Effects of Cultivation on Tuber and Starch Granule Morphometrics of *Solanum jamesii* and Implications for Interpretation of the Archaeological Record

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EFFECTS OF CULTIVATION ON TUBER AND STARCH GRANULE MORPHOMETRICS OF *SOLANUM JAMESII* AND IMPLICATIONS FOR INTERPRETATION OF THE ARCHAEOLOGICAL RECORD

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2
3 **ABSTRACT**
4 Plant species native to the American southwest may have been cultivated by indigenous
5 people, but techniques to assess the extent, timing, and impacts of early manipulation are
6 lacking. Herein we apply morphometric techniques to tubers and starch granules of the Four
7 Corners potato, (*Solanum jamesii* Torrey) to determine if cultivation, even over a relatively
8 short period of time, can be detected. When compared to wild source plants, cultivated plants
9 produced significantly larger tubers and starch granules. We suggest that, in concert with other
10 archaeological and/or ecological data, microbotanical data may aid in identifying modifications
11 to plant food resources related to strategic shifts from wild harvest to cultivation in the
12 American west.
13
14
15 **KEYWORDS**
16 Geophytes; starch granule analysis; plant domestication; Colorado Plateau; Four Corners potato
17
18
19 **1. INTRODUCTION**
20 Plant domestication and its processes, antecedents, and outcomes have been central themes in
21 archaeological research for the last century. The outcome of the domestication process is a
22 genetically modified strain of a target species that is generally more productive, easier to
23 harvest or process, resistant to environmental stressors and/or more palatable in accordance
24 with human preferences. Possessing such distinctive characteristics often allows domesticates
25 to be readily distinguished from their progenitors but the steps leading to domestication
26 (selective harvest, deliberate seed dispersal, active plant management, i.e. cultivation) can be
27 difficult to detect archaeologically.
28
29 Researchers investigating the origins of New World domesticates have examined the
30 evolutionary stages of domestication (see Meyer and Prugganan 2013) by applying various
31 techniques across a range of cultivated and/or domesticated species. One approach examines
32 the morphological and morphometric modifications of seed coat thickness and seed size (e.g.
33 Asch and Asch, 1985; Bruno and Whitehead, 2003; Fritz and Smith, 1988; Fritz et al., 2009;
34 Heiser Jr, 1985; Smith, 2006a, 2006b), others analyze the underlying genetic structure of a
35 genus or species for alterations related to favorable traits (e.g. Blackman et al., 2011; Doebley
36 et al., 2006; Harter et al., 2004; Sanjur et al., 2002; Van Heerwaarden et al., 2011; Kraft et al.,
37 2014; Hardigan et al., 2017; for a review see: Zeder et al., 2006), and less frequently,
38 archaeobotanists study changes in subcellular inclusions, such as sclerids/phytoliths and starch
39 granules (e.g. Ball et al., 2016; Holst et al., 2007; Liu et al. 2015; Perry, 2002; Piperno et al.,
40 2009) to detect human selection and intervention.
41
42 Changes in phytolith size related to cultivation have been documented in both maize and
43 *Cucurbita* spp. (Pearsall, 1978; Piperno and Stothert, 2003; Piperno et al., 2009). However,
44 geophytes often lack numerous or distinctive phytoliths. Starch granules on the other hand are

1 abundant in many of the underground storage organs (such as tubers, corms, rhizomes, etc.) of
2 geophytes, and can exhibit genus-and sometimes species-specific morphological and
3 morphometric variability. Starch granule analysis has been used as a tool to detect the
4 presence or absence of plant species *known to have been cultivated and/or domesticated* (e.g.:
5 Dickau et al., 2007; Perry et al., 2007; Piperno and Dillehay, 2008; Zarrillo et al., 2008). In such
6 cases, the wild progenitors and first phenotypes are either not examined or wholly unknown so
7 that early stages of the domestication process remain obscure. But we do not know how
8 granules respond to cultivation, in part because comparative approaches using wild progenitors
9 are lacking. Furthermore, detecting changes in granule morphology must be statistically based,
10 examining hundreds of starch granules from multiple plants and populations of a species to
11 distinguish developmental variations from genetic variations (Louderback et al., 2016). This in
12 fact may be the greatest constraint on using starch granule analysis to detect domestication;
13 the tendency to rely on small sample sizes that reflect physiological dynamics (i.e. *in vivo*
14 synthesis and utilization) rather than differences arising from modified genomes or novel
15 growing conditions.
16
17 Recent research has demonstrated that some geophytes produce morphologically distinct
18 starch granules, and that the measurable characteristics of the largest granules from a sample
19 can be used to distinguish between some species (Louderback et al., 2016) and perhaps even
20 between strains of a single species. This approach has been successfully implemented with
21 other known domesticated geophytes (*Manihot esculenta* and *Ipomoea batatas*; Perry, 2002).
22
23 While it is not currently grown as a crop, tubers of the Four Corners potato, (Fig. 1, *Solanum*
24 *jamesii* Torrey), are known to have been processed on grinding stones at a rock shelter near
25 Escalante, in southern Utah, as early as 10,900 cal B.P. (Louderback and Pavlik, 2017). At that
26 same site are small, extant populations of *S. jamesii*, occupying unusual habitat well beyond the
27 central range of the species (Figure 2; central New Mexico and Arizona), suggesting a history of
28 long-distance transport, if not cultivation. Furthermore, plants from these Escalante
29 populations have been transferred into the gardens of pioneer descendants within the last 20
30 years and tended continuously. Additional evidence from the Four Corners region suggests that
31 populations of *S. jamesii* growing in and around ancestral Puebloan habitation sites may
32 represent remnants directly descended from previously cultivated plots (Kinder et al., 2017).
33
34 Using multiple population sources we test whether tubers and starch granules from
35 propagated, cultivated, and potential cultivar remnant populations of *S. jamesii* show any
36 morphometric differences from those of distant wild populations. We use these data as a test-
37 study to determine whether tuber and starch morphometrics may be useful in determining the
38 degree of cultivation when examining micro-archaeobotanical remains from potential geophyte
39 cultivars.
40

1
2 Figure 1. Mature *S. jamesii* plant from the wild (left) and tubers of propagated *S. jamesii,* average tuber
3 length is 14.9 mm (right).
4
5
6 **2. METHODS**
7
8 **2.1 Reference materials**
9 *Solanum jamesii* is a tuber-forming herbaceous perennial that is active in the summer months
10 and highly productive. This diminutive plant begins forming progeny tubers early in the season
11 and, given ideal growing conditions, can produce up to 500 progeny tubers in one growing
12 season (Louderback and Pavlik, 2017). Perhaps due to its prolific nature and/or high nutrient
13 content, ethnographic and ethnohistoric accounts of *S. jamesii* describe consumption of the
14 species by many Native American groups as well as early pioneers and soldiers (see: Louderback
15 and Pavlik, 2017). The modern range of *S. jamesii* spans the Four Corners region of south-
16 central North America, hence the common name, Four Corners potato.
17
18 Tuber specimens for this study came from four sources: 1) wild populations in central Arizona
19 and New Mexico [n=60], 2) populations located near or within archaeological sites, termed
20 "archaeological" [n=74], 3) populations cultivated in private gardens near Escalante, Utah (for
21 ~20 years) and in Madison, Wisconsin (~20 years) or in experimental gardens in Farmington,
22 New Mexico (cultivated for ~10 years), and 4) tubers propagated for two generations in a
23 greenhouse at Red Butte Gardens in Salt Lake City, Utah [n = 19].
24
25 We collected wild and archaeologically associated tuber specimens from multiple field sites
26 during the summer/fall of 2014, 2015 and 2016 (Figure 2) as part of an ongoing systematic
27 study of the archaeobotanical, genetic, and phytogeographic characteristics of *S. jamesii*. The
28 archaeological populations are small, isolated and found to be discontinuous across the
29 landscape when sampled along a six mile transect. The wild populations however were large,
30 abundant (thousands of stems) and continuous when sampled along a six mile transect. When

1   we came across plants, we conducted an informal archaeological survey in a wide area
2   surrounding the population. Therefore, we are confident in our designations of archaeological
3   and wild populations.
4
5   Cultivated specimens grown in Farmington, NM were from the USDA Potato Genebank's core
6   collections of *S. jamesii* (Bamberg et al., 2016) [n=55]). It is not known if the practices of
7   cultivation were equivalent in the private gardens and the USDA plots. We are aware that
8   selection took place in both cases but for different purposes. In the private gardens, interviews
9   suggest a preference for larger tubers, sometimes returned to the garden, sometimes eaten,
10   but irregularly. The USDA however had various research objectives which did include selection
11   for larger tubers, but at the same time selection for other characteristics as well (e.g., freezing
12   tolerance). So it is safe to say that in both cases there was considerable manipulation but for
13   different purposes and different intensities.
14
15   The fourth source, propagated tubers, came from tubers collected near Escalante and
16   propagated for two generations in a greenhouse at Red Butte Gardens in Salt Lake City, Utah.
17   These are termed "propagated", but the original material had been archeologically associated.
18   All collected specimens from a given population were included in this study. All propagated
19   tubers grown from a single, second-generation source plant were included in this study.
20

21
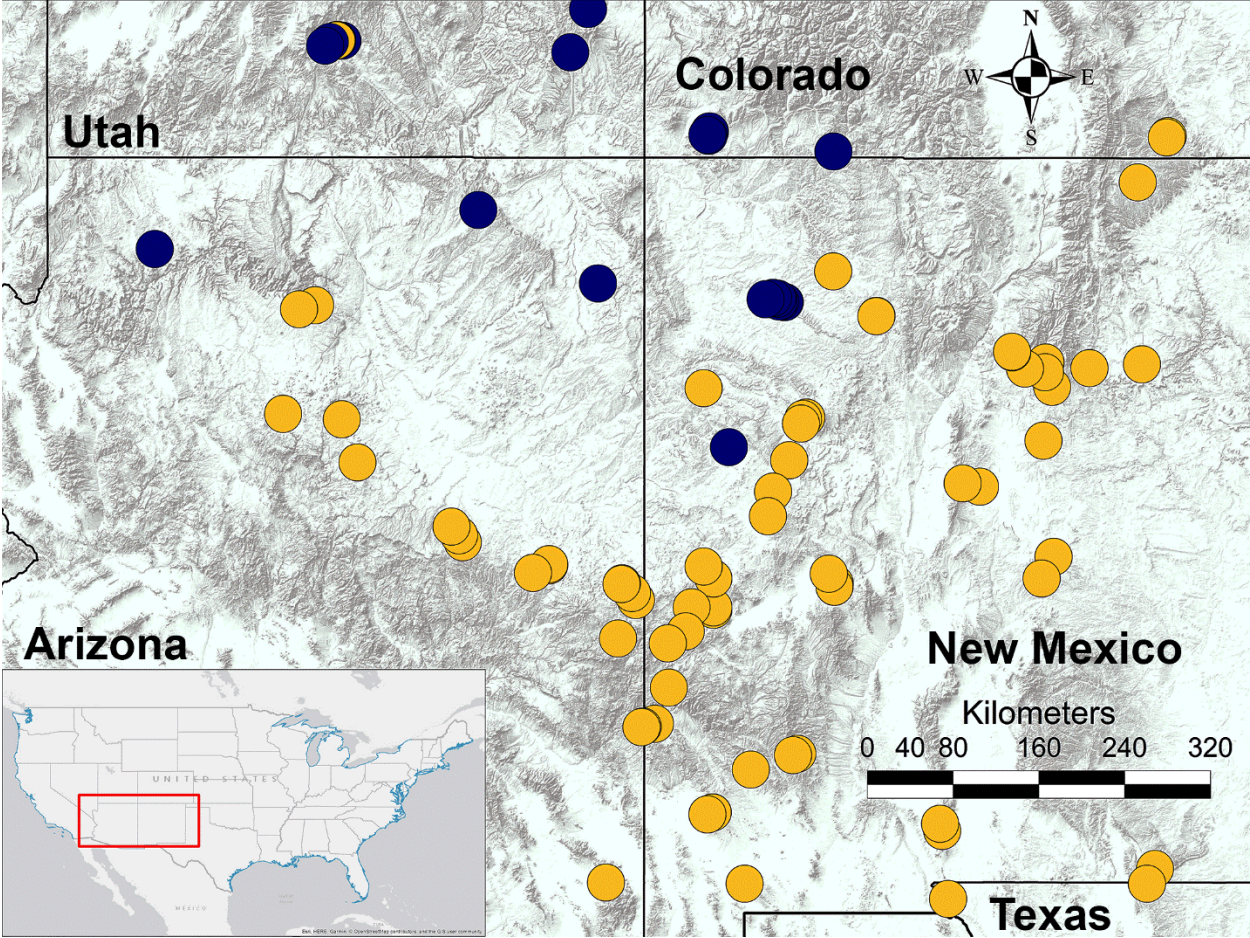
1 Figure 2. Modern distribution of *S. jamesii*. Populations associated with archaeological sites are
2 indicated by blue circles, wild populations are shown in yellow.
3
4 **2.2 Tuber size analysis**
5 To determine whether tuber size is related to cultivation, we measured tuber size (length of
6 tuber from apical bud to distal end) for field and propagated sources (n = 188). Though tuber
7 weight and/or volume may also be a relevant metric, many of the specimens in this study had
8 deflated slightly (perhaps due to water loss) by the time of measurement. Therefore,
9 measurement of length more accurately reflected tuber size, and, likely reflects mass as well. In
10 future studies, weighing tubers (after drying) during field collection is recommended. We
11 applied the Anderson-Darling normality test in the nortest package of R to measures of tuber
12 length in order to assess skewness and kurtosis within the distributions (R Core Team, 2012;
13 Thode, 2002). Distributions were non-normal both across the entire dataset and within each
14 source, so a generalized linear model (GLM) was constructed using the R stats package (R Core
15 Team, 2012) to test relationships between tuber size and source.
16
17 **2.3 Starch granule analysis**
18 *2.3.1 Starch extraction and measurement.* We randomly selected a subset of field-collected and
19 propagated *S. jamesii* specimens (n = 16) representing each of the above sources (cultivated,
20 propagated, archaeological and wild) for starch granule measurements (Table 1). Sampled
21 tubers were chosen by drawing a specimen at random from all undecayed/nondeflated
22 collected specimens of a given population/source.
23
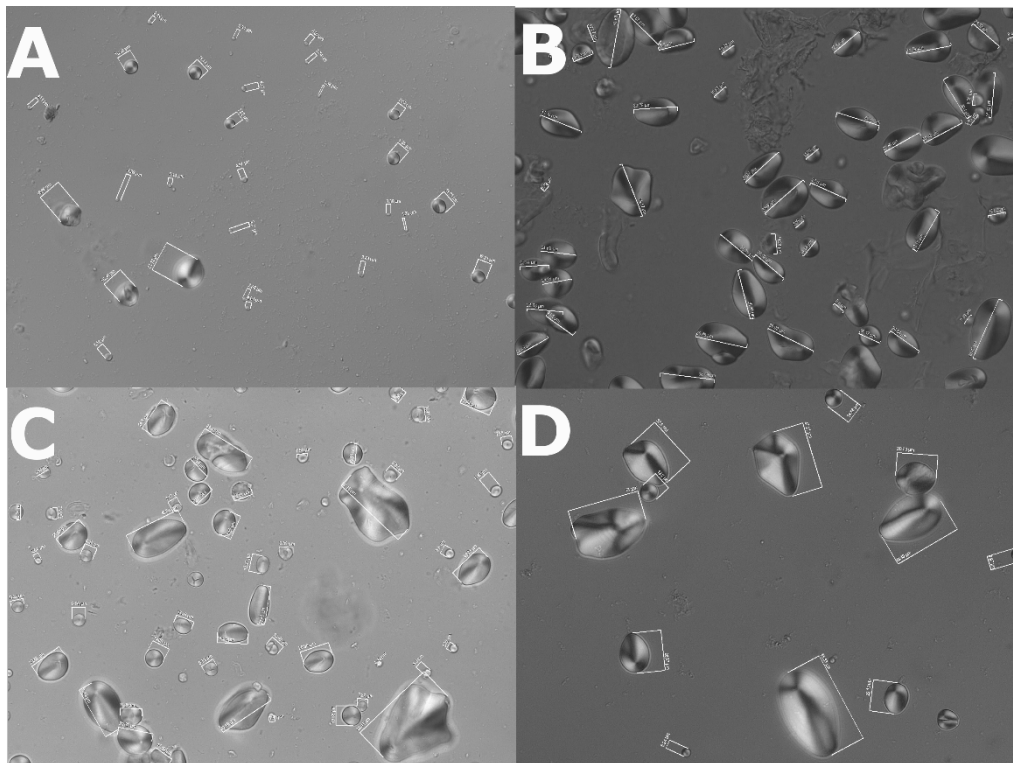
24

1 Figure 3. Photographs showing a typical slide image for each of the four sources, (a) F536 – wild, (b) EG1
2 – archaeological, (c) EG2.2 – propagated, and (d) MEGA76 – cultivated. Each box is approximately 360
3 microns across.
4
5 To isolate starch granules from each specimen for analysis, tubers were bisected with a
6 sterilized blade and the exposed tissue rubbed onto an ethanol-cleaned glass slide. A 50/50
7 glycerol and DH_2O solution was then added to the slide surface and mixed with the tissue smear
8 using a sterile pipette. Once mixed, we placed a glass cover slide over the sample, and
9 illuminated in both bright light and cross polarized light using a Zeiss Axioscope 2 transmitted
10 brightfield microscope fitted with polarizing filters and Nomarski optics. A Zeiss HRc digital
11 camera was used to capture images of starch granules at a magnification of 400x (Figure 3).
12
13 Each slide was positioned beneath the objective and tranches of starch granules were
14 photographed at different locations on the slide using a set of randomly generated X-Y
15 coordinates. In order to minimize bias, all non-compound granules at each position were
16 measured until a sample of 100 was achieved. Once an adequate number of granules were
17 photographed, we measured the maximum length of each starch granule through the hilum
18 using Zen imaging software. This process was conducted independently by two people for each
19 specimen, resulting in the measurement of 200 granules per specimen totaling 3,200 individual
20 granules (data available: Herzog et al., 2018). Length was selected as the primary measure for
21 this study based on previous research indicating that *S. jamesii* starch granules exhibit non-
22 normal distributions, with smaller granules spherical to slightly ovate in shape and larger
23 granules most often oblong (Louderback et al., 2016). These data suggest expansion is skewed
24 longitudinally. Furthermore, granule length is correlated with other morphological
25 characteristics, such as width, area, circumference (Louderback et al., 2016). For the purposes
26 of this study, other morphological traits were not determined to be useful in making inter-
27 population distinctions.
28
29 **Table 1. Tubers selected for starch granule analysis**

Specimen	Origin	Collection Location	Date Collected	Tuber length (mm)
BG1	Cultivated	Madison, Wisconsin	6/2016	15.36
DG1	Cultivated	Escalante, Utah	9/2015	10.88
DG2	Cultivated	Escalante, Utah	9/2015	13.44
MEGA076	Cultivated	Farmington, New Mexico	11/2016	16.5
DD1	Propagated	Salt Lake City, Utah	10/2015	14.77
DD1.2	Propagated	Salt Lake City, Utah	10/2015	12.34
EG2.1	Propagated	Salt Lake City, Utah	10/2015	12.49
EG2.2	Propagated	Salt Lake City, Utah	10/2015	15.00
LNC1	Archaeological	L. Navajo Canyon, Colorado	9/2015	8.28
EG1	Archaeological	Escalante Gorge, Utah	9/2015	8.22
MEVE G	Archaeological	Mesa Verde, Colorado	9/2015	15.07
NP1	Archaeological	Newspaper Rock, Utah	9/2015	9.31
VR1	Wild	Vermejo Ranch, New Mexico	8/2015	10.00
F536	Wild	Feaster, New Mexico	9/2015	10.15

| PH1 | Wild | Pecos, New Mexico | 8/2015 | 9.35 |
| PC517 | Wild | Picnic Creek, Arizona | 10/2015 | 7.67 |

2.3.2 Statistical analysis. We generated a series of models to test whether starch granule size (length in microns) was linked to tuber size, and/or source. In order to assess the normality of starch granule length distributions we tested each sample's distributions using the Anderson-Darling normality test in the nortest package of R and measured skew and kurtosis using the moments package in R (R Core Team, 2012; Thode, 2002). All of the populations exhibited non-normal granule length distributions, mostly skewed toward smaller-sized granules, a pattern observed in previous research (Louderback et al., 2016). When sources were combined, the length distribution for the entire source dataset was also non-normal (Table 3). As such we log-transformed granule length then applied a GLM for hypothesis testing.

Previous research has shown that within *S. jamesii* it is often only the largest sized granules that exhibit useful surface and structural characteristics for identification (Louderback et al., 2016). Because starch granule length distributions are positively skewed across all sources, we also constructed a GLM to compare the top 20% of the sample.


3. RESULTS

3.1 Tuber size
Tuber size progressively increased along a population source gradient from wild to cultivated (Figure 4, Tables 2 and 3). Tubers from the wild population seldom exceed ~16.5 mm, while the largest tubers from cultivated sources could reach 20 mm or more, a significant difference (Table 2). The largest tubers, on average, came from propagated sources, an outcome likely related to the greater degree of environmental control (water, nutrients, and temperature) exerted during the growing season of these plants.
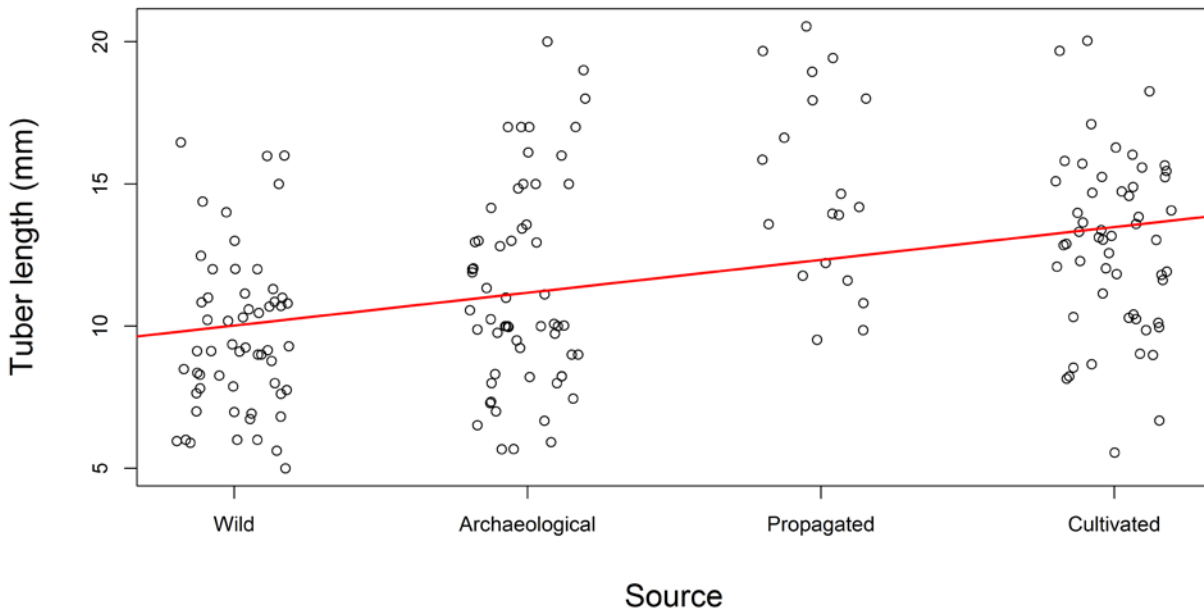
1
2 Figure 4. Scatterplot showing the relationship between tuber length (mm) and source population. Black
3 dots represent raw measurements for individual tubers. The red line represents the parameter
4 estimates of the GLM model, with the intercept as the intercept parameter (8.871; p ≥ 0; 95%
5 Confidence Interval (CI) 7.82 - 9.91) and slope as source (1.153; p ≥ 0; 95% Confidence Interval (CI) 0.76 -
6 1.54).
7

8 **Table 2. GLMs for relationships between tuber and granule size and population sources.**

Model	Variable	Estimate	SE of Estimate	Pr(>|t|)
Tuber length	Intercept	8.871	0.535	< .001***
	Source	1.153	0.201	< .001***
Starch granule length	Intercept	1.037	0.022	< .001***
	Source	0.044	0.006	< .001***
	Tuber size	-0.003	0.002	0.201
Starch granule length (top 20%)	Intercept	1.351	0.018	< .001***
	Source	0.047	0.006	< .001***
	Tuber size	0.003	0.002	0.204

9

10 **3.2 Starch morphology**
11 Starch granules from wild populations exhibited the smallest mean length size, while those
12 from cultivated source populations were largest (Table 3). The starch granule distributions from
13 wild populations contain relatively few outliers, reflecting the low degree of kurtosis, while all
14 other populations are leptokurtic indicating the presence of outliers influencing the overall
15 shape of the distributions (Table 3). Granule length distributions are negatively skewed across
16 each of the sources, weighted by an abundance of smaller sized granules (Table 3).

17 In the GLM model, log-transformed granule length was positively correlated with source

1   population but not tuber size (Table 2, Figure 5). Therefore, regardless of tuber size, starch
2   granules from the cultivated sources are larger than those from all other source categories.
3
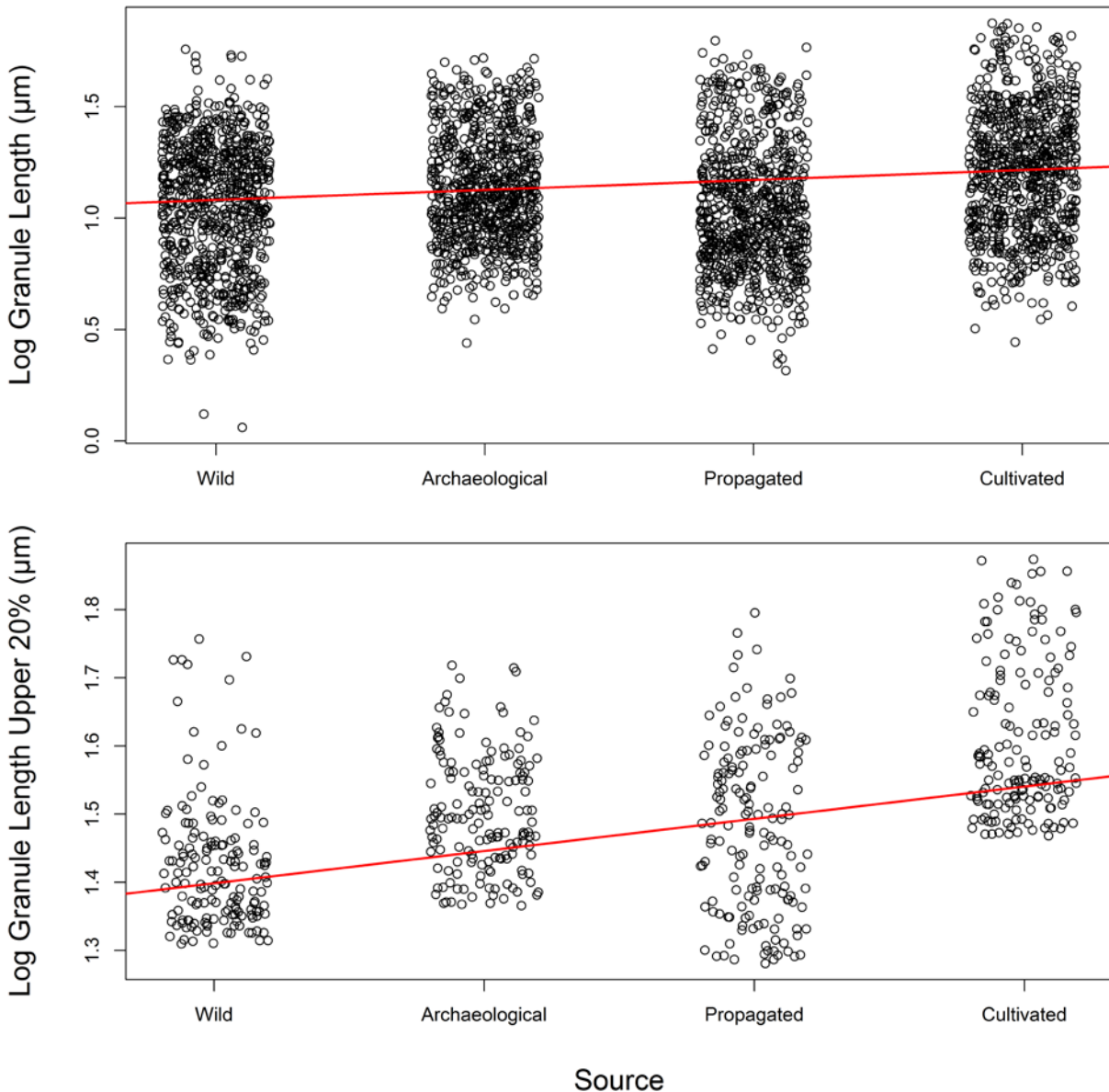
4
5   Figure 5. (Above) Scatterplot showing the relationship between log-transformed starch granule length
6   (μm) and source population. (Below) Scatterplot showing the relationship between the upper 20% of
7   log-transformed starch granule lengths (μm) and source population. Black dots represent measurements
8   for individual starch granules (above: n = 800 per source; below: n =160 per source). Red lines represent
9   the parameter estimates of the GLM model holding tuber size constant (above: Intercept 1.04, $p \geq 0$,
10   95% Confidence Interval (CI) 0.99 − 1.08; slope as source, 0.06, $p \geq 0$, 95% CI 0.03 − 0.06. below:
11   Intercept parameter 1.35, $p \geq 0$, 95% CI 1.32 − 1.38; slope as source 0.05, $p \geq 0$, 95% CI 0.04 − 0.06).
12
13   When lengths of granules within the top 20% of the sample were entered into a GLM model
14   (granule length top 20% ~ source + tuber size), differences in granule length among sources

1 became much more pronounced, and the statistical relationships stronger (Table 2).
2 Additionally, potentially diagnostic structural features, such as the narrow, unbranched
3 longitudinal fissure that is characteristic of this species, are much more common among
4 granules within the top 10 and 20% of samples in *S. jamesii* (Louderback et al., 2016).
5
6 **Table 3. Tuber length and starch granule length across source populations.**

Sample	Cultivation condition	Mean	Median	Kurtosis	Skewness
Tuber length (mm)	Wild	9.642	9.150	2.979	0.627
	Archaeological	11.320	10.080	2.451	0.517
	Propagated	14.900	14.190	1.787	0.095
	Cultivated	12.84	13.040	2.921	-0.034
Log starch granule length (µm)	Wild	1.053	1.093	2.480	-0.321
	Archaeological	1.134	1.108	2.371	0.207
	Propagated	1.042	1.006	2.607	0.346
	Cultivated	1.216	1.225	2.575	0.371
Raw starch granule length (µm)	Wild	13.88	12.39	5.521	1.228
	Archaeological	16.03	12.83	4.01	1.251
	Propagated	13.839	10.135	5.555	1.689
	Cultivated	20.170	16.81	5.260	1.474

7
8
9 **4. DISCUSSION**
10 The suite of morphological changes apparent in the seeds of domesticated plant species,
11 collectively termed 'the adaptive or domestication syndrome', has been well-documented
12 (Hammer, 1984). However, among domesticated geophytes that are vegetatively propagated
13 (i.e. cloned), populations tend to be closely related and often possess minor genetic
14 differences (Fuller et al., 2014; Zohary, 2004). Consequently, there is no widely described
15 'adaptive syndrome' for geophyte domesticates, especially in the absence of archaeological
16 plant material. Could tuber and starch granule characteristics be indicative of early stage
17 domestication?
18
19 In the present study, tubers from wild populations were, on average, three millimeters smaller
20 than those originating from cultivated plots. These data suggest that tuber size could be used as
21 an index of degree of cultivation when compared to tubers from wild populations. However,
22 vegetative materials from tuberous food sources are not often recovered from archaeological
23 deposits. When they are, they are commonly fragmentary and desiccated, preventing any
24 straightforward assessment of original size.
25
26 In lieu of preserved vegetal material, other means of detection are necessary for identifying
27 pre-domesticate root crops in the archaeological record. Past research has highlighted the

1   ability of starch granule analysis to detect the presence of geophyte residues on stone tools
2   despite their absence in macrobotanical assemblages (e.g. Dickau et al., 2007; Herzog and
3   Lawlor, 2016; Louderback and Pavlik, 2017; Messner, 2011; Perry, 2004, 2002). Therefore,
4   archaeological starch granule assemblages may provide an avenue for identifying pre-
5   domesticates and cultivars (i.e., Perry, 2002). Indeed, starch granules from cultivated
6   populations were larger than from wild populations, especially those in the top 20% of the
7   sample distribution. No starch granule from a wild population exceeded 60 microns in length
8   and only 3.5% of the granules exceeded 50 microns. However, 20% of the granules in the
9   cultivated populations exceeded 50 microns. As such, recovered archaeological starch granules
10   >50-60 microns may be an indicator of cultivation in *S. jamesii*.
11
12   Increases in tuber and granule size may be an outcome of genetically driven increases in carbon
13   fixation, sugar transport and starch synthesis. In studies of South American potato species,
14   advantageous traits appear to have been achieved via distinct pathways reliant on regional
15   adaptation rather than conserved developmental processes (Hardigan et al., 2017). Work on
16   the potato genome has identified a set of population-specific genes in *S. tuberosum* cultivars
17   that suggest selection for traits related to enlarged tubers, increase in leaf carbon fixation and
18   transport, glycoalkaloid reduction, adaptation to long-day photoperiod, and reduced sexual
19   fertility (Hardigan et al., 2017). We fully expect the same to be found for the North American *S.*
20   *jamesii*. Preliminary data demonstrate a range of tuber glycoalkaloid content across *S. jamesii*
21   populations, suggesting alternate pressures at different locations (Louderback and Pavlik,
22   2017).
23
24   Alternatively, tuber and starch granule size are also known to be linked to environmental
25   conditions (Thitisaksakul et al. 2013). For example, under drought conditions starch granules
26   are significantly smaller than those of well-watered control plants (Brooks et al., 1982). The
27   differences in tuber and granule size measured in the present study cannot exclusively be
28   assigned to genetic differences among source populations. Long-term manipulation (as in wild
29   or archaeological settings) or intensive, purposeful selection (as in an experimental garden)
30   would be required for the differences to have a genetic basis. Short-term manipulation (as in a
31   greenhouse) would more likely result in phenotypic differences, especially when water and
32   nutrient resources are provided and carbon gain is maximized. Whether these differences have
33   a genotypic or phenotypic basis, human intervention appears likely to have shaped changes in
34   potato tuber micro and macromorphology, including among populations of *S. jamesii*.
35
36   While these results provide a platform for the use of starch granule morphometrics to evaluate
37   cultivation practices in the past, we urge caution in this approach. Results presented here apply
38   only to *S. jamesii* and cannot be generalized as yet for other species. An adequate assessment
39   of wild population granule size is necessary before analyses of archaeological assemblages can
40   be undertaken. In addition, we find it inadvisable to make determinations of cultivation status
41   based on the presence and morphology of single granules or even limited assemblages. It is
42   difficult to estimate at what size an archaeological assemblage might be large enough to assess
43   these effects. It is clear that, at least in the case of *S. jamesii*, a minimum of 20% of the total
44   archaeological assemblage should be over 50 microns in size to consider cultivation a

1 possibility. In a sample of only 10 granules, such a conclusion would obviously be tenuous.
2 Ideally, starch analyses would work with hundreds of archaeological granules and be
3 considered in tandem with other archaeological and/or ecological indications of cultivation.
4
5 While it is still unknown whether extant populations of *S. jamesii* associated with archaeological
6 sites in Utah and New Mexico are remnant populations derived from cultivated strains, these
7 produced both larger tubers and larger starch granules than those with no archaeological
8 association – suggesting some form of human manipulation in the past. Another clue to
9 anthropogenic interaction is the distribution of some archaeologically associated populations.
10 Populations located furthest from the present range of *S. jamesii* (Hijmans et al., 2002) are
11 often associated with archaeological sites, suggesting transport and/or management by human
12 groups (Bamberg et al., 2016; Kinder et al., 2017; Louderback and Pavlik, 2017). Further study,
13 incorporating more populations across the entire range of the species are necessary to test this
14 hypothesis and to develop the chronology of wild plant domestication in western North
15 America.
16
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29
30

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