



Article

Ancient Genome of Broomcorn Millet from Northwest China in Seventh Century CE: Shedding New Light to Its Origin and Dispersal Patterns

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Abstract: Broomcorn millet (*Panicum miliaceum*) is among the earliest domesticated staple crops in the world's agricultural history and facilitated the development of several early agrarian cultures, particularly those originating in northern China. However, the propagation route of broomcorn millet in China from the Middle Ages to the present remains unclear. The aim of this study is to explore the genetic affinity between ancient and modern millet samples, trace the genetic origins and diffusion pathways of broomcorn millet, and provide insights into its domestication and spread. To achieve this, we sequence ancient DNA from broomcorn millet remains excavated from the Chashan Village cemetery (AD 691) in Gansu Province, China. Phylogenetic and population genetic analyses, integrating ancient and modern millet genomes, reveal a close genetic relationship between ancient millet and contemporary millet from Ningxia Province (445 km away from Chashan Village), suggesting a potential origin for the Chashan millet. This finding aligns with the tomb's epitaph, which documents the reburial of the tomb's owner, who was originally buried in Ningxia, and provides important archaeological evidence for understanding the interaction between geopolitical dynamics and the natural environment in northwest China during the late seventh century. Furthermore, outgroup-*f*₃ and *D* statistics evidence suggests substantial genetic interactions between ancient millet and modern varieties from the Loess Plateau, Huang-Huai-Hai Plain, and Northeast Plain, indicating the dispersal route of broomcorn millet, along with human migration routes, from the northwest to northern China and ultimately to the northeast region, starting from the Middle Ages onward. This study enhances our understanding of millet's genetic history, offers a novel perspective on burial archaeology, and provides valuable insights into the origins, domestication, and diffusion of broomcorn millet.

Keywords: ancient DNA; broomcorn millet; genetic diversity

1. Introduction

The origin and dissemination of agriculture provided a stable economic foundation for the development of human society and the establishment of ancient civilization [1]. China is one of the three independent centers of agricultural origin for crops in the world [2,3]. Broomcorn millet (*Panicum miliaceum*) is one of the oldest domesticated cultivated grains in East Asia, and it was one of the most important crops in the arid regions of northern China in prehistoric times. The analysis of charred plant remains and microfossils from sites such as Donghulin, Nanzhuangtou, and Shizitan about 10,000 years ago shows that millet had been domesticated in northern China [3,4]. During the Pre-yangshao period (8500–7000 BP), charred millet seeds were commonly found at sites such as Peiligang, Dadiwan, and Baijia in the Yellow River Basin. These plant archaeological discoveries indicate that millet agriculture was initially established in northern China around 7000 years ago. The middle and late period of the Yangshao culture (6000–5000 BP) experienced rapid development and extensive expansion, promoting the spread of millet crops in the Yellow River Basin to surrounding areas. Millet farming spread westward with the expansion of the Neolithic culture to the Hehuang region on the northeast edge of the Qinghai–Tibet Plateau in 5200 BP [1]. From around 5000 to 4500 BP, millet crops expanded westward with the Majiayao culture to the Hexi Corridor region [5]. During 4500–300 BP, domesticated crops and livestock from the east and west sides of the Eurasian continent spread widely with the spread of populations from their origin centers. Millet spread to Central Asia and Europe, while wheat and barley from West Asia were introduced to northwest China and widely utilized [6,7]. Archaeological studies have shown that the Hexi Corridor region played an important role in the process of cross-cultural exchange between the Eurasian continent during the prehistoric and historical periods [8,9]. It is a key area for studying the history of East-West exchange during prehistoric and historical periods [10]. Existing research mainly focuses on the prehistoric stage, while studies on crop dissemination during historical periods, especially after the Middle Ages, are relatively limited.

With the continuous development of sequencing technologies, various studies have been conducted to understand the genetic diversity and population structure of broomcorn millet [11]. In 2019, the reference genome of broomcorn millet was assembled for the first time. The genome size is approximately 923 Mb and includes 55,930 protein-coding genes and 339 microRNA genes [12]. This genome assembly provides a solid foundation for understanding the genetic basis of its adaptability and nutritional characteristics. Xu et al. used SLAF-Seq technology to sequence 106 millet samples and found significant genetic differences between cultivated and wild millet, suggesting that cultivated millet may have experienced a genetic bottleneck effect during domestication [13]. They proposed two possible dispersal routes: the “Oasis Route” and the “Steppe Route.” Chen et al. constructed the largest genetic dataset of millet to date, sequencing 516 samples and de novo assembling 32 millet genomes. Using genome-wide association studies, they identified 139 loci associated with 31 key domestication and agronomic traits [14]. Currently, population genetics research on broomcorn millet mainly focuses on modern samples, but studying ancient samples is equally essential for understanding the genetic history of millet.

The rapid advancements in ancient DNA sequencing technology have provided us with a more comprehensive and in-depth perspective for understanding the genetic evolution of different species [15,16]. These technological advances not only enhance our understanding of the history of biodiversity but also help uncover the evolution of various cultural and agricultural practices [17,18]. For example, a study on ancient grape seeds revealed the origins of grapes’ genetic diversity. The DNA analysis of these ancient grape seeds not only mapped the genetic variation of grapes but also confirmed how humans in the Middle Ages used clonal propagation techniques, such as grafting, to maintain and enhance specific grape varietal traits [19]. This finding reflects the utilization and improvement of natural resources by humans, demonstrating the close interaction between humans and nature. Additionally, a study by Wu et al. analyzed ancient DNA from bread wheat dating back approximately 3500 years in Xinjiang, China, providing

new insights into the spread and settlement of this crop in China. The results revealed the process by which bread wheat migrated from the West Asia region to China through multiple routes [20]. This information is crucial for understanding the expansion of early agricultural societies and the adaptive selection of crops. In this way, ancient DNA research not only reconstructs the biogeographical history of the past but also promotes a deeper understanding of the social structure and ecological interactions of ancient human societies. Research on ancient DNA allows us to better understand the evolutionary processes and genetic diversity of ancient organisms. For example, studying ancient human DNA can effectively explain the origins and migration patterns of modern humans [21]. Ancient DNA can reveal changes in historical populations, which may be related to climate change, environmental shifts, or human activities [22]. This helps us understand how past environments influenced biodiversity. Ancient DNA can also elucidate genetic changes during domestication, revealing the multi-stage process of domestication. Currently, published ancient DNA studies on millet mainly analyze representative sequences within the millet genome, lacking whole-genome data research. For instance, Stephen M. Richards et al. reconstructed the chloroplast sequence of millet from the Areni-1 archaeological cave site in Southern Armenia [23]. Xu et al. analyzed ribosomal DNA (rDNA) internal transcribed spacer (ITS) and external transcribed spacer (ETS) sequences of *Panicum miliaceum* remains excavated from the Xiaohe cemetery in comparison to modern landraces [24]. Whole-genome analysis can provide more comprehensive genetic information, aiding in achieving a full understanding of the genetic characteristics and evolutionary history of organisms. Therefore, it is essential to conduct whole-genome data analysis on the ancient DNA of millet.

However, current ancient DNA research mainly focuses on ancient humans and large animals, with few studies on ancient plants, primarily due to the lack of well-preserved fossils. The excavation of the Chashan Village site provides crucial archaeological materials for addressing this issue [17]. In 2019, the excavation of Tang Dynasty tombs at Chashan Village in Gansu Province, Northwestern China, uncovered nine granary bags, with bags 5, 7, and 9 containing a total of 81,901 grains of uncharred millet [25]. The primary objectives of this study are to investigate the genetic relationship between ancient millet samples from the Chashan Village tomb and modern millet varieties, and to trace the origins and diffusion patterns of broomcorn millet across China since the Middle Ages. By examining these genetic relationships, this research clarifies millet domestication, migration routes, and the broader genetic history of the crop. We sequence DNA from millet samples found in the Chashan Village tomb and integrate these data with modern datasets. Phylogenetic and principal component analyses reveal a close genetic relationship between ancient and modern millet from Ningxia. Further population structure and gene flow analyses traced the diffusion routes of millet across China after the Middle Ages. Additionally, this study provides critical archaeological evidence linking millet remains to the documented reburial of the tomb owner, who was relocated from Ningxia due to geopolitical factors, suggesting a shared genetic ancestry between the millet from Chashan and modern samples from Ningxia. This offers a novel perspective on the interaction between geopolitical and environmental dynamics in historical China. This study enhances our understanding of millet's genetic history, offers a novel perspective on burial archaeology, and provides valuable insights into the origins, domestication, and diffusion of broomcorn millet.

2. Study Area

The Chashan Village tomb site (102°22′54.3″E, 37°40′51.7″N) is located atop a mountain in Chashan Village, Qilian Town, Tianzhu Tibetan Autonomous County, Wuwei City, Gansu Province (Figure 1a,b). In 2019, the Gansu Provincial Institute of Cultural Relics and Archaeology conducted a rescue excavation at this site. The tomb is exquisitely constructed and undisturbed by looting. It features a vertical earthen pit with a long sloping tomb passage and a single brick chamber built within the pit. The coffin bed is on the west side,

containing a wooden coffin oriented north–south. To the north of the coffin, there are nine silk bags (grain sacks) containing a large number of mixed plant seeds (Figure 1c). Among these, three damaged bags contained a total of 81,901 millet seeds (Figure 1e). Additionally, a variety of burial items were unearthed, including exquisite pottery, metal vessels, lacquerware, stone tools, silk fabrics, and leather goods.

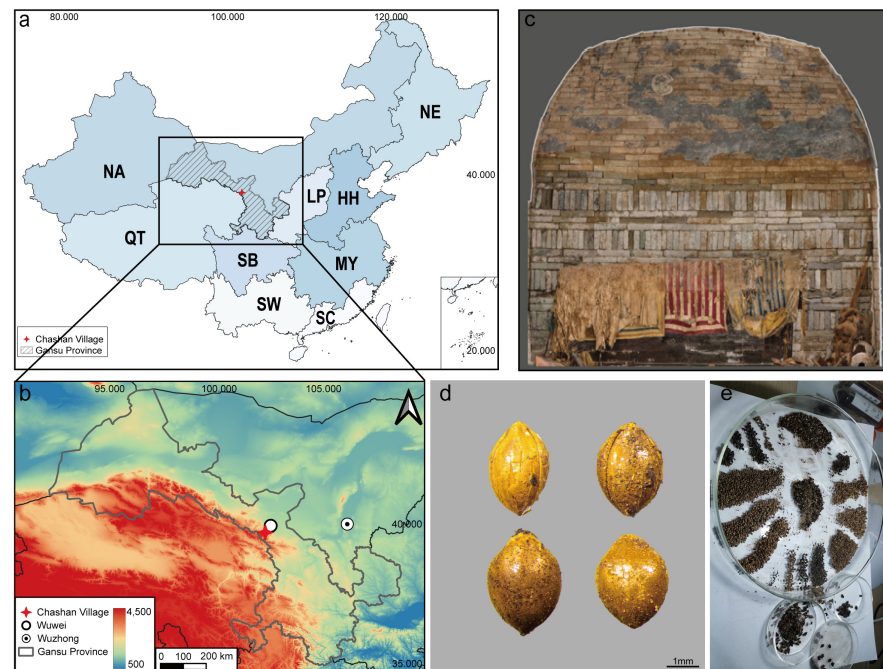


Figure 1. (a) The 9 divisions of agricultural regions in China, including the Northeast China Plain (NE), Southwest Yunnan–Guizhou Plateau (SW), Northern arid and semiarid region (NA), Southern China (SC), Sichuan Basin and surrounding regions (SB), Middle–Lower Yangtze Plain (M), Qinghai–Tibet Plateau (QT), Loess Plateau (LP), and Huang-Huai-Hai Plain (HH). (b) The location of the Chashan Village burial site. (c) West side of the tomb chamber. (d) Ultra-microscopic photos of broomcorn millet plant remains. (e) Plant remains collected from all the torn silk bags.

According to the epitaph, the tomb owner was Murong Zhi, a member of the Tuyuhun royal family, who died in Lingzhou (Ningxia Wuzhong, 445 km away from Chashan Village) in March of the second year of Tang Tianzhou (691 AD) and was reburied in the Great Khan Tomb (present-day Wuwei, Gansu) in September of the same year [26]. The tomb is located in the northern arid and semiarid region [27] at an altitude of 2672 m, with an annual average temperature and precipitation of 1.6 °C and 200–400 mm [25], respectively. The high altitude and arid environment provided favorable conditions for the preservation of plant remains, resulting in well-preserved, uncharred seeds, making them highly suitable for ancient DNA study.

3. Materials and Methods

The mixed plant seeds from the broken silk bags (wugunang, 五谷囊) in the coffin chamber were sent to the MOE Key Laboratory of Western China's Environmental System, College of Earth and Environmental Sciences, Lanzhou University. There, we classified these mixed plant seeds based on their morphological characteristics and compared them with various illustrated identification keys. All 81,901 broomcorn millet seeds were selected, and representative samples were further chosen and photographed using a stereomicroscope for high-precision identification (Figure 1d). Given the relatively small size of the millet grains (ranging from 3.06 mm to 3.31 mm), we mixed every 10 grains for DNA extraction and sequencing. A total of 5 samples were studied, labeled AN1, AN2, AN3, AN4, and AN5. To avoid contamination with exogenous DNA during the ancient

plant DNA extraction, the experiment was carried out in a dedicated paleoethnobotany lab at the Institute of Archaeological Science, Fudan University, utilizing a modified N-phenacylthiazolium bromide (PTB) column-based method [20].

The extraction protocol encompassed 5 key stages: soaking, lysis, DNA isolation, sequencing library preparation, and DNA sequencing. Initially, to dissolve and remove some soluble impurities from the surface, the seeds were soaked in 5% sodium hypochlorite solution for 10 min, followed by 2 times washing with 50% ethanol solution, and subsequently air-dried overnight and exposed to ultraviolet (UV) light within a controlled clean-room environment. For complete lysis, some 3 mm steel balls were added into a 2 mL centrifuge tube containing seeds, quickly frozen with liquid nitrogen, and then ground into powder. Then, 1.2 mL of PTB buffer (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was added to each tube and the PTB—seed powder mix was then incubated on a shaker for continuous mixing at 37 °C with a speed of 150 rpm under dark conditions for 18–24 h to facilitate lysis. The tube was then centrifuged at 14,000 rpm for 10 min. The supernatant was carefully collected and purified using a Zymo-Spin V column (Zymo Research Corporation, Irvine, CA, USA) following the default manufacturer's protocol. Further purification using a Qiagen Mini spin column (Qiagen, Hilden, Germany) was thereafter applied following the manufacturer's instruction, with eluting 50 uL final DNA extract for each tube. The DNA concentrations were measured using a fluorometer. For library preparation, we followed the protocol established by Meyer and Kircher [28] to construct libraries compatible with Illumina (Illumina Inc., San Diego, CA, USA) NovaSeq sequencing. DNA sequencing was conducted on the Illumina NovaSeq 6000 platform using PE 150 mode by Mingma (Shanghai) Biotechnology Co., Ltd., Shanghai, China.

4. Bioinformatics and Statistics

To enable genetic analysis of the population genetic patterns and evolutionary processes of ancient millet samples, we built a comprehensive reference database consisting of 225 modern broomcorn millet genomes (details of the reference database can be found in Supplementary Table S1). The database comprised 141 genomes sampled from China and 45 genomes representing the global genetic diversity. To contextualize the analysis within a broader phylogenetic framework, 39 wild broomcorn millet genomes were further added.

For the ancient sequencing data, we utilized AdapterRemoval v2 [29] to eliminate adapter sequences while simultaneously removing low-quality bases and merging overlapping reads (parameters: --trimns, --trimqualities, --mm 3, --collapse, --minalignmentlength 11, --minlength 30). Fastp 0.23.4 [30] was employed to remove duplicate sequences, poly-G tails, and reads of low complexity (parameters: --low_complexity_filter, --dedup, --dup_calc_accuracy 3, --trim_poly_g, --poly_g_min_len 6).

To reduce the potential false-positive matches, we excluded scaffold sequences from the reference genome (BC332, GCA_032594955.1), retaining only the chromosome reads, and aligned the quality-controlled ancient DNA sequences to the processed reference genome using bwa aln and samse [31]. MapDamage (version: 2.3.0a0-5175c20) [32] was used to analyze the DNA damage patterns, particularly for quantifying the deamination damage models.

For the modern millet sequencing data, the bwa mem [33] was used for mapping sequences to the reference sequence. All the generated SAM files were sorted using samtools [34] and converted into BAM format. Picard MarkDuplicates (version 3.1.1) [35] was then applied to remove any alignment duplicates. To assess the mapping quality of the ancient samples, we employed qualimap [36] for statistical analysis of the BAM files.

Before SNP calling, we used bamUtil (version 1.0.15) [37] to remove 3 bases from both ends of the reads in the BAM files to avoid the influence of DNA damage on subsequent analyses. Both the ancient and modern data were executed using samtools mpileup. The resultant vcf files were subsequently organized with BCFtools 1.19 [34] and filtered through VCFtools 0.1.16(parameters: --max-missing 0.80 --minDP 3 --maf 0.05 --mac 3 --minQ 30

--min-alleles 2 --max-alleles 2) [38]. Visualization of the SNP density distribution was accomplished via the CMplot R package (version 4.5.1) [39].

Neighbor-Joining (NJ) phylogenetic trees were constructed based on the p-distance matrices using the VCF2dis [40] and PHYLIP(version 3.69) [41] packages. The resulting phylogenetic trees were further refined and visually enhanced using iTOL (<https://itol.embl.de/>, accessed on 1 August 2024) to incorporate grouping information and color strips. Principal component analysis (PCA) was performed utilizing PLINK (version 1.90b6.21) based on the calculation of the genetic covariance matrices and visualized through the ggplot2 R package (version 3.5.1). Analysis of the population genetic structure was conducted using the Admixture program [42]. Here, we used PLINK to remove SNPs in strong linkage disequilibrium, employing a window of 50 SNPs advanced by 10 SNPs and with an r^2 threshold of 0.1 and visualized through TBtools (version 2.102). The Outgroup-f3 and D statistics were produced using the admixtools [43] R package (version 2.0.0), which allowed for the evaluation of the shared genetic drift and gene flow between populations, providing insights into historical admixture events and genetic relationships between ancient and modern millet samples.

5. Results

5.1. Ancient Dataset

In this study, we extracted DNA from a total of five ancient millet samples, with each sample comprising a mixture of 10 seeds. The average genome coverage of the different samples ranged from 0.94% to 13.35%, and the endogenous proportion ranged from 0.012% to 0.04% (Supplementary Table S2). Ancient DNA is generally highly degraded. Investigation into the DNA fragmentation and deamination damage patterns therefore enables the authentication of the ancient origination of the sequenced DNA. In all five ancient samples, the average DNA sequence lengths were 46.84, 50.86, 53.07, 50.82, and 48.78, respectively (Supplementary Figure S1), consistent with the characteristics of fragmented DNA damage. The frequency of C to T substitutions at the 5' end and G to A substitutions at the 3' end also increased when moving toward the reads' ends (Supplementary Figure S2). Both these features align with the regular fragmentation patterns of ancient DNA, confirming the authenticity of the ancient data and subsequent analysis.

5.2. Closest Genetic Affinity of Ancient Samples with Modern Samples from Ningxia

To understand the systematic evolutionary relationship between ancient millet seeds and modern seeds, we collected a total of 225 whole-genome sequencing data from public databases (Figure 2a), which were divided into Chinese wild types, Chinese domesticated types, and Eurasian types. By performing SNP calling, we obtained VCF files for a total of 230 samples, including ancient ones, and calculated the genetic distances between the samples to construct an NJ tree (Figure 2b). In the phylogenetic tree, we observed distinct clustering of the samples from each group, so we divided the tree into five clades. Clade I primarily consists of Eurasian millet samples, including a few from western China, such as Xinjiang and Tibet, which are geographically closer to Central Asia. Clade II mainly comprises Chinese wild samples. Clades III to V are all Chinese domesticated types, with ancient samples located in Clade III. By considering the regional varieties and germplasm within each clade and their geographic origins, we further encoded the regions within the clades. We found that the ancient samples are most closely related to millet from the arid and semi-arid regions of Ningxia Province in northwest China (NA16-Ningxia, NA18-Ningxia, NA19-Ningxia, NA11-Ningxia, NA12-Ningxia, NA20-Ningxia). Clades IV and V reflect significant differentiation within Chinese landraces, with Clade IV mainly consisting of millet from arid and semi-arid regions, the Loess Plateau, and the Qinghai-Tibet Plateau, and it is more closely related to Clade III. Clade V is mainly composed of millet from the Huang-Huai-Hai region, South China, and the Northeast Plain, and is genetically more distant from the ancient samples.

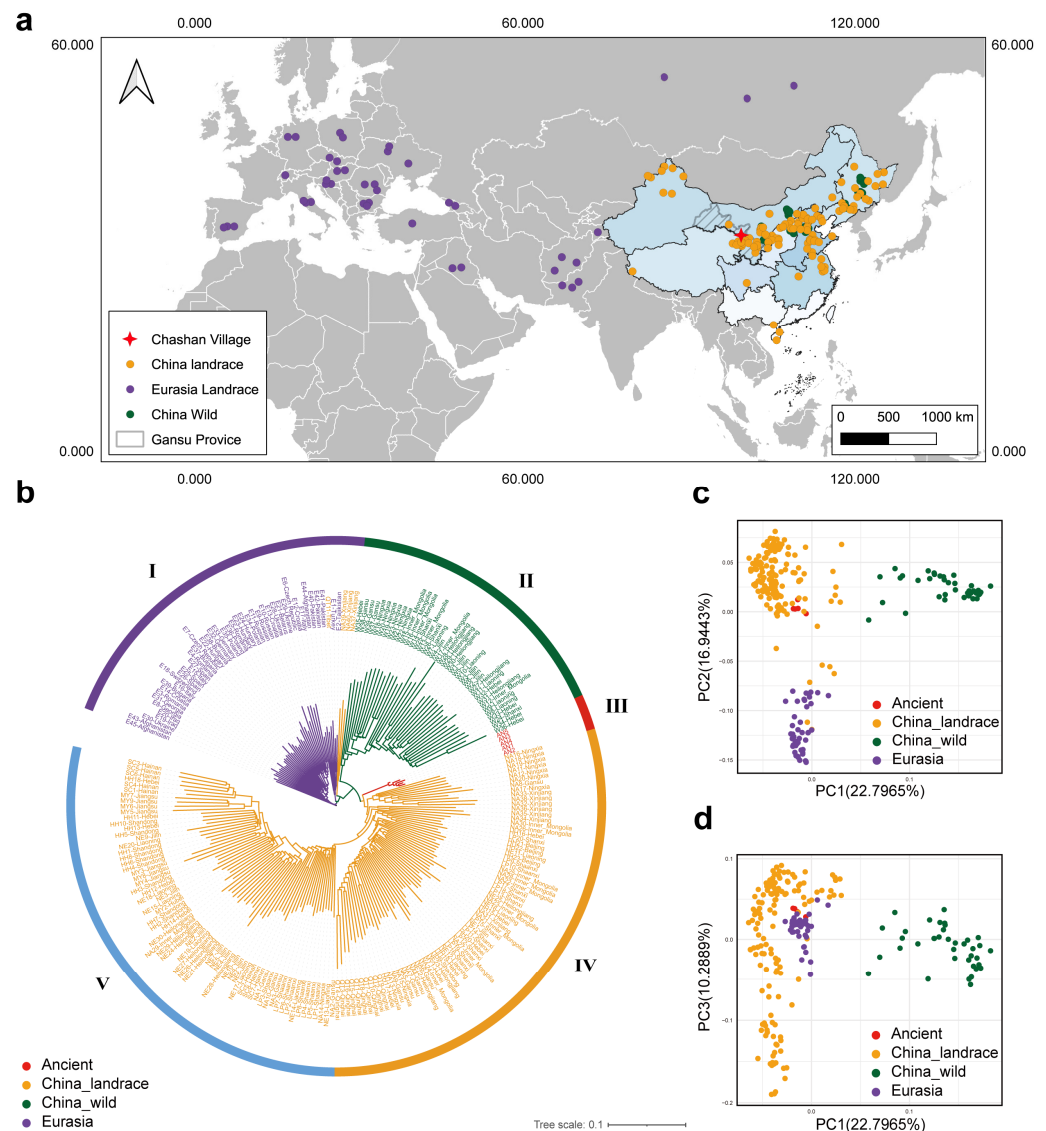


Figure 2. (a) Ancient and modern sample locations. (b) The NJ phylogenetic tree of all the samples based on the p-distance. (c,d) PCA clustering of all the samples.

To better clarify the genetic relationship between the ancient and modern samples, we performed a principal component analysis. As shown in Figure 2c, all 230 samples are divided into three groups, with the first principal component (PC1, 22.7965%) distinguishing Eurasian types, Chinese landraces, ancient Chinese types, and Chinese wild types, while the second principal component (PC2, 16.9443%) separates Eurasian types from the other three groups. We can see that ancient samples are closely related to modern Chinese landraces. In Figure 2d, the third principal component (PC3, 10.2889%) shows clear differentiation among Chinese landrace groups, and these results are highly consistent with the phylogenetic tree results.

Admixture analysis is employed to quantify the genetic admixture levels within individuals or populations. In the field of population genetics, this method is predominantly utilized to investigate the genetic interchanges among disparate groups. In the current study, the PLINK software was employed for the filtration of linkage disequilibrium in the VCF files, thereby establishing an independent core dataset for each SNP locus (Figure S3). Notably, at $K = 5$, the model demonstrated minimal cross-validation error (Figure S4), indicating optimal explanatory power regarding the genetic structure of the populations under study. At this clustering parameter, distinctive genetic structures were observed: the Chinese wild species W and the Eurasian continental species exhibited predominantly yellow

and pink regions, respectively (Figure 3). Regions such as HH, MY, SC, and NE displayed a deep green genetic composition, distinguishing these populations from other Chinese landrace subgroups and suggesting unique genetic signatures. The ancient samples predominantly exhibited a light green genetic structure, which was extensively preserved in the NA, LP, and NE groups. The genetic configuration of these ancient samples exhibited strong concordance with certain contemporary samples within the NA group, implying shared genetic ancestry or sustained genetic continuity between the ancient specimens and these modern counterparts.

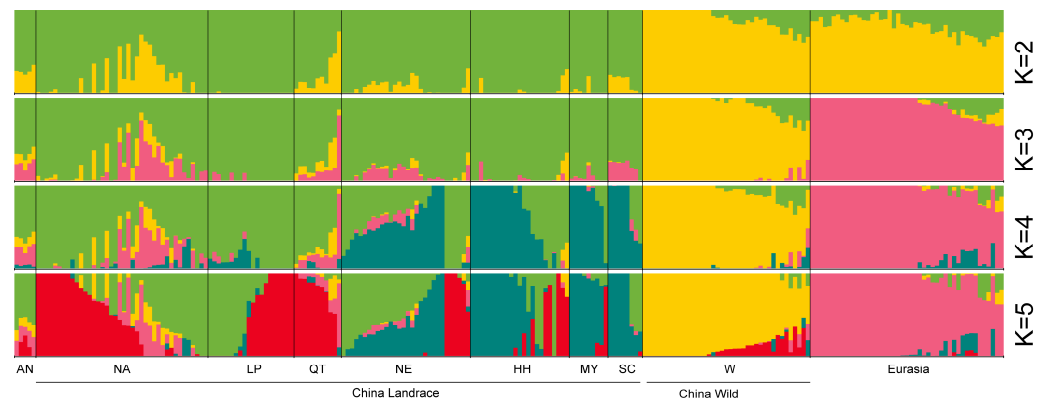


Figure 3. Population structure of ancient broomcorn millet and modern samples based on the admixture analysis.

5.3. Gene Flow from Ancient to Modern Samples

Outgroup-f3 analysis allows for the comparison of genetic similarities between different populations and is instrumental in understanding the genetic evolutionary history of a species. Here, we used Eurasian broomcorn millet, which has the greatest genetic distance from millet, as the outgroup. By comparing the Outgroup-f3 statistics of ancient samples with a modern millet panel, we found that modern samples from the Loess Plateau show the closest genetic link to ancient millet. Next are the arid and semi-arid regions, the Huang-Huai-Hai Plain area, and the Northeast Plain area. The arid and semi-arid regions cover a large area, including provinces such as Inner Mongolia, Ningxia, and Xinjiang. The Outgroup-f3 statistical values will be influenced by samples from more distant areas (Figure 4a).

To further reveal the patterns of gene flow and interaction among different populations, we customized a topological structure (pop1, pop2, ancient group, outgroup) to produce D-statistics based on autosomal genetics variants. This method enabled us to comprehend the extent of the gene exchange between different groups. To ensure the reliability and statistical significance of the results, instances where the Z-value exceeded 3 were considered to indicate substantial biological importance, as shown in Figure 4b.

Through this precise analysis, the results of the D-statistics have revealed a series of striking findings. Among the most notable is the significant shared gene flow between the ancient samples and the modern broomcorn millet from the Loess Plateau, suggesting that these two groups may have had close genetic ties historically. Additionally, gene flow is observed between the ancient samples and the modern foxtail millet samples from the Huang-Huai-Hai Plain and the Northeast Plain. However, it is noteworthy that no significant gene flow is evident between millet from South China and the middle and lower reaches of the Yangtze River and the ancient samples, while only weak gene flow exists between millet from the Tibetan Plateau and the ancient samples.

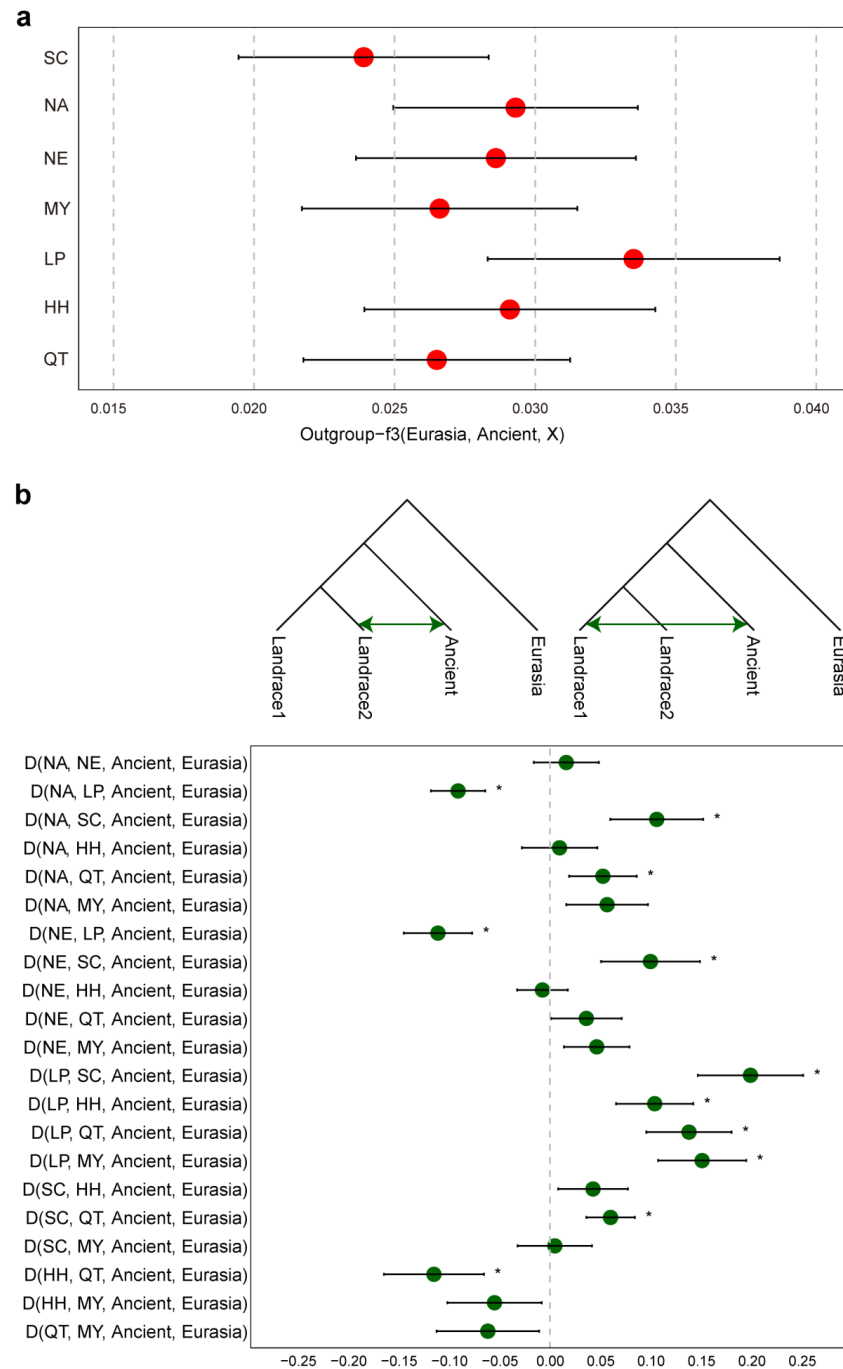


Figure 4. (a) Outgroup-f3 statistics for different millet populations. (b) D-statistic for different millet populations. All results that reach statistical significance are indicated by asterisks (corresponding $|Z| > 3$).

6. Discussion

Broomcorn millet is one of China's ancient native crops, with a cultivation history dating back over eight thousand years [44]. In China's traditional agricultural "Five Grains (wugu)", millet held a significant place, especially during the Shang and Zhou dynasties when it was a staple food [45]. Around 5000 years before the present (BP), wheat was introduced to China, gradually spreading from the northwest to the Central Plains and further promoted across a wider region during the Han Dynasty [46], replacing some existing crops, including broomcorn millet. After the Tang Dynasty, millet cultivation was gradually replaced by other crops. In the current archaeobotanical research, studies of millet after

the Middle Ages are rare, and research on ancient millet DNA is even more scarce [23,47]. Ancient DNA research can provide valuable background information on the genetics and evolution of crops. This study, through ancient DNA analysis of uncharred millet from the graves in Chashan Village, preliminarily inferred the kinship between modern representative local millet varieties and ancient millet from the graves, estimated the similarity in the population structure and allelic composition between modern and ancient samples, assessed the potential inter-population admixture, and inferred the potential dissemination pathways of millet cultivation in post-Tang Dynasty China. There are also some interesting aspects of our results that need to be addressed. Our analysis shows that the genetic relationship between China_wild and China_landrace is closer than that with the Eurasian samples. Despite the extensive geographic distribution of the Eurasian samples, their genetic diversity is lower than that of the China_landrace samples. We observed a similar pattern in our previous studies [13]. We hypothesize that this is likely due to broomcorn millet being originally domesticated in China [48] and subsequently transferred to other regions. Consequently, China preserves most of its genetic diversity. Additionally, gene flows between wild and domesticated broomcorn millet at various domestication stages may have introduced further genetic diversity. Notably, several of the Chinese landrace samples cluster with the Eurasian group, all of the individuals that were from Tibet and Xinjiang, which mark the border of China and Central Asia. This observation therefore suggests the broomcorn millet individuals sequenced in these regions are genetically closer related to the lineages that were later distributed from northern China, rather than to the originally domesticated lineages.

According to the epitaph in the Chashan Village cemetery, the owner, Murong Zhi was a member of the Tuyuhun royal family who died of illness in 691 AD in Lingzhou, Ningxia (now Wuzhong, Ningxia), and was later reburied in Liangzhou (now Wuwei, Gansu) [26,49]. Studies on plant seeds unearthed from this gravesite showed through oxygen isotopes and trace elements that the seeds did not originate from the surrounding areas of Chashan Village [25]. Our phylogenetic research found that the ancient millet had the closest genetic distance to the modern millet from Ningxia. Combining PCA and structure evidence, the ancient samples and these modern samples shared a common genetic ancestor or long-term genetic continuity, leading us to deduce that the millet unearthed from the tomb likely originated from Ningxia and possibly moved to Gansu with Murong Zhi due to geopolitical factors.

After the Tang Dynasty, political relations and military conflicts between the Central Plains dynasties and the frontier ethnic groups could have led to the movement of populations in the frontier regions, including southward migrations from the northwest, encompassing the Huang-Huai-Hai Plain. Approximately 1300 BP, the outbreak of the An-Shi Rebellion led to a large migration of people from the northwest region to the south, especially to the middle and lower Yangtze River regions (as shown in Route 1 in Figure 5) [50,51]. During the Southern Song period, the Jin Dynasty occupied the Huang-Huai-Hai Plain and the Northeast Plain, utilizing agriculture as a basis for military expansion and gradually spreading Central Plains' agricultural tools and farming techniques to the then-backward Northeast Plain [52,53]. During the Ming Dynasty (~600 BP), there were large-scale government-led migration policies that relocated the ancestors from the Loess Plateau region to the Huang-Huai-Hai region and the middle and lower Yangtze River regions [54] (as shown in Route 2 in Figure 5). However, strict control was maintained over border regions [55], limiting large-scale population movements. In the mid-Qing Dynasty, Qing policies became more flexible and open [56,57]. From the late Qing Dynasty to the Republic of China era, a large number of people from the Huang-Huai-Hai Plain migrated to the Northeast Plain, promoting the spread of Central Plains agriculture to the northeast [58] (as shown in Route 3 in Figure 5). Ancient DNA research on millet plant remains from the Chashan Village cemetery can effectively confirm the gene flow of ancient millet to the Loess Plateau, Huang-Huai-Hai area, and Northeast Plain regions, providing a new perspective on agricultural transmission and development during historical periods.

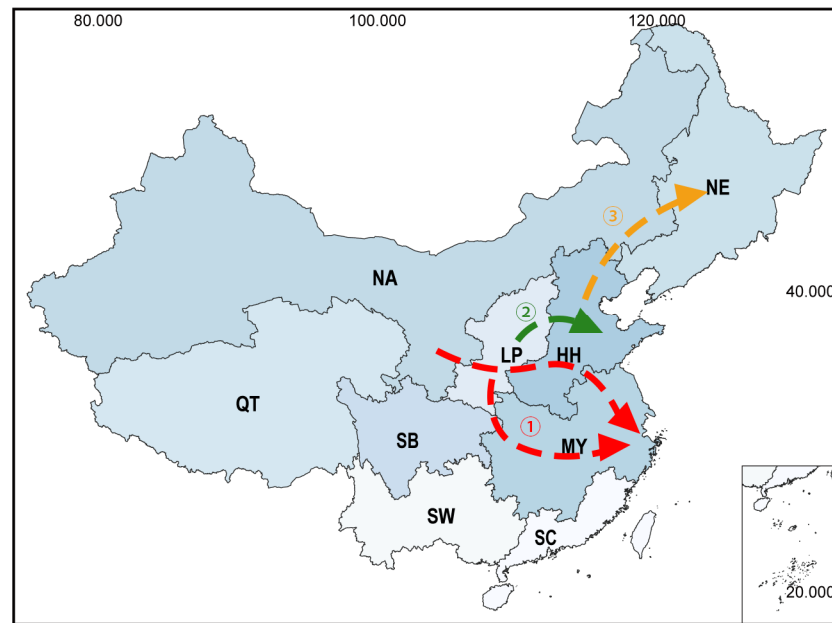


Figure 5. Historical patterns of human dispersal in China. Route 1 represents the southward migration of northern populations after the An Lushan Rebellion (~1300 BP). Route 2 traces the migration from the LP region to the HH region (~600 BP). Route 3 reflects the movement from the HH region to the NE region (~200 BP to modern times).

Ancient plant DNA research moves beyond inference to directly recover the evolutionary history of plants through the analysis of ancient genetic material [59]. This approach enables reconstruction of plant genomes across historical periods, revealing their adaptation, domestication, and dispersal. Unlike traditional methods, ancient DNA provides direct evidence of evolutionary responses to environmental pressures, climate shifts, and human influence [60,61]. This capacity to recover evolutionary trajectories allows for the identification of key genetic variations, offering deeper insights into the historical dynamics and patterns of plant evolution [62].

Analyzing ancient plant DNA from various historical periods enables the reconstruction of genetic lineages, revealing past agricultural practices and genetic exchanges. This approach extends beyond regional studies to offer a global perspective on the origins and spread of agriculture. By recovering rather than inferring evolutionary processes, we can precisely trace the diffusion of agricultural technologies, including their spread, modes, and the impacts of migration, trade, and warfare. Such research uncovers interactions between regions, demonstrating how agricultural techniques evolved globally and informing our understanding of modern agricultural systems, including crop diversity, distribution, and environmental adaptation.

Furthermore, tracking the expansion of agricultural technologies provides crucial insights into ancient agricultural origins, technological advancements, and their environmental and societal contexts. This research enhances cross-cultural comprehension of agriculture's origins and offers valuable evidence for addressing current global challenges, such as food security, crop genetic resource conservation, and sustainable agriculture.

7. Conclusions

Through population genetic studies of ancient millet seeds excavated from Chashan Village, we found that they are genetically closest to modern millet from Ningxia Province. Integrating archaeological evidence and geographical information, we infer that the millet unearthed in Chashan Village likely originated from Ningxia. There is significant gene flow between the ancient samples and modern millet from Loess Plain, Huang-Huai-Hai

Plain, and Northeast Plain, which may be associated with the migration of the Chinese population after the Tang dynasty.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14092004/s1>, Figure S1: The length plot of the ancient samples; Figure S2: The damage pattern of the ancient samples; Figure S3: The number of SNPs within a 0.1 MB window size; Figure S4: The statistics of the CV error. Table S1. The information of the modern and ancient broomcorn millet samples. Table S2. Summary of the sequence results of ancient broomcorn millet seeds. Table S3. Summary of the mapping rate of each chromosome. Table S4. Outgroup-f3 statistics for different comparisons between ancient and modern broomcorn millet. Table S5. D statistic for different comparisons between ancient broomcorn millet and modern broomcorn millet from China and Eurasia.

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