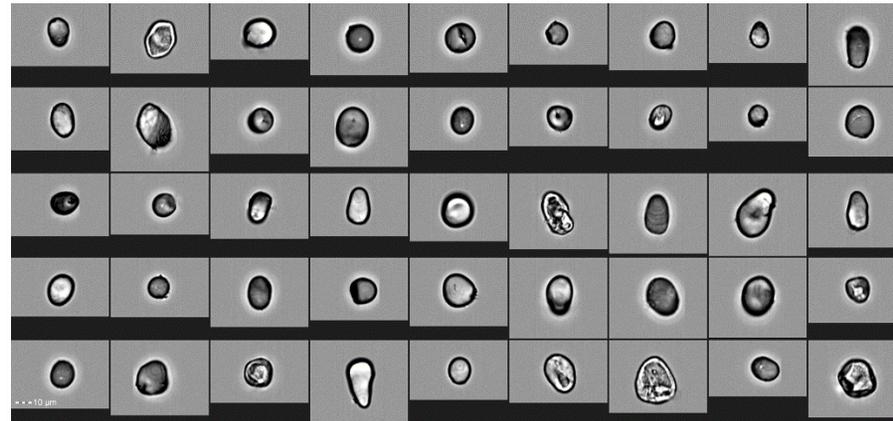
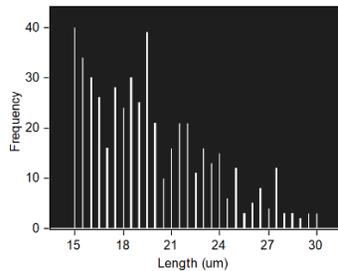


Fig. F.2.13: Descriptive statistics of length, and sample gallery for *Lilium canadense* (Canada-lily).

9.2.6.2 *Lilium michiganense* (Michigan-lily)

Michigan-lily



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
Michigan-lily	500	19.77	19	3.687	30

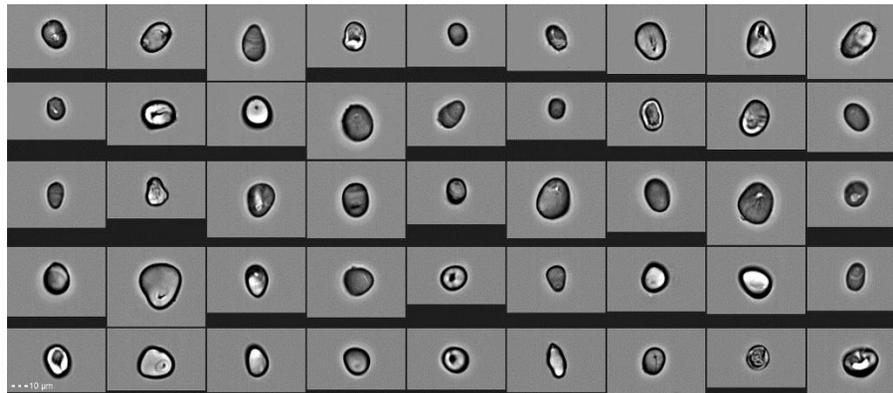
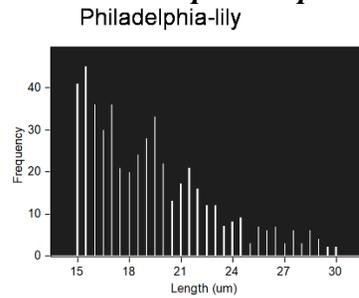


Fig. F.2.14: Descriptive statistics of length, and sample gallery for *Lilium michiganense* (Michigan-lily).

9.2.6.3 *Lilium philadelphicum* (Philadelphia-lily)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
Philadelphia-lily	500	19.33	18.5	3.637	30

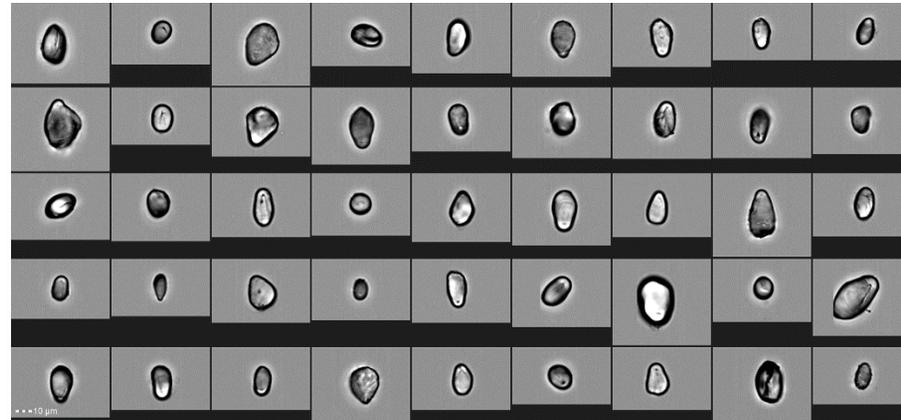
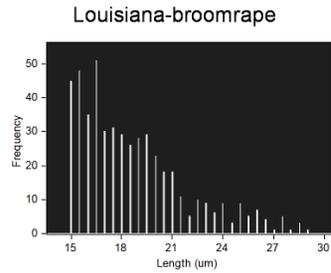


Fig. F.2.15: Descriptive statistics of length, and sample gallery for *Lilium philadelphicum* (Philadelphia-lily).

9.2.7.0 Orobanchaceae (Broomrapes)

9.2.7.1 *Orobanche ludoviciana* (Louisiana-broomrape)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
Louisiana_broomrape	500	18.67	18	3.183	29

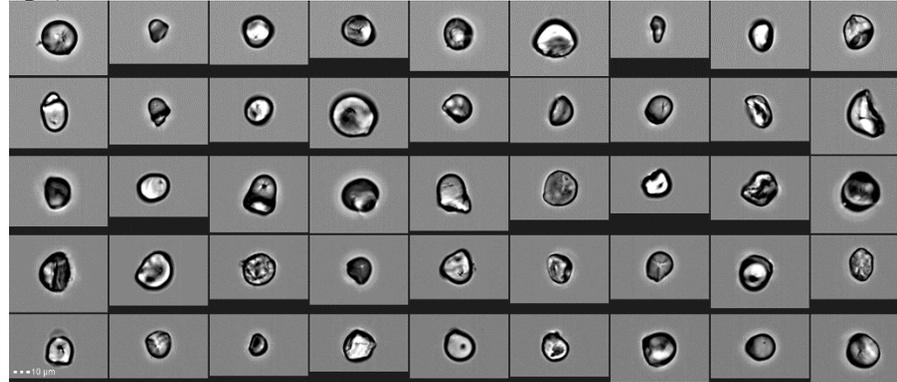
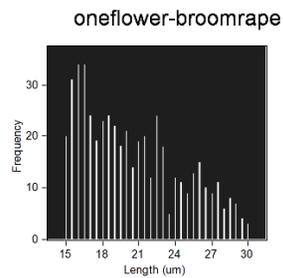


Fig. F.2.16: Descriptive statistics of length, and sample gallery for *Orobanche ludoviciana* (Louisiana-broomrape).

9.2.7.2 *Orobanche uniflora* (one-flower-broomrape)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
oneflower-broomrape	500	20.49	20	4.056	30

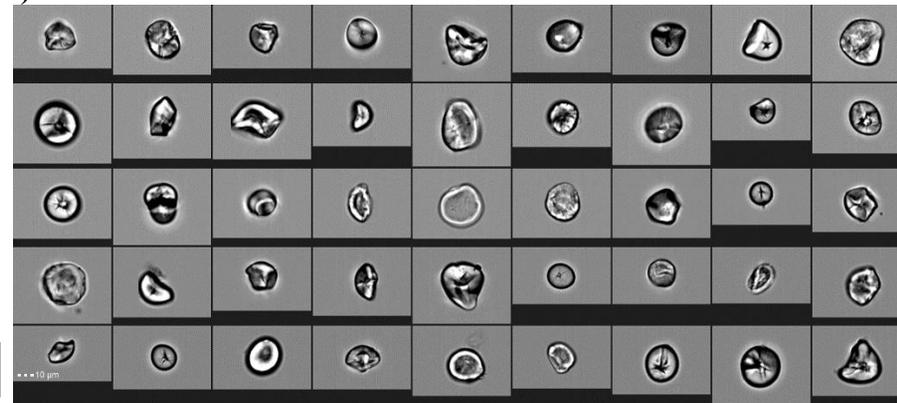


Fig. F.2.17: Descriptive statistics of length, and sample gallery for *Orobanche uniflora* (one-flower-broomrape).

9.2.8.0 Poaceae (Grasses)

9.2.8.1 *Zea mays ssp. mays* (maize)

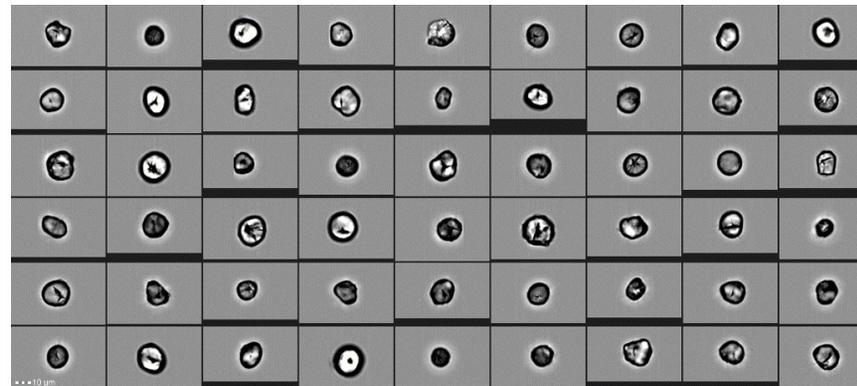
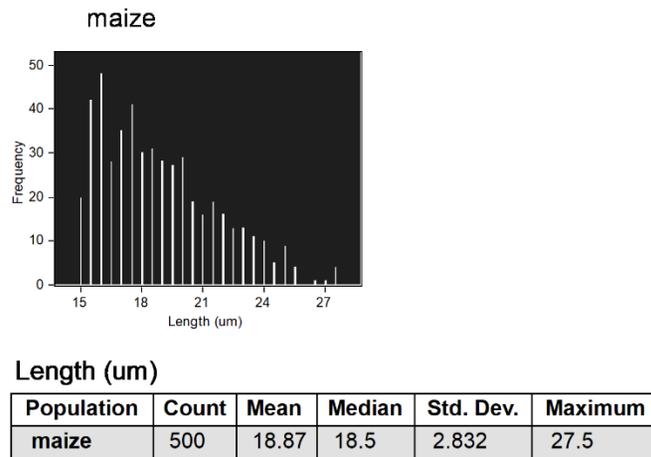


Fig. F.2.18: Descriptive statistics of length, and sample gallery for *Zea mays ssp. mays* (maize).

9.2.8.2 *Zea mays ssp. everta* (popping-corn) popping-corn

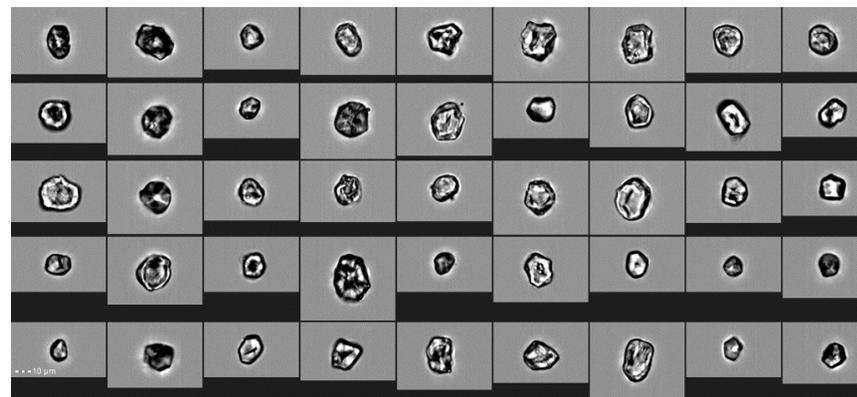
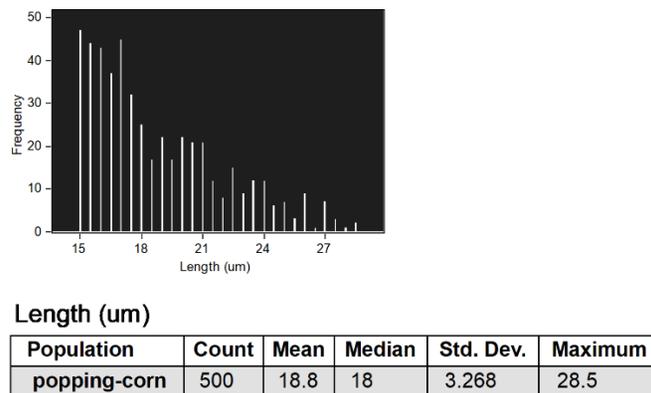
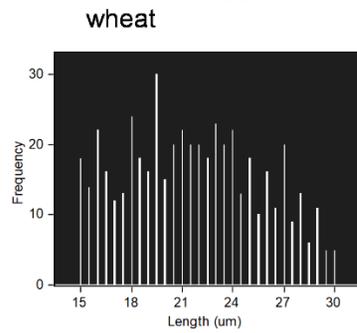


Fig. F.2.19: Descriptive statistics of length, and sample gallery for *Zea mays ssp. everta* (popping-corn).

9.2.8.3 *Triticum spp.* (wheat)



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
wheat	500	21.66	21.5	3.995	30

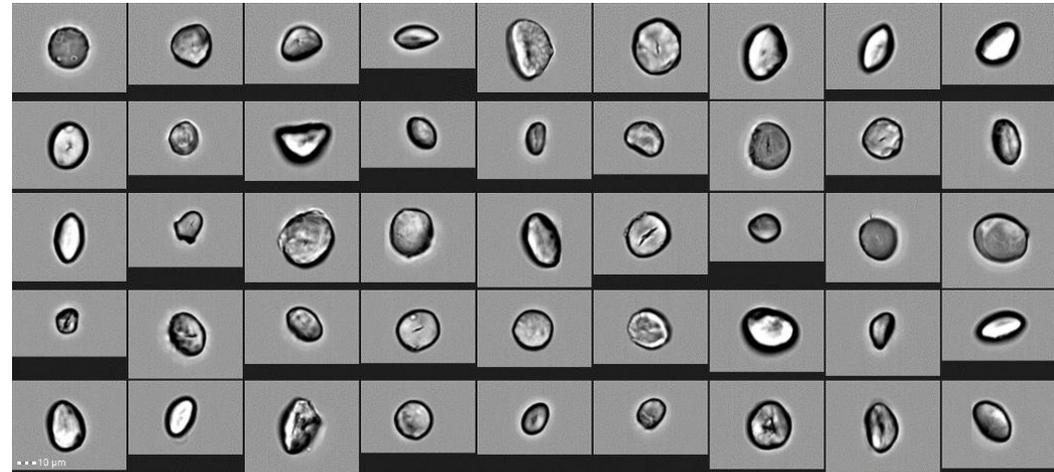
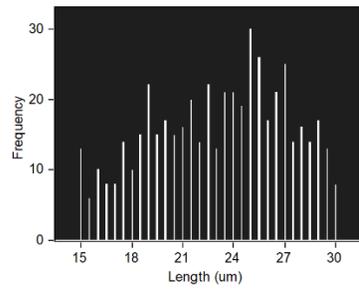


Fig. F.2.20: Descriptive statistics of length, and sample gallery for *Triticum spp.* (wheat).

9.2.9.0 Solanaceae (Nightshades)

9.2.9.1 *Solanum tuberosum* (potato)

potato



Length (um)

Population	Count	Mean	Median	Std. Dev.	Maximum
potato	500	23.08	23.5	4.002	30

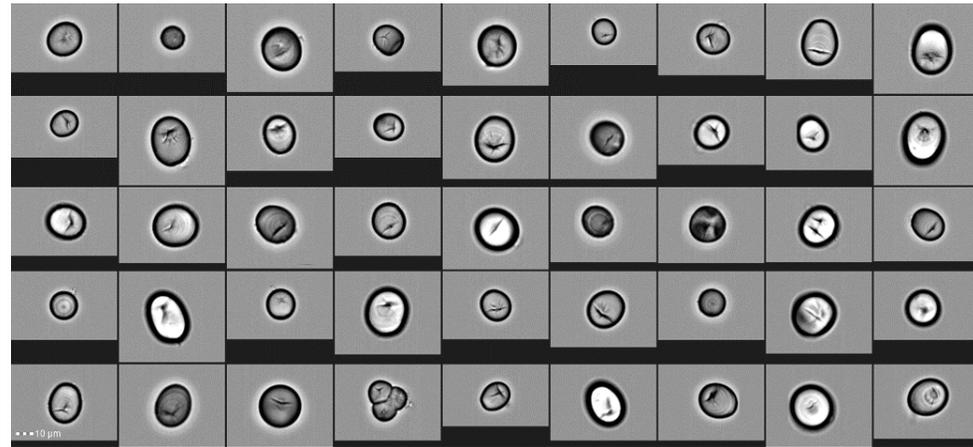


Fig. F.2.21: Descriptive statistics of length, and sample gallery for *Solanum tuberosum* (potato).

10.0 Interpretation and discussion

10.1 Comparison of classification accuracy using other statistical techniques

The method of identifying starch granules described in this chapter resulted in an average validation accuracy of 85.8% over all 17 species. This can be compared to some other published classification methods, but only in cases where data outputs are given as a percentage accuracy. Many of these methods are listed below (Table T.2.2) for comparison. This table compares the number of species studied, the sum of starch granules used for training and testing, classification method, whether the measurements from grains are collected manually by hand or automatically, the average reported classification accuracies for each method and the range of accuracies which varied between the different taxa studied.

There were four studies used to compare the results in this chapter (Torrence et al. 2004, Coster and Field 2015, Arráiz et al. 2016, and Wilson et al. 2010). Other than the research presented in this chapter, the only methods reporting species being correctly classified nearly all the time (>90%) or always were those that involved the manual collection of measurements (i.e., Torrence et al. 2004, Coster and Field 2015). Two species were reported by Coster and Field (2015), and six were reported by Torrence et al. (2004). These species were nardoo (*Marsilae drummondii*), mulga (*Acacia aneura*) (Coster and Field 2015), plantain (*Musa sp.*), horsfieldia (*Horsfieldia laevigata*), lesser-yam (*Dioscorea esculenta*), purple-yam (*Dioscorea alata*), palm-sago (*Metroxylon sagu*), and ginger (*Zingiber officinale*) (Torrence et al. 2004). Coster and Field (2015) analyzed eight species, while Torrence et al. (2004) analyzed 29, meaning roughly 20-25% of species analyzed in either study were highly (>90%) recognizable. Comparatively, the method described in this chapter includes 10 (58%) highly recognizable (>90% validation accuracy) species (see Fig F.2.4). None of the species analyzed by Coster and Field (2015) or

Table T.2.2: Comparison of statistical starch grain classification methods accuracies. Average (avg.) accuracy and accuracy (acc.) ranges are all for species level identification

author(s)	year	species (n)	grains measured (n)	classification method	automated/manual	avg. accuracy (%)	acc. range (%) ²
Torrence et al.	2004	29	1,998	multivariate analysis	manual	69 – 75 ³	1.4 – 100
Wilson et al.	2010	9	6,120	supervised learning	automated	45.6	17 – 70
Coster and Field	2015	8	1,032	geometric-morphometric analysis ⁴	manual	85.9	77.5 – 100
Arraiz et al.	2016	20	5,028	random forest tests	automated	52	23 – 76 (~80) ⁵
this study	n/a	17	8,500	image recognition	automated	85.8	19 – 99.5

² Accuracy range pertains to the differences in classification accuracies that were produced for different species within the same experiments. For example, one method may have an overall accuracy of 50% (this would be given in this table as “average accuracy”), although one species may be less identifiable (for example 25%) while another would be 75% accurate. In this table that range would be displayed as “25-75” within the “accuracy range” column.

³ A range is given for average accuracy because two separate populations of starches were tested, those with centric hila (69% correct classification on average) and those with eccentric hila (75% on average).

⁴ While all grains were traced with a digital tablet, including hilum positions, all measurements were collected using the Matlab platform. Where additional parsing was needed, then expert knowledge was also involved.

⁵ The value in brackets represents identification accuracy for some family level identifications, Poaceae (Grasses) and Fabaceae (Legumes).

Torrence et al. (2004) were analyzed in this chapter so it is difficult to make comparisons for species levels of identification.

Classification methods involving the automatic collection of measurements reported average classification accuracies of 45.6% (Wilson et al. 2010) and 52% (Arraiz et al. 2016). This is considerably lower than those involving manual collection (see Table T.2.2). Some species were more easily identified using these two automated techniques, however none were highly (with >90% classification accuracy) recognizable. The most recognizable species using Wilson et al.'s (2010) method was potato which was classified correctly 70% of the time. Potato was also analyzed in the method described in this chapter where it was highly recognizable (98.5% validation accuracy). The better performance of the method described in this chapter could be explained in part by the number of granules used to train the identification algorithms. In Wilson et al.'s (2010) research, 100 granules from each species were used to train the identification algorithm while another 5,220 granules (approx. 580/species) were used for testing. Conversely, in my own research described in this chapter, 300 granules per species were used for training, while the remaining 3,400 granules (200/species) were used for testing. It was suggested by Coster and Field (2015) that increasing the number of granules in the training group should increase the overall accuracy of an identification method. Their theory is supported by the McNemar's test that was conducted in this chapter where it was found that a statistically significant difference occurred in validation accuracies when using 100 compared with 300 training images (see section 9.1). It is also worth noting that Wilson et al.'s (2010) method used an automated shape recognition that averaged the outlines of the grains. This had the effect of turning the measured grains into average "blob-like" shapes.

An argument has been made that increasing the number of target species in classification schemes will lower identification accuracies (Mercader et al. 2018), although this pattern isn't apparent in the average accuracies provided in Table T.2.2. The methods with the highest reported average accuracies were from Torrence et al. (2004), Coster and Field (2015), and the method described in this chapter. These averages are 75%, 85.9% and 85.8% respectively, although the number of species in each study varied greatly from 29 (Torrence et al. 2004), eight (Coster and Field 2015) and 17 (this chapter). The data in Table T.2.2, however, may suggest that increasing the number of analyzed species decreases the minimum reported accuracy of a specific species, not the average classification accuracy of a method overall. Torrence et al. (2004) included the greatest number of species (n=29) and reported a minimum classification accuracy of 1.4%. My own method included 17 species had reported a minimum accuracy of 19% (Table 2.2). Coster and Field (2015) analyzed the fewest species (n=8) and reported a minimum classification accuracy of 77.5%. This pattern may suggest that increasing the number of species in any given classification scheme will increase the odds that species producing indistinguishable starches from another species will be included.

Classification methods involving the automatic collection of measurements are remarkable in that they consistently include the measurements of a greater number of granules/species when compared to manual methods. Wilson et al. (2010) measured approximately 680 granules/species; Arráiz (2016) measured approximately 250/species and my own method included the measurements of 500 granules/species. Conversely, Coster and Field (2015) measured at least 80-100 granules/species and Torrence et al. (2004) measured approximately 50-100 granules per species. This difference is likely explained by how time-consuming gathering measurements by hand can be.

10.2 Cost and reproducibility

The results from this experiment demonstrate the utility of image recognition software in its application to typological questions, such as those faced by archaeobotanists. This experiment was inexpensive, reproducible, and provided results that could be subjected to statistical testing. Besides Matlab, and a small number of inexpensive (~20 CAD/ea.) modules, all the programs necessary were free to use. The website for the institution that provided the photography (Lunenfeld-Tanenbaum Research) lists the service of the use of their MIFC at an affordable 65 or 85 CAD/hr for assisted or unassisted use of the instrument respectively (website accessed Feb 11th, 2023). The images required for this report were all collected over the span of two and a half days and included the images of ten additional species that were not included for analysis.

10.3 Future research directions

Further experimentation will be required to determine whether the method described in this chapter is practical in the identification of archaeological starch granules. Unlike the starch samples used in this experiment, archaeological materials contain significant amounts of impurities (e.g., sedimentary particles, charcoal, microdebitage, other floral and faunal remains, etc.). Since a MIFC will photograph all particles within a sample, all possible procedures must be employed to isolate the starch granules from other materials (e.g., heavy liquid flotation) (see Henry et al. 2016). Since the identification method described in this chapter is designed to identify starch grains no larger than 30 μ m, floated materials should be passed through a mesh as close to that size as is allowable. If it is possible, materials smaller than 15 μ m should also be removed using additional sieving. Following this purification process, the samples should be left suspended in distilled water so they can be photographed using a MIFC. Locating starch granules

from all the photographs taken by MIFC will require an experienced starch analyst. As was the case with the reference starch material photographed to create the classification method described in this chapter, archaeological residues will produce images of debris and other particles that will need to be removed by an analyst by hand.

Typically, starch analysts can rely on the appearance of extinction (“Maltese”) crosses when granules are viewed under cross polarized light. Although the extinction crosses of some granules are weakly visible under normal light (this was noted in Louisiana-broomrape) the phenomenon is exceedingly rare. Since MIFCs are currently incapable of photographing under cross polarized light, analysts will need to rely on other criteria to locate granules like the presence of hila, lamellae, and fissures.

Multispectral imaging flow-cytometers (MIFCs) can recognize immunofluorescent stains which could be useful in the detection of starch granules in archaeological residue samples. If an immunofluorescent stain known to bind to starch granules was applied to a sample before being photographed by a MIFC, it may simplify the differentiation of starch granules from other particles. The experimentation of using immunofluorescent staining was beyond the scope of this project, although starches are known to accept several stains. These stains include the iodine-based Lugol’s solution (Barton and Fullagar 2006), Trypan blue (Barton 2007) and Congo red (Lamb and Loy 2005). Using an immunofluorescent stain similar to Lugol’s solution would be preferable to ones which behave similarly to Trypan blue or Congo red. This is because the image recognition algorithm has been trained with images of undamaged grains, and Lugol’s solution will only stain intact starches. Conversely, Trypan blue and Congo red preferentially stain damaged grains which are often unidentifiable to species and are typically used to understand the taphonomic processes starch grains underwent (Barton and Torrence 2015). One

iodine-based immunofluorescent stain that has been used to locate starch granules in the guard cells of mustard (*Arabidopsis thaliana*) is propidium iodide (Flütsch et al. 2018). This stain may be of use when attempting to distinguish starch grains from other particles photographed using a MIFC.

Following the successful recovery and identification of starch granules from archaeological residues, the next logical step would be the comparison of identification accuracy when compared to other identification schemes, including those conducted by human analysts. A well-established method of quantitatively assigning species to starch granules is the geometric morphometric approach (GMA) (Coster and Field 2015) which has been applied to archaeological starch assemblages several times (e.g., Field et al. 2016, Lape et al. 2018, Shaw et al. 2020, Field et al. 2020). However, direct comparison with this study may not be possible because GMA has only been applied previously to Oceanian and Southeast Asian species. Comparison against identification by human analysts could be done following the method used by Doan et al. (2020). In their experiment, red blood cells were classified by two human experts as well as an image recognition algorithm. The frequency in which the two experts, and the algorithm agreed was compared with how frequently only one, or none of the experts agreed with the algorithm. Using archaeological material this would play out as allowing experts to assign species to unknown starch granules, then comparing their attributions with those made by the image recognition algorithm. Granted, it is possible (maybe even probable) that some starches recovered from archaeological materials would be from species not included in this chapter, or other classification methods.

11.0 Conclusion

The research presented in this chapter demonstrates that multispectral imaging flow-cytometry (MIFC) in conjunction with image recognition software is capable of assigning species to starch granules. The results from this novel approach are comparable and, in some cases, arguably better than other published methods of starch grain classification. Furthermore, the high-throughput nature of MIFCs could prove to be useful in speeding up the process of starch analysis, such as collection of length measurements which can be extremely time-consuming.

Some species were more readily identified than other; these included the New World cultigens maize (*Zea mays ssp. mays*) and potato (*Solanum tuberosum*). The most easily recognizable species was Canada-lily (*Lilium canadense*), an edible wild species known to have been used medicinally (Densmore 1928). The method described in this paper also illustrated that some species were difficult to distinguish from one another, the most remarkable being Michigan-lily (*Lilium michiganense*) and Philadelphia-lily (*Lilium philadelphicum*).

Further experimentation should be conducted to determine whether starches can be identified from archaeological materials using the method described in this chapter. Since this method requires the use of a MIFC, images of starches collected using conventional microscopy methods (i.e., images collected from slides) are not likely to be compatible. Starches from archaeological residues have never been photographed using MIFC. The use of immunofluorescent stains may be of utility since MIFCs can recognize these types of stains, and it is not yet possible for MIFCs to photograph particles under cross polarized light. Therefore, researchers cannot rely on the conspicuous extinction crosses to recognize starch granules.

Since the method described in this chapter collects measurements automatically, it is time efficient. The method is also inexpensive since MIFC rentals are affordable; low-end gaming laptops can be used to run classification experiments; and Matlab is a readily available, widely used program in academia. The method described in this chapter is useful for investigating patterns suggested by other researchers such as whether using larger numbers of training images will produce better classification results. It can also be used to investigate the effect of increasing the total number of studied species on average classification accuracies.

Image recognition software is in no way a replacement for archaeological specialists. The methodology presented in this chapter describes the creation of a digital reference collection which requires academic scrutiny to accept or reject identifications. Furthermore, the recovery of starches from archaeological residue samples will likely be technically difficult and require a thorough understanding of starch grain anatomy. The method described in this chapter holds promise to speed up starch grain analyses, as well as providing measurable data outputs. This is necessary to add validity to interpretations and identifications made by ancient starch analysts. To date there are few other statistical approaches at our disposal.

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Appendices

A.1.0 Appendices for Chapter One: Ancient starch research literature review

A.1.1 Co-citation maps

While conducting the background research for the literature review in chapter one of this thesis two co-citation maps were created to help visualize the sampled articles. This was done to facilitate the identification of research streams or other patterns. Co-citation maps can be created automatically by HistCiteTM software (Garfield 2009) and are useful in identifying articles that contain related content. This is done by comparing the references cited by each article and comparing them to the references cited by each other article. This information is accessed from a *.txt file (the database file which includes all the sampled articles' metadata) that can only be downloaded from the WoSTM website. This *.txt file lists each individual cited reference for each sampled article which is how HistCiteTM can access this information automatically. It is also therefore possible to include journal articles (book chapters, dissertations, site reports, etc.) outside of what is available through WoSTM if a researcher were to type out the information for each cited reference by hand. There are at least two weaknesses of taking that approach. First is the required amount of time that would be necessary to input all that information accurately. Secondly, at least some degree of personal bias would certainly be reinserted into the literature review if the researcher chooses to include additional titles.

To identify articles with similar content, HistCiteTM software can compare the references cited by each of the sampled articles, and then plot them as nodes along an x-axis that has no unit value. The distance between two articles on the x-axis is not scalar, rather it represents the relative similarity of their cited references. Since the x-axis has no unit, it does not increase in

value as the reader moves from left to right. Instead, articles become more dissimilar in their cited references as they move further away from each other along this axis in either direction. Although this method isn't perfect, the logic behind comparing the cited references is that it is most likely that articles sharing a relatively high number of cited references are more similar in content than articles that share a relatively small number of cited references. This can help researchers find groupings of similar articles which can then in turn be compared to identify significant themes, trends, or research streams within the sampled articles.

In addition to displaying the articles as nodes along the x-axis of a co-citation map to display their theoretical similarity in content, HistCiteTM software will also plot these articles by their publication year along the y-axis. Unlike the unitless x-axis of co-citation maps, the y-axis is scalar and so distances shown along that axis are absolute and not relative.

To add another layer of comprehension, HistCiteTM will change the size of each article's node based on either its Local Citation Score (LCS) or Global Citation Score (GCS). These terms are defined as the number of times a particular article was cited within and outside the sample of articles respectively (Maditati et al. 2018). This can be used to approximate each article's impact. For the two co-citation maps created for the literature review in chapter one of this thesis, one was created to show the LCS (Fig. F.A.1.1) and the other was created to show the GCS (Fig. F.A.1.2).

I modified the co-citation maps by outlining articles using a colour coded system that represented seven different broad themes. These themes are all common to ancient starch research, but realistically other groupings could have been chosen and so these should be considered arbitrary. The groupings found in these co-citation maps (Figs. F.A.1.1, F.A.1.2)

were also used to categorize articles in the content matrices (A.1.2). These categories are described in the table (Table A.1.1) below.

Table T.A.1.1: List of categories used in the construction of the co-citation map and descriptions for each category.

Name	Colour	Description
Earliest evidence	yellow	Articles written on the earliest evidence of a specific cultigen or cultivation technique within a region.
Paleoenvironment	blue	Articles written to better understand how events such as glacial maxima affected cultivation choices.
Foodways	navy blue	Articles written about collecting archaeological data regarding foodways. This category is like earliest evidence in that the intention is to find evidence of food-use. The major difference is that articles within the foodways category do not claim to be the oldest recovered remains.
Taphonomy	purple	Articles written in to track the affect that taphonomic processes (like fermentation) have on the appearance of starch granules.
Contamination	baby blue	Articles written about methods to control for the contamination of starch samples.
Dental calculus	red	Articles written about the recovery of starch granules from human teeth.
Morphometrics	green	Articles written about methods to identify species of starch granules recovered from archaeological materials.

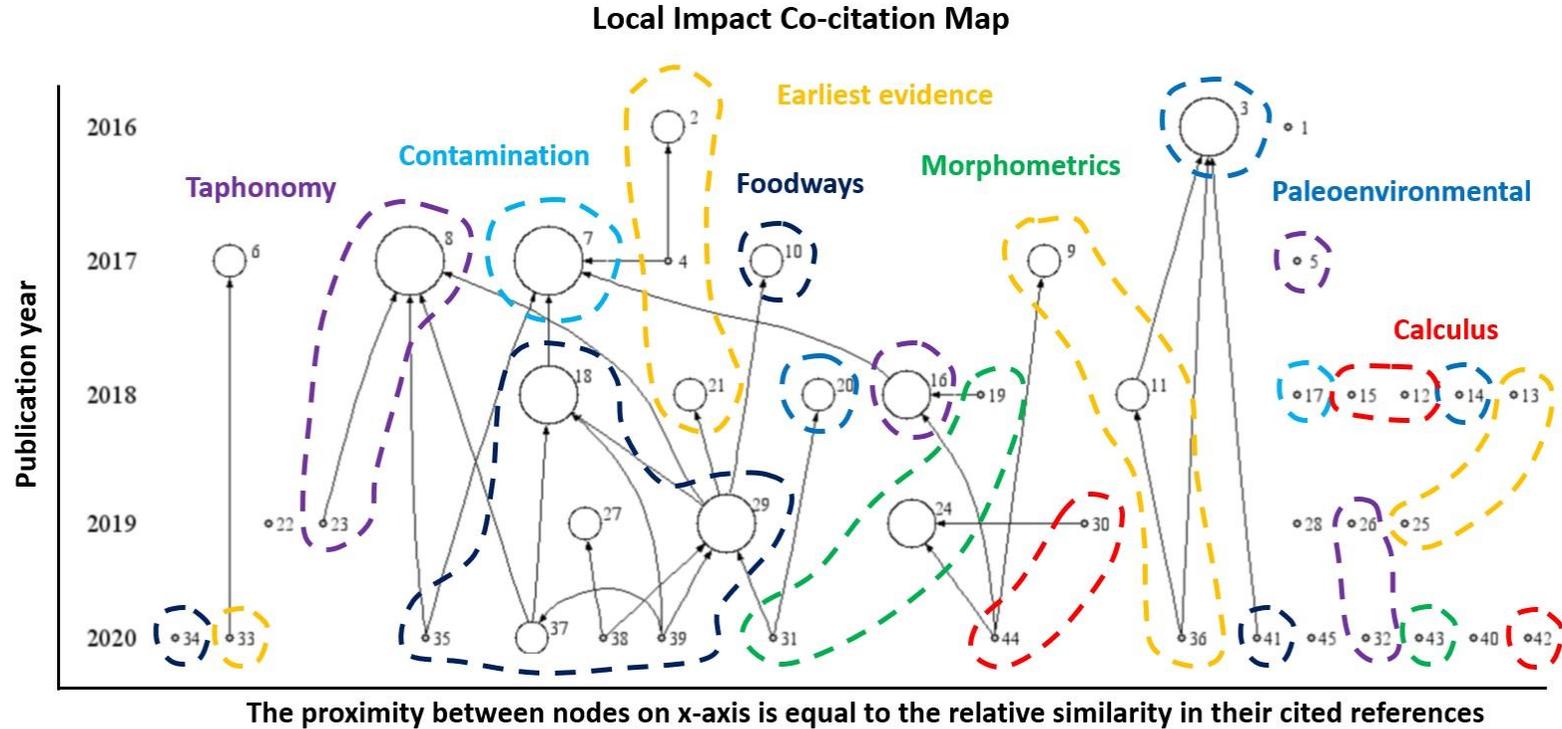


Fig. F.A.1.1: Co-citation map of the 45 articles sampled in this study. The relative size of each node is proportional to its Local Citation Score. Articles represented by each are: (1) Price (2016), Rumold and Aldenderfer (2016), (3) Field et al. (2016), (4) Louderback and Pavlik (2017), (5) Liu et al. (2017), (6) Huard and Burley (2017), (7) Mercader et al. (2017), (8) Wang et al. (2018), (9) McGovern et al. (2017), (10) Cagnato and Ponce (2017), (11) Lape et al. (2018), (12) Copeland and Hardy (2018), (13) Albert et al. (2018), (14) Yang et al. (2018), (15) Cristiani et al. (2018), (16) Mercader et al. (2018a), (17) Tavarone et al. (2018), (18) Ciofalo et al. (2018), (19) Mercader et al. (2018b), (20) Farley et al. (2018), (21) Zarillo et al. (2018), (22) Hayes et al. (2019), (23) Ma et al. (2019), (24) Gismondi et al. (2019), (25) Larbey et al. (2019), (26) Hanson et al. (2019), (27) Liu et al. (2019), (28) Liu et al. (2019), (29) Ciofalo et al. (2019), (30) D’Agostino et al. (2019), (31) Wan et al. (2020), (32) Johnson and Marston (2020), (33) Burley et al. (2020), (34) Berube et al. (2020), (35) Garcia-Granero (2020), (36) Shaw et al. (2020), (37) Ciofalo et al. (2020), (38) Li et al. (2020), (39) Berman and Pearsall (2020), (40) Chen et al. (2020), (41) Field et al. (2020), (42) Goude et al. (2020), (43) Brown and Louderback (2020), (44) D’Agostino et al. (2020), and (45) Perez et al. (2020).

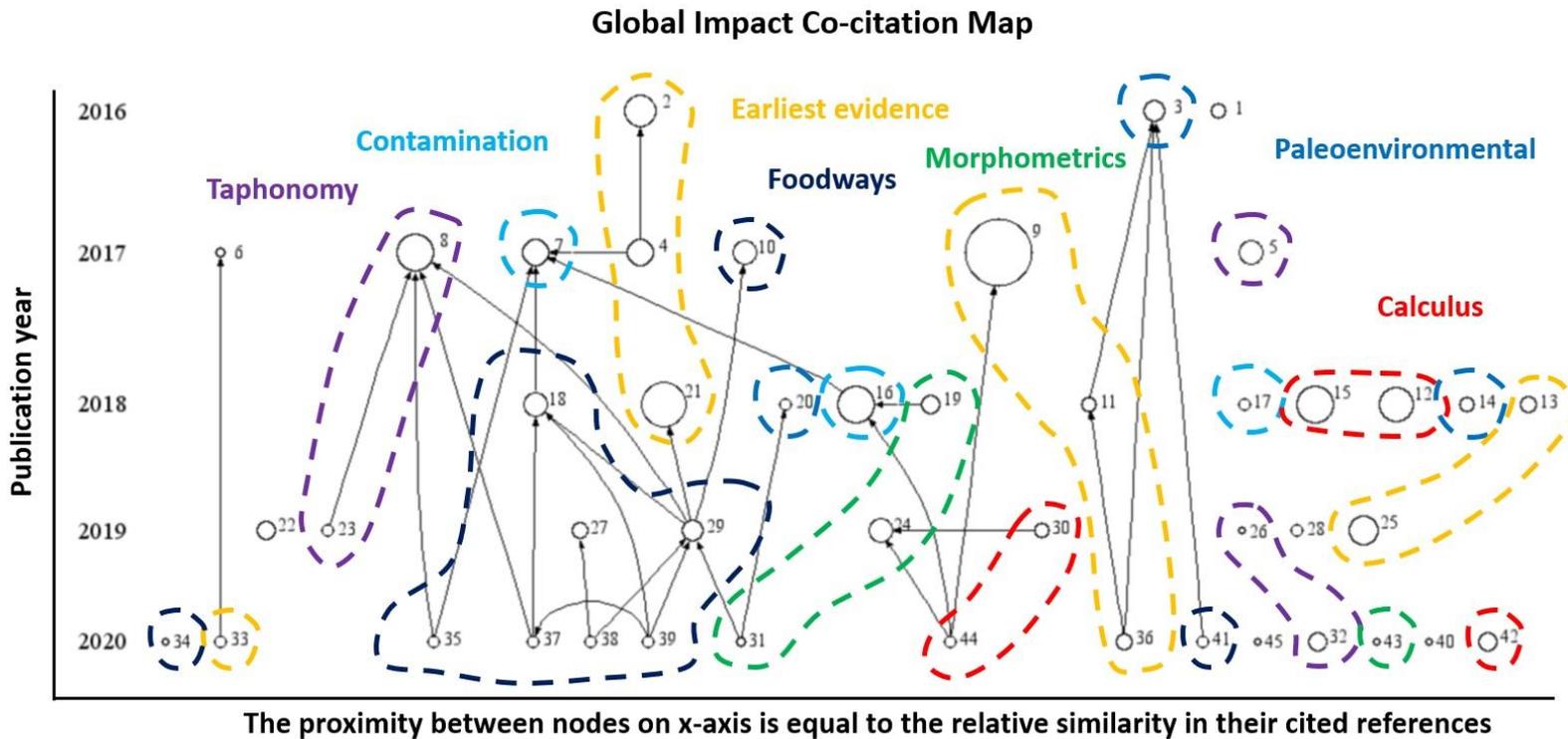


Fig. F.A.1.2: Co-citation map of the 45 articles sampled in this study. The relative size of each node is proportional to its global impact. Articles represented by each are: (1) Price (2016), (2) Rumold and Aldenderfer (2016), (3) Field et al. (2016), (4) Louderback and Pavlik (2017), (5) Liu et al. (2017), (6) Huard and Burley (2017), (7) Mercader et al. (2017), (8) Wang et al. (2018), (9) McGovern et al. (2017), (10) Cagnato and Ponce (2017), (11) Lape et al. (2018), (12) Copeland and Hardy (2018), (13) Albert et al. (2018), (14) Yang et al. (2018), (15) Cristiani et al. (2018), (16) Mercader et al. (2018a), (17) Tavarone et al. (2018), (18) Ciofalo et al. (2018), (19) Mercader et al. (2018b), (20) Farley et al. (2018), (21) Zarillo et al. (2018), (22) Hayes et al. (2019), (23) Ma et al. (2019), (24) Gismondi et al. (2019), (25) Larbey et al. (2019), (26) Hanson et al. (2019), (27) Liu et al. (2019), (28) Liu et al. (2019), (29) Ciofalo et al. (2019), (30) D'Agostino et al. (2019), (31) Wan et al. (2020), (32) Johnson and Marston (2020), (33) Burley et al. (2020), (34) Berube et al. (2020), (35) Garcia-Granero (2020), (36) Shaw et al. (2020), (37) Ciofalo et al. (2020), (38) Li et al. (2020), (39) Berman and Pearsall (2020), (40) Chen et al. (2020), (41) Field et al. (2020), (42) Goude et al. (2020), (43) Brown and Louderback (2020), (44) D'Agostino et al. (2020), and (45) Perez et al. (2020).

A.1.2 Content matrices

In preparation for the literature review in chapter one, content matrices were created according to Maditati et al.'s (2018) methodology with minor modification. In their review article, they created a chart that included journal article title, author(s), publication year, key words, researcher question(s), methodology, theory, article category, sub-category and key findings. The content matrices included in this appendix cover all the same information except that category and sub-category are grouped together. This was done because at times there was no need for a sub-category. When sub-categories were used, they were often unique and written to remind myself of a major theme within the article. For example, articles describing the earliest evidence of a specific cultigen would be categorized as “earliest evidence (name of cultigen).” Likewise, sub-categories would also at times be the name of a geographic region (e.g., contamination (East Africa)) or of a time period (e.g., paleoenvironment (Holocene)). Also, when article touched on topics from other categories, the main topic was listed first and the less central topic was placed in parentheses. For example, contamination (taphonomy). These charts allowed me to review the content of each journal article at-a-glance, while also providing the ability to easily reorganize and classify articles if desired.

There is no way to automatically (ie. use statistical techniques) to pull out the actual meaning of any given journal article, and to group them together with others that are deemed similar. Even with the aid of co-citation mapping and the construction of content matrices, it was still necessary to read all the sampled articles in their entirety to properly understand where they fit within the literature as-a-whole.

Table T.A.2.1: Title and keywords

#	Author	Year	Title	Keywords
1	Price	2016	A field processing model that accounts for central place labor	Optimal foraging theory; Field processing; Ethnoarchaeology; Indonesia; New Guinea; Sago
2	Rumold, Aldenderfer	2016	Late Archaic-Early Formative period microbotanical evidence for potato at Jiskairumoko in the Titicaca Basin of southern Peru	Microbotanical starch analysis; <i>Solanum tuberosum</i> ; plant domestication; South-Central Andes; Food production
3	Field et al.	2016	Human-environment dynamics during the Holocene in the Australian Wet Tropics of NE Queensland: A starch and phytolith study	Australia; Rainforest; Archaeology; Holocene; Phytoliths; Ancient starch; Niche construction
4	Louderback, Pavlik	2017	Starch granule evidence for the earliest potato use in North America	Potato; <i>Solanum jamesii</i> ; Starch granule analysis; Tuber use; Colorado Plateau
5	Liu et al.	2017	Usewear and residue analyses of experimental harvesting stone tools for archaeological research	Experimental archaeology; Usewear analysis; Starch analysis; Phytolith analysis; Millet cultivation; Starch in leaves
6	Huard, Burley	2017	On the question of <i>Anadara antiquata</i> bivalve scrapers in the archaeological record of Tonga	<i>Anadara antiquata</i> ; shell tools; experimental archaeology; starch; Tonga
7	Mercader et al.	2017	Starch contamination landscapes in field archaeology: Olduvai Gorge, Tanzania	n/a
8	Wang et al.	2017	Identifying ancient beer brewing through starch analysis: A methodology	Ancient beer; Fermentation; Starch granules; Archaeological residues
9	McGovern et al.	2017	Early Neolithic wine of Georgia in the South Caucasus	Neolithic; wine; viticulture; Georgia; Near East

#	Author	Year	Title	Keywords
10	Cagnato, Ponce	2017	Ancient Maya manioc consumption: starch grain evidence from Late to Terminal Classic occupation at La Corona, northwestern Peten, Guatemala	Maya archaeology; Archaeobotany; Starch grain analysis; Root crops; Manioc; Manihot esculenta Crantz; Terminal Classic
11	Lape et al.	2018	New data from an open Neolithic site in Eastern Indonesia	Neolithic; agriculture; archaeology; Island Southeast Asia; eastern Indonesia; Maluku
12	Copeland, Hardy	2018	Archaeological starch	starch; archaeology; human evolution; dental calculus
13	Albert et al.	2018	Earliest microbotanical evidence for maize in the Northern Lake Michigan Basin	n/a
14	Yang et al.	2018	Critical role of climate change in plant selection and millet domestication in North China	n/a
15	Cristiani et al.	2018	Dental calculus and isotopes provide direct evidence of fish and plant consumption in Mesolithic Mediterranean	n/a
16	Mercader et al.	2018	Exaggerated expectations in ancient starch research and the need for new taphonomic and authenticity criteria	biomolecular archaeology; ancient starch; taphonomy; authenticity criteria; elemental and structural characterisation
17	Tavarone et al.	2018	Cleaning protocol of archaeological dental calculus: A methodological proposal for vegetable micro remains analysis	contamination; dental calculus; human skeletal remains; starch grains
18	Ciofalo et al.	2018	Determining Precolonial botanical foodways: starch recovery and analysis, Long Island, the Bahamas	Starch analysis; Foodways; Caribbean archaeology; Microlith; Shell

#	Author	Year	Title	Keywords
19	Mercader et al.	2018	Morphometrics of starch granules from sub-Saharan plants and the taxonomic identification of ancient starch	starch granule morphometry; reference collection; starch identification; ancient starch research; sub-saharan ethnobotany; unique identifiers
20	Farley et al.	2018	A Late Holocene palaeoenvironmental reconstruction of Ulong Island, Palau, from starch grain, charcoal, and geochemistry analyses	Starch; Palau; Archaeology; Archaeobotany; Geochemistry; Pacific
21	Zarrillo et al.	2018	The use and domestication of Theobroma cacao during the mid-Holocene in the upper Amazon	n/a
22	Hayes et al.	2019	Integrating SEM-EDS in a sequential residue analysis protocol: Benefits and challenges	Reflected light microscopy; Transmitted light microscopy; Scanning electron microscopy; Functional analysis; Residue extraction; Experimental archaeology; Stone tools
23	Ma et al.	2019	Morphological changes in starch grains after dehusking and grinding with stone tools	n/a
24	Gismondi et al.	2019	Starch granules: a data collection of 40 food species	Amylose content; botanical atlas; electron scanning microscopy; light microscopy; starch granule morphology
25	Larby et al.	2019	Cooked starchy food in hearths ca. 120 kya and 65 kya (MIS 5e and MIS 4) from Klasies River Cave, South Africa	Palaeolithic; Starch diet; Tuber-parenchyma; Micro-context; Klasies
26	Hanson et al.	2019	Acorn processing and pottery use in the Upper Great Lakes: an experimental comparison of stone boiling and ceramic technology	Acorns; Quercus; Tannin leaching; Adoption of pottery; Stone boiling; Experimental archaeology; Upper Great Lakes; Foodways

#	Author	Year	Title	Keywords
27	Li et al.	2019	New insights into the grinding tools used by the earliest farmers in the central plain of China	Neolithic archaeology; Chinese archaeology; Jiahu grinding tools; Use-wear analysis; Tool function
28	Liu et al.	2019	Exploitation of job's tears in Paleolithic and Neolithic China: Methodological problems and solutions	Coix lacryma-jobi; Starch granules; Phytoliths; Charred seeds; Use-wear analysis; Experimental archaeology
29	Ciofalo et al.	2019	Late Precolonial culinary practices: starch analysis on griddles from the Northern Caribbean	Starch analysis; Foodways; Caribbean archaeology; Griddles; Manioc; Cultural niche construction; Culinary practices
30	D'Agostino et al.	2019	Lifestyle of a Roman Imperial community: ethnobotanical evidence from dental calculus of the Ager Curenensis inhabitants	Dental calculus; Cereals; Non-dietary micro-remains; Secondary metabolites; Light microscopy; Gas chromatography mass spectrometry; Passo Corese
31	Wan et al.	2020	Morphological analysis of starch granules through discriminant method and its application in plant archaeological samples	archaeobotany; ancient recipes; neolithic revolution; geometric characteristic; microfossils; residue analysis
32	Johnson, Marston	2020	The experimental identification of nixtamalized maize through starch spherulites	Nixtamalization; Starch granule; Starch spherulite; Experimental archaeology; Food processing; Maize; Nejayote
33	Burley et al.	2020	Earliest Evidence for Pit Cultivation Provides Insight on the Nature of First Polynesian Settlement	pit cultivation; first Polynesian settlement; Lapita; Tonga archaeology; plant microfossils
34	Berube et al.	2020	Paleoethnobotanical evidence of Early Formative period diet in coastal Oaxaca, Mexico	Agriculture; Mesoamerica; Paleoethnobotany; Early Formative Period; Maize; Beans; Phytoliths; Starch grains; Stable isotope analysis; Ceramics; Lithics

#	Author	Year	Title	Keywords
35	Garcia-Granero et al.	2020	From Storage to Disposal: a Holistic Microbotanical Approach to Domestic Plant Preparation and Consumption Activities in Late Minoan Gypsades, Crete	Foodways; Starch grains; Phytoliths; Minoan pottery; Cereals; Phoenix dactylifera
36	Shaw et al.	2020	Emergence of a Neolithic in highland New Guinea by 5000 to 4000 years ago	n/a
37	Ciofalo et al.	2020	Starchy shells: Residue analysis of precolonial northern Caribbean culinary practices	Starch analysis; Caribbean; Archaeology; Culinary practices; Shell artefacts; Foodways; Archaeobotany
38	Li et al.	2020	Plant Foods and Different Uses of Grinding Tools at the Neolithic Site of Tanghu in Central China	Plant foods; use-wear; starch grain; Peiligang Culture; grinding tools
39	Berman, Pearsall	2020	Crop dispersal and Lucayan tool use: investigating the creation of transported landscapes in the Central Bahamas through starch grain, phytolith, macrobotanical, and artifactual studies	Caribbean archaeology; Microliths; Shell tools; Calathea latifolia; Cucurbitaceae; Manihot esculenta; Plant translocations
40	Chen et al.	2020	Last meals inferred from the possible gut contents of a mummy: a case study from Astana cemetery, Xinjiang, China	Ftir analysis; archaeobotany; turpan; Astana cemetery; Gaochang people; diet; gut contents
41	Field et al.	2020	Functional studies of flaked and ground stone artefacts reveal starchy tree nut and root exploitation in mid-Holocene highland New Guinea	ancient starch; archaeology; Holocene; Papua New Guinea; residue and usewear; stone mortar
42	Goude et al.	2020	New insights on Neolithic food and mobility patterns in Mediterranean coastal populations	ancient DNA; dental calculus; marine resources; radiocarbon date; stable isotopes

#	Author	Year	Title	Keywords
43	Brown, Louderback	2020	Identification of starch granules from oak and grass species in the central coast of California	Starch granule analysis; Archaeobotany; California Indians; Acorns; Oak (<i>Quercus</i> spp.); Triticeae grasses (<i>Elymus elymoides</i>)
44	D'Agostino et al.	2020	Investigating Plant Micro-Remains Embedded in Dental Calculus of the Phoenician Inhabitants of Motya (Sicily, Italy)	tartar; secondary metabolites; gymnosperm products; palaeodiet; nutritional ecology; Punic archaeology
45	Perez et al.	2020	Cobbles, tools, and plants: techno-functional variability within lithic industries of complex societies in Central Coast, Peru	Techno-functional approach; Lithic industries in complex societies; Peruvian Central Coast; Plant micro-remain analysis; <i>Caesalpinia spinosa</i>

Table T.A.2.2: Research question(s) and key finding(s)

#	Author	Year	Research questions	Key findings
1	Price	2016	Was sago processed at home or in the field?	Sago was likely processed in the field, which explains why it is seldom recovered from settlements
2	Rumold, Aldenderfer	2016	Can starch grain analysis be used to elucidate the domestication trajectory of potato?	This report demonstrates the utility of their methodology in assessing the timing, mode, and context of potato origins
3	Field et al.	2016	How were tropical rainforests exploited during the late Holocene?	Both locations indicate a human presence in early Holocene, coincident with re-establishment of rainforests in the area
4	Louderback, Pavlik	2017	What was the importance of potatoes in ancient North American diets?	Potato saw at least intermittent use between ca. 11k-7kya in southern Utah

#	Author	Year	Research questions	Key findings
5	Liu et al.	2017	Can use-wear, and starch analysis be used to detect early domestication processes?	Stone tools show diverse polishes and striations from cereal processing. Also, large non-transient starches were located in leaves and stems
6	Huard, Burley	2017	Can shell scraper use be detected using use-wear and starch analyses?	The use-wear can be identified, and starches should be recoverable
7	Mercader et al.	2017	Can the contaminant starch from the landscape be quantified?	Seven starch types constituted the contamination landscape
8	Wang et al.	2017	Can fermentation be detected in ancient starch granules?	The paper describes a method of identifying fermentation in ancient starches
9	McGovern et al.	2017	Were Neolithic ceramics recovered from Georgia the earliest, reliably dated evidence of wine fermentation?	The ceramics were in fact the oldest reliably dated evidence of fermentation in Near East
10	Cagnato, Ponce	2017	What role did manioc play in the diet of Late to Terminal Maya at La Corona?	It is argued that manioc wasn't a famine food, as is sometimes the assumption
11	Lape et al.	2018	What can be learned about the Neolithic foodway through starch grain analysis?	Many types of starches were identified, such as sweet-potato and musa
12	Copeland, Hardy	2018	This article reviews archaeological starch, and its ability to preserve. I also posed a new hypothesis, that a combination of archaeological, physiological, and genetic evidence can be used to elucidate human evolution	Starch analysis, especially from dental calculus, holds theoretical potential to answer questions of human evolution
13	Albert et al.	2018	What is the timing of the introduction of maize in the Northern Lake Michigan Basin?	Maize was in the Northern Lake Michigan Basin by 200 BCE, ca. 800 years earlier than macrobotanical evidence suggests
14	Yang et al.	2018	How and why were millets selected for domestication?	During LGM wild wheats and millets were exploited, but in Mid Holocene, a change in environment made millet more favourable

#	Author	Year	Research questions	Key findings
15	Cristiani et al.	2018	Were plants and fish a significant part of the Mesolithic, European diet?	People consumed fish and plant foods, which challenges other interpretations that people primarily consumed terrestrial animals
16	Mercader et al.	2018	Why does starch survive for millenia?	Further research needs to be done to understand the survivability of starches
17	Tavarone et al.	2018	Can contamination be controlled for using an alternate method of dental calculus, starch grain extraction?	Contamination can be reduced using this alternate method
18	Ciofalo et al.	2018	Can starch grain analysis be used to validate or challenge ethnohistoric reports about pre-Colonial foodways?	Results provide insight into regionally-specific use of maize, manioc, and coontie
19	Mercader et al.	2018	It attempted to explore diagnostic power of unique classifiers, morphotypes shared between taxa and addressing minimum counts necessary to capture polymorphism	98 unique identifiers were found in 9 families
20	Farley et al.	2018	Can an environmental sequence be created using starch granules?	Changes in cultigen exploitation was detected, as well as a marked change upon contact with Europeans
21	Zarrillo et al.	2018	Can starch grain analysis be used to elucidate the domestication trajectory of cacao?	Their results suggest Ecuador could have been the first center of cacao domestication
22	Hayes et al.	2019	What are the challenges and benefits of using SEM-EDS microscopy in starch residues and use-wear analyses?	Both methods have benefits and challenges. Results are most powerful when combining both techniques
23	Ma et al.	2019	Can the taphonomic transformations of starch from dehusking and grinding be quantified in millet?	Grinding could be detected, but dehusking could not

#	Author	Year	Research questions	Key findings
24	Gismondi et al.	2019	The starch granules of 40 plants are described, after being observed using SEM and conventional microscopy	Some characteristics are conserved within species, though variation always exists between grains
25	Larby et al.	2019	Can starch granules be detected in middle stone age (~60kya) archaeological residues, corresponding to duplication of starch enzyme encoding genes in H. sapiens?	Humans at this location were cooking starch rich plants ~60kya
26	Hanson et al.	2019	Was pottery adopted, in part to leach tannins from acorns in the Upper Great Lakes?	Simmering within ceramic vessels resulted in more complete tannin leaching when compared with stone boiling
27	Li et al.	2019	What can use-wear analysis inform us about 17 grinding stones previously analyzed for starches?	There were differences in use-wear patterns that corresponded to differences in starch grains previously described
28	Liu et al.	2019	What can starch grain and use-wear analysis inform us about the domestication trajectory of Job's-tears	Job's-tears were exploited in Upper Paleolithic by 28000 cal BP, but disappeared in north China during Younger Dryas. These returned to use after 8000 cal BP.
29	Ciofalo et al.	2019	Can archaeobotanical analysis give a better picture of pre-colonial foodways when compared with ethnohistoric reports?	Results expose cultural niches, different adaptation strategies, and associated culinary practices
30	D'Agostino et al.	2019	What can the analysis of dental calculus inform us when reconstructing the lifestyle of the Roman imperial community of Ager Curenensis?	Both food and non-food molecules recovered from dental calculus provide evidence for a broad diet, including plants and animals

#	Author	Year	Research questions	Key findings
31	Wan et al.	2020	Can image and discriminant analysis be conducted to accurately, and quickly identify unknown starch granules?	This method was faster and more accurate than using conventional microscopy
32	Johnson, Marston	2020	Can nixtamalization be identified through residue analysis of starch granules?	Nixtamalization can be identified in the archaeological record by the observation of starch spherulites
33	Burley et al.	2020	What can starch grain analysis tell us about the earliest recorded pit cultivation in the earliest Polynesian settlement?	First people in Polynesia grew taro, banana, candle-nut, pandanus, and coconut
34	Berube et al.	2020	Can archaeobotanical analysis be used to investigate the dietary and possibly medicinal practices between Archaic and Formative contexts?	Analysis identified four families (Malvaceae, Dioscoreaceae, Fabaceae and Poaceae, including maize). Results complement existing lithic and isotopic data for the region
35	Garcia-Granero et al.	2020	What can starch analysis inform us about the food cooking and storage processes	Results suggest cereals were stored dehusked, then later cooked together with non-staple plants
36	Shaw et al.	2020	Was there an independent Neolithic "revolution" in New Guinea, prior to the arrival of the Lapita?	The evidence supports the theory that a Neolithic revolution occurred in New Guinea ~1000 years before the arrival of Lapita
37	Ciofalo et al.	2020	Can starch analysis be used to validate or challenge tool functions described in the ethnohistorical record?	Results show that the shell tools were used to process a variety of different plant resources, unlike what is reported ethnohistorically
38	Li et al.	2020	What can starch grain analysis inform us about the use of grinding stones recovered from a Neolithic site in northern China?	Results show that the grinding stones were primarily used to process grains, but also acorns and other plants to a lesser degree. One stone was used to process bone
39	Berman, Pearsall	2020	Can starch grain analysis expand our understanding of Lucayan transported	Similarities in plant remains and lithic types in the Bahamas suggests shared plant-related food preparation and cooking practices

#	Author	Year	Research questions	Key findings
			landscape, plant food preparation, and cooking practices?	
40	Chen et al.	2020	What plant remains are in the possible gut contents of the mummy from the Astana cemetery?	The gut contents had starch from millets and wheat
41	Field et al.	2020	What can use-wear and starch analysis inform us about the development of agriculture during the early Holocene in New Guinea?	A type of nut and a tuber were found to have been processed
42	Goude et al.	2020	What can be learned about the diet, mobility, social organization, and environmental exploitation patterns of early Mediterranean farmers?	These farmers ate a wide breadth of plant foods (cereals and wild)
43	Brown, Louderback	2020	Can three species of oak and one species of wild rye be differentiated based on morphological features?	Taxonomic diagnosis of studies species can be done with a measurable level of confidence
44	D'Agostino et al.	2020	What can be learned about the exploitation of food and medicinal plants from dental calculus	Wheats and animal derived foods were eaten
45	Perez et al.	2020	What is the degree of technological and functional variability between Maranga lithic industries?	A preference for an alternating debitage system was identified in the Intermediate Period. Toward the Late Horizon there is a possible standardization in the use of <i>Caesalpinia spinosa</i> , a plant used in the production of gums and tannins

Table T.A.2.3: Method, theory and category

#	Author	Year	Method	Theory	Category (sub-cateorgy)
1	Price	2016	Metcalf and Barlow's field processing model	optimal foraging theory	Not categorized (sago)
2	Rumold, Aldenderfer	2016	Starches were recovered from ground stone tools and compared with published reference works and a preliminary comparative study	n/a	Earliest evidence (potato)
3	Field et al.	2016	Two proxies: starch and phytolith sequences were analyzed over 2000 year sequence in two difference locations	n/a	Paleoenvironment (geometric-morphometric)
4	Louderback, Pavlik	2017	Starch granules were recovered from stone tools and compared to reference material using a morphometric approach	n/a	Earliest evidence (potato)
5	Liu et al.	2017	milletts were processed using stone tools, and use-wear, and starches were analyzed	n/a	Taphonomy (experimental)
6	Huard, Burley	2017	A. antiquata shells were used to process starchy plants, then tools were analyzed	n/a	Not categorized (use-wear)
7	Mercader et al.	2017	Starches were recovered from the environment, and quantified	n/a	Contamination (experimental)
8	Wang et al.	2017	Seventeen domesticated and wild plant species were fermented using ethnographic techniques	n/a	Taphonomy (experimental)
9	McGovern et al.	2017	Chemical analysis was corroborated by archaeobotanical (pollen, starch, macros) analyses	n/a	Earliest evidence (wine)
10	Cagnato, Ponce	2017	Starch grains recovered from grinding stones and ceramic sherds	n/a	Food-ways (Terminal Classic)

#	Author	Year	Method	Theory	Category (sub-cateorgy)
11	Lape et al.	2018	This was a site report. The flora section included a starch analysis on several ceramics, which used Coster and Field's (2015) methodology	n/a	Earliest evidence (nutmeg)
12	Copeland, Hardy	2018	Literature review, and then formation of hypothesis	n/a	Calculus (human evolution)
13	Albert et al.	2018	Phytolith and starch analysis of carbonized food recovered from ceramics, and radiocarbon dating	n/a	Earliest evidence (maize)
14	Yang et al.	2018	Starch data were compared from archaeological sites dating from LGM-Mid Holocene.	n/a	Paleoenvironmental
15	Cristiani et al.	2018	Dental calculus was analyzed for fish and starch remains	n/a	Calculus (Mesolithic)
16	Mercader et al.	2018	Detailed discussion of diagenesis and biochemistry of starches	n/a	Taphonomy
17	Tavarone et al.	2018	The starches recovered from calculus was compared using different methods	n/a	Contamination (calculus)
18	Ciofalo et al.	2018	Shell and lithic tools were analyzed for starch	n/a	Food-ways (pre-colonial)
19	Mercader et al.	2018	Very meticulously measured morphologies of starch granules using low-throughput microscopy, including 3D microscopy.	n/a	Morphometrics (Africa)
20	Farley et al.	2018	A sediment core sample was analyzed for charcoal, starch and geochemical content, ranging from 3000 BP to 1783 AD	n/a	Paleoenvironmental (Holocene)
21	Zarrillo et al.	2018	Three proxies: starch grain analysis, absorbed theobromine residues and aDNA analysis	n/a	Earliest evidence (cacao)
22	Hayes et al.	2019	They analyzed 44 chert tools from the TraceoLab	n/a	Not categorized (ESEM)
23	Ma et al.	2019	Milletts were processed using stone tools, and changes in morphology was described	n/a	Taphonomy (experimental)

#	Author	Year	Method	Theory	Category (sub-cateorgy)
24	Gismondi et al.	2019	Images of 450 granules per species were observed. Fifty random grains were measured for length	n/a	Morphometrics
25	Larby et al.	2019	Sediments from a hearth were sampled and analyzed for starches, macro botanicals and parenchyma	n/a	Earliest evidence (Middle Stone Age)
26	Hanson et al.	2019	The efficacy of tannin leaching was compared between simmering in a ceramic vessel and stone boiling	n/a	Taphonomy (experimental)
27	Li et al.	2019	Grinding stones were observed under magnification, and their use-wear was recorded	n/a	Food-ways (use-wear)
28	Liu et al.	2019	Use-wear analysis	n/a	Not categorized (job's-tears)
29	Ciofalo et al.	2019	Clay griddles were compared from three sites	n/a	Food-ways (pre-colonial)
30	D'Agostino et al.	2019	Microscopy and gas chromatography were used on residues recovered from dental calculus	n/a	Calculus (Roman)
31	Wan et al.	2020	ImageJ and SPSS were used to measure and run statistical analyses on the starch granules of three species: wheat, millet and yam	n/a	Morphometrics
32	Johnson, Marston	2020	Maize was cooked with slaked lime and then photographed using polarized light and electron microscopy	n/a	Taphonomy (experimental)
33	Burley et al.	2020	Sediments from the pit cultivation were sampled 5 times and the starches were analyzed using conventional microscopy techniques	n/a	Earliest evidence (pit cultivation)
34	Berube et al.	2020	Phytoliths, starch, and macro botanicals analyzed	n/a	Food-ways (Formative)
35	Garcia-Granero et al.	2020	Ceramics were analyzed for starch and phytoliths	n/a	Food-ways (Minoan)
36	Shaw et al.	2020	Coster and Field's (2015) geometric morphometric method was used	n/a	Earliest evidence (pre-Lapita)

#	Author	Year	Method	Theory	Category (sub-cateorgy)
					Neolithic)
37	Ciofalo et al.	2020	Shell artifacts typically associated with manioc peeling were analyzed for starch	n/a	Food-ways (precolonial)
38	Li et al.	2020	Use-wear and starch analyses were conducted on grinding stones	n/a	Food-ways (Neolithic)
39	Berman, Pearsall	2020	Multi-proxy: starch, phytolith, macrobotanical and artifact studies	n/a	Food-ways (precolonial)
40	Chen et al.	2020	Multi-proxy: starch, phytolith, and macrobotanical	n/a	Not categorized (gut contents)
41	Field et al.	2020	Starches were recovered from stone bowls (mortar) and identified using Coster and Field's (2015) method	n/a	Foodways
42	Goude et al.	2020	Multiproxy: starch, isotope, calculus, microremains, aDNA	n/a	Calculus
43	Brown, Louderback	2020	Measurement of reference material and statistical analyses	n/a	Morphometrics
44	D'Agostino et al.	2020	Multiproxy: starch, pollen, gas chromatography	n/a	Calculus
45	Perez et al.	2020	Reference collection creation, recovery of starch from "transformative surfaces" of artifacts and taxonomic identification	Techno-functionalism	Food-ways (Holocene)

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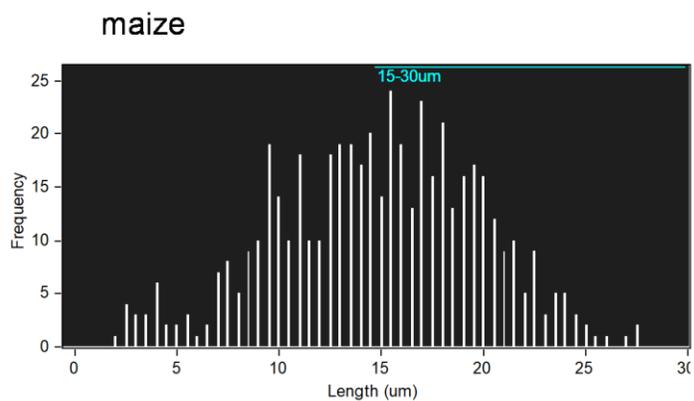
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A.2.0 Appendices for Chapter Two: Application of deep learning to the classification of some key plant taxa in eastern North America

A.2.1 Testing accuracy of length measurements taken from MIFC by comparing maize lengths to those published by Messner (2011)

The lengths of 500 maize (*Zea mays ssp. mays*) granules were selected from the maize images collected from the multispectral imaging flow cytometer (MIFC) and lengths were measured using the customized “mask” described in chapter 2 (see Section 8.3). Unlike the maize (or indeed any other species of) starch granules selected for image recognition algorithm training and descriptive statistics, the images selected here were measured regardless of length (i.e., these did not range from only 15 to 30 μm). The mean was calculated to be 14.82 μm (Fig. F.A.2). This is consistent with Messner’s (2011) key that reports the means 12.46 μm , 14.6 μm , and 15.58 μm for indian-graves-flint, Parker’s-flint, and Winnebego-flint maize cultivars respectively. The minimum length of maize was found to be 2 μm , which is slightly (2-3 μm) shorter than the maize reported by Messner (2011). Likewise, the maximum length reported here was 27.5 μm , which is approximately 5.5 μm longer than any maize in his identification key. The difference in maize length between what is observed here (Fig. F.A.2), and Messner’s (2011) key could be explained by differences in sample size. As far as I am aware, there is no published study which includes the measurements of more than 300 granules per species, and so it is reasonable to assume the 500 measurements here would capture a greater number of outliers.



Length (um)

Population	Count	% Gated	Mean	Median	Std. Dev.	Minimum	Maximum
maize	500	100	14.82	15	5.022	2	27.5
15-30um & maize	260	52	18.74	18.25	2.738	15	27.5

Fig. F.A.2: The distribution of lengths as a histogram, and descriptive statistics for 500 maize starch granules. Descriptive statistics also given for granules ranged 15-30 μ m.

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