



Short Communication

New empirical evidence from ancient foxtail millet seeds and panicles reveals phenotype divergence during its dispersal

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Foxtail millet is one of the earliest domesticated cereal crops in the world. Its domestication has been traced back to the early Holocene (10,000–8000 cal a BP), first appearing in the basins of the Yellow River and Western Liao River [1]. Dryland farming dominated by foxtail millet was ultimately established in the Central Plains during the middle Holocene (6000–5500 cal a BP) [2]. This agricultural system served as the vital subsistence basis for demographic growth, a rise in urbanization, and the formation and continuous development of Chinese civilization. This cereal had traditionally been a major and valuable staple-food crop in northern China. In the late Holocene, along with human migration and innovative agricultural technology communication, foxtail millet cultivation expanded outward on a large scale and to a wide range of new habitats far away from its original center of domestication [3]. This expansion not only covered broad geographical regions of varied natural ecological features in China and other adjacent countries of East Asia, but even stepped westward into the South Asian Sub-continent and spatially remote western Europe [4]. The early dispersal of foxtail millet was a key component of ancient food globalization wave in the Old World, and generated profound effects on the dietary composition of indigenous inhabitants, the

traditions of crop cultivation, and the structure of agricultural economies in the accessible Eurasia areas [5]. In India, Pakistan, Afghanistan, and certain countries in southwest Asia and eastern Europe, foxtail millet has been cultivated since the late Holocene period and remains a primary minor crop at present.

However, the fundamental information on early foxtail millet diffusion, involving a detailed timing sequence and spatial dispersion pattern, is vague and lacks support from precise and quantitative analyses of radiocarbon data set, even in China, where the most abundant findings of foxtail millet exist. Relative dates or absolute calendar ages of other dated materials instead of direct dating of foxtail millet are commonly applied to reconstruct the probable long-term chronological sequences of foxtail millet dispersal, which are visibly short of reliability. In addition, when foxtail millet spread out of its domestication center, it had to face markedly different environments. In-depth issues underlying the superficial information of dispersal timing and routes, such as the expression of phenotype variation for response and adaptation to new habitats in addition to the effect of phenotype divergence on the role of foxtail millet in a new agriculture cultivating structure, have seldomly been explored and pinpointed. Affected by conditions of taphonomy and preservation, most foxtail millet remains have only been charred seeds, with evolutionary phenotype traits being finitely recorded in these seeds. However, in this study, apart from measuring the quantitative traits of a large num-

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ber of seeds, well-preserved desiccated foxtail millet panicles unearthed in the arid environment of Xinjiang are reported. The remarkable discovery of panicles with more key agronomic traits allows us to propose new and innovative perspectives concerning the underlying issues, like the diversified evolution trajectory of foxtail millet and its agricultural effects.

For addressing these issues, all 214 published radiocarbon dates of foxtail millet seeds in China were collected, and 207 of them were selected (Table S1 online). Other seven dates deriving from six sporadic sites in southern China were not employed. In total, 238 direct dates of foxtail millet from 150 sites, including 207 dates previously published and 31 dates newly determined in this study (Table S1 online), were compiled and grouped into six different geographical sub-regions, covering Southwest China, Northwest inlands, the Western Liao River, the lower, middle and upper Yellow River (Fig. 1a and Fig. S1 online). A Bayesian model was used to quantitatively reconstruct the timing of the first appearance event of foxtail millet in these regions and rank the sequential order of the timing [6]. The dispersal timing and routes of foxtail millet were then determined based on the solid chronological sequences and reliable Bayesian analysis results of the data set (Fig. S1 and Table S2 online). Two major routes of the westward spatial expansion were significantly outlined. The first one is the “Northwest Route” (Fig. 1a), illustrating that foxtail millet was moved from the Central Plains through the lowlands and valleys of the tributaries of the middle Yellow River to Gansu-Qinghai regions located in the upper Yellow River (4945 cal a BP) (Table S2 online), and then continued to extend along the Hexi Corridor to Northwest inlands (4705 cal a BP) (Table S2 online). The second one refers to the “Southwest Route” (Fig. 1a), depicting foxtail millet from eastern Gansu region were translocated along the ethnographic Tibetan-Yi Corridor to mountainous southwest China (5145 cal a BP) (Table S2 online). These probable processes of spatial expansion have also been supported by other multidisciplinary evidence involving molecular biology as well as linguistic and typology or provenance analyses of archaeological objects [7,8]. Both routes, following the “Road of Heat and Water”, tended to traverse areas of low altitude with warmer climate and accessible water resources, bypassing the high elevations with low growing degree days (GDDs) in the northeast Tibetan Plateau especially in the settings of fluctuating decrease of temperature in the late Holocene or the vast Gobi Desert in Inner Mongolia with severe water stress. Overall, these routes created a camper-shaped pattern of dispersion (Fig. 1a). The spatial range of the arrival and diffusion routes of foxtail millet were distinctly constrained by heterogeneous and challenging ecological barriers. The large-scale expansion did not present an undifferentiated “wave of advance” pattern.

Subsequently, this research highlights the phenotype variation associated with foxtail millet adaptation to a primary factor of natural selection, namely precipitation, as it diffused along the Northwest Route, from humid or semi-humid East Asian Summer Monsoon (EASM) areas to the arid Central Asia drylands dominated by the Westerlies. All 16,130 charred seeds without distinct distortion obtained from 28 sites (Fig. 1b and Fig. S2, and Table S3 online) with sufficient GDDs for foxtail millet growth and across a significant precipitation gradient in northern China were measured in two dimensions: length (L) and width (W). The size and shape of these seeds were indicated by using area ($S = \pi \times L/2 \times W/2$) and L/W ratio respectively, and then were calculated. Immature seeds were excluded based on the criteria established in the measurement methodology of this study (Supplementary materials online) and other data published elsewhere [9]. Here, the assemblage of each site comprised at least 60 mature seed grains, highly exceeding the threshold value of the minimum sample size of 30, ascertained by the analyses of mod-

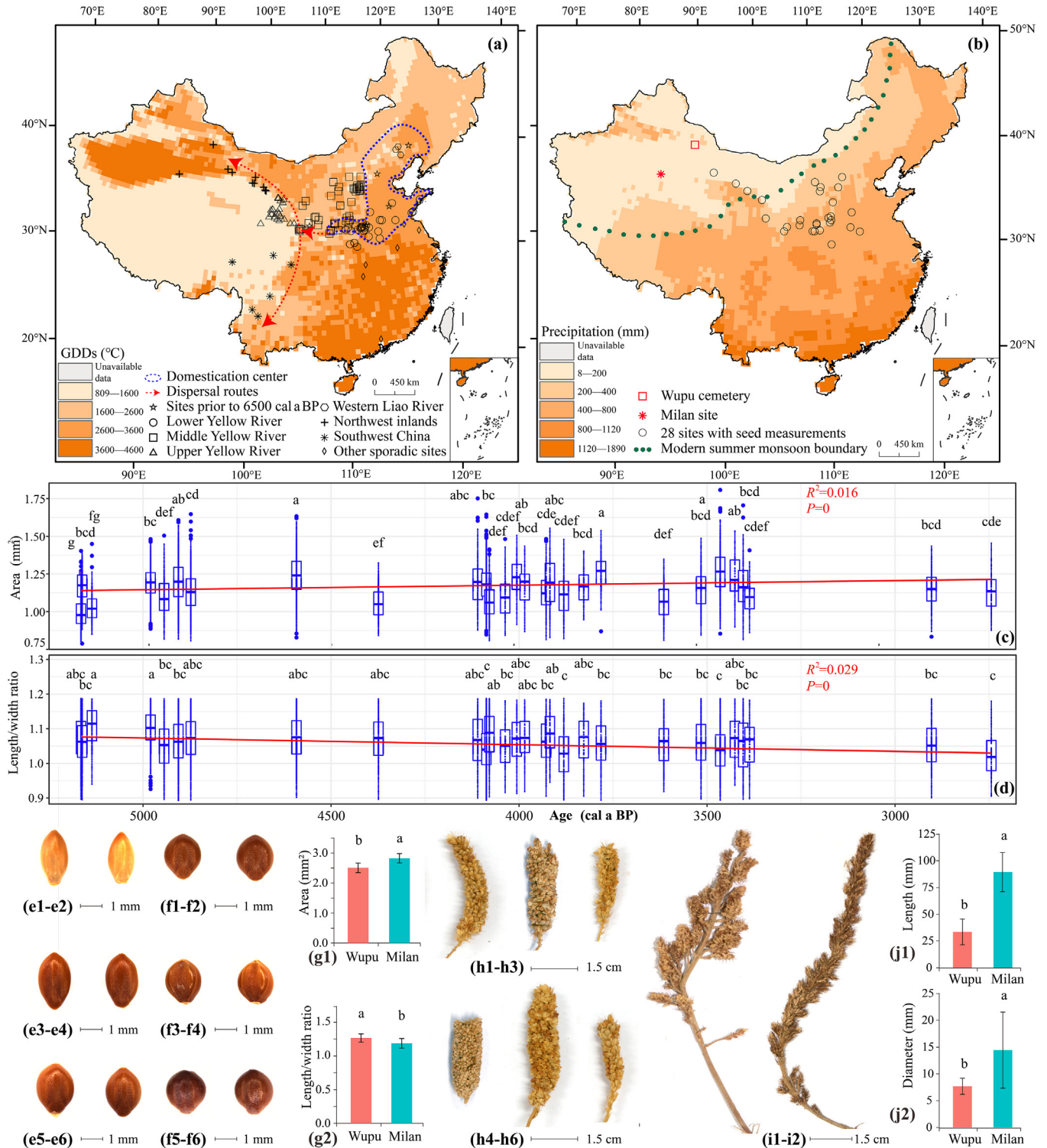
ern seed measurements (Supplementary materials online). Morphometric data of 12,977 mature seeds in all were sifted and statistically analyzed (Table S3 online). The results reveal that, although complicated and irregular differences exist among the size and shape of each seed assemblage (Tukey’s HSD test, $P < 0.05$) and the metrical data of these two quantitative traits fluctuated within a relatively large range (Fig. 1c, d), the average measured value varied slightly, such as 0.99–1.26 mm² of average area and 1.02–1.12 of average L/W ratio (Fig. 1c, d, and Tables S4 and S5 online). Integrated with the time scale of each seed population (Table S3 online), the measurements had leveled off following 5165 cal a BP, and a directional variation trend is absent (low r value) (Fig. 1c, d), indicating that foxtail millet seeds had not evolved to be much larger or rounder over time. The statistical characteristics of the data set strongly demonstrate that the phenotype of foxtail millet seeds generally exhibited diverse but relatively stable quantitative traits as it adapted to multiple precipitation locales during its dispersal process. This scenario reveals an essential characteristic of foxtail millet phenotypic evolution in the post-domestication diversification stage. The metrical data set also implies additional and potentially important agronomic information; that is, the yield improvement of foxtail millet in the late Holocene may mainly have depended on an increase in the number of seed grains.

In addition to the measurements of charred seeds, all metrical data of 7275 desiccated seeds, including 5751 mature ones derived from the Wupu and Milan sites in the extreme arid Hami and southeastern Tarim Basins of Xinjiang (Fig. 1e, f, and Tables S3 and S4 online), also support the conclusion that the variability of the size and shape in foxtail millet seeds are relatively small, shown by the area and L/W ratio of 2.49 ± 0.15 mm², 1.30 ± 0.07 and 2.82 ± 0.15 mm², 1.18 ± 0.07 of the Wupu and Milan seeds respectively (Fig. 1g, Tables S4 and S6 online). However, in contrast to the seeds, the agronomic traits of the intact desiccated foxtail millet panicles in Wupu and Milan sites first reported here are significantly different (Fig. 1h–j, and Tables S7 and S8 online). In total, 109 Wupu panicles (2715 cal a BP) were recovered, and 52 intact ones were measured at 33.50 ± 12.03 mm in length and 7.68 ± 1.50 mm in diameter (Fig. 1j, and Tables S7 and S8 online). These very short panicles are erect, columnar, and compact (Fig. 1h), the phenotype being similar to those of modern *Moharia* varieties [10], widely cultivated in Central Asia and southeast Europe. However, the panicles are discrepant in terms of the number of tillers. At most one tiller present in the base of an individual plant stand from Wupu and tiller do not appear in most of the plants (Fig. S3 online). On the contrary, a plant of *Moharia* usually develops an average of 12 tillers. The millets of Wupu are more comparable to those of modern Baltistan landraces [11]. Plant architecture is typically of low stature with one or no tillers, and presence of a small compact panicle. The landraces are currently cultivated in the Baltistan region and the Hunza Valley located in the northern mountains of Pakistan [11]. It should be noted that most of Wupu panicles are fully headed and their sizes are fully established because seeds are produced in the basal, middle, and apical part of the panicles, although many of seeds are not fully filled (Fig. 1h). All five Milan panicles (1126 cal a BP) were hand-picked, and four of them were measured, the length being 89.45 ± 18.42 mm and the largest diameter 14.43 ± 7.08 mm (Fig. 1j, and Tables S7 and S8 online). The complex architecture consisting of the second and third branches is present in the spindle-like and nodding pendulous Milan panicles (Fig. 1i). The phenotype of these large panicles with the amounts of seed grains resembles the Maxima varieties [10], a major variety intensively cultivated in East Asia. The discovery of these two evident phenotype differentiations of diversified panicles breaks through the

pre-existing limited recognition of foxtail millet phenotypic evolution, which has been only summarized from the traits of seeds to date.

The production of short-sized Wupu panicles is likely driven by a mechanism in phenotypic plasticity. In the Hami Basin, the temperature in summer and early autumn seasons is high and water resources are deficient during the entire vegetative and reproduc-

tive growth periods of foxtail millet. In order to promptly diminish the risks of this extremely hot and dry environmental stress, the sessile foxtail millet plants will adjust their physiological, biochemical, and morphological traits [12]. This plasticity and flexibility contribute to the survival of the species, yet may lead to a reduction in the performance of other agronomically desirable traits, such as generation of very small panicles and low stature



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(Fig. S3 online). Modern experiments in environmental control of foxtail millet growth are in favor of this assumption in plasticity [13]. Another driving factor is probably the high density of plant stands. An agro-pastoral subsistence economy was an optimal strategy adopted by the Wupu community [14]. In warm seasons from late spring to early autumn, the major labors of local inhabitants were engaged in transhumant livestock herding in distant wild pastures. Laborious and time-consuming farmland management, such as weeding and thinning seedlings, were not fully conducted. Many ethnological investigations of modern Xinjiang agro-pastoralists and archaeological findings in other synchronous sites also verify this phenomenon. With a lack of weeding or thinning, the density of plant stands increases sharply in a unit area. The incongruous population structure will intensify conspecific competition among different plant stands of foxtail millet or interspecific competition between the crop and weeds for the essential survival resources. These agro-ecological conditions may give rise to production of shorter panicles as well. Therefore, this study interprets the Wupu panicles as an expression of a special ecotype/landrace under new challenging natural and artificial selective pressures (Fig. 1h). In contrast, Milan foxtail millet with much larger and sophisticated panicles is a highly modified and newly introduced variety (Fig. 1i). Despite the similar arid environment between Wupu and Milan, there was a complete water conservancy facility set up at the Milan site, allowing for the provision of a stable water supply for crop cultivation. Additionally, written records of inscribed wooden slips record that some Milan inhabitants were experienced full-time farmers, mainly engaged in agriculture. They were familiar with phenology, intensive cultivation, and field management of crops. These factors contribute to the promotion of the agronomic performance of foxtail millet.

In the arid areas of Xinjiang and Inner Asia during the Bronze and early Iron Ages, the number of discoveries of foxtail millet seeds has been few and the archaeological sites with foxtail millet findings are sporadic [15]. Another intriguing phenomenon is that the dates of foxtail millet remains have proved to be much later than those of broomcorn millet, another representative of the East Asian crop package. Two conundrums persist: (1) the delay in the timing of foxtail millet diffusion across Inner Asia; (2) the diminution of foxtail millet from a high-profile, desirable, and major cereal crop for sustaining the agricultural system in EASM areas to a relatively undervalued minor crop not widely selected and cultivated by local inhabitants in arid Inner Asia in Westerly areas. Low GDDs in highland pastures, hyper-aridity in lowlands, pastoral cultural contexts, and baking cuisine traditions have been regarded as principal external factors for this diminution. In terms of archaeobotanical evidence, early Wupu foxtail millet panicles are very short in size and tillers are absent from most of the plant specimens (Fig. S3 online). Thus, the total amount of seed grains is

small and the agricultural yield appears to be very low. With low yield due to adaptation to the particularly natural and anthropic factors in Xinjiang, the Wupu ecotype offers crucial and practical plant specimens for resolving the afore-mentioned conundrums. In light of the ancient foxtail millet panicles, a certain level of production of this cereal was maintained in spite of being cultivated in a marginal and fragile ecological niche. The comparatively high performance of resiliency, flexibility, and resistance to abiotic stress will render foxtail millet as a potential primary cereal crop in marginal environments for reducing food security risk and enriching agrobiodiversity in response to modern ongoing global climate changes.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Author contributions

Guilin Zhang, Xinying Zhou, Xiaoqiang Li, and Hongen Jiang designed the research. Xinying Zhou, Guilin Zhang, Yongqiang Wang, Zhihao Dang, Wenying Li, Xue Shang, Jixiang Song, Tao Chen, Keliang Zhao, Huan Liu, and Hongen Jiang conducted archaeological surveys and collected plant samples from sites. Michael Spate, Alison Betts, Hai Xu, and Xianzhu Wu provided essential suggestions and revisions of the manuscript. Shaobo Sun and Guilin Zhang constructed the geographical distribution of modern climate elements. Jing Wang and Guilin Zhang contributed to the statistical analyses of metrical data. Guilin Zhang and Shanxia Zhang performed the Bayesian chronological model. Guilin Zhang, Xinying Zhou, and Hongen Jiang wrote the manuscript. All authors discussed and approved the manuscript.

Appendix A. Supplementary materials

Supplementary materials to this short communication can be found online at <https://doi.org/10.1016/j.scib.2022.08.015>.

Fig. 1. Dispersal routes, phenotype variation of charred seeds, desiccated seeds, and panicles of foxtail millet. The grid data set of growing degree days (GDDs) and precipitation were calculated using average daily temperature and precipitation from May to September between 2001 and 2011 at 2472 meteorological stations (China National Meteorological Information Center). The horizontal resolution is $0.5^\circ \times 0.5^\circ$. The climate data of Taiwan Island is currently not available. (a) GDDs distribution: star shapes from north to south represent the Xinglonggou, Donghulin, Yuezhuang, Zhuzhai, and Dadiwan sites with the dates of broomcorn millet or wild plant seeds prior to 6500 cal a BP, where foxtail millet seeds appeared but were not directly dated. Other shapes mark 156 sites with 245 direct dates of foxtail millet in China. The dotted arrows indicate “Northwest Route” and “Southwest Route”, emanating from the middle Yellow River basin. (b) Precipitation distribution: hollow dots indicate 28 sites with charred seed measurements presented in this study. The small green dots indicate the modern summer monsoon boundary. (c, d) Statistical comparison of area and L/W ratio of charred mature seeds ($n = 12,977$, Tukey’s Honest Significant Difference test, $P < 0.05$) from 28 sites ranked in a diachronic sequence. The long red lines in transverse direction illustrate the variation tendency of seed size (c) and seed shape (d) over time (linear regression fitting, see R^2 value). The seed assemblages from early to late are Manan ($n = 832$), Xinghua ($n = 329$), Xinglefang ($n = 146$), Beishantou ($n = 1093$), Guangcun ($n = 277$), Dadiwan ($n = 1714$), Xishanping ($n = 913$), Nanzuo ($n = 1002$), Beiniu ($n = 260$), Mozuizi ($n = 169$), Xihetan ($n = 3260$), Xiahe ($n = 267$), Taomugang ($n = 67$), Xinzhang ($n = 112$), Shimao ($n = 67$), Qiaocun ($n = 152$), Jijiazhuang ($n = 109$), Zhaimao ($n = 171$), Taosi ($n = 80$), Zhukaigou ($n = 145$), Zhangdeng ($n = 216$), Qinweijia ($n = 292$), Donghuishan ($n = 669$), Xiaozhang ($n = 75$), Yingshuwo ($n = 172$), Dongxiafeng ($n = 82$), Donggao ($n = 210$), and Shenmingpu ($n = 96$). (e) Desiccated immature seeds (e1–e4) and mature seeds (e5 and e6) from Wupu cemetery. (f) The dorsal and ventral view of Wupu mature seeds (f1–f4) and the dorsal view of Milan mature seeds (f5 and f6). (g) Statistical test results of area and L/W ratio between Wupu ($n = 93$) and Milan ($n = 5658$) mature seeds (Tukey’s HSD test, $P < 0.05$). (h–j) Wupu panicles ($n = 52$, h1–h6), Milan panicles ($n = 4$, i1 and i2), and data analyses of their length and diameter (j1 and j2, Tukey’s HSD test, $P < 0.05$).

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