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Documenting contamination in ancient starch laboratories

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ABSTRACT

Ancient starch analysis is an important methodology for researching ancient ecology, plant use, diet, and tool function; particularly in the deep past when other proxies may not survive. Establishing the authenticity of ancient starch is therefore a major concern for researchers. Despite decades of archaeological application, there are currently no empirically-tested procedures for systematically assessing and reducing intra-laboratory contamination. At the Universities of Oxford and Calgary, we have tested laboratory consumables, airborne contaminants, and decontamination techniques (oxidisation, boiling, autoclaving, torching) to establish contamination sources, types and quantities, as well as the most effective methods of destroying them. In our laboratories, we found that (i) contaminant starches represent a restricted range of types, (ii) many commonly used consumables including non-powdered gloves and Calgon are starch-rich, (iii) passive slide traps often used to test for airborne contaminants generate unreliable proxies and unacceptably low statistical confidence, and (iv) decontamination procedures using weak acids and bleach are largely ineffective. This collaborative study has allowed us to identify and reduce the risk of contamination and to develop better internal authenticity criteria for future ancient starch studies conducted in our laboratories.

1. Introduction

Whether the objective is to reconstruct ancient ecology, diet, or tool function, inferences made from starch analysis depend on authenticating genuine archaeological molecules; a concern shared with other fields of molecular palaeontology (Waggoner, 2001; Pääbo et al., 2004; Yang and Watt, 2005; Leonard et al., 2007). This is in addition to confirming that there is potential for a millennial durability of polysaccharides, for example through controlled laboratory experimentation and chemo-profiling (Barton et al., 1998; Haslam, 2004; Barton, 2009; Gupta et al., 2009), and employing quantitative approaches to taxonomic identification (Torrence et al., 2004; Tong et al., 2008; Wilson et al., 2009). Even though archaeological collections employed for starch analysis may be contaminated during survey, excavation, packing, and curation (e.g. Barton, 2007; Wesolowski et al., 2010; Hart, 2011), these are outside the scope of this article, where we concentrate on contamination avoidance in ancient starch laboratories.

Although post-excavation contamination has been studied by several authors (Loy et al., 1992; Barton et al., 1998; Loy and Barton, 2006; Williamson, 2006; Crowther and Haslam, 2007; Wadley and Lombard, 2007; Laurence et al., 2011; Langejans, 2012), there are currently no reproducible baselines to assess pollutants, vectors, and the efficacy of common decontamination techniques. Loy and Barton (2006) and Messner (2011) recommended several remedial measures: (i) airborne contaminants entering via unfiltered air or open windows should be monitored by placing slide 'traps' around the laboratory, (ii) water used in analytical procedures should be distilled and filtered at 0.22 μm, (iii) food should never enter the laboratory and be removed from hands by washing, and, (iv) non-powdered gloves should be worn during analytical procedures. Other precautions to avoid contamination include preparing modern reference materials in a separate location to reduce the potential for cross-contamination (Loy et al., 1992; Pearsall et al., 2004; Wesolowski et al., 2010), using fresh disposable consumables (Allen and Ussher, 2013), and cleaning non-disposable apparatuses between uses with bleach or vinegar (Henry, 2012) or by sonication (Li et al., 2010; Yang and Jiang, 2010). Others may use a direct flame, steam or boiling water (Perry et al., 2007; Zarrillo

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et al., 2008; Li et al., 2010; Yang et al., 2012). In general, however, the description of laboratory anti-contamination protocols tends to be brief and lacking in the reproducible detail that allows for an independent evaluation of how effective the controls being utilised really are. For example, while the use of 'powder-free' gloves is common-place in ancient starch analysis, it is acknowledged that they are not necessarily starch-free but only that they have less starch (Campbell et al., 1984; Makela et al., 1997; Phillips et al., 2001; Loy and Barton, 2006; Messner, 2011). And while it may be somewhat usual to monitor the laboratory environment for airborne starches through passive horizontal traps (Parr, 2002; Nugent, 2006; Laurence et al., 2011; Messner, 2011), there is no discussion of how the choice of trap types, their placement, quantities, and testing routine would achieve an acceptable confidence level.

From 2007 to 2009 the old Tropical Archaeology Laboratory 'TAL' at the University of Calgary started a systematic study of consumable contamination, environmental pollutants, and laboratory decontamination techniques. By 2010, emerging results revealed that widely used anti-contamination protocols were unable to capture the full contamination picture for this laboratory, thus requiring outside cross validation. A concurrent programme of tests was then performed at the University of Oxford's Ancient Starch Laboratory (Research Laboratory for Archaeology and the History of Art: 'RLAHA') from 2011 to 2012. The aim of this collaboration between Canada and the United Kingdom was to establish sources of modern starch, their types and quantities, and to evaluate methods for reducing contamination risk in these two laboratories.

2. Methods

Three main variables were tested during the intra-laboratory contamination study reported here: (i) consumables and reagents, (ii) the laboratory environment including horizontal and vertical surfaces and air-supplies, and (iii) decontamination methods. RLAHA and TAL mirrored each other's methodologies to the extent possible, but nevertheless introduced variations in the experimental set up to adapt it to each laboratory's environment and to represent a larger array of work conditions.

2.1. Microscopy

To assess the presence and nature of specific contaminants, RLAHA employed a compound light microscope (SP400) at magnifications ranging 100–600×. All slides were scanned through transects at 100× under plane- and cross-polarised light. Morphometric analysis was carried out directly through the microscope at 400× and 600× and on photographic images using the software ImageJ 1.46r. The TAL group used a system microscope (Olympus BX51, 200×–400×). Inspection and counting were done under polarised light; regular light microscopy and differential interference contrast (D.I.C.), which greatly enhances contrast and resolves fine structural details. Close examination of starch characteristics and sizes were conducted directly through the microscope and on photographic frames obtained through the image processing software Image Pro-Plus 5.1. Individual starch granules were described according to standard morphological and metric variables including shape (three-dimensional where rotation of the granules was possible); length (the maximum dimension on any axis) and width (the widest point perpendicular to the length); hilum position (centric, eccentric, highly eccentric); form and perpendicularity of the extinction cross arms; presence and form of vacuoles, fissures, lamellae and facets; surface texture; degree of birefringence; and any other distinctive features (e.g. Perry et al., 2006; Torrence, 2006; Holst et al., 2007; Yang et al., 2012; Liu et al., 2013). Starches were then grouped into morphotypes based on shared morphological features, and these were qualitatively compared to modern counterparts from our reference collections or published descriptions (e.g., Reichert, 1913; Ugent et al., 1982; Cortella and Pochettino, 1994; Jane et al., 1994; Pearsall et al., 2004; Piperno et al., 2004; Holst et al., 2007; Yang et al., 2012; Yang and Perry, 2013).

Physical alterations to individual granules were also noted, including mechanical damage (e.g., broken granules, cracking, rough surface texture or wrinkling, enlarged hilum, 'scooping' at the hilum; loss of birefringence) (e.g., Baldwin et al., 1995; Babot, 2003; Chandler-Ezell et al., 2006; Perry et al., 2006; Perry, 2007; Vinton et al., 2009; Liu et al., 2013), partial or complete gelatinisation from exposure to heat and moisture, which causes swelling and loss of birefringence (e.g., Duodu et al., 2002; Babot, 2003; Chandler-Ezell et al., 2006; Barton, 2007; Henry et al., 2009; Crowther, 2012), and enzymatic damage (exo-corrosion of the granule outer layers, or surface pores and channels resulting from endo-corrosion) (e.g., Gallant et al., 1992; George et al., 1995; Moorthy and Mathew, 1998; Numfor et al., 1995; Samuel, 1996a, 1996b; Barton, 2007). Granules that showed no evidence of physical alteration, or only very minor indications such as highly localised loss of birefringence without any clear indications of mechanical damage or swelling associated with gelatinisation, were classified as native granules. Both laboratories also recorded whether granules occurred individually or in clusters, and if they were attached to hairs, fibres, pollen grains or other extraneous particles, which may facilitate airborne transport by piggy-backing (cf. Schäppi et al., 1999; Laurence et al., 2011).

2.2. Consumables

We focused our tests on consumables in ready-to-use form, rather than items that were cleaned and re-used within a laboratory setting. For all analyses where water was required both groups employed water purified on-site through a Millipore Milli-U system that uses reverse osmosis, ion exchange and activated carbon (RO/DI). For microslides, we scanned 4–5 cm^2 from the centre of one surface at 100–200×, without a coverslip. Contaminants were extracted and concentrated by rinsing with water or sonicating (5–15 min) and centrifuging (3000 rpm, 5 min). In most cases, RLAHA recovered starches from 100% of the surface area of the sampled item, or 100% of the interiors of containers such as tubes or polyethylene bags, while TAL conducted smaller spot samples. Subnatants were pipetted onto a sterilised microslide and scanned without a cover slip (RLAHA) or with an autoclaved/boiled cover slip (TAL). Interior surfaces of tested consumables were filled with water and sonicated prior to pouring into sterilised centrifuge tubes and concentrating via centrifugation.

In Oxford, unopened batches of all consumables were used to ensure that they had not been exposed previously to contaminants within the laboratory. Sterilised stainless steel forceps were used to handle materials. Disposable gloves were not worn as they were not easily sterilised, and our hands were washed regularly throughout the tests, using synthetic towels for drying. The testing sequence began with microscope slides and Milli-U water, which were integral to all later testing procedures. All other glassware and consumables were sterilised immediately prior to use (see decontamination tests below) with 5% NaOH and/or boiling water, then rinsed with fresh Milli-U water. When filtration itself was being tested (e.g., sodium polytungstate recycling at RLAHA), we filtered the solution sequentially through 11 μm (Whatman Grade 1 qualitative), 2.5 μm (Whatman Grade 5 qualitative) and 0.7 μm (Whatman GF/F glass microfiber) filters.

In Calgary, we tested new batches of consumables and used forceps to handle samples. However, we wore VWR polyethylene disposable gloves, which tested negative in multiple contamination tests over several years. When direct manual contact was required, we wore autoclaved gloves: see below. All instruments and glassware were autoclaved (Tuttnauer 2340M) at 134 °C for 2 h and 30 psi after one use, and kept in a closed cabinet between uses. For sterilisation, we ensured that steam, heat, and pressure affected the equipment evenly and kept a consistent air gap in between the items. When covering beakers or dishes, we used sterilised petri dishes as covers. Alternatively, we used Parafilm, but in this case we tested a control every tenth sample we ran. Centrifuge tubes (50 ml) were autoclaved prior to use. Our testing sequence started with microscope slides previously autoclaved. We used a permanent marker to delineate a safety field on every slide, so that prior to mounting any substance, slides were inspected under the microscope at 200× to confirm that no starch was present. Water was filtered by reverse osmosis and deionisation yielding 0 Total Dissolved Solids (TDS). TAL has always employed EDTA salts for clay dispersal and prepared our stock solution with boiled RO/DI water. Our glycerol was reagent-grade quality bi-distilled (187 °C, 20 mmHg) in the Organic Chemistry Laboratory at the University of Calgary from animal fat under the direction of Dr T. Back. All glycerol-based traps were screened in their totality prior to set up. At TAL, we only tested fresh sodium polytungstate, not filtered product.

2.3. Environmental aspects

Four different types of tests were conducted to assess the types and rates of environmental starch contaminants occurring within our laboratories and associated workspaces: (i) horizontal slide traps; (ii) vertical slide traps; (iii) indoor air quality tests (TAL only); and (iv) miscellaneous tests of cleaning equipment, mats, floor surfaces, shoes, etc. (TAL only).

Horizontal (passive) traps captured starches landing on microscope slides coated with a drop of glycerol (4 cm^2). Running time was 72 h, and they were set up to test variables such as work days versus non-work days and, in the case of RLAHA, before and after laboratory cleaning. Slides were placed at various locations around the laboratories and at different heights (at RLAHA: floor level; work level [60–90 cm above floor level] and shelf level [140–200 cm]; at TAL: 100 cm and 200 cm above floor level) and inside cupboards, in order to trace variations in starch accumulation across different work and storage spaces. The quantity of slides placed around the workspaces varied, but for horizontal traps involved 25 per test in the Ancient Starch Laboratory at RLAHA (2.66 slides/m^2) and 75 in the 'old' starch laboratory at TAL (1.17 slides/m^2).

Vertical traps employed 'stick-to-it' 4 cm^2 adhesive samplers (SKC™) and consisted of stationary and mobile tests. Normally, stationary tests were run for 72 h (range: 3–72 h) and were placed on walls, door and other vertical surfaces around the laboratories. In addition, to control for the impact of motion on contamination rates, TAL conducted a mobile vertical analysis using stick-to-it samplers attached to the lapels of eight individuals working in five departments across three buildings throughout the University of Calgary campus and had them walk at regular speed while going about their business as usual for a period of 3 h.

TAL also carried out a limited indoor air quality assessment in which contaminants were collected directly from the circulating air system. A suction pump running at constant flow (15 L per min) and pressure (10 psi) was connected through a hose to a leak-free cassette recorder (SKC, Versatrap) with its mouth placed 5 cm away from the air vent that was being tested. The air flow thus hit a sticky slide-trap where contaminants >1.5 μm were entrained, ready for counting and identification under the microscope. We tested a total of 13 air supplies. Average running time was 30 min per test (range: 10–240 min; total cumulative: 880 min). The volume of air screened per 30 min test was 450 L; about 37.5% of the total volume emitted by one vent in the same time (1200 L). The turnover rate of indoor (old to fresh) air replacement fluctuated from 10% to 60% per day in the three buildings we studied, although the building where TAL is located experiences complete air stagnation overnight.

Miscellaneous environmental tests at Calgary encompassed stationary and mobile materials (Table 6). For hard surfaces, we scraped off the top <1 mm from an area of 4 cm^2 with a new, autoclaved blade onto a sterilised, pre-checked microscope slide. The sample was then mounted with glycerol/water and covered with a slip. Fabric pieces from mops, brooms, and carpets (1 g total) were mixed with 50 ml of water and soaked overnight while shaken at 200 rpm. Contaminants in the water were centrifuged (3000 rpm, 5 min) and sampled for inspection (aliquot: two drops).

At Oxford, the environmental tests focused on the Ancient Starch Laboratory (ASL; 9 m^2), which is a self-contained room located in RLAHA's dedicated archaeological science research facility (other laboratories at RLAHA are used for isotope studies, radiocarbon dating, chromatography, tephrochronology, and petrographic microscopy, among other applications). The ASL was established about six months prior to the start of the contamination study, before which the room was used on an irregular basis as an archaeology bio-containment room for preparing modern biological samples (principally bone and animal tissues). When it was converted to the Ancient Starch Laboratory, all previous samples, consumables and equipment except for fixtures and fridges were removed, a top-down clean of all surfaces including walls, floors, benches and fridges was performed (although wearing gloves and using paper towels), and only the equipment and fresh consumables needed to perform ancient starch studies were introduced. No modern starch reference materials were analysed or stored in the room, and access was restricted to ancient starch personnel (at the time, only AC and MH). Prior to conducting the contamination study, standard cleaning procedures for ASL workspaces involved frequent wiping of all work surfaces with paper towel and household bleach (5%) or surface detergent (Decon90) as well as regular wiping of the floor. No cleaning was performed at any time by external personnel. In compliance with general laboratory health and safety regulations as well as best practice ancient starch procedures, food stuffs were never stored or consumed in the room.

In addition to the ASL, four other rooms at RLAHA were tested using horizontal and vertical traps (Fig. 1A): the balance room (12 m^2) that adjoins the ASL and provides the only access point, also used for weighing samples and reagents; the ancient diet laboratory (58 m^2), which is a large open space used primarily for isotopic studies but also by us to prepare modern starch reference samples and for oven-drying ancient starch samples; the water purification room (16 m^2); and an access corridor (10 m^2). Three rounds of tests were conducted at RLAHA; the first two involved all five rooms and tested weekday versus weekend variables, while the third round was only conducted in the Ancient Starch Laboratory and took place after a complete top-down clean of all horizontal and vertical surfaces using synthetic cloths (rather than paper towel), tap water and without the use of gloves.

At the University of Calgary, the stationary horizontal and vertical tests focused on the 'old' ancient starch laboratory (64 m^2) (Fig. 2). In addition, we conducted air quality tests across 15 ± 1% of the Archaeology department's space (Earth Sciences -ES-building: 7th–8th floors); that is, seven rooms from the 7th floor (267 m^2) and seven from the 8th floor (226 m^2). For comparison, we studied

Fig. 1. Plan of RLAHA and the Ancient Starch Laboratory (ASL), Oxford, showing results of horizontal passive slide traps on A) work days, B) non-work days (ASL only), and C) after cleaning (ASL only); key in A) applies to all.

two additional rooms lower in the same building (ES 3rd, 5th floor), plus four rooms from two other buildings (Biological Sciences, Chemistry).

2.4. Decontamination protocols

Oxford conducted two experiments to study the efficacy of using oxidising chemicals to decontaminate consumables and work surfaces by destroying native starch granules. The first experiment tested hydrogen peroxide (H_2O_2 6%, 10%, 20% and 30%), bleach (NaOCl 12.5%), sodium hydroxide (NaOH 5%) potassium hydroxide (KOH 5%), and acetic acid ($C_2H_4O_2$ 5%) on separate slides supporting ~50 μg of starch from potato (*Solanum tuberosum*), taro (*Colocasia esculenta*), and yam (*Dioscorea* sp.). Slides were monitored microscopically for up to 1 h, during which time progressive changes to starch morphology and birefringence were recorded.

The second eradication test determined whether exposure to either 5% NaOH or boiling water followed by sonication is sufficient to not only destroy starches but also remove any residue of them from consumables. Individual slides coated with ~50 μg of starch from seven modern crops (chickpea, *Cicer arietinum*; potato; tapioca, *Manihot esculenta*; quinoa, *Chenopodium quinoa*; rice, *Oryza sativa*; durum wheat, *Triticum durum*; maize, *Zea mays*) were immersed in beakers of either 5% NaOH or boiling water for 5 min, then in beakers of fresh Milli-U water and sonicated for a further 5 min. They were then rinsed, dried in a covered beaker, stained with iodine potassium-iodide (IKI, 0.1%) and examined microscopically for any remaining native or gelatinised starch granules. The NaOH sterilisation procedure was then tested on 20 fresh microscope slides from two different brands, and the boiling water procedure was tested on 50 pipette tips, the same batches of which had previously registered starch contaminants.

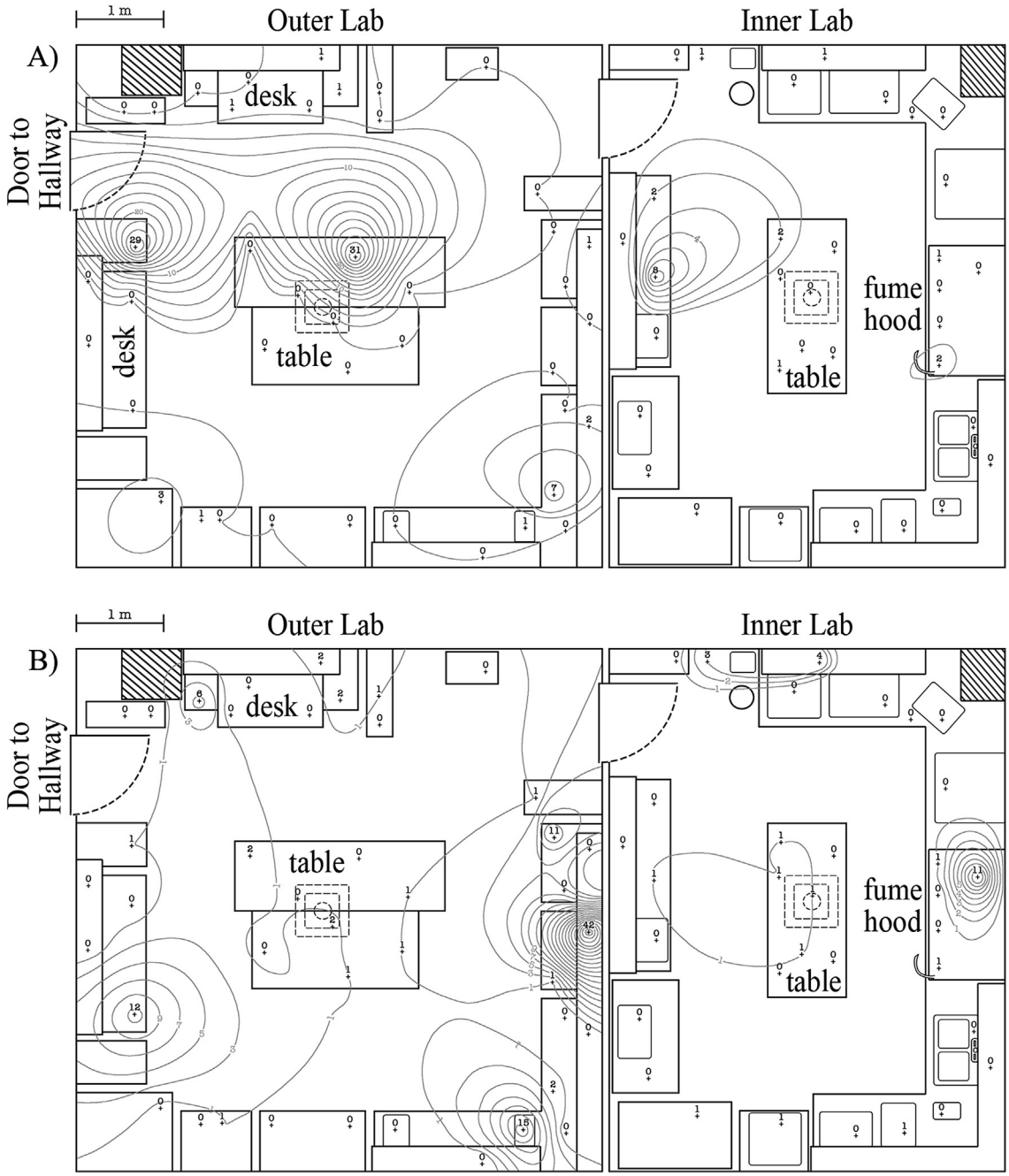

Fig. 2. Plan of TAL, Calgary, showing results of horizontal passive slide traps on A) non-work days and B) work days.

The RLAHA decontamination experiments were undertaken in a physically separate laboratory space using modern reference starches derived from pulverised seeds or tubers stored in 70% ethanol, small aliquots of which were removed by pipette as required. Bulk or commercial starch powders, on the other hand, were not (and never have been) used for any of our experimental or reference purposes, as the starches can easily become airborne and contaminate workspaces.

In Calgary, decontamination experiments concentrated on four aspects of starch eradication: 1) the effect of household bleach on wiping laboratory surfaces, 2) boiling equipment in excess water, 3) the autoclaving of heat-resistant laboratory materials, and 4) the torching of metal tools. We tested the effect that 4 ml of bleach (NaOCl 6%) has on starch eradication (4 ml is the amount required to coat 400 cm^2 from a laboratory bench). To measure this effect, 0.004 g of starch from maize, wheat (*Triticum* spp.), quinoa, waxy barley (*Hordeum vulgare*), rice, tapioca, arrowroot (*Maranta arundinacea*), wrinkled pea (*Pisum sativum*), potato, and sweet potato (*Ipomoea batatas*) were placed on microscope slides, then exposed to bleach for 30 min twice, then examined microscopically. For boiling, we brought 100 ml of RO/DI water to the boil. We then coated a metal spoon with starch from the species being tested, left it in the beaker for 1 h, and then inspected both the water and the spoon. The materials we autoclaved included piston-driven, polypropylene maxi-pipettes, tweezers, glassware, brushes, crucibles, mortar, and pestles. Metal spoons were subjected to butane

Table 1
Total starch-types documented across all contamination tests.

	Type I	Type II	Type III	Other	Total
RLAHA	1244	343	13	579	2179
TAL	604	1005	12	17	1638
Total	1848	1348	25	596	3817
%	48.41	35.32	0.65	15.61	100.00

torching. The starch sample was placed in the centre of the spoon and the flame was maintained one inch away from it for 30 s (range: 5–30 s). The TAL decontamination tests followed precautions similar to those employed at RLAHA and starch powders were not volatilised in areas where they could contaminate our samples.

3. Results

3.1. Typology

This study detected modern starch contamination in the form of 3817 granules; of which Oxford documented 2179 and Calgary 1638 (Table 1). Based on basic differences in shape, size, hilum location, and texture these contaminants were classified in three main types. The largest group (85% of the evidence; Tables 1 and 2) includes Types I (~49%) (Fig. 3) and II (~36%) (Fig. 4), and a much lower quantity represents Type III (Fig. 5), and unclassified granules.

3.1.1. Type I

The majority of granules recovered during this study were small- (5–10 μm maximum length) to medium-sized (10–25 μm maximum length), with sub-rounded shapes (orbicular, orthogonal, ovate, bell) and a centric hilum that often displayed a small vacuole (hole) or fissure (transverse slit, Y-, X- and stellate-shaped) (Fig. 3a–u, Fig. 6).

3.1.2. Type II

The second most common type comprised medium- (10–25 μm) to large-sized (>25 μm) granules (average size 20 μm) of circular or sub-circular shapes with centric hila in plan view (Fig. 4a–p), and lenticular (discoidal) shapes and a distinctive equatorial groove in three-dimensional view (Fig. 4g). Many starches of this type displayed lamellae (Fig. 4a, d, f, h–m) and surface depressions (Fig. 4b, d).

3.1.3. Type III

The third type is much less frequent than the other two. It comprises medium to large-sized (15–58 μm), ellipsoidal/ovate granules with strong birefringence, marked lamellae and highly eccentric hila (Fig. 5a–g).

The taxonomic identification of starch morphotypes is a subject of scholarly debate that lies outside the main scope of this paper and is secondary to the contamination scenarios presented here; nevertheless, attempting to identify the botanical sources in our sample helps understand the provenance of contaminants. It has been suggested that granule shape, size, and hilum position discriminate root starches from those found in grains, beans, and peas (e.g. Reichert, 1913; Jane et al., 1994). Some authors (e.g. Yang et al., 2012) support that Poaceae starches are easily distinguishable from those in other economically significant families. Several researchers (e.g. Pearsall et al., 2004; Piperno et al., 2004; Perry et al., 2006; Holst et al., 2007; Zarrillo et al., 2008) have also proposed that subfamily, tribe, and genus identification within the grasses is possible on simple shape grounds that separate the Triticeae tribe from all others, including all starches from the subfamily Panicoideae. Furthermore, ancient maize research suggests that species (even varieties) could be identified according to various two- and three-dimensional surface features and metric parameters (Table 2: e.g. Type 1 'double border' for 'hard endosperm' maize cf. Fig. 3d, f, h; Type 1 'bumped surfaces' and 'white dot' for 'soft endosperm' maize cf. Fig. 3e, i, l, Fig. 6). Contaminant Types I and II would therefore be consistent with commonly utilised identification criteria for seed/grain starches; and Type III for tubers (e.g. Reichert, 1913; Jane et al., 1994; Yang et al., 2012; among others). Type I morphometrics overlap with widely used identification standards in ancient maize research for grass (Poaceae) seeds, specifically those within the Panicoideae sub-family (Pearsall et al., 2004; Piperno et al., 2004; Perry et al., 2006; Holst et al., 2007; Zarrillo et al., 2008). Moreover, if we were to follow the criteria listed by Cortella and Pochettino (1994), Pearsall et al. (2004), and Holst et al. (2007) (Table 2), our sample would then contain maize (*Z. mays*); with hard and soft endosperm varieties. Type II includes starches with features that several authors consider typical of the Triticeae (Evers and Bechtel, 1988; Lineback and Rasper, 1988; Piperno et al., 2004; Henry and Piperno, 2008; Henry et al., 2011; Yang et al., 2012; Liu et al., 2013; Yang and Perry, 2013), including the various surface depressions (Fig. 4b, d) and lamellae (Fig. 4a, d, f, h–l), as well as the centric pleats (Fig. 4a,c), that Piperno et al. (2004), Yang et al. (2012) and Yang and Perry (2013) present as diagnostic of the genera *Triticum* (wheat), *Hordeum* (barley), *Aegilops* (goatgrass) and *Secale* (rye). Type III includes granules identified by Jane et al. (1994) and Reichert (1913) in roots and tubers, while Ugent et al. (1982) found them in potatoes (*S. tuberosum*).

While the majority of granules discovered were native, modified types were also present (Fig. 3p–u, 4h–p, 5f, g). Observed features included cracking (Fig. 5f, g), tearing/breakage (Fig. 4h), rough surface texture (Fig. 4l, o, p), scooped or depressed centres (Fig. 3s–

Table 2
Morphometric characteristics of the three main starch types (I–III) identified in this study and their possible taxonomic sources.

Starch type	Ugent et al., 1982	Jane et al., 1994	Cortella and Pochettino, 1994	Pearsall et al., 2004	Piperno et al., 2004	Holst et al., 2007	Yang et al., 2012
Type I: Centric, small to medium size							
Orbicular/orthogonal/ovate/bell	n/a	Grain	n/a	Poaceae	Poaceae	Poaceae	Poaceae
Orthogonal, fissured, double border	n/a	n/a	*Zea mays*, hard endosperm	*Zea mays*, hard endosperm	n/a	*Zea mays*	Poaceae
Orbicular/psilate/bumped/hilum (white dot)	n/a	n/a	*Zea mays*, soft endosperm	*Zea mays*, soft endosperm	n/a	*Zea mays*	Poaceae
Type II: Centric, medium to large size							
Lenticular, dimpled surfaces, lamellae, central pleat	n/a	Grain	n/a	n/a	*Triticum*, *Hordeum*, *Aegilops*	n/a	Triticeae, *Aegilops*
Type III: Eccentric, large size							
Ellipsoidal (lamellae), ovate	*Solanum tuberosum*	Root, tuber	n/a	n/a	n/a	n/a	n/a

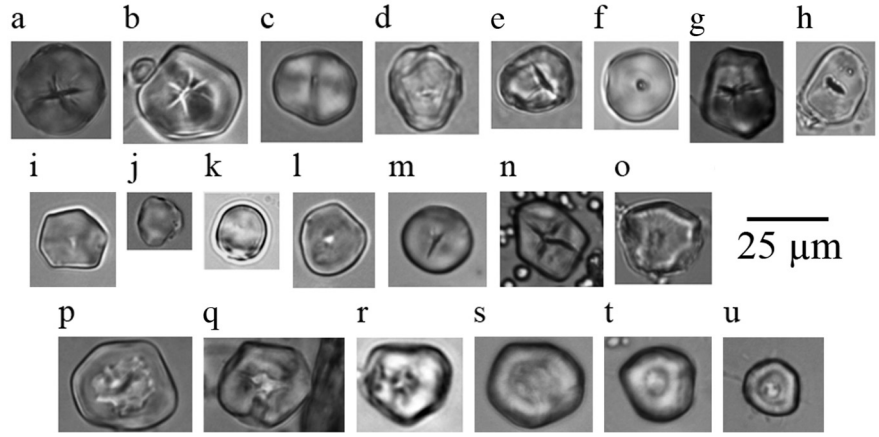

Fig. 3. Examples of Type I starch granules documented in the contamination study. a–o) native (unmodified) granules; p–u) modified granules (p–r: enlarged hilum; s–u: scooped out centre typical of grinding damage to maize starch, e.g., Perry et al., 2006).

u), enlarged hilum (Fig. 3p–r), loss of birefringence (Fig. 4n, p), partial gelatinisation (partial loss of birefringence and limited swelling) (Fig. 4l–p), and enzymatic alteration (characterised by corrosion holes/pores: Fig. 4i; exposed/corroded lamellae: Fig. 4j–k; and weak birefringence). At RLAHA, over 30% of the starches exhibited modifications, while at TAL, only 3% of the starches displayed damage.

3.2. Consumables

Laboratory consumables (Table 3) have high contamination potential, with Oxford recording 1391 contaminant granules. Almost three quarters (~72%) of the classified granules recovered from laboratory consumables derive from Type I sources, while one quarter (~26%) comes from Type II (Table 3). Type III starches are not a significant contaminant (<1%). Although the amount of consumable analysed was sometimes larger than the portion of that consumable that would be likely to come in contact with ancient starch samples, it is noticeable that many 'powder-free' gloves are contaminated, as well as glycerol, microslides, parafilm, pipette tips, sodium polytungstate, paper towels, lens tissue, sample bags, centrifuge tubes, cling film, and plastic weighing trays. The degree of contamination varied, in some cases quite widely, depending on the brand of consumable tested by each laboratory, although as we discuss in Section 4.2 below, the different testing methods used by each laboratory may have impacted some of these results. 'Calgon', a sodium hexametaphosphate preparation employed for clay dispersion, was confirmed positive by both RLAHA and TAL (Figs. 3o and 4d, m, n, 5g).

3.3. Environmental aspects

Airborne, modern starch grains landing on laboratory surfaces are pervasive ($n = 1016$; Table 4) with Type I starches amounting to more than three quarters of both Oxford and Calgary's assemblages; these were consistently followed by Type II starches in a 5:1

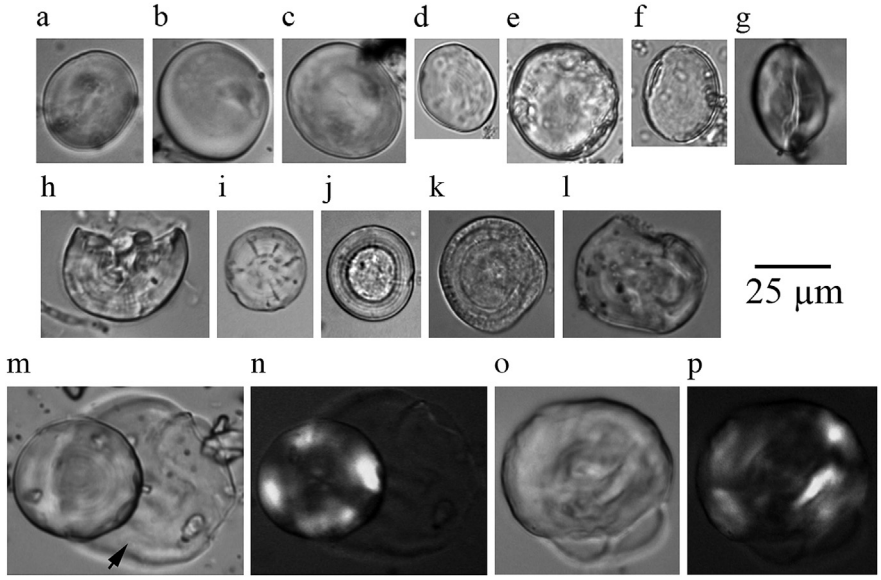

Fig. 4. Examples of Type II starch granules. a–f) native granules in plan view; g) native granule in side view showing flattened (lenticular) three-dimensional shape and equatorial groove; h–p) modified granules (h: torn/broken; i–k: channels and exposed lamellae typical of enzymatic corrosion; l: rough surface texture and distortion typical of partial gelatinisation of Triticeae starch during cooking, e.g., Henry et al., 2009; m–n: native granule attached to a gelatinised granule shown in plane (m) and cross-polarised light (n); o–p: distorted morphology and loss of birefringence typical of partial gelatinisation.

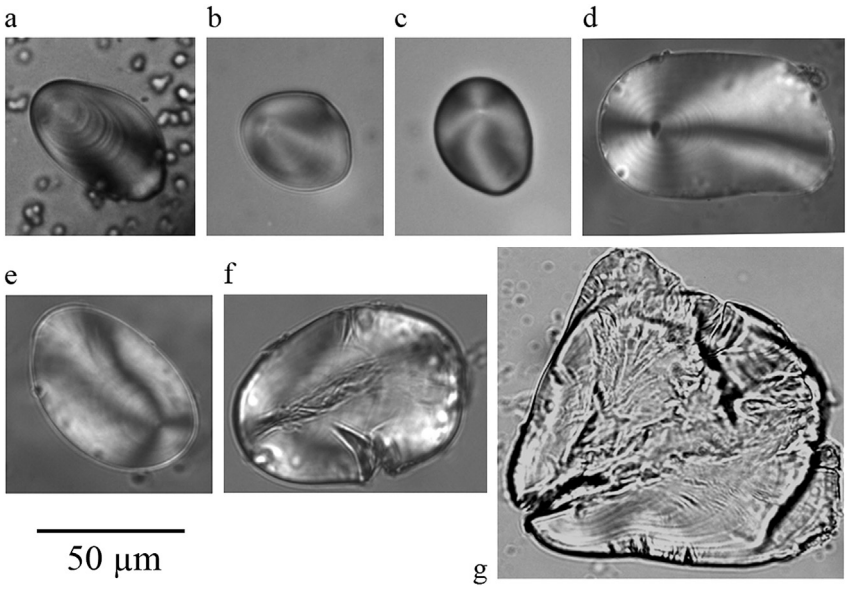

Fig. 5. Examples of Type III starch granules. a–e) native granules; f–g) modified granules (f: cracking; g: cracking and morphological distortion).

ratio. Direct sampling of air circulation vents at the University of Calgary showed that a single vent has the potential to mobilise 41 starch granules per hour, or 986 granules per day. Cloud contamination, to which laboratory personnel are exposed as they walk around campus, is 24 times higher than that recorded in any other form of passive trap, totalling 725 granules with a large majority being Type II. Building materials, garments, and university furnishings are heavily contaminated by both Type I and II starches ($n = 428$).

Horizontal drop-of-glycerol passive traps ($n = 267$) yielded a total of 1016 granules (Table 4). At RLAHA, 117 traps captured a total of 788 starch granules. Of these, 98 traps recorded starches while 19 showed no sign of contamination. Prior to cleaning, the highest average number of granules was detected in the Ancient Starch Laboratory (average 12/trap), where they were concentrated in the fume hood (24/trap), at floor level (18/trap), and on the main workbench (12/trap) (Fig. 1A, B). The lowest quantities in the Ancient Starch Laboratory were recorded inside the cupboards (1/trap). Higher spaces such as shelves generally also showed lower accumulation levels. Starch granules were less common in the other rooms (<4/trap), except for the weighing room, which recorded 13/trap in the first testing round versus 4/trap in the second round. Overall, ~90% of traps in the other rooms held fewer than nine granules, compared to 55% in the Ancient Starch Laboratory. After removing all contaminated consumables (particularly gloves and paper towels) and cleaning all surfaces, fewer starch granules (2/trap) were recorded in the Ancient Starch Laboratory, with the majority still occurring in the fume hood (Fig. 1C). We noted that 20% of granules were attached to hairs and fibres, which may have facilitated their airborne movement. Clusters (two or more granules) represent 58% of our finds, with a maximum number of 16 granules ($n = 2$). More than three quarters of the entire airborne assemblage consists of Type I starch granules (77%), while less than one-sixth (~15%) derives from Type II. Type III is represented in very low percentages (<1%).

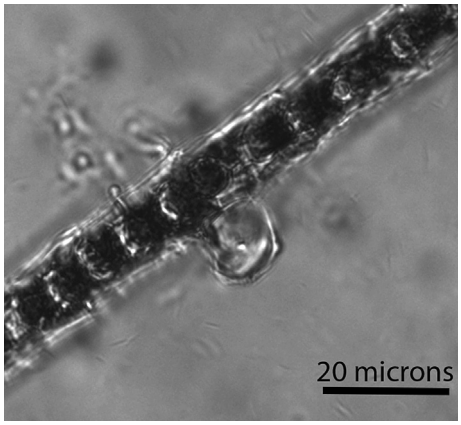

Fig. 6. Type I starch granule identified as *Zea mays* (maize) 'piggy-backing' on a hair, recovered in the airborne tests at Calgary (mobile SKC 'stick-to-it', vertical sampler, ES 7th floor, see Table 5, row 13).

At TAL, 50 out of the 150 horizontal glycerol traps demonstrated the presence of starch ($n = 228$ granules), while the remaining 100 showed no traces (Table 4). Twenty-eight contaminated traps had a single starch grain present, nine had two present, two had three starch grains present, and two had 11 present. Single traps holding four, six, seven, eight, 12, 15, 29, 31, and 42 starch grains comprise the remainder of the contaminated trap sample. We note that 95% of our traps held fewer than nine starch grains. Clusters ($n = 7$) represent 50% of our finds, with the highest occurrence at 42 granules (range: 3–42). More than three quarters of the assemblage consists of Type I granules (~80%), while less than 20% have come from Type II starches. Other types were rare (<1%).

The distribution obtained is distinctly and irretrievably non-normal (extreme values result in skewed distribution), so a robust categorical data-based formula was employed to calculate the sample sizes needed to reproduce the results obtained reliably, assuming these results could represent true contamination patterns. The formula used is:

$$n = \left(\frac{\sqrt{pq}(t)}{\mathrm{ER}}\right)^2$$

Where n is the sample size to be calculated, p is the proportion (in this case, the proportion of uncontaminated samples—0.6668), q is $1 - p$, t is the t score for a 95% confidence level (1.96 in this

Table 3
Results of consumable contamination test.

Item	RLAHA	Unit/Amount	Type I	Type II	Type III	Other	Total	TAL	Unit/Amount	Type I	Type II	Type III	Other	Total
Calgon	Fisher Scientific S/4120/60, 10336340	100 g	43	10	1	0	54	Commercial provider	1 g	25	3	2	0	30
EDTA disodium salt	–	–	–	–	–	–	–	J.T. Baker, VWR 6381-92-6	1 g	0	0	0	0	0
Ethanol	VWR prolabo technical grade denatured 95%, 20827.365	500 ml	0	0	0	0	0	–	–	–	–	–	–	–
Filter paper	Whatman Grade 1 qualiative, 150 mm diam	1	0	0	0	0	0	–	–	–	–	–	–	–
Filter paper	Whatman Grade 5 qualitative, 125 mm diam	1	1	0	0	0	1	–	–	–	–	–	–	–
Filter paper	Whatman GF/F glass microfiber circles, 90 mm diam	1	0	0	0	0	0	–	–	–	–	–	–	–
Foil, aluminium	Commercial provider	250 cm^2	13	5	0	1	19	Commercial provider	4 cm^2	0	0	0	0	0
Gloves, 'powder-free'	Nitrile medium Aloe, Fisher Scientific, FB51965, 11762779	1	58	10	0	2	70	Positive Touch™ Powder-Free Gloves CAPH8844	1	0	0	0	0	0
Gloves, 'powder-free'	Nitrile large Fisher Scientific, FB69264, 11542723	1	19	2	0	3	24	VWR Polyethylene gloves, 32915-268	1	0	0	0	0	0
Gloves, 'powder-free'	Nitrile large Aloe Fisher Scientific, FB51967, 11772779	1	28	18	0	6	52	–	–	–	–	–	–	–
Gloves, 'powder-free'	Nitrile large Fisher Scientific, FB69263, 102238821	1	26	0	0	2	28	–	–	–	–	–	–	–
Gloves, 'powder-free'	Evergreen Sensa latex large, EPF2104	0.5	261	53	0	7	321	–	–	–	–	–	–	–
Gloves, nylon	Nylon glove liner, re-useable, laundered by commercial provider	1	6	3	6	461	476	–	–	–	–	–	–	–
Glycerol	VWR Prolabo Analar Normapur 99.5% bidistilled analytical grade, 24388.260	40 cm^2	1	0	0	0	1	MP Biomedicals ultrapure glycerol, VWR IC1106650	736 cm^2	0	0	0	0	0
Kimwipes	–	–	–	–	–	–	–	White, supplied by Kimberly–Clark 344133	10 cm^2	0	0	0	0	0
Microslides	VWR 611-0117	26	5	0	0	0	5	VWR 48312-501	19	0	0	0	0	0
Microslides	Thermoscientific Menzel-Gläser, VWR 631-1303	7	1	0	0	0	1	–	–	–	–	–	–	–
MU water	Purified on site with Millipore Milli-U system	1000 ml	0	0	0	0	0	–	–	–	–	–	–	–
Packaging microslides, plastic casing	–	–	–	–	–	–	–	Packaging from VWR 48312-501	4 cm^2	0	0	0	0	0
Packaging microslides, carton	–	–	–	–	–	–	–	Packaging from VWR 48312-501	4 cm^2	1	0	0	0	1
Parafilm	Parafilm M	50 cm^2	21	2	0	1	24	Parafilm M, VWR 52859-079	4 cm^2	0	0	0	0	0
Pipette - flint glass, disposable	–	–	–	–	–	–	–	VWR 53499-632	5	0	0	0	0	0
Pipette tips	VWR 1–200 μl, VWR 613-0246	50	67	6	0	5	78	–	–	–	–	–	–	–
Pipette, transfer, polypropylene	–	–	–	–	–	–	–	VWR 16001-190	1	0	0	0	0	0
Polyethylene sample bag	280 × 205 mm, ziplock	5	0	1	0	0	1	U-line, 220 × 150 mm, ziplock	1	0	0	0	0	0
Polyethylene sample bag	116 × 90 mm, ziplock	5	0	0	0	0	0	–	–	–	–	–	–	–
Polyethylene sample bag	56 × 56 mm, ziplock	5	0	0	0	0	0	–	–	–	–	–	–	–
Sodium polytungstate	Sometu-Europe, powder, SPT1	100 g	24	5	0	18	47	Poly-Gee, powder, SP006	5 g	0	0	0	0	0
Tissue, lens	Whatman Grade 105, 150 × 100 mm	1	20	2	0	1	23	–	–	–	–	–	–	–
Towels, cloth	–	–	–	–	–	–	–	Commercial provider	10 cm^2	0	0	0	0	0
Towels, paper	Brown, interfold, Kimberly–Clark	575 cm^2	28	16	0	2	46	White, Kimberly–Clark 0100520	10 cm^2	0	0	0	0	0
Tubes, centrifuge	VWR, polypropylene 50 ml, 525-0403	4	9	1	0	0	10		1	0	0	0	0	0

							BD Falcon, polypropylene 50 ml 21008-951						
Tubes, centrifuge	–	–	–	–	–	–	BD Falcon, polypropylene 15 ml 62406-200	1	0	0	0	0	0
Weighing boats	250 ml VWR 611-0095	5	0	0	0	0	30 ml 16005-128	1	0	0	0	0	0
Weighing boats	100 ml VWR 611-0094	5	2	0	0	2	–	–	–	–	–	–	–
Weighing paper	–	–	–	–	–	–	VWR 12578-165 10 × 10 cm	1	0	0	0	0	0
Wrap, plastic, cling-on	No brand	300 cm^2	4	97	5	2	No brand	4 cm^2	0	0	0	0	0
		Total	637	231	12	511		Total	26	3	2	0	31
		%	45.79	16.61	0.86	36.74	100.00	%	83.87	9.68	6.45	0.00	100.00

Table 4
Results of horizontal passive slide trap tests.

Lab	Context	Traps (n)	Type I	Type II	Type III	Other	Total
RLAHA	Work day	46	242	42	0	25	309
RLAHA	Weekend	46	329	63	1	37	430
RLAHA	After cleaning	25	36	7	0	6	49
		Total	607	112	1	68	788
		%	77.03	14.21	0.13	8.63	100
TAL	Work day	75	127	4	1	1	133
TAL	Weekend	75	55	39	0	1	95
		Total	182	43	1	2	228
		%	79.82	18.86	0.44	0.88	100

example), and the desired error range (ER) is 5%. The result indicates that a sample size of 342 traps would be needed to obtain a representative sample of the degree of contamination at TAL at the $p \leq 0.05$ confidence level.

TAL also tested starch from drop-of-glycerol traps against the level of activity (Fig. 2A, B). One round was conducted while students were in classes on campus and while the laboratory was in use (Fig. 2B). The second round was conducted when classes were not in session and most students stayed away from campus and the laboratory was not in use (Fig. 2A). We found that levels of contamination increased significantly (Pearson Chi-square = 4.320; $p = 0.038$) in the total sample during the period when classes were in session and the laboratory was in active use. The effect of activity levels was not significant when the room samples were considered individually. Proximity to doors increased starch counts by a factor of three. TAL confirmed RLAHA's observation that the fume hood concentrates contaminants (Fig. 2B).

None of the vertical, 'stick-to-it' passive adhesive samplers tested positive at RLAHA, but they did at the Earth Sciences building from the University of Calgary: one Type III granule from the 8th floor and 31 Type I granules from the 7th floor. On the other hand, vertical lapel adhesive samplers used on the move recorded cloud contamination as the subject walked around campus (Table 5). These mobile vertical traps showed starch attached to hairs and fibres (<1% of the total contaminated assemblage) (Fig. 6) (cf. Schäppi et al., 1999). At the University of Calgary, environmental samples from cleaning utensils (broom, mop), garments, wall paint, carpets, mats, shoe soles, as well as floor and ceiling tiles (Table 6) yielded 428 starch granules dominated by Type I and Type II granules.

Limited air quality assessment at Calgary detected 226 granules over 880 min (Table 7), and one test demonstrated that five granules can land in a cassette sampler within 10 min (see Table 7, row

Table 5
Results of vertical lapel adhesive samplers at Calgary (locations indicate the areas where the subject walked during the test period).

Univ of Calgary, location	Type I	Type II	Type III	Other	Total
ES stair shaft	1	0	0	0	1
ES 8th floor	1	0	0	0	1
Social Sciences	1	0	0	0	1
ES 8th floor	1	0	0	0	1
ES stair shaft	1	0	1	0	2
ES 3rd floor	1	0	1	0	2
Social Sciences	1	0	1	0	2
ES 8th floor	2	0	1	0	3
Biological Sciences	1	4	0	0	5
Science B	6	0	0	0	6
Chemistry	22	38	4	0	64
Science A	1	168	0	0	169
ES 7th floor	4	464	0	0	468
Total	43	674	8	0	725
%	5.93	92.97	1.10	0	100.00

Table 6
Results of miscellaneous environmental tests at Calgary (e.g. cleaning utensils, mats, floor surfaces, shoes).

Location	Type I	Type II	Type III	Other	Total
ES 806, office counter	0	0	0	1	1
ES 824, carpet	0	0	0	1	1
ES 8th floor, ceiling tile	0	1	0	0	1
TAL employee no. 1, shoe sole	2	0	0	0	2
TAL employee no. 2, shoe sole	0	3	0	0	3
Wall paint	3	0	0	0	3
TAL floor mat	4	1	1	0	6
TAL employee no. 3, shoe sole	15	0	0	0	15
Cleaning gear, mop	10	7	0	0	17
TAL employee no. 4, shoe sole	19	0	0	0	19
ES 830, floor tile	1	151	0	0	152
Cleaning gear, broom	200	8	0	0	208
Total	254	171	1	2	428
%	59.35	39.95	0.23	0.47	100.00

14). Because we captured 37.5% of the air being produced by a vent per hour, the normalisation needed to express true contamination figures is achieved by multiplying hourly starch counts by 2.66 (the volume of air produced by one vent is approximately 57,600 L per day or 2400 L per hour). These results show that a single air vent can mobilise between 2 and 113 granules per hour, or as many as 2712 granules per day (average = 986 granules). While the majority of tests indicated that single vents emitted ≤10 granules per hour, several showed much higher spikes (18, 20, 24, 30, 54 and 113 granules per hour), including at locations such as TAL that otherwise produced ≤10 granules per hour in repeat tests. These patterns indicate that starch granule emissions can fluctuate randomly, making predictions of where and when high counts will be detected difficult without constant air quality monitoring.

3.4. Decontamination protocols

RLAHA noted that only 5% NaOH and 5% KOH caused near instantaneous and complete destruction of native starch granules for all three species tested, causing them to fully gelatinise (swell, lose form and birefringence, and in some cases leach or solubilise) (Table 8). Thirty per cent (30%) H_2O_2 and 12.5% bleach were more variable, taking from around 5–30 min to completely destroy starches, depending on the crop. Weaker solutions of hydrogen peroxide took at least 30 min to achieve complete gelatinisation, with the 6% solution having no effect on potato or yam starches within an hour. Starch granules exposed to 5% acetic acid appeared unchanged after 1 h. Our subsequent tests using 5% NaOH or boiling water and sonication to eradicate starches from slides showed that both these treatments were highly effective at destroying native starch granules. However, iodine staining revealed that intact gelatinised granules (n = 10) remained on the wheat slides, and amylose/amylopectin residues still adhered to all others except for maize.

TAL noted that exposure to household bleach (6% sodium hypochlorite) failed to destroy native granules from any of the ten plant species utilised; often preserving the full morphometric spectrum. The highest eradication was achieved with wheat, while the lowest was with corn (Table 9). Much better results were accomplished with excess boiling water and autoclaving (136 °C) for 2 h, with no native grains being preserved; even though the autoclave allows for the formation of spherulites after thermal events (Singh et al., 2010) and some starches are known to resist the high temperature-pressure of autoclaving (Escarpa et al., 1996; Skrabanja and Kreft, 1998). The use of butane torches to decontaminate metal tools shows that repeated incinerating bouts shorter than 5 s at 2 min intervals fail to eradicate corn, sweet potato, and arrowroot; but the method succeeded when incineration was maintained for 30 s.

Table 7
Results of air quality assessment at Calgary.

Location	Sampling minutes	Type I	Type II	Type III	Other	Total
ES 747	30	0	0	0	1	1
ES 747	30	0	0	0	1	1
TAL	30	0	1	0	0	1
ES 859	30	2	0	0	0	2
TAL	30	2	0	0	0	2
ES 830	30	2	1	0	0	3
ES 830	30	1	2	0	0	3
TAL	30	3	0	0	0	3
ES 747	60	4	0	0	0	4
ES 3rd floor	60	3	1	0	0	4
ES 714	30	5	0	0	0	5
ES 847	30	4	1	0	0	5
TAL	10	4	1	0	0	5
TAL	240	5	1	0	0	6
Chem 114	30	6	0	0	3	9
Bio 564	30	9	1	0	0	10
Chem 29	30	5	0	0	5	10
ES 824	30	12	0	0	0	12
Bio 196	30	18	6	0	3	27
ES 7th floor	60	14	99	0	0	113
	Total	99	114	0	13	226
	%	44	50	0	6	100

Table 8
Time taken for oxidising agents and acetic acid to gelatinise starch granules.

Starch	30% H_2O_2	20% H_2O_2	10% H_2O_2	6% H_2O_2	12.5% NaOCl	5% NaOH	5% KOH	5% $C_2H_4O_2$
Potato	5 min	40 min	>1 h	>1 h	5 min	<15 s	<15 s	>1 h
Taro	5 min	30 min	55 min	45 min	30 min	<15 s	<15 s	>1 h
Yam	30 min	55 min	55 min	>1 h	5 min	<15 s	<15 s	>1 h

4. Discussion

4.1. Modern starches may cause false positives

The presence of modern contaminant starches on consumables and in the laboratory environment opens the possibility for such starches to be misidentified as ancient if recovered incidentally during laboratory processing. The contaminant assemblages in our laboratories display morphometric features that are consistent with what others identify as (i) maize (*Z. mays*, including hard and soft endosperm varieties), (ii) wheat and its close relatives (*Triticum*/*Hordeum*/*Aegilops*/*Secale*), and (iii) potato (*S. tuberosum*).
These three botanical sources have widespread industrial

Table 9
Number of intact starch granules remaining after exposure to 6% bleach for 30 min.

Starch	Granules (intact)
Wheat	3
Potato	5
Quinoa	10
Arrowroot	18
Rice	125
Waxy barley	181
Cassava	195
Sweet potato	295
Wrinkled pea	957
Corn	5230

applications, including in the manufacture of paper, textiles, and cosmetics, and as a lubricant in the manufacture of plastics such as disposable gloves (Radley, 1976; Campbell et al., 1984; Makela et al., 1997; Ellis et al., 1998; Phillips et al., 2001; Loy and Barton, 2006). They are therefore the most parsimonious sources for the contaminant starch assemblages, but we are cautious about making such specific taxonomic identifications when we cannot yet reliably exclude other species with overlapping starch morphologies. Researchers have noticed that successful taxonomic discrimination is most accurate at the family and genus levels rather than species, given the ambiguity introduced by multiplicity and redundancy, and overlapping morphometrics across geographically disparate taxa (Lentfer et al., 2002; Torrence et al., 2004; Langejans, 2006; Lentfer, 2009; Yang et al., 2012). Unlike in ancient starch studies, where taxonomic identifications of granules with overlapping morphologies can often be constrained by the range of species expected to occur in the archaeological context under analysis, modern contaminants could potentially derive from any starch source around the world used for industrial purposes or introduced from food sources (for example, on shoes or clothing). Morphological redundancy between contaminant types and target species should also be considered more broadly when assessing the potential for false-positives caused by modern contaminants. For example, many wild and domesticated plants from across the world produce starch grains that overlap in part with some of the morphometrics classified here as Type I.

The presence of modified starch types in the contaminant assemblages indicates that the condition of granules alone cannot necessarily be taken as an indicator of authenticity in our studies. The observed modifications, which included evidence of mechanical alteration (e.g., broken granules, cracking, scooping at the centre, partial loss of birefringence), heat treatment (partial or complete loss of birefringence accompanied by indications of swelling) and also enzymatic decay (surface pores and exposed lamellae), which possibly derived from industrial extraction and treatment (e.g., Harbers, 1975; Sujka and Jamroz, 2007), overlap with the morphological criteria utilised in ancient starch research as evidence of prehistoric culinary processing (see Section 2.1; also Babot, 2003; Perry et al., 2006; Perry, 2007; Henry et al., 2009; Liu et al., 2013; Yang and Perry, 2013). For ancient starch studies, qualitative and quantitative patterns in both an ancient starch assemblage and the archaeological context(s) under study, as well as comparison with intra-laboratory contamination datasets that include negative controls, may assist in more securely linking these types of damage to ancient culinary processing activities.

4.2. Consumables cannot be assumed to be starch-free

Oxford's results clearly illustrated that many consumables commonly employed in ancient starch laboratories could have a significant contamination potential. The much lower numbers from Calgary's TAL could be a reflection of cleaner products (different manufacturers, variable starch contamination of consumable processing plants), spurious chance, or an artefact of sample size. Indeed, the amount of each consumable analysed at TAL was always lower than RLAHA's, in accordance with the idea that only a part of a given item is in contact with a sample during ancient starch extractions. These two laboratories show, regardless, that consumable contamination varies widely within and between laboratories, and must therefore be monitored internally as part of each laboratory's authentication procedures.

Two items, Calgon and non-powdered gloves, deserve particular mention, as standard brands of these were confirmed to be starch-positive by our laboratories. Calgon (sodium hexametaphosphate) is recommended for use as a deflocculant in ancient microfossil research, in part because it has been shown to not harm ancient starches (Coil et al., 2003; Torrence and Therin, 2006). Our results indicate, however, that it could have a high risk of contaminating them; the different brands tested in our laboratories, for example, contained between 5 and 30 starch granules in 1 g of powder. Our testing shows that safer alternatives to clay dispersal include EDTA disodium salt (J.T. Baker, MSDS no. E0174). Fresh sodium polytungstate powder used by RLAHA for heavy-liquid starch separations also registered significant quantities of contaminant starches (Table 3).

Our results are consistent with numerous non-archaeological studies that show non-powdered disposable gloves to be not necessarily 'starch-free' (e.g., Campbell et al., 1984; Makela et al., 1997; Newsom and Shaw, 1997; Phillips et al., 2001; see also Henry et al., in press). Corn starch is commonly used as a lubricant during the manufacture of disposable gloves, and is removed from 'powder-free' types post-manufacture by washing; a process that can potentially leave some starch residue. Several types of disposable gloves tested by RLAHA showed starch contaminants, predominantly Type I, which are typical of corn (maize). While we cannot recommend for analysts in our laboratories to stop wearing gloves for procedures that require them as a health and safety measure, the risk could be reduced by: (i) using brands that are routinely tested for starch contaminants and produce negative results, (ii) avoiding touching samples directly and using sterilised forceps for holding or manipulating, and (iii) using gloves that can withstand sterilisation via long autoclave cycles.

Looking across our results more broadly, the use of modern starch in industrial manufacturing processes appears to be a major source of contamination for a number of other consumables we tested. Starch is commonly used in the manufacture of paper products (Kraak, 1993; Maurer and Kearney, 1998; Lawton, 2000; Maurer, 2009), for example, and RLAHA's results showed both the paper towel and lens cleaning tissue used in their laboratory to be particularly problematic. Owing to their potential starch contents, use of similar paper products will be restricted or excluded from our laboratories until suitable 'starch-free' types are found. Likewise, we can conclude that samples in our laboratories can be safely covered with sterilised petri dishes but not necessarily with parafilm, foil, or cling film, the surfaces of which can also be starch-rich depending on the brand. Plastic and glass consumables such as pipette tips, centrifuge tubes and microscope slides used in our laboratories also tested positive for contamination, and will therefore always be decontaminated prior to use in our ancient starch studies (see Section 4.4). While we registered negligible amounts of starch contamination in our glycerol, which is commonly used in starch analysis as a mounting medium for slide preparation, we note that a yeast-activating medium rich in starch (usually corn starch) is often added to glycerol that is synthesised from animal fat by hydrolysis (Virto et al., 1991; Wang et al., 2001). Future testing of glycerol manufactured by this method would be useful to determine if such brands pose a contamination risk. As an added precaution, TAL uses glycerol that is bi-distilled by the University's Organic Chemistry Laboratory to ensure it is free of starch contaminants.

4.3. How can we test for environmental contamination and what are the dispersal vectors?

Our combined results show that small numbers of passive traps (vertical or horizontal) are unreliable contamination proxies and generate unacceptably low statistical confidence for tracking airborne contamination in our laboratories. The distribution of starch landing across both laboratory spaces was non-normal; large counts were recorded in certain hotspots while other areas of the

laboratories recorded none, and the hotspots shifted erratically between tests depending, among other factors, on the type and level of activity within the laboratories. Therefore, deploying small numbers of traps around a laboratory to monitor airborne contaminants can generate false-negative results, leading to a failure to reject the null hypothesis that background contamination is absent. In fact, our calculations show that, for a laboratory such as TAL, more than 300 slides are needed to obtain a representative sample of the degree of contamination in a 72 h time period. Unfortunately, even this logistically unrealistic exercise could be proven futile, because by the time passive traps start showing contaminants, the work space is likely to have already been largely compromised. Indeed, this was the case at the old ancient starch laboratory at the TAL, where in the absence of proper containment and micron air filtration, a single air vent has the potential to propel more than 1000 starch granules per day. This problem was also apparent at RLAHA, which, despite having micron air filters, is nevertheless connected to other rooms, open to shared air circulation, and unable to reject contaminants via positive air flow. Oxford and Calgary agree that small granule numbers per aliquot of ancient samples recovered under these laboratory conditions could therefore be due entirely to contamination. Yet, small yields are often the norm (cf. Haslam, 2004). At the same time, adopting a granule threshold (e.g. 10 granules per sample, based on our observation that >90% of horizontal passive traps held fewer than 9 starch grains) as an authenticity criterion would be indiscriminately simplistic. Use of negative controls that are subjected to exactly the same treatments as ancient samples at all stages of storage, extraction, processing and analysis, should therefore play a critical role in assessing the likely extent of contamination during any given procedure (Boyadjian et al., 2007; Langejans, 2010; Monnier et al., 2012). Effective use of such controls in terms of the number used per ancient sample must be carefully considered in the context of each study and the specific laboratory protocols employed; for example, taking into consideration the complexity of each procedure and the degree to which ancient samples are 'exposed' to potential contaminants during processing.

In addition to air vents, granules may become airborne by attaching themselves to fibres and hairs (Newsom and Shaw, 1997), along with consumables and people. TAL tested the hypothesis that the analyst's motion is a major dispersal agent and contamination vector. During high activity times in TAL's rooms, starch contamination was concentrated around work stations and the fume hood (Fig. 2B), with a smaller peak at the outer room entryway. During low activity times, contamination decreased and concentrated in areas most affected by the ventilation system (Fig. 2A). Similar observations were made at RLAHA (Fig. 1), where the consistently high starch counts detected in the fume hood were most likely the result of airborne starches being pulled from the room and concentrated in the cabinet, which uses a 'push–pull' displacement air flow system to move air through the hood and out through the exhaust system at the back. The small size of RLAHA's Ancient Starch Laboratory probably also meant that contaminant starches became very concentrated in the confined work space; an issue potentially compounded by the long-term use of 'non-powdered' gloves and paper towels, which are likely to have been major vectors for introducing airborne starch contaminants. Significantly, Oxford showed lower starch counts after a major top-down clean of the laboratory using synthetic cloths rather than paper towel and after gloves stopped being used (Fig. 1C). No pollen grains or phytoliths showed up in any of our environmental tests, even though they were conducted during the peak pollen season (April–June). This observation suggests that insignificant quantities of external pollutants were entering the rooms through windows, which were never opened at RLAHA and did not exist at TAL, or through the air system at RLAHA, which is HEPA filtered. Ongoing monitoring of the other rooms tested at RLAHA, taking into account their range of uses, potential contaminant vectors, and cleaning regimes, is needed to understand broader patterns of the accumulation and turnover of starch contaminants in those connected workspaces.

4.4. What is the efficacy of commonly used decontaminating techniques?

RLAHA's tests indicate that rinsing or soaking in 5% sodium hydroxide is a highly effective method for destroying native starch. Future tests with weaker solutions might be useful to determine a 'safer' concentration for routine laboratory use; for example Ragheb et al. (1995) report that gelatinisation of starch by sodium hydroxide occurs immediately at a concentration of 1.2%, while gelatinised pastes can be obtained at >4%, but we are confident that 5% instantly destroys native starches. Boiling in excess water for more than 30 min and autoclaving at 136 °C for 2 h were also highly effective, and possibly safer for not involving caustic chemicals. Mixed results were obtained for other reagents, including bleach, hydrogen peroxide, and acetic acid, suggesting that they are not effective at decontaminating at the concentrations tested in this study. Other published experiments have also demonstrated that native starch granules can survive after at least 24 h of exposure to hydrochloric acid (10% and 2.2 mol/L respectively; Henry and Piperno, 2008; Xia et al., 2010), suggesting that acid hydrolysis in general is not an effective decontaminant. Also, whilst chemical decontamination works in structurally destroying native starch, this method involves gelatinisation (which also occurs during cooking, e.g., Duodu et al., 2002; Henry et al., 2009; Crowther, 2012) and we found that gelatinised granules as well as amorphous starch residues stainable with iodine can remain, even after cleaning consumables by sonication. This method may therefore present a problem for studies that are investigating ancient cooked starches. Torching reusable tools under flame or incandescent heat sources remains unreliable for bouts shorter than 30 s.

5. Conclusions

Documenting contamination in ancient starch laboratories is an important step toward developing adequate and reproducible protocols. To this end, this paper has provided a comprehensive snapshot of contamination in two ancient starch laboratories over a short study period, allowing us to identify the main sources, types and vectors of contaminants affecting our laboratories at this time. Our results highlight the fact that, although a similar range of contaminants were encountered, there was still considerable variation within and between the laboratories, as would be expected given the unique workspaces and settings involved, the different brands of consumables used, internal cleaning procedures, and other independent variables. We do not assume that similar types, sources or rates of contamination occur in other ancient starch laboratories. As a general point, however, we argue that the potential for contamination of ancient samples cannot be fully assessed until these factors are systematically understood and controlled for in an individual laboratory setting; a process that should also be ongoing and reported as a matter of routine. Intra-laboratory contamination datasets should serve as an additional line of evidence to the archaeological and contextual controls that are routinely drawn upon during ancient starch studies to assess the provenience of starch granules and their archaeological authenticity. Our results suggest that we need to be particularly vigilant when there is overlap between target archaeological species and external contaminant types, and in cases involving small ancient starch yields.

The primary aims of this study were to identify effective strategies for contamination management, and to revise our laboratory protocols to ensure that better quality control, results, and legitimacy criteria are employed. In light of our results, we therefore recommend that ancient starch practitioners:

1. Employ demonstrably effective decontamination methods such as boiling (>30 min), autoclaving (136 °C, 2 h) or rinsing with sodium hydroxide (5%) for heat- and chemical-resistant consumables (e.g., glass, plastic, metal), ensuring any remaining gelatinised residue is removed.
2. Routinely check that all reagents, tools, and other laboratory materials utilised are starch-free.
3. Wear starch-free, full protective clothing.
4. Use negative controls during storage, processing, and analysis of ancient starch samples to trace the potential rates and types of external contamination during laboratory procedures.
5. Test whether their laboratories have efficient air filtration/containment.
6. Collect baseline air quality data from before/after all extractions.
7. Perform regular and systematic top-down cleaning of all spaces before conducting extractions, using methods shown to be effective at removing surficial contaminants.
8. Routinely publish the results of their contamination control studies, to allow the ancient starch research community to identify and avoid problematic consumables and practices.

Further measures that could help control contaminants include setting up barrier curtains between open spaces, using sticky mats to capture hairs, fibres, and contaminants as people stand on them, using disposable plastic runners daily so that technicians do not propel floor contaminants up as they walk around benches, and banning outside cleaning personnel. As a final measure, for example, Calgary has adopted an approach similar to ancient DNA and forensics by converting the old starch laboratory to a clean room facility with separate, sealed venues, filtered air (HEPA Class = H14), positive pressure, constant/controlled air flow, and very restricted access.

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