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RESEARCH ARTICLE

Ancient Genomes Reveal Population Interaction at China's North–South Boundary

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Abstract

The boundary zone between northern and southern China has long served as a key arena of cultural interaction, yet the genetic history of populations in this region remains poorly characterized. Here we present genome-wide ancient DNA data from three individuals recovered at the Wenmiao site in Xuzhou, Jiangsu Province, situated at the intersection of Shandong, Henan, Anhui, and Jiangsu. To our knowledge, these represent the first genomic data from this pivotal region. Despite the region's cultural diversity, the Wenmiao individuals show striking genetic similarity to contemporaneous and earlier populations from Shandong on the eastern coast of China, without additional signals of admixture from southern groups as well as agricultural populations from Central Plain. This genetic homogeneity contrasts with the archaeological evidence of cultural convergence, suggesting that the spread of material culture into Xuzhou did not necessarily involve substantial movement or integration of genetically distinct populations. Our findings highlight the importance of distinguishing cultural from genetic processes in reconstructing the history of interaction zones in China.

Keywords: Ancient DNA, Shang-Zhou Period, Dawenkou Culture, Population History.

1. Introduction

Recent advances in ancient DNA (aDNA) research have markedly deepened our understanding of population differentiation and admixture across China over the past 10,000 years. Genome-wide studies have revealed complex patterns in northern China: populations of the Yangshao culture in the Yellow River Basin laid the genetic foundation for later Chinese groups, yet Late Neolithic genomes show additional input from southern lineages, shaping the Longshan populations and diffusing across northern China[1,2]. In eastern coastal Shandong, early Neolithic groups displayed distinctive genetic features, while Dawenkou populations in the Late Neolithic received substantial influences from the Central Plains[3,4]. In contrast, populations from the Amur region retained strong continuity with the ancient Northeast Asian gene pool for more than 14,000 years[5]. In the northwestern frontiers, populations show remarkable genetic continuity while simultaneously reflecting complex admixture between eastern and western Eurasians[6-9]. In the south, genomes from Yunnan document deeply divergent southern East Asian ancestry (e.g., "Basal Asian Xingyi") and links to Hòabìnhian foragers of mainland Southeast Asia[10,11]. Ancient individuals from coastal Fujian reveal affinities to present-day Austronesian-speaking groups[12], while populations from Guangxi, Guizhou, and Sichuan bear signatures of multiple East Asian ancestries[11,13,14]. Collectively,

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these findings demonstrate that China’s population history was shaped by parallel developments and intricate interactions between northern and southern genetic lineages throughout prehistory and history.

Despite these advances, important gaps remain. China’s vast geography, ecological diversity, and cultural heterogeneity mean that many transitional zones between major archaeological cultures remain poorly characterized at the genomic level. Previous studies have largely focused on cultural core areas, while the genetic features of borderland regions—and their role in mediating cultural transmission and demographic interaction—are less well understood. Systematic investigation of such cultural crossroads is therefore essential for reconstructing the processes that underpinned cultural exchange and population dynamics. Xuzhou in Jiangsu Province represents one of the most critical of these crossroads (Figure 1). Situated at the junction of Jiangsu and Anhui to the south, Shandong to the east, and Henan to the west, the region has long been recognized as a meeting ground between the Central Plains, the eastern coast, and the lower Yangtze. Archaeological evidence documents sustained cultural convergence from the Late Neolithic through the Han period, with overlapping influences from multiple traditions[15,16]. From a cultural–archaeological perspective, Xuzhou lies at the intersection of the Haidai, Central Plains, and Jianghuai–Lower Yangtze cultural spheres: it was shaped by Dawenkou and Longshan traditions from the north and east, by the Yangshao–Longshan sequence from the west, and by Late Neolithic developments such as Lingjiatan and Liangzhu to the south[15,16]. These findings highlight the region’s role as a zone of persistent cultural overlap and interaction.

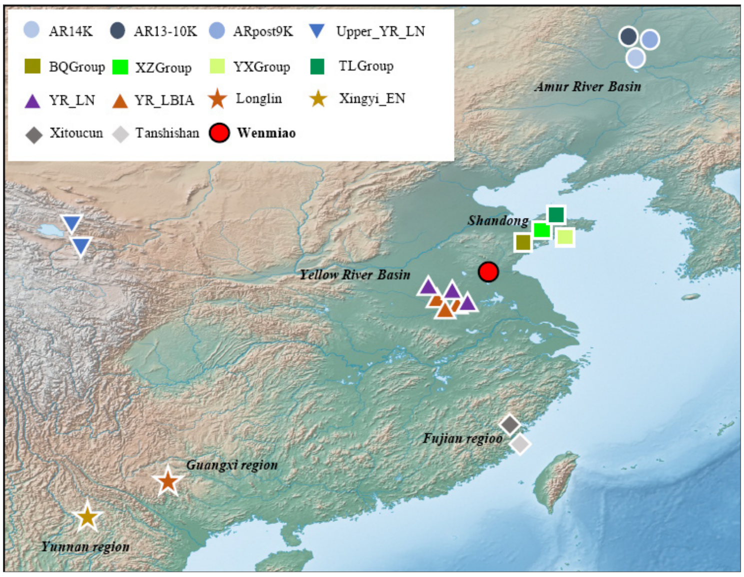


Figure 1. *Geographic location of the Wenmiao site and relevant ancient populations.*

Yet, whether such cultural hybridity also reflected demographic processes remains unresolved. Ancient DNA evidence from surrounding regions—including Shandong, Henan, Anhui, and southern Jiangsu—has been reported, but Xuzhou itself has remained a blank spot on the genomic map. This absence leaves key questions unanswered: What genetic components were present in Xuzhou populations during prehistory? How did they relate to groups in the Central Plains, Shandong, and the Jianghuai–Yangtze region? To what extent did cultural exchange involve actual migration and admixture? Here we address these questions through genome-wide analysis of three individuals from the Western Zhou period excavated at the Wenmiao site in Xuzhou. By integrating these new data with genomic datasets from both northern and southern China, as well as other parts of East Asia, we provide the first direct genetic insights into this pivotal cultural frontier.

2. Materials and Methods

2.1 Archaeological Context

The Wenmiao Subterranean City site is located on the east side of the intersection of Pengcheng Road and Heqing Road in Gulou District, Xuzhou City, within the former campus of Xuzhou No. 2 Middle School. The excavation area lies in the southwest corner of the project block, covering 900 m². The site preserves thick cultural deposits with a broad chronological span, extending from the Western Zhou to the Ming dynasty. Excavation yielded a series of important discoveries, including Ming-period bridge foundations and wharves, river channels dating to the Song–Yuan period, and architectural foundations from the Western Han through Wei–Jin periods. Of particular significance was the discovery of a Western Zhou cemetery more than 10 meters below the modern surface—the first such find within the Xuzhou urban area—which fills a major gap in local archaeology and provides critical evidence for locating the Song polity of Pengcheng and tracing the city's historical development.

The Western Zhou cemetery lies in the northern sector of the Wenmiao Subterranean City site, at the confluence of the ancient Si and Bian rivers. The burials were cut beneath Warring States strata and were deposited in a floodplain environment. All tombs are rectangular shaft pit burials filled with gray-brown clay mixed with fine sand. The pit walls show simple trimming, and wooden coffins were used, though they have largely decayed. Owing to the deep burial and waterlogged conditions, human skeletal remains were relatively well preserved. All individuals were interred in extended supine position, with variation in hand placement defining three burial styles: hands crossed on the chest, hands crossed on the abdomen, or hands crossed behind the back. Except for M5, which was oriented to the south, all burials were oriented to the north. The tombs are generally small in size: the largest, M11, measured 2.8 m in length and 1.7 m in width (4.76 m^2), while the smallest, M6, measured only 1.8 × 0.52 m (0.9 m^2). The main grave goods assemblages consist of ceramic tripods (li), basins (yu), stemmed dishes (dou), and jars (guan), with a small number of bronze implements such as axes, chisels, and adzes. These assemblages and vessel types closely resemble those of Phases IV–VI of Western Zhou cemeteries in the Fenghao region, dating the Xuzhou cemetery to the middle to late Western Zhou. Based on burial form and associated grave goods, the tomb occupants appear to have been of modest social status, likely associated with handicraft production or other artisanal activities.

2.2 Ancient DNA Extraction and Library Preparation

Three ancient human samples were processed in dedicated clean-room facilities at the ancient DNA laboratory of Sichuan University, following established protocols for DNA extraction and library construction[8]. Prior to sampling, skeletal elements were decontaminated by wiping with 5% bleach and irradiating with ultraviolet light for 30 minutes on each side. For each tooth, fine powder was generated using a dental drill (Dremel, USA). Approximately 50 mg of powder was digested overnight at 37 °C in a buffer consisting of 900 µl of 0.5 M EDTA (Sigma-Aldrich), 16.7 µl Proteinase K (Sigma-Aldrich), and 83.3 µl ddH_2O (Thermo Fisher, USA). DNA was extracted from the digested supernatant using MinElute silica spin columns (QIAGEN, Germany) with a customized binding buffer (5 M guanidine hydrochloride, 40% isopropanol, 90 mM sodium acetate, and 0.05% Tween-20). After two washes with PE buffer (80% ethanol), DNA was eluted in 100 µl TET buffer (QIAGEN, Germany). From 20 µl of extract, we constructed double-stranded, dual-indexed libraries treated with partial uracil-DNA glycosylase (UDG). Blunt-end repair was carried out using T4 Polynucleotide Kinase (0.5 U/µl; Thermo Fisher) and T4 DNA Polymerase (0.08 U; Thermo Fisher) for 15 minutes at 25 °C. Libraries were purified on MinElute columns and eluted in 18 µl TET buffer. Illumina adapters (0.25 µM adapter mix) were ligated with Quick Ligase (NEB) in a 40 µl reaction, followed by purification. The fill-in reaction was performed with 1X isothermal buffer, 0.4 U/µl Bst polymerase (NEB), and 250 µM dNTPs (Thermo Fisher) for 30 minutes at 37 °C and 20 minutes at 80 °C. Indexed libraries were amplified using unique dual index combinations, purified with AMPure XP beads (Beckman Coulter), and quantified on a Qubit 2.0 fluorometer (Thermo Fisher). Sequencing was carried out on an Illumina HiSeq X10 platform (Annoroad, China) with a 150-bp paired-end design. Sequence data were demultiplexed allowing for one mismatch across the dual 8-bp index sequences.

2.3 Sequencing and Data Processing

Raw sequencing reads were demultiplexed, allowing for one mismatch across the dual 8-bp index sequences. Adapter sequences were trimmed with AdapterRemoval v2.3.3 [17], and overlapping read pairs were merged. The resulting sequences were aligned to the human reference genome (hs37d5, GRCh37 with decoy sequences) using BWA v0.7.17[18]. PCR duplicates were identified and removed with DeDup v0.12.8[19]. To mitigate the impact of postmortem cytosine deamination on downstream analyses, we additionally produced "trimmed" BAM files by soft-masking the terminal 10 bp of each read using the trimbam function in bamUtils v1.0.15[20], guided by the observed misincorporation profiles of each library. SNP genotyping on the 1240k panel [21,22] was performed using the pileupCaller program (https://github.com/stschiff/sequenceTools), sampling a single base with Phred quality ≥30 to generate pseudodiploid calls. For transition SNPs (C→T and G→A), trimmed BAM files were used, whereas for transversions, untrimmed files were applied.2.3 Genetic sexing and uniparental haplogroup assignment

2.4 Genetic Sexing and Uniparental Haplogroup Assignment

Molecular sex was inferred by comparing coverage of the X and Y chromosomes relative to the autosomes[23]. For mitochondrial DNA, consensus sequences were reconstructed with Geneious v11.1.3[24], and haplogroups were assigned using HaploGrep3 (https://haplogrep.i-med.ac.at/) based on PhyloTree build 17.

2.5 Population Structure Analysis

To investigate population structure, we performed principal component analysis (PCA) with smartpca v16000[25]. The reference dataset included 2,077 present-day Eurasian individuals from the HumanOrigins panel and 266 East Asian individuals from the 1240k panel. Projection of the ancient samples was conducted using the options lsqproject: YES and shrinkmode: YES. To explore allele sharing, we calculated outgroup f3 statistics to assess the extent of shared genetic drift between the target individuals and other Eurasian populations since their divergence from an African outgroup. The f4 statistics were also computed using the f4mode: YES option in ADMIXTOOLS[26]. These analyses were conducted with qp3Pop v435 and qpDstat v755. Admixture modeling of the Wenmiao genomes was performed using the qpAdm framework (qpAdm v810)[21]. For these models, we employed a panel of outgroups from the 1240k dataset curated in the Allen Ancient DNA Resource (AADR): Mbuti.DG, Israel_Natufian, Villabruna, Tianyuan, Iran_Ganj_Dareh_Neolithic, Mixe.DG, Ami.DG, Onge.DG, Ust_Ishim.DG, Kostenki14, Papuan.DG, and Anatolia_Neolithic.

3 Results

3.1 Ancient Genome Data Production from the Wenmiao Site

We generated genome-wide data for three individuals excavated from the Wenmiao site using the 1240k SNP capture array, preparing a single Illumina library for each. The specimens yielded high proportions of endogenous human DNA, ranging from 53% to 90%, reflecting excellent DNA preservation (Table 1). Multiple lines of evidence confirmed the authenticity of these data. All libraries showed nucleotide misincorporation patterns characteristic of ancient molecules, with elevated cytosine-to-thymine substitutions at terminal positions (Figure S1). Estimates of exogenous modern human contamination remained consistently below 5% (Table S1). After stringent filtering, we obtained haploid genotype calls for 427,156 to 660,012 autosomal SNPs on the 1240k panel (Table 1). These new data were subsequently merged with published genome-wide datasets of both present-day and ancient individuals from the "HumanOrigins" and "1240k" references. For downstream analyses, the Wenmiao genomes were organized into comparative groups primarily according to archaeological period, geographic origin, cultural attribution, and genetic characteristics. This framework allowed systematic evaluation of their affinities in relation to ancient East Asian populations.

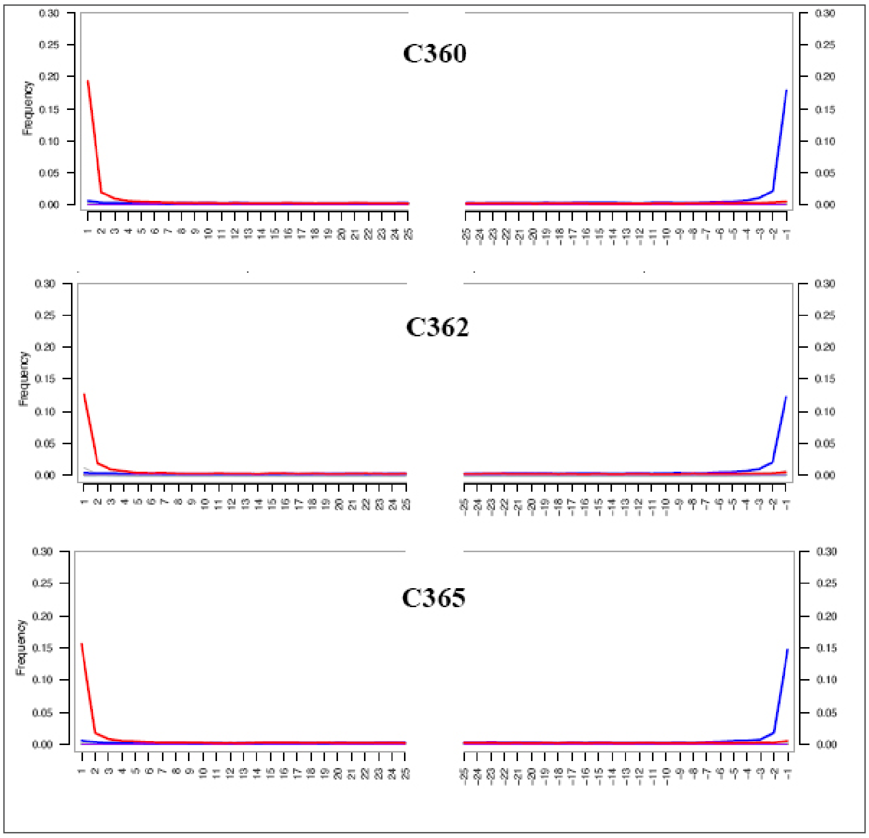


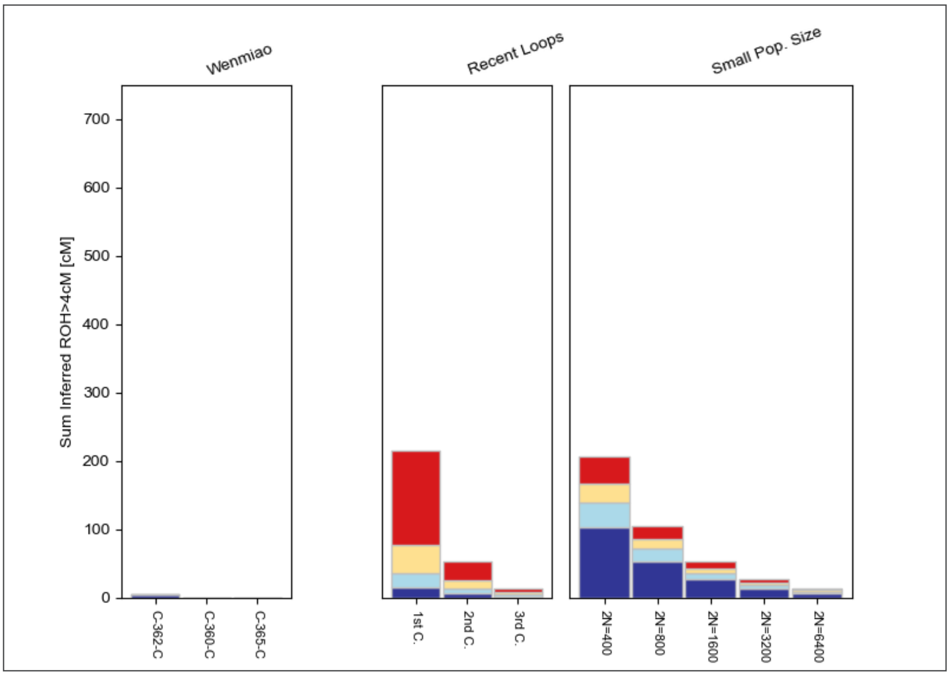
Table 1. *The summary of Wenmiao samples reported in our study.*

Lab ID	Sample ID	Total Reads	pr.endo	auto. Covage	Genetic Sex	Mt haplogroup	Y haplogroup	x-Contam	mt-Contam (95% CI)	1240k SNPs
C360	M1	6091076	53.10%	0.644	F	D4b2	-		0.01 (0.00–0.02)	427156
C362	M12	4352113	90.98%	1.542	M	C7a1	O2a2b1a1a-F5	0.007 ± 0.0025	0.01 (0.00–0.02)	660012
C365	M3	4771525	57.34%	0.916	F	A6	-		0.01 (0.00–0.02)	584195

3.2 Sex Determination, Uniparental Marker Analysis, and Kinship

To determine the biological sex of the three individuals from the Wenmiao site, we assessed relative sequence coverage of the X and Y chromosomes. Individual C362 displayed nearly identical coverage on both X and Y (0.51 and 0.51, respectively), consistent with a male assignment, whereas C360 and C365 exhibited elevated X coverage (0.45 and 0.69) with negligible Y reads, indicating they were female (Table 1). Mitochondrial haplogroup diversity was observed among the three genomes, including D4b2, C7a1, and A6 (Table 1). Haplogroup D4b2 has previously been reported in ancient individuals from Shandong and Henan in northern China, while C7a1 also occurs in late Neolithic Shandong populations. Haplogroup A6, in contrast, has been detected in Bronze and Iron Age individuals from northwestern China, particularly in Xinjiang. The only male carried Y-chromosome haplogroup O2a2b1a1a-F5, a lineage widely represented among both ancient and present-day northern Chinese groups (Table 1). Collectively, these uniparental markers indicate strong genetic connections between the Wenmiao individuals and populations from northern China across different periods.

Kinship estimation revealed no relationships closer than the second degree, excluding the presence of close familial ties among the sampled individuals (Table S2). Runs of homozygosity (ROH) analysis further demonstrated that none of the Wenmiao genomes harbored long ROH segments (>20 cM), providing no evidence of recent parental relatedness or consanguineous mating (Figure. S2).



3.3 Genetic profile of the Wenmiao individuals

To explore the genetic background of the Wenmiao individuals from Xuzhou, we first projected them onto the top two principal components (PC1 and PC2) of present-day Eurasian genetic variation. All three individuals fell within the genetic variation of modern East Asians, particularly along PC1, consistent with an overall East Asian genetic profile and providing a basis for further analyses of their specific affinities (Figure 2A). To further examine population structure within East Asia, we expanded the comparison to 18 present-day East Asian populations. The first two principal components distinguished several groups, including Tungusic speakers (Ulchi, Hezhen, and Xibo), Tibetans, and indigenous Taiwanese groups (Ami and Atayal) (Figure 2B). The Wenmiao individuals, situated in the geographic transition zone between northern and southern China, did not cluster with previously reported southern populations (e.g., Fujian, Guangxi, and Yunnan). Instead, they fall closer to ancient northern Chinese groups, particularly clustering with individuals from Shandong dating to the dynastic period (Shandong_CD) and with agricultural populations from the Yellow River Basin (YR), including Late Neolithic groups associated with the Longshan culture (YR_LN) and Late Bronze to Iron Age individuals (YR_LBIA) (Figure 2B). By contrast, they were more distant from other northern populations, including Neolithic Shandong groups, ancient populations from the Amur River Basin dating from ~14,000 BP onward, and ancient groups from northwestern China.

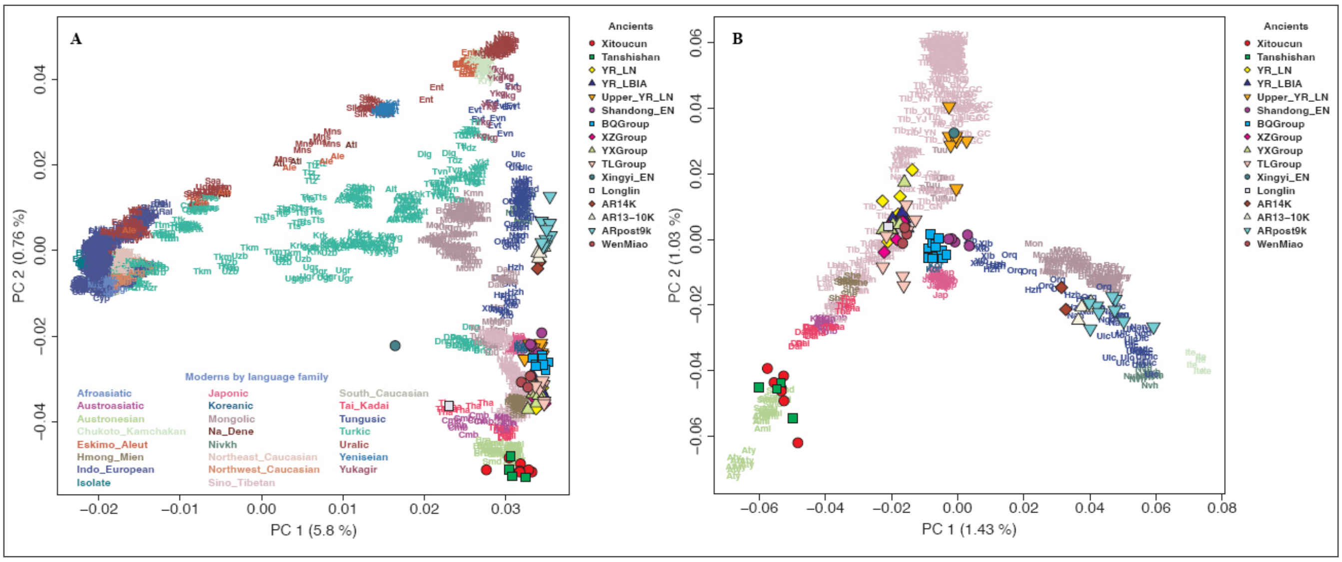


Figure 2. *The PCA results of the Wenmiao populations in our study. (A) the PCA constructed from present-day Eurasians; (B) the PCA constructed from present-day Eastern Eurasians. The Wenmiao individuals (Wenmiao) and other ancient individuals in the literature are projected onto the top PCs.*

Outgroup *f3* statistics showed strong allele sharing between Wenmiao and ancient populations from the Central Plains, including YR_LN, Miaozigou_MN, and YR_LBIA. However, the highest affinity was observed with Shandong populations spanning different periods, notably historical groups (XZGroup, YXGroup, TLGroup) and the Dawenkou-associated Neolithic BQGroup. These findings indicate genetic influences from both the Central Plains and Shandong coastal regions (Figure 3). However, relative to Shandong_CD groups, the Wenmiao individuals formed clades with several Shandong populations and did not show additional affinity with YR-related groups (Figure 4A-C). Similarly, compared with YR_LN and YR_LBIA, Wenmiao did not display enhanced allele sharing with Shandong groups (Table S3). Interestingly, in comparison with early Neolithic Shandong (Shandong_EN), the Wenmiao individuals exhibited additional naaffinity with populations from southern China and Southeast Asia—a pattern also observed among historical Shandong groups (Figure 4D). To further investigate ancestral sources, we modeled the Wenmiao individuals using qpAdm. Our results show that one-way models with YR-related populations alone failed to provide adequate fits, while models with Shandong_CD groups produced acceptable results (Table S4). Two-way models incorporating YR-related and Shandong_CD populations also yielded fits with $P > 0.05$, but the admixture coefficients were associated with very large standard errors, in some cases exceeding the estimated proportions (Table S5). In contrast, models using Shandong_EN as one source and either Central Plains or southern East Asian populations as the second source generated stable and well-fitting results (Table S5). This pattern mirrors previous findings for Shandong_CD populations, suggesting that both Wenmiao and Shandong_CD groups carried comparable ancestral components. Taken together, the Wenmiao individuals from the northern–southern boundary zone show close genetic similarity to historical Shandong populations, reflecting cross-regional homogeneity. Their ancestry can best be described as a mixture of early Shandong populations and farming groups from the Yellow River Basin.

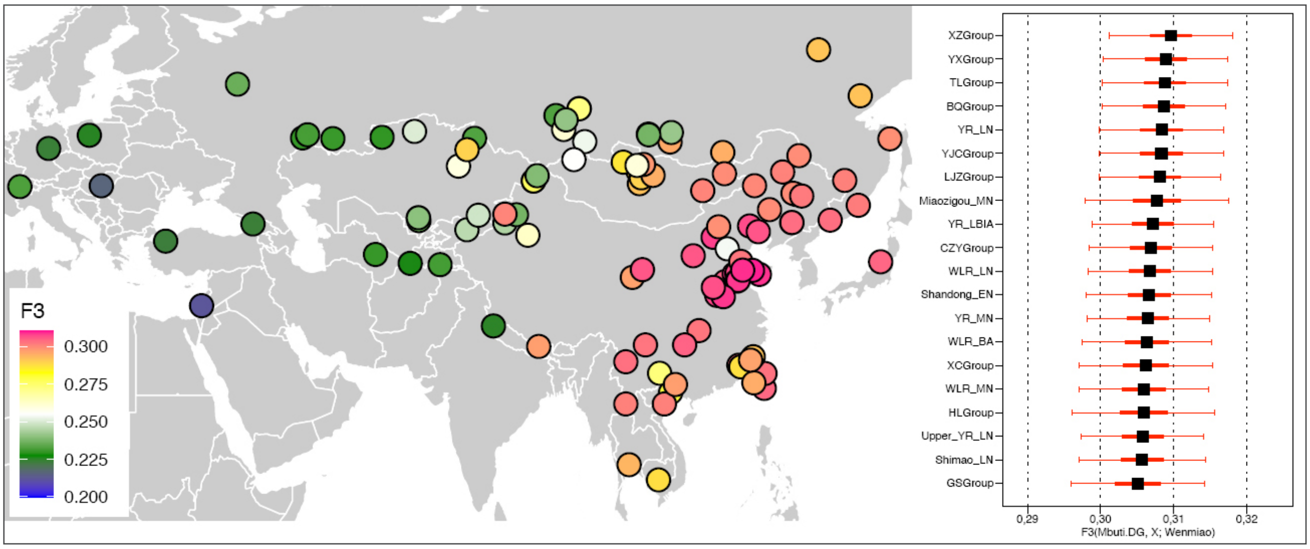
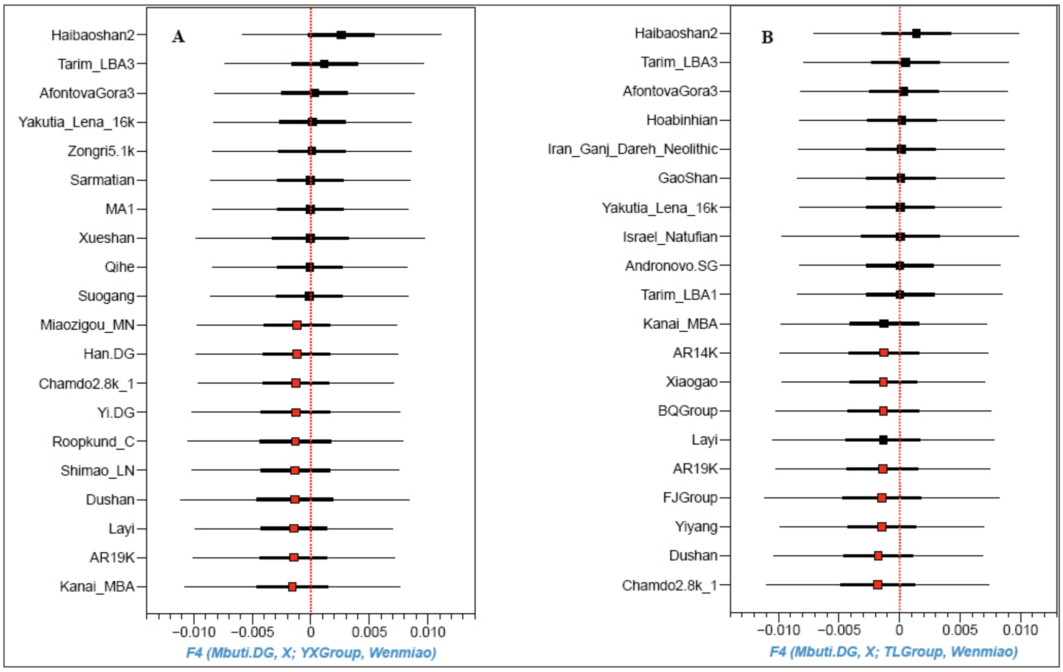


Figure 3. *Genetic affinity between Wenmiao and present-day populations from Eurasia*

4. Discussion

The Wenmiao genomes provide new insights into the role of Xuzhou in the demographic history of ancient China. Archaeologically, Xuzhou has long been regarded as a cultural frontier linking the Central Plains, Shandong, and the lower Yangtze[15,16]. Yet our genomic analyses demonstrate that this cultural plurality did not translate into broad genetic admixture. The Wenmiao individuals show no detectable input from southeastern coastal or Central Plains populations (Figure 3 and Table S4). Instead, their genetic profile is dominated by affinities to Shandong groups, indicating that demographic connections were more constrained than archaeological evidence alone would suggest. This finding has important implications for understanding the role of Shandong populations. Previous research has emphasized the relative isolation of Shandong groups, in contrast to the extensive demographic impacts of Central Plains farmers and southern lineages[3]. Our results challenge this view by showing that Shandong ancestry extended southward into Xuzhou by the Western Zhou period. This demonstrates that Shandong was not merely a regional isolate but also a contributor to broader demographic processes in northern China. The contrast between cultural and genetic evidence at Xuzhou highlights a wider pattern in East Asian prehistory: material culture could circulate widely without substantial gene flow, while demographic influence was more selective and regionally channeled. In this case, Xuzhou functioned as a hub of cultural interaction, but genetically it was integrated primarily into the Shandong sphere. Recognizing Shandong as an active source of genetic influence revises prevailing models of population structure in early China and underscores the need to account for multiple demographic centers beyond the Central Plains.



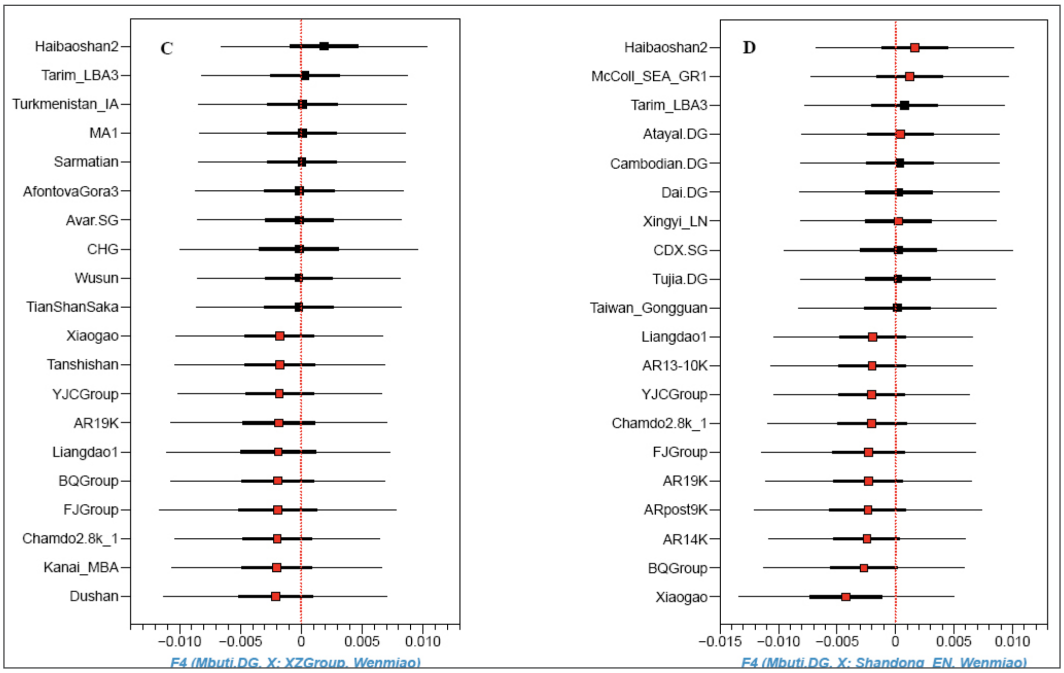


Figure 4. *Genetic affinity between Wenmiao and ancient populations from Shandong. (A) The f4 (Mbuti.DG, X; YXGroup, Wenmiao). (B) The f4 (Mbuti.DG, X; TLGroup, Wenmiao). (C) The f4 (Mbuti.DG, X; XZGroup, Wenmiao). (D) The f4 (Mbuti.DG, X; Shandong_EN, Wenmiao).*

Author Contributions

Conceptualization, F.Z. Writing original draft preparation, W.M., and F.Z. Source, Q.W., and M.W. Data analysis, J.D. Editing, rewriting, and supervision, W.M., F.Z., and J.D. All authors have read and agreed to the published version of the manuscript.

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Resource Availability

The aligned sequences (BAM) and genotype files are available through the Genome Sequence Archive in the BIG Data Center (https://bigd.big.ac.cn/gsa-human; accession number: PRJCA045808). All software used are freely available online.

Declaration of Interests

The authors declare no competing interests.

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