Agricultural origins and the isotopic identity of domestication in northern China

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Stable isotope biochemistry ($\delta^{13}C$ and $\delta^{15}N$) and radiocarbon dating of ancient human and animal bone document 2 distinct phases of plant and animal domestication at the Dadiwan site in northwest China. The first was brief and nonintensive: at various times between 7900 and 7200 calendar years before present (calBP) people harvested and stored enough broomcorn millet (*Panicum miliaceum*) to provision themselves and their hunting dogs (*Canis* sp.) throughout the year. The second, much more intensive phase was in place by 5900 calBP: during this time both broomcorn and foxtail (*Setaria viridis* spp. *italica*) millets were cultivated and made significant contributions to the diets of people, dogs, and pigs (*Sus* sp.). The systems represented in both phases developed elsewhere: the earlier, low-intensity domestic relationship emerged with hunter–gatherers in the arid north, while the more intensive, later one evolved further east and arrived at Dadiwan with the Yangshao Neolithic. The stable isotope methodology used here is probably the best means of detecting the symbiotic human–plant–animal linkages that develop during the very earliest phases of domestication and is thus applicable to the areas where these connections first emerged and are critical to explaining how and why agriculture began in East Asia.

East Asia | millet | Neolithic | origins of agriculture | stable isotope biochemistry

It is widely believed that East Asian agriculture evolved in isolation from early agricultural developments elsewhere around the globe, producing a developmentally distinct suite of domesticates including rice, broomcorn millet, foxtail millet, pigs, dogs, and chickens (1–11). Although the details of this East Asian agricultural revolution are cloudy, existing evidence points to 2 historically-independent evolutionary phenomena rooted in separate and ecologically distinct parts of mainland China: a rice-based system in the warm-humid south and a millet-based system in the cold-arid north (7, 8, 12, 13) (Fig. 1). There is some support for an alternative idea that the millet-based system is merely the "northern phenotype" of the southern rice-based system (14, 15), holding that as the southern rice-based system spread toward the more arid north, rice farmers already familiar with the drought-tolerant wild ancestor (*Setaria viridis*) of foxtail millet (*Setaria viridis* spp.*italica*) increasingly adopted it to compensate for lower rice productivity. In this view, agriculture everywhere in East Asia (excepting New Guinea) arrives via migration from the Yangzi River core in a Neolithic farming diaspora that explains the modern distribution of language families throughout the region (14, 16).

New data from locations well beyond the lower Yangzi (17–19) suggest the transition to agriculture was less unified and much more complex than suggested by this single-origin, or East Asian "Garden of Eden" model. Most notably, a second drought-tolerant annual grass, broomcorn millet (*Panicum miliaceum*) appears to have been cultivated as early as rice and foxtail millet in areas hundreds to thousands of kilometers beyond the southern, humid Yangzi River region, suggesting that East Asian

agriculture evolved in many different places almost simultaneously, under different natural and social circumstances, and likely by different processes.

Unfortunately, we know very little about the domestication of broomcorn millet in northern China, only that it appears early and suddenly from an as-yet-unidentified wild progenitor and is gradually replaced by foxtail millet (20–22). Plant domesticates are typically identified by measuring the degree to which they have been modified from their wild type (*SI Text*), but these data are rare in the East Asian record. Few samples represent the earliest domesticated forms, fewer still have been dated directly. The problem, however, is not one of preservation but of method. The standard molecular and morphological indices of domestication are unlikely to catch the initial stages of domestication before the strength of human selection has had time to register (*SI Text*), nor do they speak directly to the importance of different domesticates in the diet. Stable isotope biochemistry makes both possible, and we use it here to document the initial establishment of domestic symbioses between people, millet, dogs, and pigs at the Neolithic site of Dadiwan in northwest China.

Dadiwan

Dadiwan is the westernmost expression of early agriculture in northern China (17, 22). The site produced China's earliest painted pottery and is the earliest and type site of the Laoguantai cultural tradition, which extends from Dadiwan south to the Qinling mountains and east down the Wei River (12). Although Laoguantai is considered an independent development (23), it shares basic similarities in pottery (cord marked), architecture (round houses), and site plan (scattered dwellings) with other "pre-Yangshao" Neolithic complexes of the Huang He drainage (Cishan, Peiligang, and Houli). All were eventually replaced by the distinctive Yangshao and other contemporary Neolithic traditions between 6800 and 6000 calendar years before present (calBP).

The Neolithic farming sequence at Dadiwan begins at 7900 calBP and can be divided into 2 distinct phases separated by ≈700 years: Phase 1 (pre-Yangshao/Middle Neolithic), from 7900 to 7200 calBP; and Phase 2 (Yangshao/Late Neolithic), from 6500 to 4900 calBP (*SI Text*). As in other early millet sites, carbonized broomcorn millet macrofossils are present at Dadiwan but rare in the older Phase 1 deposits (21, 22) and tell us almost nothing about the domestication, use, or importance of
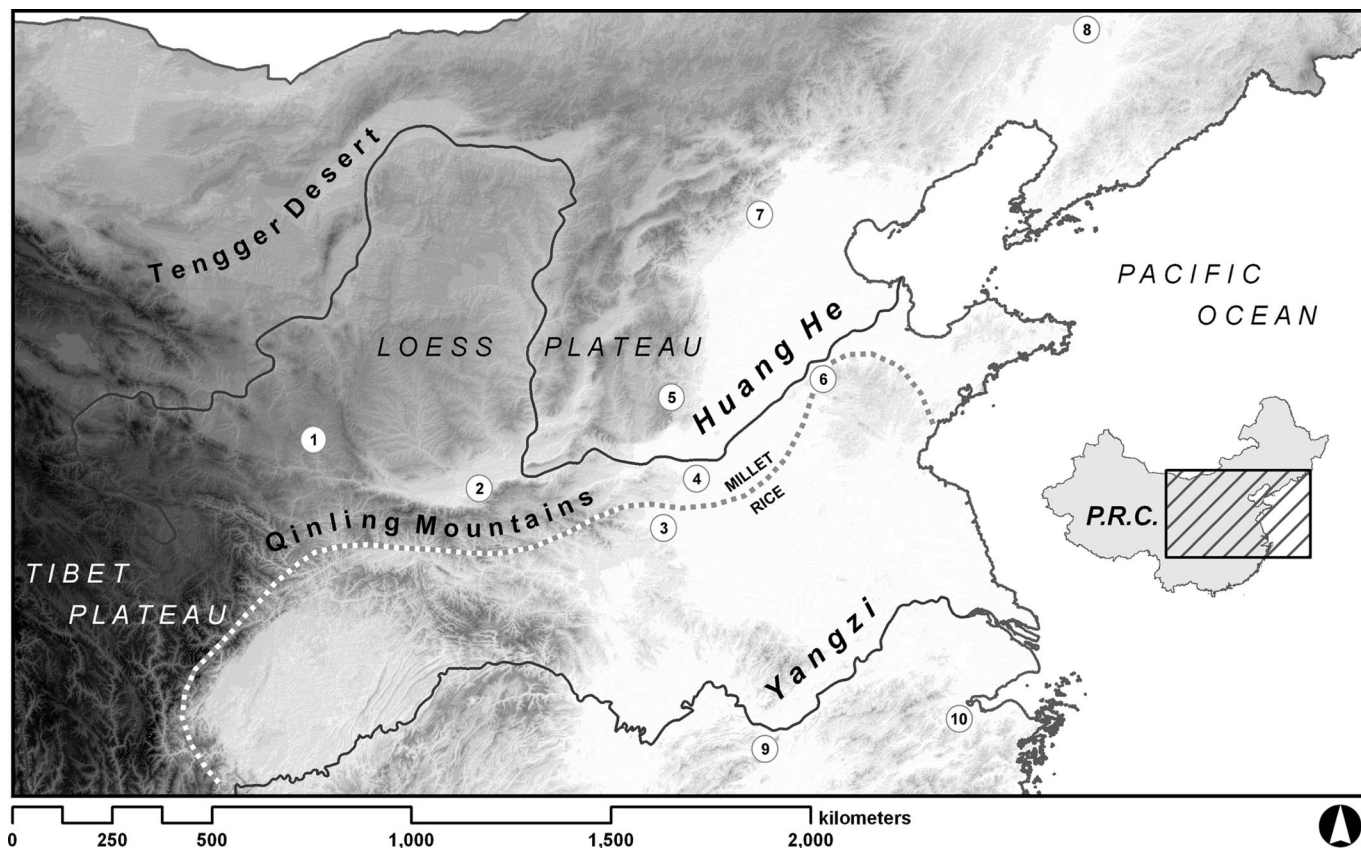

Fig. 1. Archaeological sites where early plant or animal domestication has been proposed. ① Dadiwan. ② Baijia. ③ Jiahu. ④ Peiligang. ⑤ Cishan. ⑥ Yuezhuang. ⑦ Nanzhuangtou. ⑧ Xinglongwa. ⑨ Diaotonghuan. ⑩ Kuahuqiao. The margin between rice and millet farming is approximate. P.R.C., People's Republic of China.

the species. Accordingly, we trace the evolution of broomcorn millet agriculture by observing the effect of its direct and indirect consumption, through stable isotopes, using carbon and nitrogen isotope values from Dadiwan Phase 1 and 2 human and animal bone samples selected from Gansu Museum and Lanzhou University collections on the basis of cultural affiliation, species, and bone preservation (Table S1). Seventy-four collagen samples were prepared and analyzed for their carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition (see *Methods* and Table S1). Of these, 34% were dated directly by accelerator mass spectrometry to corroborate cultural and temporal affiliations assigned by the excavators (*SI Text*, Table S2, Fig. S1). Obviously, it would be nice to have samples from the late Pleistocene—early Holocene occupation of Dadiwan, but suitable plant, animal, and human remains from this era have not been found (17); indeed, we were unable to obtain any Phase 1 human bone. Despite these gaps, the Dadiwan isotopic data date back to the very beginnings of agriculture in northwest China, allowing us to follow and evaluate human diet and human–plant–animal mutualism as it unfolded in this very important independent agricultural center.

Domestication and Stable Isotope Biochemistry

To evaluate early domestication with stable isotope biochemistry, potential domesticates must differ isotopically from other wild taxa so that the consumers of the potential domesticates will also be isotopically distinct from consumers of other taxa. Because the millets of northern China are C_4 grasses, characterized by high $\delta^{13}C$ values, heavy millet consumers will stand out only if local vegetation was in the past dominated by C_3 plants characterized by relatively low $\delta^{13}C$ values. Although their

productivity varies as a function of temperature and precipitation, C_4 plants are rare in northern latitudes (24–27), and north China is no exception: C_4 plant growth is largely confined to summer months (25), comprising <10% of perennial terrestrial vegetation (28). At Dadiwan, therefore, high $\delta^{13}C$ values in bone collagen would indicate heavy consumption of C_4 plants whose availability was extended by human planting, tending, storage, and related activities. Because bone collagen is replaced slowly throughout the life of an organism, isotopic values represent an average of dietary patterns over many years. Humans with high $\delta^{13}C$ values must have stored and consumed large quantities of C_4 grain or kept and consumed animals with $\delta^{13}C$ values elevated by year-round provisioning with the grain or hay of C_4 plants and/or the meat or waste of animals whose $\delta^{13}C$ values were elevated for the same reason. Any C_4 plant will elevate skeletal $\delta^{13}C$, but in northern China only millets attracted such attention. Certainly at Dadiwan, the archaeobotanical records from Phase 1 and Phase 2 are dominated by millets and contain very little else (21, 22). Alone, high $\delta^{13}C$ values in bone collagen do not confirm domestication or even cultivation of C_4 plants, but at Dadiwan they do reveal the intensive human selection on otherwise rare plant populations (most likely millet) that might generate the morphological and molecular attributes of the domestication syndrome (*SI Text*). The connection between millet use and elevated values of skeletal $\delta^{13}C$ is well established for the Phase 2 Yangshao millet farmers throughout the Huang He drainage. By ≈6000 calBP, human, dog, and pig remains from these late Neolithic sites display uniformly high $\delta^{13}C$ values (29–31), attesting to a millet-anchored symbiotic mutualism already entrenched enough to alter human settlement patterns profoundly and to produce important morphological change in the species involved.

What little data we have for the earliest food-producing systems of north China show the same connection between millet use and elevated $\delta^{13}C$ values. In far northeast China during the Xinglongwa period (≈8100–7200 calBP), for example, high $\delta^{13}C$ values in human skeletal elements go hand in hand with the carbonized remains of 2 types of millet (*Panicum* and *Setaria*) (18, 32). By contrast, south of the Huang He at Jiahu (≈9000–7800 calBP), low $\delta^{13}C$ values in human skeletal elements agree with an archaeobotanical record that includes rice and other C_3 species but not millet (33). In the lower Huang He drainage at Houli culture sites like Xiaojingshan and Yuezhuang, $\delta^{13}C$ values suggest that C_4 plants were a minor component of a diet dominated by C_3 plants (34), despite the presence of both rice and millets (35). Last, a few human samples from the middle Huang He site of Baijia (≈7500–6500 calBP) point to a very mixed C_3/C_4 diet (31) and at least occasional use of C_4 plants, almost certainly millet, although it is impossible to be sure because plant remains were not recovered from the site. Close to the northern margin of early rice farming, Baijia's mixed C_3/C_4 signal might reflect millet compensating for diminishing rice production, as predicted by the Garden of Eden single-origin dispersal hypothesis, but it might just as easily reflect a nonintensive phase of early millet cultivation. Either way, the connection between millet use and skeletal $\delta^{13}C$ in north China is clear.

$\delta^{15}N$ furnishes a second, quite different line of evidence regarding early millet farming systems. Because $\delta^{15}N$ values increase by $\approx3\%o$ with each trophic step in a food web, the $\delta^{15}N$ value from an organism indexes its position in the food chain. For dogs and pigs, "ascending the early farming food chain" meant becoming more heavily dependent on domestic sources of protein (e.g., meat table scraps, offal, and miscellaneous domestic waste including human feces), thus elevating the $\delta^{15}N$ value of their tissues. Pigs and dogs whose diet was further augmented with millet grain as slop or table scraps, or that consumed meat scraps or domestic waste from animals fed with millet grain or hay, should have simultaneously elevated $\delta^{13}C$ values. Accordingly, the position of dogs and pigs in the web of early food production should be marked by an isotopic gradient from wild types with relatively low $\delta^{13}C$ and $\delta^{15}N$ values to domesticated types with high $\delta^{13}C$ and $\delta^{15}N$. This relationship should register as a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ in archaeological samples of human and animal bone. Together, these 2 lines of evidence comprise the isotopic identity of domestication, which is useful for evaluating domestication where archaeological plant or animal remains are scarce or otherwise uninformative.

Results

As expected, relatively low $\delta^{13}C$ and $\delta^{15}N$ values characterize the wild prey at Dadiwan. Phase 1 and 2 ungulates, birds, and a single bear show that from ca. 7900–4900 calBP local vegetation was predominantly C_3 (Fig. 2*A*, Table S1, and Fig. S1). Likewise, 2 of the Phase 1 dogs and all 4 of the Phase 1 pigs display this C_3-based diet (Fig. 2*B*). Three of the Phase 1 dogs, however, display simultaneously high $\delta^{13}C$ and $\delta^{15}N$ values that clearly distinguish them from their wild counterparts. The bimodal distribution of isotopic values for Phase 1 Dadiwan dogs reveals 2 distinct groups: wild-foraging dogs captured, and perhaps eaten by human hunters; and dogs that lived with, hunted with, and were likely provisioned by humans. The latter camp-fed, behaviorally-domestic dogs consumed millet in quantities possible only through association with humans that selectively harvested and stored millet. Dogs provisioned with millet also consumed animal products in far greater quantities than did dogs living on wild forage. Consumption of human feces alone would not likely lead to such differences, and because there is no animal source to account for their elevated $\delta^{13}C$ levels during Phase 1, camp dogs appear to have been fed or were tolerated in such
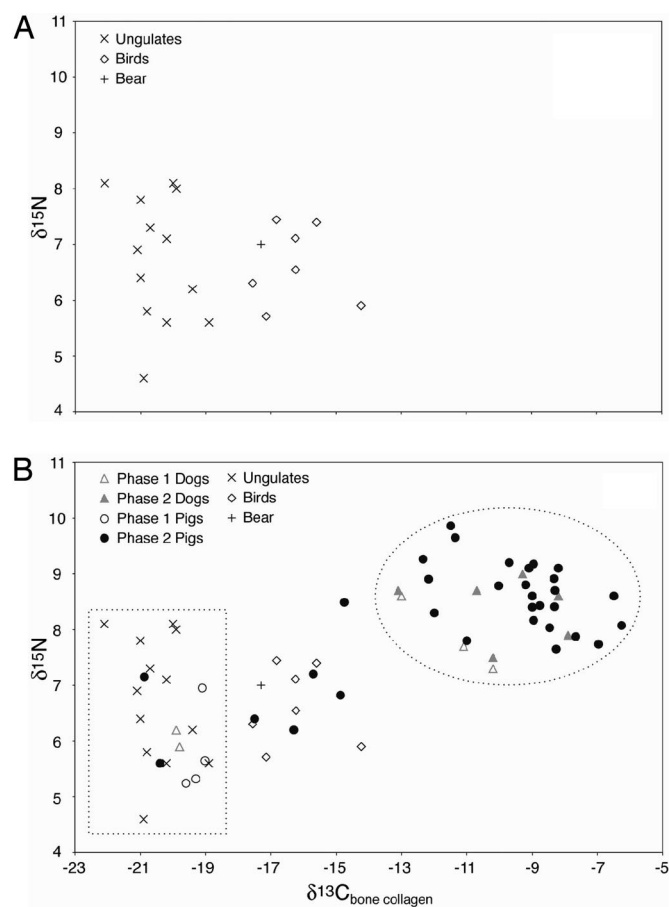

Fig. 2. The isotopic identity of domestication at Dadiwan. (*A*) Low $\delta^{13}C$ values in wild-foraging taxa establish the dominance of C_3 plants in the landscape. (*B*) Positive correlation between high $\delta^{13}C$ and high $\delta^{15}N$ illustrates life within the domestic sphere. Gradation between wild and domestic illustrates the plasticity of early farming systems. Phase 1 dogs ($\triangle$) within the dotted oval are the earliest examples of domestication in northwest China. Ungulates include deer (*Cervus* sp. and *Moschus* sp.) and cattle (*Bos* sp.) but not pigs. Birds have been tentatively identified as *Gallus* sp., bear as *Ursus arctos* (22).

proximity to be able to scrounge a combination of millet, most likely grown by humans, and meat obtained in the wild by humans, likely with their aid. Whether the high $\delta^{13}C$ values in the Phase 1 dogs reflect intensive harvest of natural (wild) or managed (domestic) stands of millet is unclear, because the current study straddles the tentative divide between the two. What is clear is that the bimodal distribution of $\delta^{13}C$ values in dogs results from an exposure to millet that is impossible for animals living without human help. Here, the persistent and intensive human use of both dogs and millet reveals the circumstances expected of early experiments with plant and animal domestication.

Directly dated between 7560 and 7160 calBP (Table S2 and Fig. S1), these Phase 1 Dadiwan dog bones provide the earliest evidence for an integrated system of food production in northwest China (Fig. 2*B*).* These are the oldest samples we were able to analyze, so it is possible the food production system they

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*Dogs may have been domesticated earlier. The numerous Phase 1 "wild" dogs were at minimum "camp followers" and certainly benefited from a close association with humans, who may have found them useful to have around, in the hunt, for example. Still, the Dadiwan data show that dogs moved closer to humans about the same time humans moved closer to millet.
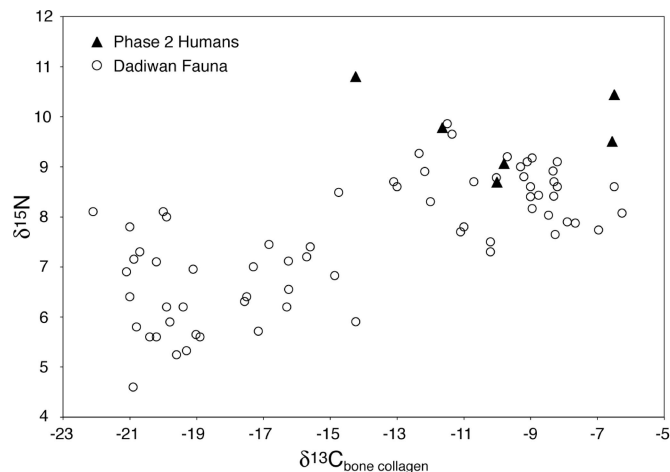
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Fig. 3. Human diet during the Yangshao Neolithic at Dadiwan. As regular consumers of C₄ plants with regular access to animal products (likely including the flesh of animals that also eat C₄ plants), humans clearly reside within the domestic sphere.

Fig. 4. A chronology of Holocene occupation and domestication at Dadiwan. This summed probability distribution of calibrated radiocarbon dates (Table S3) provides a rough proxy for occupation intensity at the site. ① Hunter–gatherers arrive at Dadiwan. ② The earliest evidence for domestication of dogs and broomcorn millet. ③ The site is largely abandoned. ④ Intensive agriculture involving dogs, pigs, broomcorn, and foxtail millet arrives with the Yangshao Neolithic. ⑤ The site is abandoned again.

document extends further back, to the very beginning of Phase 1. Either way, it was very short-lived: there is little evidence of human occupation at Dadiwan, or anywhere else in the western Loess Plateau, after 7200 calBP.

The much more intensive farming system that appears when Dadiwan was reoccupied in Phase 2 features both broomcorn and foxtail millet (21, 22), camp-fed dogs and, for the first time, pigs dependent on a camp diet of millet and extra meat (Fig. 2*B*). All Phase 2 dogs ($n = 6$) display the typical camp diet (high $\delta^{13}C$ and $\delta^{15}N$), suggesting dogs had been wholly drawn into the domestic farming sphere (Fig. 2*B*). Phase 2 pigs show a similar isotopic pattern, with high $\delta^{13}C$ and $\delta^{15}N$ values appearing for the first time ≈5800 calBP, suggesting their domestication sometime between 7200 and 5800 calBP. Some Phase 2 pigs ($n = 4$), however, have low $\delta^{15}N$ and $\delta^{13}C$ values, showing they foraged and were presumably taken in the wild (Fig. 2*B*). These and several individuals with intermediate $\delta^{13}C$ and $\delta^{15}N$ values representing either wild animals that raided millet fields or free-range animals occasionally provisioned with millet and meat demonstrate quite clearly that pigs had not as yet been as fully incorporated into the domestic farming sphere as dogs. As with most of the phase 2 pigs ($n = 25$) and all of the Phase 2 dogs ($n = 6$), Phase 2 humans ($n = 6$) also have high $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 3) suggesting the same heavy reliance on millet and domestic animals seen elsewhere in the Yangshao Neolithic culture area (29–31). As a result, a $\delta^{13}C$ and $\delta^{15}N$ biplot for all specimens from both Dadiwan phases ($n = 74$) shows the expected trajectory of millet domestication and farming in northern China; humans, dogs, and pigs with high $\delta^{13}C$ also had higher $\delta^{15}N$ values, suggesting that when they ate more millet they also ate more meat (Figs. 2*B* and 3). Together, $\delta^{13}C$ and $\delta^{15}N$ illustrate the strength of the relationship between people, plants, and animals, providing a stand-alone index of domestication, against which the morphological and molecular attributes of plant or animal domestication can be compared. A summed probability distribution (36) of all known Holocene radiocarbon dates from the Dadiwan site (Table S3) provides a chronology of occupation and food production in the western Loess Plateau (Fig. 4).

Implications

That only dogs were provisioned with meat and millet in Phase 1, and pigs only later, in Phase 2, suggests that broomcorn millet was initially targeted in an economy that emphasized hunting.
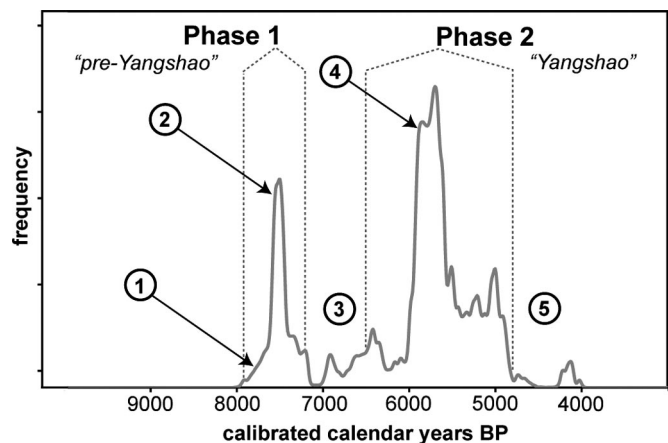
This idea is consistent with the Phase 1 archaeological record, notably the abundant remains of large wild animals (red deer, sika, musk deer, and pig), makeshift dwellings, impoverished middens, and sparse archaeological assemblages (22), suggesting intermittent occupation and a continuing need for regular moves between seasonal camps. Millet farming seems to have made a fairly small contribution to this economy. Apart from a few carbonized seeds (21, 22), dogs provide the only concrete evidence of its contribution, and the Phase 1 archaeological assemblage and its mixture of wild and camp-fed dogs indicates occasional, pragmatic, and opportunistic food production, resembling the "low-level" food production that so frequently characterizes agricultural origins in various independent centers around the world (37).

The early Holocene climate of northwest China was episodically harsh enough (38, 39) to encourage short-term use of low-return plants by hunter–gatherers living in north China's more marginal environments. Severe cold-dry intervals like the hemispheric climatic anomaly at 8,200 calBP (40) may have forced some living in the deserts on either side of the upper Huang He to move southward into the western Loess Plateau (17). Evidence for this is particularly compelling at Dadiwan. Phase 1 microblades and microblade cores made from exotic raw materials are entirely without precedent in local lithic technology, suggesting that the earliest food producers at Dadiwan were immigrant hunter–gatherers from the arid north where this microlithic technology is common (41, 42).

Given the long coassociation between humans and dogs in northern Eurasia (43), dogs were likely essential to the hunting system of these early millet farmers, and their experiments with quick-growing, storable plants were probably motivated by the need to provision their dogs as much as themselves whenever more traditional resources were scarce. Because *Panicum* grows faster and is more cold- and drought-tolerant than other candidate crops (44), it is an ideal crop for mobile hunter–gatherers attracted to flexible resources requiring minimal investments, and limited delays. We suggest this complementarity both enabled and promoted numerous independent experiments with *Panicum*-based food production across northern China and, perhaps more generally, throughout Eurasia (45, 46). Although climate in the deserts north of the Loess Plateau would have held millet productivity in check, it likely flourished when exploited

in settings like the upper Huang He near Dadiwan, where summer monsoon rains were more reliable than in the desert north. That even these very modest experiments were relatively short-lived again suggests a connection with hunting; food production was too costly and inhibited mobility too much for hunters except during short periods of extreme hardship, a short-term solution to short-term deficits within a generally stable economy of hunting and gathering.

By contrast, the millet farming system that appears when Dadiwan is reoccupied in Phase 2 is full time, intensive, and attended by the classic Yangshao cultural package: hard-fired pottery, square houses, and moat-enclosed village plan (22). The remains of wild game (including pigs with low $\delta^{13}C$ and $\delta^{15}N$ values) show that hunting persists well into the late Neolithic, but Dadiwan's Phase 2 occupants were clearly farmers heavily dependent on millet and the animals provisioned with it, living a sedentary lifestyle that entailed stable annual cycles of field preparation, cultivation, harvest, processing, storage, and protection. This Phase 2 domestic agricultural system is clearly part of the Yangshao Neolithic of the Huang He drainage (2, 4, 29–31). Equally clear is that it does not originate at Dadiwan. Foxtail millet, the featured Yangshao species, was not a part of Dadiwan's Phase 1 system, and although it was the species of choice in other early millet farming locations (e.g., Peiligang and Cishan), Yangshao did not arise from any of these but from another, as-yet-unidentified, early farming complex.

In combination with those previously available, the Dadiwan data presented here illustrate adaptive programs that developed independently around 2 different plants, in what were almost certainly quite different subsistence-settlement systems across an area of >1 million km^2. All of the early millet farming systems so far documented, including Dadiwan, were short-lived and none is the source of the Yangshao Neolithic system that would in very short order replace all of the early, low-level millet farming systems of the Huang He drainage. This replacement was likely quick and easy because economies based on mobile hunting support only small populations with little ability to defend the large territories required to maintain them. When larger groups require less land to support themselves (as expected under agricultural food production) they invade easily and expend less to hold their ground.

The key to explaining the rapid diffusion of agriculture in East Asia is in identifying those places where larger groups capable of territorial maintenance emerge and in discussing why they expand. If agricultural food production is central to this expansion, methods for establishing the timing and intensity of the stable interactions between people, plants, and animals are essential for tracking it. The methodology illustrated here provides for this, and with it we can begin to evaluate the competing hypotheses for the origins of agriculture in East Asia.

Methods

For $\delta^{13}C$ and $\delta^{15}N$ analysis, a ≈100-mg sample of compact bone was removed from each specimen with a low-speed cutting tool. Bone fragments were cleaned of sediment and demineralized in 0.5 N hydrochloric acid (HCl) for ≈12–15 h at 5 °C. The resulting material was treated repeatedly with a chloroform/methanol (2:1) mixture to remove lipids and then lyophilized. Dried samples (≈0.5 mg) were sealed in tin boats and analyzed with a Carlo-Erba elemental analyzer (NC 2500) interfaced with a Finnegan Delta Plus XL mass spectrometer (Carnegie Institution of Washington).

Isotopic results are expressed as δ values, $\delta^{13}C$ or $\delta^{15}n = 1,000 \times [(R_{sample}/R_{standard}) - 1]$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone for carbon and atmospheric N_2 for nitrogen. The units are expressed as parts per thousand or per mil (‰). The within-run standard deviation of an acetalinide standard was ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. As a control for the quality of bone collagen, we measured the carbon-to-nitrogen ([C]/[N]) ratios of each sample to test the possibility that isotopic values were altered postmortem. The atomic C/N ratios of all bone collagen samples are 2.9–3.4 (Table S1), well within the range that characterizes unaltered collagen (47). Duplicate isotopic measurements were performed at the Center for Arid Environment and Paleoclimate Research at Lanzhou University on ≈90% of all unknown samples, yielding a mean absolute difference of 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ values.

Human and animal bone collagen samples were prepared for accelerator mass spectrometry radiocarbon analysis (48) at the Center for Accelerator Mass Spectrometry of Lawrence Livermore National Laboratory. Raw bone samples were crushed and demineralized in 0.5 N HCl at 5 °C for ≈12–16 h. The resulting organic matter was twice rinsed with deionized H_2O, and gelatinized in 0.01 N HCl at ≈60 °C for ≈16 h. These gelatinized samples were filtered with glass microfiber filters, then ultrafiltered with centrifugal filters to remove low molecular weight (≈30 kDa) fragments, then vacuum-concentrated. Collagen was then combusted to CO_2 and graphitized for accelerator mass spectrometry. All dates reported are in calBP. All radiocarbon age estimates on bone are based on a 5,568 half-life, are $\delta^{13}C$-corrected, include a background subtraction based on similarly and simultaneously-prepared ^{14}C-free bone, and have been calibrated to 2σ range by using OxCal 4.0 (49) and the INTCAL 04 calibration curve (50) (Table S2).

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