Early millet use in West Liaohe area during early-middle Holocene

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Abstract It is generally recognized that millet agriculture originated in northern China. However, the domestication process of foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum miliaceum*) is still poorly understood. Based on statistical and morphological analyses of ancient millet starch grains, a tangible hypothesis has been proposed for the long-term domestication of green foxtail millet (*S. viridis*). However, the hypothesis requires validation by evidence from more regions and more archaeological finds. The West Liaohe region is one of the earliest regions of millet cultivation. Here, we report ancient starch grains recovered from 12 stone grinding tools from eight sites of the Xiaohexi culture (before 8.5 ka BP), Xinglongwa culture (8.2–7.4 ka BP), Zhaobaogou culture (7.0–6.4 ka BP), and Hongshan culture (6.5–5.0 ka BP) in the West Liaohe region of China. Our results indicate that the proportion of millet starch grains with wrinkled surfaces and rough edges, which are diagnostic of wild millet grasses, decreased from 13.0% to 3.4% from the Xiaohexi culture to the Hongshan culture. Millet starch grains measuring >16.8 μm , a size class recorded only in domesticated foxtail millet, increased from 55.0% to 62.1%. These millet data imply that the process of millet domestication in the West Liaohe region began in the Xiaohexi period and continued up to the Hongshan period.

Keywords Plant domestication, West Liaohe region, Agricultural origin, Neolithic Age, Starch grain analysis

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1. Introduction

The domestication of wild plants is a key issue in the study of agricultural origins (Diamond, 2002). It is generally believed that plant domestication occurred in three phases: initial gathering, followed by the tending of wild stands, and then cultivation (Tanno and Willcox, 2006; Fuller et al., 2009). The traits of wild plants changed during the process of long-term planting, and domesticated traits began to ap-

pear. Based on the key changes in the spikelet during the course of domestication, researchers have found that the domestication processes of barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*) were very slow, occurring over a period of at least 2000–3000 years (Tanno and Willcox, 2006; Fuller et al., 2009).

Northern China is thought to be the centre of origin of foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum miliaceum*). The earliest plant macroremains and microremains of the foxtail millet and broomcorn millet were recovered from the North China Plain (Lu et al., 2009; Yang et al., 2012a; Zhao, 2014). When did humans start gathering

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and growing wild species of millets? How long did it take for the domesticated traits of these millets to appear after they were first grown? These processes are still unclear. Yang et al. (2012a) recovered and analysed ancient starch grains from residues on the surfaces of unearthed artefacts from the Nanzhuangtou site (before 11 ka BP) and the Donghulin site (11–9.5 ka BP) on the North China Plain. They found that the wild properties of the millet starch grains gradually decreased over 1500 years (11–9.5 ka BP), as the properties of domesticated millet starch grains gradually increased. The millet starch grains from the Nanzhuangtou and Donghulin sites allow us to formulate the hypothesis that the process of domestication of foxtail millet was protracted; the foxtail millet was still undergoing domestication during the period 11–9.5 cal ka BP. However, this hypothesis must be verified with further research.

Carbonized seeds of foxtail millet and broomcorn millet with domesticated characteristics, from about 8000 years ago, were recovered at the Xinglonggou site (8.0–7.5 ka BP) in the West Liaohe region. As well as discoveries of a diversity of ancient human agricultural tools and utensils of daily life and reconstruction of palaeo-ecological environment, scholars believe that the West Liaohe region is one of the important regions in which Chinese millet agriculture originated (Zhao, 2006; Jones and Liu, 2009). Therefore, 12 slabs and mullers used in the early-middle Holocene (8.5–5.0 ka BP) that were excavated from eight archaeological sites in the West Liaohe region were examined with a starch grain analysis to provide more evidence for the millet domestication hypothesis described above.

2. Archaeological sites and study area

The West Liaohe region (41°–45°N, 117°–124°E) is a ma-

jor region of ancient human activity in northeast China, with two notable features. One is the food-processing tools, including slabs and mullers, pottery for cooking, and carbonized millet seeds from the early-middle Holocene, that are often found widely in the archaeological sites in this area, (Yang, 2008; Suo and Li, 2014; Zhao, 2006, 2014), which are highly significant in study on the origins of millet agriculture. The other is that the archaeological cultural sequence and lineage relationships have been established in this region from the early to late Holocene, causing it to be rated one of the six major archaeological cultural areas (Su and Yin, 1981). The archaeological cultures of the West Liaohe area during the early-middle Holocene include the Xiaohexi culture (earlier than 8.5 ka BP) (Liu, 2006; Yang and Lin, 2009), the Xinglongwa culture (8.2–7.4 ka BP) (Zhao, 2004; Liu, 2006), the Zhaobaogou culture (7.0–6.4 ka BP) (Liu and Dong, 1996; Liu, 2006), and the Hongshan culture (6.5–5.0 ka BP) (Zhao, 2005). Eight typical sites from the West Liaohe area, in the early-middle Holocene, were selected as the study sites (Table 1 and Figure 1).

3. Materials and methods

3.1 Materials

Two slabs and ten mullers retrieved from eight archaeological sites in the West Liaohe area were chosen to examine by starch grain analysis (Table 1 and Figure 2). One slab and three mullers were collected from the surface of the sites of Aohanyingzidong and Luojiayingzi, whereas all the other grinding tools were excavated from cultural layers dating back to 8.5–5.0 ka BP. Dust was collected from the storerooms where the grinding stools were curated as the contamination control samples.

Table 1 Data on the stone grinding tools from early-middle Holocene in the West Liaohe area

Archaeological culture	Site	Stone tool type	Stone tool no.	Length (cm)	Width (cm)	Context	Age (ka BP)	Laboratory no.
Xiaohexi culture	Beishandong	slab	150426-0256	12.5	11.7	layer	>8.5	XHX 1
	Beishanxi	muller	150426-0258	6.6	7.2	layer		XHX 2
Xinglongwa culture	Aohanyingzidong	slab	no number	17.5	11.3	collection	8.2–7.4	XLW 1
		muller	no number	16.4	7.3	collection		XLW 2
	Aohanyingzixi	muller	150426-0366-50001	8.4	3.7	layer		XLW 3
Zhaobaogou culture	Toudaozhangfang	muller	150426-0024-5001	24.9	5.1	layer	7.0–6.4	ZBG 1
	Jianguo	muller	150426-0326-5002	18.6	6.5	layer		HS 1
		muller	150426-0328-5002	18.5	8.3	layer		HS 2
Hongshan culture	Luojiayingzi	muller	no number	9.7	4.6	collection	6.5–5.0	HS 3
		muller	no number	6.3	5.1	collection		HS 4
	Sanjianfang	muller	150426-0319-5001	13.6	8.3	layer		HS 5
		muller	150426-0321-5001	10.4	7.3	layer		HS 6

Figure 1 Locations of the study area and archaeological sites. The archaeological sites of the Xiaohexi culture and Xinglongwa culture are indicated with red and blue triangles, respectively. The archaeological sites of the Zhaobaogou culture and Hongshan culture are indicated with red and blue circles, respectively.

3.2 Methods

(1) Samples were collected from the used and unused facets of 12 stone grinding tools and examined by starch grain analysis. In total of 24 samples were collected. The protocols for starch grain analysis have been presented previously (Wan et al., 2012a; Yang et al., 2012a, 2015). The process is described briefly below. The used and unused facets of each grinding tool were cleaned with ultrapure water and a nylon brush to remove any dust that had adhered during storage, and were then shaken separately in an ultrasonic bath. Each ultrasonicated mixture was then transferred to a test tube and centrifuged. A solution of 6% H_2O_2 was used for the oxidative breakdown of some of the larger charred particles. A solution of 10% HCl was then used to remove calcium impurities. The resulting sediment was mixed with a 5% solution of sodium hexametaphosphate (Calgon) to deflocculate the clay minerals. Finally, the starch grains were isolated with heavy liquid CsCl (density, 1.8 g/cm^3). The recovered residue was mounted on a slide in a solution of 10% glycerine and 90% ultrapure water. The slide was sealed with nail polish and the starch grains were observed, measured, and counted under a microscope (Machine model: Olympus BX-51).

(2) A dust sample (0.5 g) collected from the room where the grinding tools were stored was also subjected to a starch grain analysis. Details of the starch grains extracted from the dust sample have been presented previously (Yang et al., 2012a, 2014, 2015). A solution of 6% H_2O_2 was added to the dust to release any starch grains and to oxidize any

Figure 2 Stone grinding tools from the early-middle Holocene in the West Liaohe area. (a), (c) slab; (b), (d)–(l) muller. White dots and arrows indicate sampling locations. Scale bar: 5 cm.

extraneous organic material. A 5% Calgon solution was added to disperse the dust and remove the clay. A heavy liquid solution of CsCl (density, 1.8 g/cm^3) was used to extract the starch grains. The starch grains were then observed, measured, and counted under a microscope (Machine model: Olympus BX-51).

(3) Modern reference collections. The identification of the starch grains was based on one-on-one comparisons of the ancient starches with those from over 200 Asian species housed in modern reference collections, including 50 genera in the families of Poaceae, Leguminosae, Fagaceae, published images and data (Yang et al., 2009a, 2009b, 2010, 2012b; Li et al., 2010; Wan et al., 2012b; Yang and Perry, 2013). Starch grain identification was also based on a previous classification of millet crops that included morphological and statistical analyses of the starch grains from millets that had been shelled and ground in a simulation experiment (Ma, 2014). After the starch grains derived from the seeds of 31 modern millet samples were analysed, a dichotomous key was established to define the diagnostic morphological characteristics of the starch grains, including those of wild millets, which are small particles with wrinkled surfaces and rough edges. Only domesticated foxtail millet has starch grains measuring $>14.0\text{ }\mu\text{m}$ (Figure 3) (Yang et al., 2012b). The morphologies and sizes of the millet starch grains change negligibly after shelling. After grinding, the average length of the starch grains increases by 1.2 times and their cross-extinction weakens (Figure 3) (Ma, 2014). In summary, two key standards were defined. 1. Wild millets have small particles (the length is usually below $10\text{ }\mu\text{m}$ and no more than $14\text{ }\mu\text{m}$), wrinkled surfaces, and rough edges. 2. Only domesticated foxtail millet has

Figure 3 Starch grains from foxtail millet, broomcorn millet and their wild relatives. (a) Foxtail millet (*Setaria italica*) collected from Hebei Province, size range 5.3–15.4 μm and mean diameter 8.0 ± 3.1 μm ; (b) broomcorn millet (*Panicum miliaceum*) collected from Gansu Province, size range 5.0–10.0 μm and mean diameter 6.8 ± 1.8 μm ; (c) green foxtail (*S. viridis*) collected from Shanxi Province, size range 5.1–11.0 μm and mean diameter 7.8 ± 1.6 μm ; (d) green foxtail (*S. viridis*) collected from Hebei Province, size range 5.2–14.0 μm and mean diameter 8.2 ± 1.6 μm ; (e) *P. bisulcatum* collected from Beijing City, size range 5.1–9.3 μm and mean diameter 6.9 ± 1.2 μm ; (f) *S. pumila* collected from Shandong Province, size range 5.3–8.2 μm and mean diameter 6.6 ± 0.7 μm ; (g) palmgrass (*S. palmifolia*) collected from south China, size range 5.1–7.7 μm and mean diameter 5.7 ± 0.8 μm ; (h), (k) and (n); (i)–(j), (l)–(m), and (o)–(p). starch grains with greater lengths, broken surfaces, and weakened cross-extinction from the foxtail millet (*S. italica*) collected from Henan Province, size range 5.5–27.4 μm and mean diameter after grinding 11.8 ± 2.9 μm ; broomcorn millet (*P. miliaceum*) collected from Shanxi Province, size range 5.8–15.8 μm and mean diameter after grinding 8.8 ± 2.2 μm , and green bristlegrass (*S. viridis*) collected from Beijing City, size range 5.5–12.3 μm and mean diameter after grinding 8.1 ± 2.1 μm). Scale bar: 20 μm .

starch grains measuring >16.8 μm after it is processed with stone grinding tools.

4. Results

No starch grains were recovered in the control samples of the dust in the storerooms in which the 12 grinding tools were curated. The 24 samples extracted from the 12 grinding tools yielded a total of 643 starch grains, of which, identifiable 629 can be classified into type A–D, and the remaining 14 that could not be identified because of damage or an absence of identifiable characteristics are grouped into type E.

Type A starch grains. The common features of type A grains ($n=594$) were their polyhedral shape, centric hilum

Figure 4 Starch grains retrieved from stone grinding tools in the West Liaohe area during the early-middle Holocene. (a)–(c) type A; (d) type B; (e), (f) type C; (g) type D; (h), (i) starch grains with broken surfaces; (j), (k) starch grains with weak cross-extinction. Scale bar: 20 μm .

and absence of lamellae. Some had deeply crossed or winged to Y-shaped fissures (Figure 4a, b). When the grain was rotated, the shape was still polyhedral. When we considered these properties with the range of their diameters, we identified the type A grains as from foxtail millet, broomcorn millet and *Setaria* spp. We also noted that the type A grains could be divided into three subtypes based on differences in their sizes, surface morphologies and fissure types: type A1 grains, with wrinkled surfaces and rough edges, which are features diagnostic of wild millet grasses (Figure 4c); type A2 grains, measuring >16.8 μm , a size class only recorded in domesticated foxtail millet; and type A3 grains including the remaining starch grains (Table 2).

Type B starch grains. Twenty-six starch grains were ovoid, and were characterized by eccentric hilum, no fissures and no lamellae (Figure 4d). The archaeological starch grains in type B were identical to modern grains from acorns (Yang et al., 2009b) (Table 2).

Type C starch grains. Eight starch grains had an irregular lenticular shape (such as an ellipse or semicircle) with a centric hilum and craters on the surface. The starch grains were olivary and had a longitudinal dent when rotated (Figure 4e, f). Type B grains are similar to the starch grains of *Hordeum* spp., studied by Yang and Perry (Yang and Perry, 2013), and therefore we inferred that the type C grains are from *Hordeum* spp. (Table 2).

Type D starch grains. The type D grains included only one elliptical starch grain, which was extracted from a mullet (laboratory no. HS 2) from the Jiangou site of the Hongshan culture. The grain showed radiating fissures with a long elliptical shape (Figure 4g), especially under polarized light. The length of the starch grain was 25.4 μm . These features are consistent with those of starch grains from food legumes (Wang et al., 2013). Therefore, we inferred that the type D grain was from a food legume.

Table 2 Data for the starch grains from stone grinding tools in the West Liaohe area during the early-middle Holocene

Laboratory no.	Location ^{a)}	A			Size range and mean diameter of starch grain (μm)	B	Range and mean diameter of starch grain (μm)	C	Range and mean diameter of starch grain (μm)	D	Range and mean diameter of starch grain (μm)	E	Total
		A1	A2	A3									
XHX 1	U	3	12	10		5		2		0		0	32
	N	1	6	5	8.6–25.6	1	10.8–21.5	0	18.7–23.2	0	0–0	0	13
XHX 2	U	7	20	15	17.4±3.5	6	15.4±4.8	0	21.0±2.3	0	0	1	49
	N	2	10	9		3		0		0		1	25
XLW 1	U	3	19	8		3		1		0		3	38
	N	2	12	13		2		1		0		3	33
XLW 2	U	2	14	11	9.2–27.8	2	11.4–20.6	0	19.2–21.5	0	0–0	0	29
	N	1	6	2	17.8±3.4	1	16.0±3.1	0	20.4±1.2	0	0	0	10
XLW 3	U	7	18	3		2		0		0		1	31
	N	3	15	6		1		0		0		1	26
ZBG 1	U	2	20	10	11.4–26.8	0	0–0	0	0–0	0	0–0	0	32
	N	0	16	10	18.3±4.2	0	0	0	0	0	0	0	26
HS 1	U	3	31	15		0		2		0		1	52
	N	0	5	5		0		0		0		0	10
HS 2	U	1	32	18		0		0		1		0	52
	N	0	7	5		0		1		0		0	13
HS 3	U	2	20	12	7.5–31.1	0	0–0	0	18.7–27.5	0	25.4	1	35
	N	1	15	10	18.0±3.6	0	0	0	19.9±4.0	0	25.4	0	26
HS 4	U	1	18	13		0		0		0		1	33
	N	1	15	9		0		0		0		0	25
HS 5	U	2	18	7		0		0		0		0	27
	N	1	10	2		0		1		0		0	14
HS 6	U	0	8	2		0		0		0		1	11
	N	0	0	1		0		0		0		0	1
Total		44	354	196									
			594			26		8		1		14	643

a) U indicates the used facet of the stone tool; N indicates the unused facet of the stone tool.

5. Discussion

5.1 Contamination control in the analysis of ancient starch

When using ancient starch grains from residues on artefacts to analyse their functions and the utilization of plants by ancient humans, the common practice is to collect the dust in the storeroom where the artefacts are curated (Wan et al., 2012a; Yang et al., 2012a, 2014, 2015), residues from both the used and unused surface of the artefacts, and nearby sedimentary samples (deposits in immediate proximity to the artefacts) to control contamination (Barton, 2007). In this experiment, no starches were recovered from the dust in the storeroom, indicating that the residues on the grinding tools were not contaminated from this source. No sedimen-

tary samples were obtained because the grinding tools had been excavated or collected before this study. However, we extracted samples from the used and unused surfaces of each grinding tool and compared the starch grain assemblages separately to determine the possibility of contamination from deposits of surrounding environment. Table 2 shows that far fewer starch grains were extracted from the unused surfaces of the 12 grinding tools than from the used surfaces of the tools. This general pattern was common across all the grinding tools (see Table 2). The differences in the numbers of grains obtained from the used and unused surfaces of the grinding tools suggest that most of the starch grains collected from the grinding tools were related to their use. Obvious signs of damage (e.g., broken surfaces (Figure 4h, i) and blurred extinction areas (Figure 4j, k) were simi-

lar to the characteristics of starch grains after they were shelled and ground in previous simulation studies (Ge et al., 2010; Ma, 2014), indicating that the grains on the grinding tools were preserved during the use of the tools.

5.2 Early millet use in the West Liaohe area during the early-middle Holocene

From the number of starch grains extracted from the residues on the surfaces of the grinding stones used in the West Liaohe area during the early-middle Holocene, we inferred that the main plant utilized was millets. Of all the plants utilized, millet accounted for 84.0% in the Xiaohexi culture, 87.4% in the Xinglongwa culture, 100% in the Zhaobaogou culture, and 97.0% in the Hongshan culture (Table 2), indicating that millets occupied an important position among the ancient plant-based foods consumed in the West Liaohe area in the early-middle Holocene.

The millet starch grains extracted from residues on the surfaces of stone grinding tools were studied statistically based on the distinguishing criteria established with a dichotomous key and a simulation analysis of millet plants (Yang et al., 2012b; Ma, 2014). The results showed that the wild millet starch grains decreased from 13.0% to 3.4%, whereas the domesticated type increased from 55.0% to 62.1% (Table 2 and Figure 5) during the 3500 years from the early to the middle Holocene. Based on the carbonized broomcorn millet obtained by flotation from the Nanwanzibei site of the Xiaohexi culture (Sun, 2015), we consider

that the domestication of millet began in the Xiaohexi culture. The results of our millet starch grain analysis were compared with the results from the Nanzhuangtou site in Hebei Province (38% wild and 46.8% domesticated millets) and the Donghulin site in Beijing (32.3% wild and 36.2% domesticated millets in the early period and 14.9% and 51.4%, respectively, in the late period) (Yang et al., 2012a). The proportions of wild millet starch grains were lower, whereas the proportions of the domesticated type were higher in our study, which probably indicates that the degree of millet domestication in the Xiaohexi, Xinglongwa, Zhaobaogou and Hongshan cultures was higher than the degree of Nanzhuangtou and Donghulin sites. Evidence from plant macroremains from the West Liaohe area, obtained by flotation, indicated that the morphology and size of the foxtail and broomcorn millet from the Xinglongwa site of the Xinglongwa culture differed from those of the green foxtail and their modern corresponding species (Zhao, 2004). The foxtail millet and broomcorn millet unearthed from the Duliyingzi, Halahaiwa, and Xiaoshandegou sites of the Zhaobaogou culture and the Weijiawopu site of the Hongshan culture were clearly identified as domesticated varieties (Sun, 2015). In particular, the morphology and size of the millet starch grains from the Weijiawopu site were basically consistent with those of modern grains (Sun et al., 2012; Sun and Zhao, 2013). The results of our starch grain analysis based on plant microremains support the evidence of plant macroremains, and also indicate that the process of millet domestication proceeded gradually over a long period.

Figure 5 Proportional changes in millets in the West Liaohe area during the early-middle Holocene. Brown indicates the proportion of starch grains with wrinkled surfaces; blue indicates the proportion of starch grains with smooth surfaces; red indicates the proportion of starch grains > 16.8 μm; green indicates the proportion of starch grains < 16.8 μm.

5.3 Utilization of plants other than millet in the West Liaohe area during the early-middle Holocene

Many plants contain little or no starch, including soybeans (*Glycine max*) and most fruits and vegetables. Some plants are also edible without grinding, including berry plants, such as the wild grape (*Vitis* spp.). Therefore, the starch grains extracted from stone grinding tools only reflect some of the plants processed by humans, and reflect far from the whole range of the plants used other than millet.

Besides the carbonized foxtail millet and broomcorn millet found in the West Liaohe area, apricot (*Armeniaca sibirica*), salsola (*Salsola collina*), and *Artemisia sieversiana* from Chahai and the Nanwanzibei sites of the Xiaohexi culture (Suo and Li, 2008; Sun, 2015), mallow plants (*Malva*) from the Duliyingzi, Halahaiwa, and Xiaoshandegou sites of the Xinglongwa culture (Sun, 2015), and food legume seeds from the Weijiawopu site of the Hongshan culture (Sun et al., 2012; Sun and Zhao, 2013). The largest numbers of starch grains in the residues on the surfaces of the grinding tools were from acorns, then from *Hordeum*, and then from individual food legumes (see Table 2). These starch grains, occurring with those from millets, supplement the research findings from plant macroremains, and also demonstrate the diversity of the ancient plants used in the West Liaohe area.

As millet agriculture gradually developed in the West Liaohe area, the contents of these other plant starch grains declined, from 16% in the Xiaohexi culture to 12.6% in the Xinglongwa culture. No accompanying plants were found

during the Zhaobaogou culture, and the proportion of *Hordeum* spp. and food legumes was 3% in the Hongshan culture (Table 2 and Figure 6). Therefore, the proportions of accompanying plants declined gradually from the Xiaohexi culture, to the Xinglongwa culture, and then to the Hongshan culture. These changes in the plants accompanying millet agriculture are probably related to the increasing proportions of millet in the diets of ancient humans. Moreover, the methods of food processing used and the environmental changes that influenced the types and distributions of the available plant resources cannot be ignored, and the influence of these specific factors requires further investigation. The lack of starch grains from accompanying plants in the period of the Zhaobaogou culture can probably be attributed to statistical error caused by the very small sample (one muller, 58 starch grains).

6. Conclusion

In this study, ancient starch grains from millets, acorns, barleys, and leguminous plants were recovered from 12 stone grinding tools excavated from the sites of Xiaohexi culture, Xinglongwa culture, Zhaobaogou culture, and Hongshan culture. A statistical analysis of the occurrence of the grains showed that the proportion of millet starch grains with wrinkled surfaces and rough edges, diagnostic of wild millet grasses, decreased from 13.0% to 3.4% between 8.5 ka BP and 5.0 ka BP. At the same time, millet starch grains measuring >16.8 μm , a size class recorded only in domesticated

Figure 6 Proportional changes in the plants accompanying millet agriculture in the West Liaohe area during the early-middle Holocene. Brown indicates the proportion of millet starch grains; blue indicates the proportion of starch grains from accompanying plants.

foxtail millet, increased from 55.0% to 62.1%. These results indicate that the process of millet domestication in the West Liaohe region began in the period of Xiaohexi culture. Our results provide new evidence for the previously proposed hypothesis of a long process of millet domestication.

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