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Genomic dynamics of the Lower Yellow River Valley since the Early Neolithic

Highlights

- Neolithic Yellow River farmers largely influenced Shandong during the Dawenkou period
- A demic succession from Dawenkou to Shandong Longshan
- Central Plain immigrants mixed with local population during the Shang to Zhou Dynasties
- Ancient Shandong populations resemble modern ones since the Qin to Han Dynasties

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In brief

Du et al. report 69 ancient genomes and 325 modern individuals from Shandong, revealing a tripartite genetic structure during the Dawenkou period. The Longshan population inherited the genetic legacy, and after mixing with migrants from the Central Plain during the Shang to Zhou Dynasties, the ancient Shandong population began to resemble modern ones.

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Genomic dynamics of the Lower Yellow River Valley since the Early Neolithic

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SUMMARY

The Yellow River Delta played a vital role in the development of the Neolithic civilization of China. However, the population history of this region from the Neolithic transitions to the present remains poorly understood due to the lack of ancient human genomes. This especially holds for key Neolithic transitions and tumultuous turnovers of dynastic history. Here, we report genome-wide data from 69 individuals dating to 5,410–1,345 years before present (BP) at 0.008 to 2.49× coverages, along with 325 present-day individuals collected from 16 cities across Shandong. During the Middle to Late Dawenkou period, we observed a significant influx of ancestry from Neolithic Yellow River farmers in central China and some southern Chinese ancestry that mixed with local hunter-gatherers in Shandong. The genetic heritage of the Shandong Longshan people was found to be most closely linked to the Dawenkou culture. During the Shang to Zhou Dynasties, there was evidence of genetic admixture of local Longshan populations with migrants from the Central Plain. After the Qin to Han Dynasties, the genetic composition of the region began to resemble that of modern Shandong populations. Our genetic findings suggest that the middle Yellow River Basin farmers played a role in shaping the genetic affinity of neighboring populations in northern China during the Middle to Late Neolithic period. Additionally, our findings indicate that the genetic diversity in the Shandong region during the Zhou Dynasty may be linked with their complex ethnicities.

INTRODUCTION

Located in the Yellow River (YR) Delta, Shandong Province lies on China's east coast and was key to the rise of ancient Chinese civilization. Benefiting from enduring and sustained agriculture in the YR basin, ancient Shandong could feed a large population, and modern-day Shandong remains a highly populated region of 102 million or approximately 1% of the global population. Yet population dynamics in Shandong from the Neolithic transition through the present day remain poorly understood. This is especially true for the key episodes from prehistoric to historical periods in Shandong, such as the Dawenkou period, Shandong Longshan period, Shang Dynasty, Zhou Dynasty, and Han Dynasty.

Building on evidence from abundant archaeological excavations, five successive Neolithic and Bronze cultures have been identified in Shandong: Houli culture (6,300–5,400 BCE), Beixin culture (5,400–4,200 BCE), Dawenkou culture (4,200–2,600 BCE), Shandong Longshan culture (2,600–2,000 BCE), and Yueshi culture (1,900–1,500 BCE). Of these, the Middle and Late Dawenkou (3,500–2,600 BCE) is an exceptionally important period of Shandong prehistory, with archaeobotanical, zooarchaeological, and stable isotope analysis of human remains indicating the expansion of sedentary cultivation of millets and the pig rearing industry flourished[1–3] and substantial increase in the Dawenkou population, particularly in the Late Dawenkou.[4,5] Late Dawenkou is seen as a critical transitional period[6] when specialized handicraft industry and production developed,[5] and walled settlements first appeared.[7] Following the Late Dawenkou, the Shandong Longshan culture exhibited more pronounced social stratification.[2] Compared with archaeological findings, limited genetic sampling has hindered our understanding of ancient population dynamics for prehistoric Shandong. The only genome-wide study[8] on the region has focused on a single individual from the Late Paleolithic to Early Neolithic period at Bianbiandong and three Houli culture sites. This study confirmed that Early Shandong individuals shared genetic similarities with Neolithic northern East Asians, Siberians, and Tibetans. Analysis of ancient mitochondrial DNA (mtDNA) has suggested an increase in haplotype variety in Shandong after around 2,600 BCE, indicating an influence from pre-Longshan populations that persists in modern northern populations.[9] Looking at northern China as a whole, previous research[10] has shown that middle YR farmer-related ancestry expanded into regions such as the upper YR, Shaanxi, Inner Mongolia, and the West Liao River (WLR) during the Middle to the Late Neolithic period. This genetic pattern aligns with archaeological evidence[11,12] of the significant role played by the Yangshao culture in the middle YR region. However, there is a gap in our understanding of the genetic structure and the impact of YR-related ancestry on the lower YR region, particularly during key Neolithic transition periods such as the Dawenkou culture.

The Shang (1,600–1,046 BCE) and Zhou (1,046–256 BCE) Dynasties represent the flourishing of China's Late Bronze culture and its transformation from the Bronze Age to the Early Iron Age.[13] The Shang Dynasty witnessed the emergence of China's written characters alongside traditional rituals and administration.[14] Shandong's western region (especially approaching northern Henan and southern Hebei) was a thriving center[15] even before the Shang dynastic polity emerged. Within the Early Shang Dynasty, two of the five capital shifts[16] most likely occurred within Shandong (referring to the *Bamboo Annals* 竹书纪年). In the Zhou Dynasty, to strengthen administration and cope with the need to control vast territories acquired through successive military conquests, the Zhou Kings deployed the settlements controlled by designated lineages. They authorized many establishments for their aristocratic families and followers, *Zhuhou* (诸侯, regional lords), and devised a political system, the *Fengjian* (封建, demarcation and authorization of establishments) system.[17,18] This situation caused people (such as *Zhuhou's* families, soldiers, and slaves) from the Central Plain to migrate to *Sifang* (四方, four directions),[19] for instance, the east direction of territories in today's Shandong. For some powerful states, the surrendering Shang aristocrats and their slaves from Henan were also allocated to *Zhuhou*.[20,21] In Shandong, Lü Shang (吕尚) was appointed to Qi State (齐国) and Duke of Zhou (周公旦) to Lu State (鲁国). This is where the origin of Shandong's nickname, "Qilu" (齐鲁), comes from.

The Han Dynasty (206 BC to 220 AD) spanned over four centuries and is considered one of the most influential dynasties in Chinese history. During this period, advanced agricultural techniques, booming handicraft industries, and flourishing trade all contributed to the steady increase of population in Shandong. For example, the *Shih Chi* (史记) and *Hanshu* (汉书) described that the city of Linzi emerged as a prominent commercial center, boasting a population of over 1 million people, a number comparable to that of ancient Rome.[22] At the same time, the massive growth in population, increasing urbanization, stable currency system, and the development of the Silk Road promoted international trade and exchanges between the Han Empire and Eurasian countries, and influences spread as far as Parthia and Rome.[23,24]

To date, there have been no reports of ancient human genomes from key periods in Shandong's prehistory and history. To better characterize the genetic dynamics of the Shandong population, we report genome-wide data from 69 ancient human individuals retrieved from 18 different archaeological sites across the Shandong and northern Jiangsu provinces. Our dataset densely covers a time span ranging from the Neolithic Dawenkou culture to historical time, including Dawenkou (n = 29), Longshan (n = 13), Shang to Zhou (n = 11), Han to Sui (n = 12), and Song to Ming (n = 4). Additionally, we produced new genome-wide data from 310 modern-day individuals from sixteen cities across Shandong. This new dataset allows us to analyze genetic changes within the context of the region's extensive archaeological and historical records. Our study focuses on three main themes: (1) the population structure of Shandong during major Neolithic transitions, (2) the impact of Neolithic YR-related ancestry on Shandong populations, and (3) the influence of historically documented events on Shandong's genetic makeup.

RESULTS

Ancient genome-wide data from the Shandong Province

Genome-wide data were obtained from 69 ancient individuals at 18 sites in Shandong Province, with coverage ranging from 0.008- to 2.49-fold (Figure 1A; Table 1; Data S1A). Calibrated

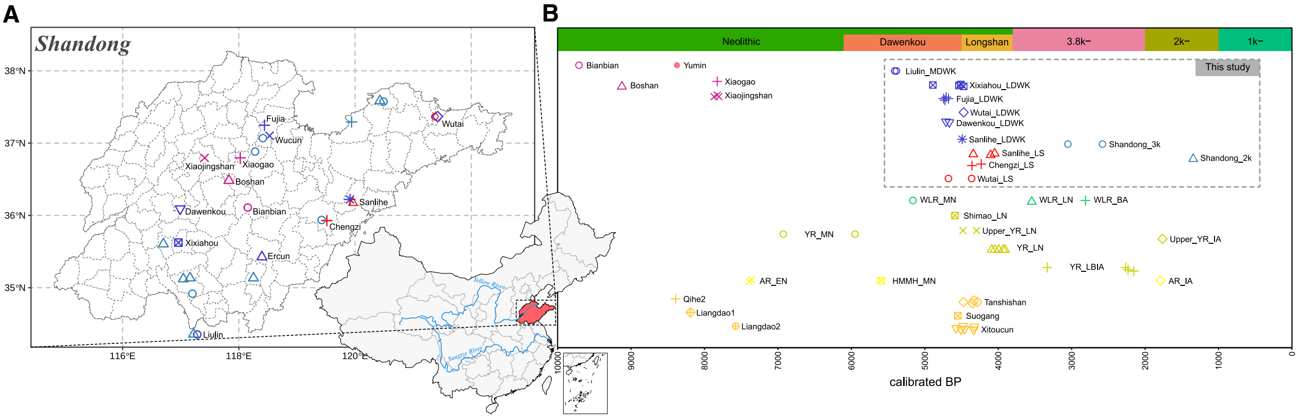

Figure 1. Geographic location and dates of ancient individuals

(A) Location of archaeological sites in Shandong. The names of pre-historical sites are labeled. Four previously published samples (Bianbian, Boshan, Xiaogao, and Xiaojingshan) are also labeled on the map.

(B) Calibrated radiocarbon dates of ancient samples in China. Important published samples from China are presented. The new individuals reported in this study are indicated in the dotted box.

See also Figures S3 and S4 and Data S1A and S1B.

carbon dating placed these individuals between 5,410 and 1,345 years before present (BP) (Figure 1B; Table 1; Data S1B). Samples were filtered through several steps. The authenticity of ancient genome-wide data was verified by identifying the presence of post-mortem patterns of ancient DNA (Data S1C). Kinship was calculated using READ.[25] In total, four pairs of individuals with second or under-second-degree relatives were detected, and individuals with lower coverage were removed from downstream analyses (Data S1D). Modern human contamination was assessed using mitochondrial-based schmutzi for all individuals and X chromosome-based ANGSD for all male individuals: <2% for mtDNA and <3% for X chromosome DNA contamination was estimated (Data S1A and S1E). We trimmed 2–11 bp from each end of reads according to the C>T and G>A misincorporation patterns to ensure the transition rate at each end was lower than 3% (Data S1C). Then, pseudo-haploid calls for 69 individuals were generated using a 1,240,000 panel. After removing individuals with close kinship and low coverage, 64 ancient individuals covering 32,351–1,090,325 single-nucleotide polymorphisms (SNPs) with a median coverage of 402,070 SNPs (Data S1A) were used for downstream analyses. In this study, we collected 325 samples of modern individuals from sixteen cities in Shandong and genotyped using Illumina BeadChip, covering 699,537 SNPs. We retained the bi-allelic SNP sites and had a minor allele frequency larger than 0.1%. After quality control and filtering out first- and second-degree relatives, we published genome-wide data in a collection of 687,295 SNPs for 310 individuals. We merged the dataset with our ancient individuals for further analysis.

Intensified connections among Shandong populations over time

In this study, we initially conducted principal-component analysis (PCA) to examine the overall genomic structure of the ancient Shandong populations. We plotted our individuals with other ancient East Asians onto the PCA result calculated by modern East Asians (Figures 2 and S1). The PCA result showed a triangular pattern, with modern populations from Northeast Asia (NEA), Southeast Asia (SEA), and Tibetan at each vertex. Ancient individuals from Shandong primarily fell along a cline formed by ancient YR and Southeast Asian populations. Notably, previously reported ∼8,000 years BP Shandong hunter-gatherers (SD_HG)[8] showed extra genetic affinity with the Amur River (AR) region populations, such as ancient individuals from the Early Neolithic AR (AR_EN) and DevilsCave. However, ancient populations from this study differed from local hunter-gatherers and formed four clusters in the PCA result. Specifically, individuals from the Fujia, Wucun, Xixiahou, and Dawenkou sites from the Dawenkou period clustered together and were genetically closest to Middle Neolithic YR (YR_MN) farmers. Individuals from the Sanlihe, Chengzi, and Wutai sites from the Dawenkou period formed another cluster and showed a genetic affinity with SEA populations. Additionally, individuals from Liulin from the Dawenkou period and Wutai from the Longshan period fell between these clusters. Historical populations from Shandong, primarily those dating to 3,000–1,000 years BP, began to cluster together and showed genetic similarities with Late Neolithic and Late Bronze to Iron Age YR farmers (YR_LN and YR_LBIA), eventually leading to the formation of modern Shandong populations. We also found that the genetic homogeneity of the Shandong group increased over time, as evidenced by a decrease in genetic differentiation (fixation index, F_{ST}) from 0.041 during the Dawenkou period to 0.004 during the historical period (Data S1F).

Genetic influence of YR farmers and Southeast Asian populations on Shandong Dawenkou populations

Flourishing for approximately 1.5 millennia, the Dawenkou culture played a crucial role in the Neolithic transition in the YR basin and throughout China. During this period, sedentary cultivation of millets and pig rearing thrived, leading to significant growth in the Dawenkou population.[1–3] Archaeological discoveries also have revealed extensive cultural interactions between the Yangshao and Dawenkou cultures. This is evidenced by the

Table 1. Summary of ancient and modern samples reported in this study

Group label	Site names (sample size)	Archaeological culture	Date range
Liulin_MDWK	Liulin (3)	Middle Dawenkou	3,623–3,365 cal. BCE
Ercun_MLDWK	Ercun (1)	Middle and Late Dawenkou	3,600–2,600 BCE
Xixiahou_LDWK	Xixiahou (4)	Late Dawenkou	3,013–2,472 cal. BCE
Fujia_LDWK	Fujia (10)	Late Dawenkou	2,891–2,581 cal. BCE
Wucun_LDWK	Wucun (3)	Late Dawenkou	3,000–2,600 BCE
Dawenkou_LDWK	Dawenkou (3)	Late Dawenkou	2,883–2,577 cal. BCE
Sanlihe_LDWK	Sanlihe (2)	Late Dawenkou	2,625–2,469 cal. BCE
Wutai_LDWK	Wutai (1)	Late Dawenkou	2,578–2,463 cal. BCE
Wutai_LS	Wutai (6)	Longshan	2,871–2,299 cal. BCE
Sanlihe_LS	Sanlihe (4)	Longshan	2,466–1,984 cal. BCE
Chengzi_LS	Chengzi (1)	Longshan	2,453–2,064 cal. BCE
Shandong_3k	Chengzi (1), Wucun (2), Liangchun (3), Xisanjia (2), Dongkangliu (3)	–	1,300–221 BCE
Shandong_2k	Fengshan (1), Xisanjia (3), Xujiaying (3), Xiyanchi (2), Dongxiaogong (2), Jianyaomiao (1)	–	202 BCE–589 CE
Shandong_1k	Dongguandongnan (3)	–	1,080–1,644 CE
Shandong Han	Cities: Binzhou (19), Dezhou (20), Dongying (19), Heze (19), Jining (19), Laiwu (20), Liaocheng (20), Linyi (18), Qingdao (20), Rizhao (19), Taian (20), Weifang (19), Weihai (20), Yantai (19), Zaozhuang (20), Zibo (19)	–	modern

discovery of new artifact types, such as colored pottery bowls (彩陶钵) and colored pottery basins (彩陶盆), as well as decorative styles like the coiling linked-hook pattern (回旋钩连纹) and petal-pattern (花瓣纹) on pottery in Dawenkou culture.[26] Meanwhile, in the Yangshao culture, the influence of Dawenkou was also evident, such as the Dawenkou kettles, *zun* (尊)-wine vessels, *dou* (豆)-stemmed bowls and cups.[26] Regarding the influence of southern China, archaeological evidence of rice cultivation[27,28] and Songze-Liangzhu-related artifacts[26] were abundant in Dawenkou culture. We used genetic data here to infer if there were YR farmers and Southeast Asian-related influences on Shandong Dawenkou populations.

We initiated our analysis with outgroup-f_3 in the format of f_3(YR farmers, ancient Shandong populations; Mbuti) to evaluate the relationship between our Shandong populations and SD_HG compared with YR farmers from the Central Plain. Our results indicate that our Shandong populations share more ancestry with YR farmers than SD_HG (Data S1G). Given the differences in genetic compositions between YR farmers and SD_HG (Data S1H), we then utilized quantitative f_4 in the format of f_4(Mbuti, References; Shandong populations, YR_MN) to compare the genomic profiles of Shandong populations with YR_MN using a reference set that included 35 representative modern and ancient populations from East Asia (Figure 3; Data S2). Our ancient populations from the Dawenkou period could generally be divided into three sub-groups. First, populations from Ercun and Xixiahou sites showed the closest genetic affinity with YR_MN, with Z scores of f_4 ranging from -1.77 to 0.76 (represented by Xixiahou_LDWK), forming a group we refer to as "Dawenkou_YR." Second, populations from Fujia, Wucun, and Dawenkou sites showed an extra genetic affinity with local hunter-gatherers (SD_HG, including Bianbian, Boshan, Xiaogao, and Xiaojingshan), forming the "Dawenkou_HG" group, evidenced by Z scores of f_4(Mbuti, Boshan; Fujia_LDWK/Wucun_LDWK/Dawenkou_LDWK, YR_MN) ranging from -4.64 to -2.98 and Z scores of f_4(Mbuti, Xiaogao; Fujia_LDWK/Wucun_LDWK/Dawenkou_LDWK, YR_MN) ranging from -5.02 to -2.27. Third, populations from Wutai and Sanlihe sites showed a strong genetic affinity with SEA-related populations, forming the "Dawenkou_SEA" group, evidenced by Z scores of f_4(Mbuti, Ami; Wutai_LDWK/Sanlihe_LDWK, YR_MN) = -4.94 and -4.25, respectively. Interestingly, the earliest populations in this study, Liulin_MDWK ($\sim$5,400 years BP), showed genetic affinities with both SD_HG and SEA-related populations, with the Z scores of f_4(Mbuti, Xiaogao/Xitoucun; Liulin_M, YR_MN) = -3.69 and -4.68, respectively. This result also indicates that the genetic influence of SEA-related ancestry could date back to at least $\sim$5,400 years BP in Shandong. A previous study found that YR_LN derived more SEA-related ancestry than YR_MN.[10] To further distinguish whether the SEA-related ancestry represents actual SEA influence in Shandong or pseudo-SEA influence mediated by the middle YR populations. We performed the f_4 test in the format of f_4(Mbuti, SEA; Shandong, YR) (Data S2; Figures S2A and S2B). Liulin_MDWK and Sanlihe_LDWK have

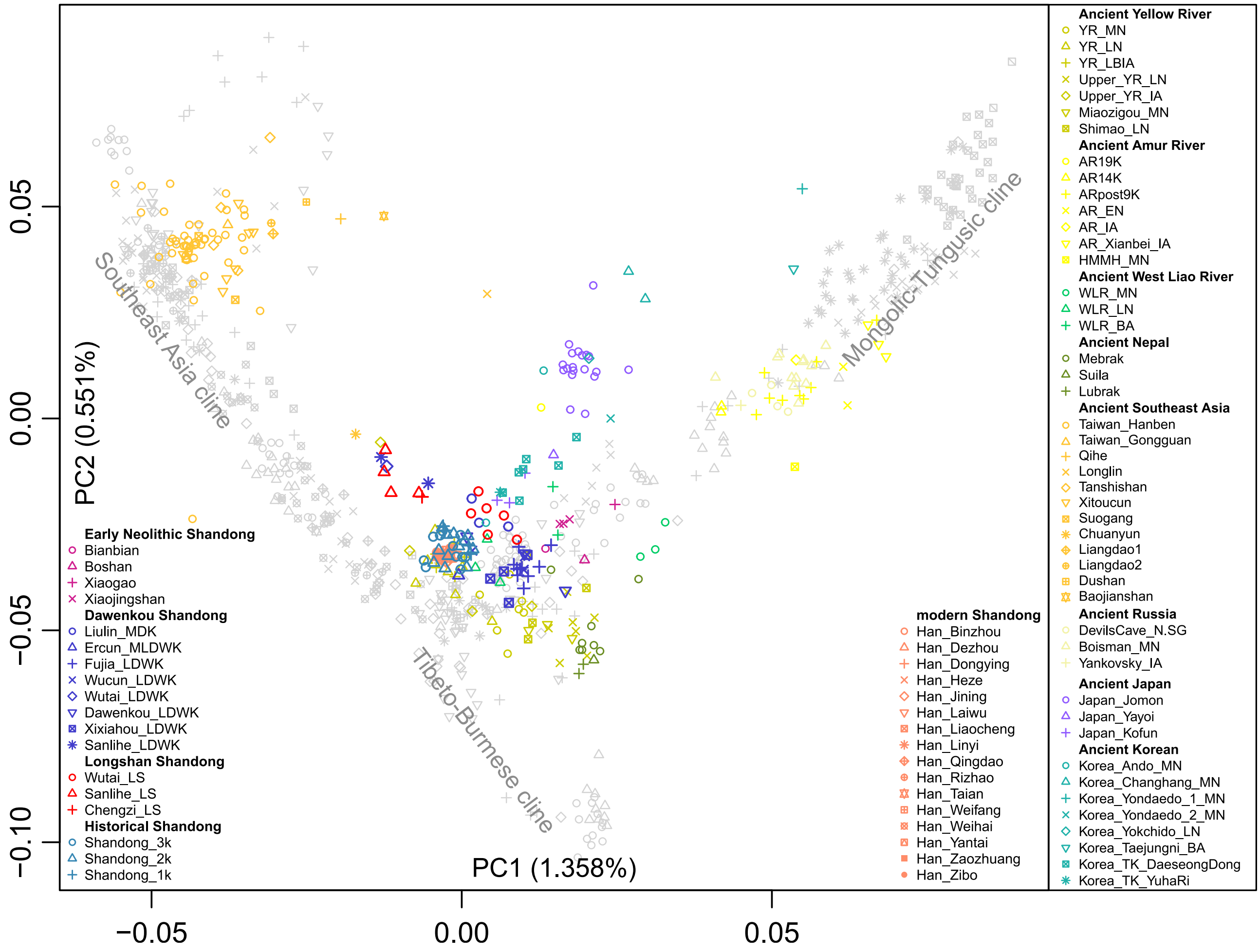

Figure 2. Principal-component analysis plot

Principal-component analysis (PCA) plot of ancient and present-day East Asians.
See also Figure S1.

a significant affinity with SEA-related populations compared with YR_MN and YR_LN. These results indicate that, among these populations, at least part of the SEA-related ancestry derived from the influence of SEA populations rather than pseudo-SEA influence entirely mediated by the middle YR population.

We then used *qpAdm* to model the admixture proportion of these ancient populations from the Dawenkou period (Figure 4). Starting from Ercun_MLDWK and Xixiahou_LDWK, who shared the closest genetic affinity with YR_MN, we found that both individuals exhibited 100% of ancestry derived from YR_MN, representing YR_MN-related populations in Shandong (Data S3A). Due to the limited sample size of Ercun_MLDWK, we focused on Xixiahou_LDWK as the representative of YR_MN-related ancestry for modeling other Dawenkou populations. This initial genetic profile closely resembled published Yangshao individuals (YR_MN) from the middle YR basin, indicating a demographic expansion of middle YR agriculturalists eastward into Shandong during the Middle-Late Neolithic period. In the Dawenkou_HG population, in addition to the YR ancestry mentioned earlier, they also retained local HG-related ancestry (represented by Boshan) dating back to the Early Neolithic Age. This mirrored the admixture dynamics of these two ancestries between the YR and local HG. The Dawenkou_SEA group, on the other hand, not only exhibited more YR ancestry but also showed an additional SEA ancestry, indicating a genetic influx from southern China to Shandong. Furthermore, the Liulin population was found to have 87.1% YR ancestry and 12.9% SEA ancestry, suggesting the SEA influence could be at least dating back to 3,500–3,000 BCE. The location of Liulin in the south likely allowed for gene flow from SEA populations (Data S3B).

Genetic legacy of Dawenkou to Longshan populations in Shandong

Around 4,600 years BP, the Shandong region saw a shift from the Dawenkou culture to the Longshan culture. This transition represented both the inheritance and development of Dawenkou cultural characteristics,[26] but it was unclear if the transition was accompanied by a population change. Through f_4 analysis, we observed two distinct patterns in Shandong Longshan populations (Figure 3; Data S2I–S2K). Sanlihe_LS and Chengzi_LS, which displayed similarities to Sanlihe_LDWK from the Dawenkou era, both indicated a genetic connection to

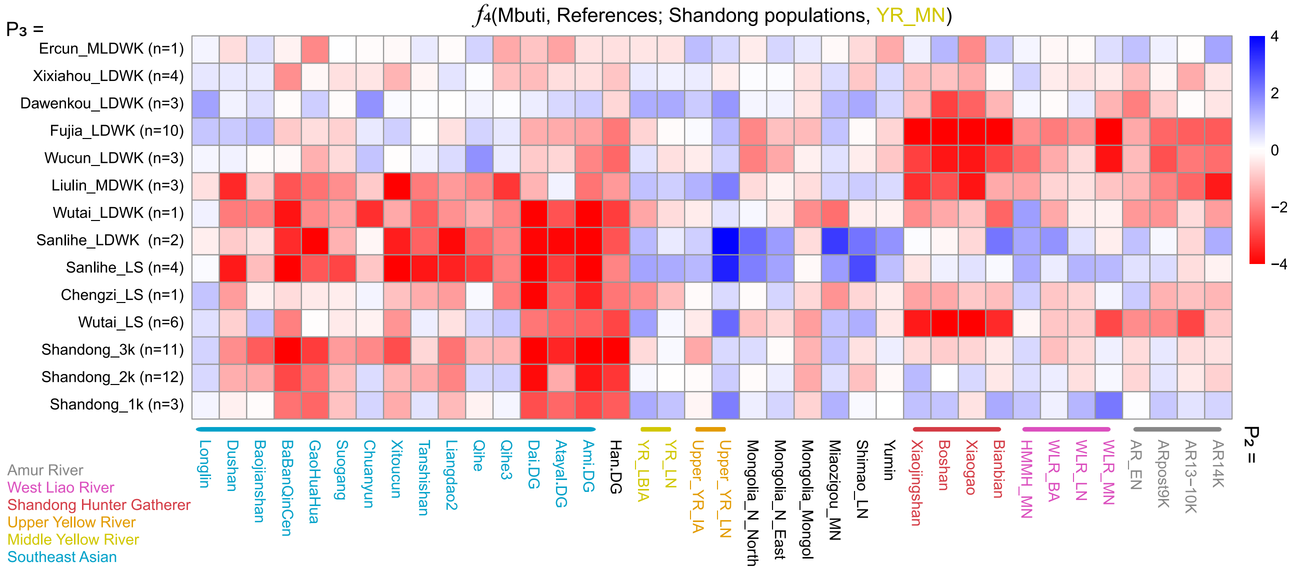

Figure 3. Heatmap of f_4 test

Result of f_4-statistics test in the form of f_4(Mbuti, References; Shandong populations, YR_MN). Gradation of colors shows the value of Z scores.
See also Figure S2 and Data S2.

Southeast Asian populations, i.e., f_4(Mbuti, Ami; Sanlihe_LS/Chengzi_LS, YR_MN) < 0 ($Z < -3$). Conversely, Wutai_LS resembled Fujia_LDWK from the Dawenkou period, all showing a stronger genetic link to SD_HG, i.e., f_4(Mbuti, SD_HG; Wutai_LS, YR_MN) < 0 ($Z < -3$). Using *qpAdm*, we further determined that Sanlihe_LS and Chengzi_LS derive 100% of their ancestry from Sanlihe_LDWK, while Wutai_LS has 85.2% ancestry from Fujia_LDWK (Figure 4; Data S3C). Therefore, the Longshan populations inherited the genetic legacy (85.2%–100%) of Dawenkou, suggesting a demic succession from Dawenkou to Shandong Longshan. These findings also align with archaeological data.

Genetic influence of the Late Bronze to Iron Age YR farmer on historical Shandong populations

In historical time, we could observe from the f_4 result that the genetic legacy of SD_HG was purged from our populations and showed no significant Z score compared with YR_MN in f_4 test in the format of f_4(Mbuti, SD_HG; Historical Shandong, YR_MN), with Z scores ranging from -0.81 to 1.36 (Figure 3; Data S2). Meanwhile, they showed no significant Z score compared with YR_LBIA in the f_4 test in the format of f_4(Mbuti, References; Historical Shandong, YR_LBIA), with Z scores ranging from -2.45 to 1.92 (Figure S2C; Data S2). Besides, we found that historical Shandong samples had an extra affinity with YR_LBIA compared with prehistoric Shandong through f_4 in the format of f_4(Mbuti, YR_LBIA; Historical Shandong, prehistoric Shandong) (Data S1I). These results indicate the influence of the Late Bronze to Iron Age YR region on historical Shandong populations. To further explore this influence, we utilized populations related to the Longshan culture from both Henan (YR_LN and YR_LBIA) and Shandong (Sanlihe_LS and Wutai_LS) to model the admixture proportions of our historical populations. Our analysis revealed that historical Shandong populations could be modeled as derived 60.8%–63.1% of their ancestry from Henan Longshan culture-related YR_LBIA, with the remaining ancestry coming from local Longshan culture-related Sanlihe_LS (Figure 4; Data S3D).

Furthermore, the *qpAdm* model revealed the fine genetic structure within the Shandong_3k group. Based on the admixture fractions of Central Plain (YR_LBIA), the Shandong_3k populations were categorized into three groups: (1) Dongkangliu_3k and Liangchun_3k with 100% YR_LBIA ancestry; (2) Wucun_3k and Chengzi_3k with a mix of YR_LBIA and local Shandong Longshan ancestry; and (3) Xisanjia_3k with 100% local Shandong Longshan ancestry.

Modern Shandong populations clustered closely with historical populations in the PCA plots. We were able to model modern Shandong populations by introducing 100% ancestry from either Shandong_2k or Shandong_1k populations using *qpAdm* (Figure 4; Data S3E). This suggests a continuity of genetic heritage in Shandong populations over time.

DISCUSSION

Genetic affinity in northern China during the Middle-Late Neolithic: The influence of middle YR farmers

During the Middle to Late Neolithic period, the primary archaeological cultures in northern China were concentrated in the YR basin and the WLR region.[5] Specifically, these cultural sequences included the Majiayao culture (3,300–2,500 BCE) in the upper YR, the Yangshao culture (5,000–3,000 BCE) and Miaodigou II culture (3,000–2,500 BCE) in the middle YR, the Dawenkou culture (4,200–2,600 BCE) in the lower reaches, and the Hongshan culture (4,500–3,000 BCE) and Xiaoheyan culture (3,000–2,600 BCE) in the WLR region. Two key trends can be observed in the archaeological materials[5]: first, the Neolithic culture in each region became more complex and gave rise to unique local civilizations; second, the range of these regional

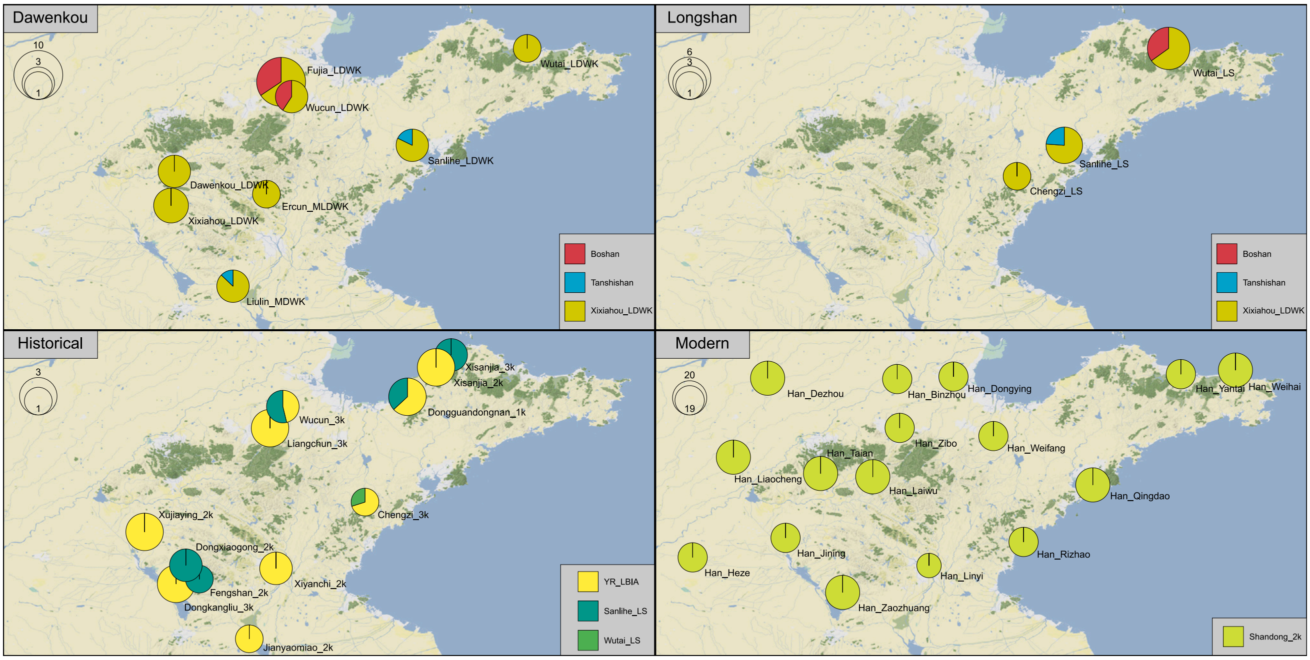

Figure 4. Admixture proportion of ancient Shandong populations

Ancestry proportion estimated for ancient Shandong populations via *qpAdm*. See also Data S3 for details.

cultures expanded, and cultural interactions strengthened. Among these cultures, the Central Plain culture from the middle YR played a vital and leading role,[11] as evidenced by the widespread distribution of painted pottery with coiling linked-hook and petal patterns. This pottery originated from the Yangshao culture and later spread throughout the YR, WLR, and even the Yangtze River Basin.[12] This phenomenon, known as the Central Plains-centric model of double flower pattern (重瓣花朵式假说), proposed by Yan Wenming,[11] highlights the cultural influence of Central Plain region.

Our study utilized the *qpAdm* model to identify a tripartite genetic structure during the Dawenkou period in Shandong, consisting of Dawenkou_YR, Dawenkou_HG, and Dawenkou_SEA. Compared with published local HG individuals (dating to 7,545–5,721 BCE),[8] our findings reveal a significant genetic shift in Shandong's ancestry during this period, with all individuals exhibiting YR-related ancestry (ranging from 58.6% to 100%), indicating a strong influence from middle YR farmers prior to 2,700–2,600 BCE. Due to the lack of contemporaneous genomes from the Central Plain during the Middle to Late Dawenkou period, we were unable to determine whether the westward expansion of the Dawenkou culture, as indicated by archaeological findings,[26] involved population migration. Therefore, we cannot rule out the possibility of migration toward the West. The Late Dawenkou period saw cultural innovations linked to population migration and genetic admixture from Neolithic farmers in the middle YR region, with an additional gene flow (16.7%–24% SEA ancestry) from southern China. Furthermore, consistent with archaeological evidence, our ancient DNA data also support the population's genetic legacy from Dawenkou to Shandong Longshan.

Previous research[10] has shown a substantial genetic flow from the middle YR to surrounding regions within a 600 km radius during the Middle to Late Neolithic era. Using *qpAdm* modeling, it was estimated that individuals from various cultural groups received a significant genetic contribution from the YR. For example, individuals from the Qijia culture in the upper YR region during the Late Neolithic period had received an estimated genetic contribution of 80.4%, while those from the Miaozigou culture in Inner Mongolia during the Middle Neolithic period had a contribution of 80%. Similarly, individuals from the Shimao culture in Shaanxi Province had an ancestry of 79%, and Hongshan culture and Hamin culture individuals from Western Liao River had an ancestry of 60.3% and 24.9%, respectively. Additionally, analysis of ancient mtDNA revealed a close maternal relationship between the Qingtai (from Central Plain Yangshao culture, ~3,500–3,000 BCE) and Shandong populations.[29] Genetic evidence suggests that farmers growing foxtail millet in the middle YR basin influenced neighboring populations, leading to the increased genetic similarity in northern China during the Middle to Late Neolithic period. These findings coincide with the Central Plains-centric model of the double flower pattern hypothesis.[11]

Population migration and admixture between the Central Plain and Shandong during the Late Bronze to Iron Age

Since around 1,500 BCE, there has been a noticeable shift in ancestry toward Late Bronze to Iron Age YR farmers from the Central Plain (Figures 2 and S1) and genetic similarities between historical Shandong individuals and modern populations. The genetic makeup of Shandong_3k-1k closely resembles ancient Central Plain populations from the Shang to Warring States period (YR_LBIA) and modern Shandong and Northern Han populations.

Historical evidence supports connections between the Central Plain and Shandong. First, the Shang's basic social organization had strong military aspects, oracle records abound in mentions of military campaigns, and the Shang rulers had ample force at their command both externally and internally.[14] Most of the *Fangguo* (方国, regional states) located in today's Shandong Province had a subtle relationship with the Shang Court, and they were sometimes friends and sometimes enemies.[30–32] Whether it was wars or alliances, these activities indirectly promoted the integration of ethnic groups. Additionally, military campaigns led by figures such as King Wu of Zhou (周武王), Duke of Zhou, and King Kang of Zhou (周康王) resulted in large-scale movements of people from the middle YR region into Shandong. The implementation of the *Fengjian* system in the Early Zhou Dynasty (~1,000 BCE) further drove population migrations, with aristocratic families, soldiers, and slaves from Shaanxi and Henan settling in Shandong.[20,21] The policy against same-surname marriage[33,34] (同姓不婚) imposed by the ruler of Zhou promoted intermarriage between Central Plain immigrants and local *Dongyi* (东夷),[35–37] leading to extensive population admixture in Shandong.[20] Moreover, according to *Shih Chi*, the Qin state's annexation of weaker territories in the upper to middle YR region during 301–251 BCE forced refugees into the Qi state for asylum.[23,38] These historical events illustrate the complex dynamics of population movements and admixture between the Central Plain and Shandong over time.

We observed the genetic diversity in the Shandong_3k group, which may be related to the complex ethnicities of Shandong,[39] as historical records show that supporters of the King of Zhou were located in central and southwest Shandong, such as Lu, Qi, and Xue (薛), while the eastern region was home to local *Dongyi* states, such as Lai (莱) and Ju (莒).[20,39] The Dongkangliu[40] site's proximity to the Xue capital and the Liangchun[41] site's proximity to the Qi capital align with their 100% YR_LBIA ancestry. In comparison, the Xisanjia[42] site's proximity to the Lai capital corresponds to its 100% local Shandong Longshan ancestry. Further research is needed to explore the relationship between state affiliations and genetic ancestry in a larger sample size.

Demic succession in Shandong populations over two millennia

Over the past 2,000 years, ancient Shandong populations have gradually changed to closely resemble modern Shandong populations, with both groups now being genetically indistinguishable. During the Qin and Han Dynasties, particularly the Han Dynasty, ancient China exhibited strong centralized institutions that facilitated genetic exchange within Shandong and across the country. However, following the Han Dynasty, Shandong's population suffered significant losses[43] due to political instability, conflicts, and warfare during the Wei (220–266 CE), Jin (266–420 CE), and Northern and Southern Dynasties (420–589 CE). Nomadic groups from the eastern steppes later gained power in the region, such as the Xiongnu nobles founding the Former Zhao (304–329 CE), Murong Xianbei establishing the Former Yan (337–370 CE), and Yuwen Xianbei creating the Northern Zhou (557–581 CE). The impact of these non-Han rulers on the population of Shandong remains poorly understood. So, we also examined the genetic contributions of North Asian steppe nomads, such as the Xiongnu and Xianbei,[44–46] to the gene pool of Shandong. However, research findings suggest that Jin Dynasty individuals did not display more affinity with NEA-related ancestry than Han Dynasty individuals (Data S1J), indicating that historical records may have exaggerated the influence of ancient nomads in Shandong.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
    - Lead contact
    - Materials availability
    - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
    - Archaeological background of studied sites
    - Dawenkou Culture
    - Liulin (刘林) site
    - Andi Ercun (岸堤二村) site
    - Fujia (傅家) site
    - Wucun (五村) site
    - Dawenkou (大汶口) site
    - Xixiahou (西夏侯) site
    - Sanlihe (三里河) site
    - Wutai (午台) site
    - Longshan Culture
    - Wutai (午台) site
    - Sanlihe (三里河) site
    - Chengzi (呈子) site
    - Historical period sites
    - Background for modern populations
- METHOD DETAILS
    - Radiocarbon dating of sample materials
    - Ancient DNA extraction and library preparation
    - Nuclear SNPs capture and sequencing
- QUANTIFICATION AND STATISTICAL ANALYSIS
    - Sequence data processing
    - Authentication of Ancient DNA
    - Genetic Sexing and uniparental haplogroup assignment
    - Data merging
    - Kinship detection
    - Principal Components Analysis
    - Pairwise F_{ST} Genetic Distance
    - *f*-statistics
    - Admixture modelling

SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

S.W., C.-C.W, H.L., G.C., and L.J. conducted the project and conceived the idea. P.D., M.W., Z.S., J.T., Bo Sun (author 6), Bo Sun (author 7), P.W., J.X., H.M., S.X., B.W., D.G., Y.M., P.S., X.R., Y.X., B.Z., X.C., K.W., X.M., Z.D., D.L., Y.P., B.L., Y.Z., and X.Z. collected the samples and conducted the experiments. K.Z., C.S., G.H., Y.T., H.B., Y.Y, L.W., J.G., X.Y., R.W., H.M., and C.-C.W. analyzed the data. P.D., K.Z., Z.H., S.X., E.A., X.Q., S.H., C.-C.W., and S.W. wrote the paper. P.D., K.Z., E.A., and S.W. wrote and edited the supplementary text. All the authors revised the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Ancient skeletal element	This study	See Data S1A for Individual ID
Chemicals, peptides, and recombinant proteins		
Ethanol	Sinopharm	100092008
NaClO	Sinopharm	80010428
0.5 M EDTA, pH 8.0	Thermo Fisher	AM9262
5 M NaCl	Invitrogen	AM9760G
1 M Tris-HCl, pH 8.0	Sangon Biotech	B548127
20× SSC buffer	Thermo Fisher	AM9770
20% (wt/vol) SDS solution	Thermo Fisher	AM9820
Proteinase K	Merck	539480
Silica magnetic beads	G-Biosciences	786-915
Guanidine hydrochloride	Sigma Aldrich	50933
Isopropanol	Sigma Aldrich	67-63-0
Sodium acetate	Sigma Aldrich	S2889
Tween-20	Sigma Aldrich	P9416
ATP solution (100 mM)	Thermo Fisher	R0441
dNTP mix (25 mM each)	Thermo Fisher	R1121
T4 polynucleotide kinase (10U/µL)	Thermo Fisher	EK0031
T4 DNA polymerase (5U/µL)	Thermo Fisher	EP0062
Bst enzyme	New England Biolabs	M0275S
Q5 High-Fidelity DNA polymerase	New England Biolabs	M0491S
BSA 20mg/mL	New England Biolabs	B9000S
T4 RNA ligase reaction buffer	New England Biolabs	B0216L
Klenow fragment	Thermo Fisher	EP0052
FastAP thermosensitive alkaline phosphatase	Thermo Fisher	EF0651
T4 DNA ligase (30U/µL)	Thermo Fisher	EL0013
T4 DNA ligase (5U/µL)	Thermo Fisher	EL0012
AccuPrime Pfx DNA polymerase	Thermo Fisher	12344-024
Dynabeads MyOne Streptavidin C1	Life Technologies	65001
Agencourt AMPure XP beads	Beckman Coulter	A63881
Critical commercial assays		
MinElute PCR Purification Kit	QIAGEN	28006
Quick Ligation Kit	New England Biolabs	M2200
MGI Easy Universal Library Conversion Kit (App-A)	MGI	1000004155
Twist Ancient Human DNA Panel	Twist	106658
Twist Mitochondrial Panel	Twist	102039
Twist Universal Blockers	Twist	100767

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Twist Binding and Purification Beads Kit	Twist	100984
Twist Hybridization and Wash Kit	Twist	101026
Twist Wash Buffers	Twist	100846
Deposited data		
BAM files and genotype calls reported in this paper have been deposited in the GSA-Human (https://ngdc.cncb.ac.cn/gsa-human/)	This paper	HRA007869
Software and algorithms		
AdapterRemoval v2.3.1	Schubert et al.[47]	https://github.com/MikkelSchubert/adapterremoval; RRID:SCR_011834
BWA v0.7.17	Li and Durbin[48]	https://bio-bwa.sourceforge.net/; RRID:SCR_010910
bamUtil v1.0.14	https://github.com/statgen/bamUtil	https://github.com/statgen/bamUtil
DeDup v0.12.3	Peltzer et al.[49]	https://github.com/apeltzer/DeDup
pileupCaller	https://github.com/stschiff/sequenceTools	https://github.com/stschiff/sequenceTools
PMDtools	Skoglund et al.[50]	https://github.com/pontussk/PMDtools
Schmutzi v1.5.5.5	Renaud et al.[51]	https://github.com/grenaud/schmutzi
ANGSD v0.931	Korneliussen et al.[52]	http://www.popgen.dk/angsd/index.php/ANGSD; RRID:SCR_021865
HaploGrep2	Weissensteiner et al.[53]	https://haplogrep.uibk.ac.at
Yleaf	Ralf et al.[54]	https://github.com/genid/Yleaf
IGV	Thorvaldsdottir et al.[55]	https://igv.org/; RRID:SCR_011793
EIGENSOFT v7.2.1	Patterson et al.[56]	https://github.com/DReichLab/EIG; RRID:SCR_004965
ADMIXTOOLS v7.0.2	Patterson et al.[57]	https://github.com/DReichLab/AdmixTools/; RRID:SCR_018495

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Shaoqing Wen (wenshaoqing@fudan.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA007869), which is publicly accessible at GSA-Human: https://ngdc.cncb.ac.cn/gsa-human.
- Haploid genotype data of ancient individuals in this study on the 1240K panel are available in the EIGENSTRAT format from the following link: https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data.
- This paper does not report the original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Archaeological background of studied sites

We collected 69 ancient samples from 18 sites (Figure 1; Table 1; Data S1A and S1B). Approval for their use was curated by co-authors and obtained with permission from the respective provincial archaeology institutes or universities that managed the samples. Permission and oversight were also provided by the institutional review board of the Ethics Committee for Biological Research at Fudan University.

Located in the Yellow River delta, Shandong Province lies on China's east coast and has witnessed the rise of ancient Chinese civilisation. According to archaeological insights, the region is generally referred to as the Haidai region,[26] centred around Mount Tai in the lower reaches of the Huai and Yellow River valleys. Chronologically, the Haidai region includes the Neolithic and Bronze Ages and expanded across the entire Shandong, the northern Jiangsu and Anhui, eastern Henan, southeastern Hebei and the southern territory of the Liaoning Peninsula. Entering the historical time, the Shang and Zhou dynasties gradually strengthened the administration of Shandong. After the Qin and Han dynasties, Shandong became a prefecture or province for the central authorities. In the following sections, we briefly introduce the archaeological backgrounds of the studied sites in Shandong. Our ancient individuals cover a period ranging from the Neolithic Dawenkou Culture to historical time, including Dawenkou (n=29), Longshan (n=13), Shang to Zhou (n=11), Han to Sui (n=12) and Song to Ming (n= 4).

Dawenkou Culture

Based on archaeological research,[26] the Dawenkou cultural sphere is divided into eight smaller areas (Figure S3A), including south-central Shandong, northern Jiangsu, southeastern Shandong, Weihe river (潍河) basin (this area is centred in the Jiaolai Plain (胶莱平原), where it is situated between the Jiaodong mountains and hills and the south-central Shandong mountains and hills), north-central Shandong, northwestern Shandong, Jiaodong Peninsula and Southwestern Shandong (the border areas of Shandong, Henan and Anhui provinces). Six vessel types from twenty sites were selected from these eight areas according to typological seriation and stratigraphical relationships. This analysis divided Dawenkou culture into three development phases, six stages, and eleven periods. According to ^{14}C dates, early, middle and late development phases date to 6100-5500, 5500-5000, and 5000-4600 BP. Fifteen regional types are divided: 1) early development phases: Wangyin, Liulin and Zijingshan; 2) middle development phases: Dawenkou, Huating, Wucun, Chengzi and Beizhuang; 3) late development phases: Xixiahou, Lingyanghe, Shangzhuang, Sanlihe, Yangjiaquan, Zhaozhuang and Weichishi. There are eight Dawenkou Culture sites in this study:

Liulin (刘林) site

The Liulin site is located in Pizhou City, northern Jiangsu Province. In 1960 and 1964, the site was excavated by the Nanjing Museum twice.[58,59] A total of 197 tombs belonging to Dawenkou were found and could be classified into two phases (early and late). According to typological seriation and stratigraphical relationships, this site could be archaeologically dated to early to middle Dawenkou. The Liulin was a representative site in the northern Jiangsu region and referred to as the "Liulin type". In our study, three individuals produced enough data for analysis, and two were AMS-dated.

T404M177: 3516-3365 cal BCE (4650±30 BP, Beta-680952)
Missing grave number-2: 3623-3372 cal BCE (4700±30 BP, Beta-680953)

Andi Ercun (岸堤二村) site

Andi Ercun (Ercun in the main text) site is near Andi Ercun village, Yinan County, Linyi City, southeastern Shandong. This site was first excavated in 2019, and two burial sites were found there. Five individuals were initially screened, and only one (M2 north) was analysed in this study due to the poor preservation of the other individuals. Then, the individual (M2 north) was directly radiocarbon dated twice, but no data was produced due to poor preservation. However, based on archaeological culture sequencing, this site belonged to the middle and late Dawenkou Culture (about 3600-2600 BC),[60] especially the combination of typical potteries (Figure S4), like a grey *gu*-shaped cup (ancient wine goblet), grey *dou* (pedestal cups), orange *dou*, pot-shaped *ding* (tripod), and tripod bowl.

Fujia (傅家) site

The Fujia site is in Fujia village, Guangrao County, Dongying City, north-central Shandong Province. This site has been excavated several times, and at least 343 burials have been identified.[61] In this study, 11 individuals belonging to the late Dawenkou Culture were sequenced. Among these samples, two individuals (C40601 and C40604) were dated by AMS at Beta Analytic, Miami, USA and two other individuals (C40602 and C40603) were dated by AMS at Lanzhou University in Lanzhou, China.[62] Stable isotope analysis of human and pig remains suggests the community was largely millet agriculture-based.[61] Fujia community behaves with distinct local archaeological and cultural characteristics and seems more egalitarian. Most tombs contain fewer burial goods and no observable wealth differences among individuals in this cemetery. In addition, unlike most Dawenkou cemeteries, these burials were oriented in two major directions: northeast (NE) and southeast (SE). No significant differences in burial treatment between the NE and SE-oriented groups can be discerned.

GF T6M195: 2883-2632 cal BCE (4170±30 BP, Beta-463741)
GF M149: 2891-2640 cal BCE (4190±30 BP, LZU21671)
M495: 2887-2636 cal BCE (4180±30 BP, LZU21672)
GF T7M17: 2871-2581 cal BCE (4130±30 BP, Beta-463742)

Wucun (五村) site

The Wucun site is in Wucun village, Guangrao County, Dongying City, north-central Shandong. This site is about 4km from the Fujia site, and these two sites are contemporary to the late Dawenkou Culture. The archaeological culture appearance between them is also very similar. According to archaeological research, the Wucun is a significant site in northern Shandong and presents distinct local features in the unearthed objects, known as the "Wucun type". Since 1980, about 106 burials have been excavated.[63] The period of this site dates from late Neolithic culture to the Han Dynasty. According to archaeological culture age and stratigraphy, we selected three individuals from the Late Dawenkou Culture and two others from the Late Shang to Zhou Dynasty (around 1300-256 BC) and obtained enough genome coverage for analyses.

Dawenkou (大汶口) site

The Dawenkou site was near the Dawenhe River (大汶河) in Ningyang County, [64,65] Taian City, Shandong Province. This site was excavated three times in 1959, 1974, and 1978. In 1959, 133 tombs were unearthed and grouped into three phases. Our individuals were all collected from the first excavation. A wealth of grave goods with typical features was unearthed, and the “Dawenkou culture” was initially named after this site. This site was referred to as “Dawenkou type” and could be representative of middle to late Dawenkou culture in south-central Shandong. Then, we briefly introduce the “Dawenkou type” in burial styles, customs, sacrificial methods and grave goods. Most burial styles are vertical earthen pit tombs with rectangular floor plans. These tombs are mostly single interments, while the heads of occupants were oriented in the east direction. In burial customs, dental extraction and occipital deformity are frequently present at Dawenkou habitants. In sacrificial aspects, pig skulls, tortoise shells and mussel shells were found at the site. In addition to pottery works, roe deer canines, personal adornments, bone artefacts and stonewares were discovered. Judging from the unearthed objects, most of the wealth was concentrated in the hands of the few, and this wealth gap between rich and poor gradually got bigger in the late Dawenkou period. For example, the M13 may be a large joint tomb for husband and wife, which is 3.4 m long, 1.9 m wide, and 1.47 m deep. A large wood container is 2.7 m long and 1,3 m wide. A wealth of grave goods were unearthed, including ceramic objects like a tripod (*ding*) and stemmed cups (*dou*), stone tools, bone artefacts like ivory long-tubes (象牙琮) and ivory carving tube (象牙雕筒) and fourteen pig skulls. In our study, three individuals (M1 south, M13 female and M30) were sequenced and were AMS dated to late Dawenkou

M1 south: 2868-2577 cal BCE (4120±30 BP, Beta-668462)
M13 female: 2883-2632 cal BCE (4170±30 BP, Beta-668522)
M30: 2868-2577 cal BCE (4120±30 BP, Beta-680271)/2864-2500 cal BCE (4100±30 BP, LZU231181)

Xixiahou (西夏侯) site

The Xixiahou site is around 9 km southeast of Qufu County town (now Qufu City), [66,67] Jining City, Shandong province. In 1962 and 1963, the Shandong Institute of Cultural Relics and Archaeology conducted an archaeology project at the site. A total of 32 tombs belonging to the late Dawenkou were excavated in this archaeological campaign. Our materials were all collected from the first excavation. The Xixiahou site has emerged as the earliest empirical evidence for the stratigraphical seriation between Dawenkou and Shandong Longshan culture. Therefore, this finding determined the relative chronology between these two important archaeological sequences in Shandong. The Xixiahou was a key site in south-central Shandong and was known as the “Xixiahou type” in the late Dawenkou period. The covering areas of the Xixiahou type are broadly in line with the Dawenkou type. Based on archaeological comparisons, the Xixiahou type inherited archaeological features from the Dawenkou type and grew into the Longshan “Yinjiacheng type”. Like the Dawenkou, popular burial forms were single interments with extended supine positions and oriented east. The deceased people retained the dental extraction and occipital deformity customs and held the roe deer canines. In this study, four individuals were AMS-dated and produced genomic data.

DQXM1: 2837-2472 cal BCE (4050±30 BP, Beta-679457)
DQXM2: 2844-2474 cal BCE (4060±30 BP, Beta-668468)

DQXM3: 2625-2469 cal BCE (4030±30 BP, Beta-680272)/2840-2495 cal BCE (4070±20 BP, LZU231221)
DQXM9: 3013-2889 cal BCE (4320±30 BP, Beta-668463)

Sanlihe (三里河) site

The Sanlihe site[68] is around 2km south of Jiaoxian County town (now Jiaozhou City), Qingdao City, Shandong Province. The site is located in the southeast of Jiaolai Plain (胶莱平原) and around 10km southeast of the Jiaozhou Bay (胶州湾) (belonging to the Yellow Sea). This site was excavated by the Shandong Archaeological Team, Institute of Archaeology, Academy of Social Sciences in 1974 and 1975. A total of 66 tombs belonging to the late Dawenkou and 98 tombs to Shandong Lonshan were unearthed. Many of the silos for storing grains were found, indicating the flourishing of the agricultural industry. In burial customs, deceased people held the roe deer canines and strip-shaped mussel products. In addition to pig lower jawbones, numerous fishbones, gastropod shells, and mussel shells were found, and these pointed to fishing and gathering marine resources playing important roles in subsistence. In particular, substantial jade wares were found in 25 tombs,[69] accounting for 36% of all tombs. Thirty-five jades were discovered at this site, including a *xuanji*-shaped jade *huan* (璇玑形玉环) (a jade with a round bore, and three serrations at the edge), jade arrows (quadrilateral and rounded awl-like objects and four jade arrows), clasps, ear ornaments, tubes, *huan*, and saddle-shaped and horse hoof-shaped decorative pieces The *xuanji*-shaped jade *huan* would have been a deity served by female shamans. In contrast, twelve of the jade arrows were probably plugs to be placed in the mouths of the deceased, embodying the shamanic concept that ghosts and deities devoured jade. On the other hand, some styles of jades were similar to those of Liangzhu culture, such as awl-like jades, which hint at the cultural interaction with southern China.[70,71] Moreover, the Sanlihe site could represent the late Dawenkou culture in the Weihe river basin, called the "Sanlihe type". This type inherited archaeological features from the "Chengzi " and grew into the Longshan "Yaoguanzhuang type". In our study, two individuals produced genomic data for analyses; one was AMS-dated.

M296: 2625-2469 cal BCE (4030±30 BP, Beta-680273)/2840-2495 cal BCE (4070±20 BP, LZU231222)

Wutai (午台) site

The site of Wutai[72] is in a scattered plain surrounded by low hills in Yantai City in the eastern Shandong Peninsula. It is about 4.5 km from the north coast, and the Guangdang River runs by it towards the northeast into the sea. This site was excavated twice by the Yantai Municipal Museum in 2011 and 2013, uncovering more than 1000 m^2 out of its total covering area of c. 10 ha. A total of 45 tombs were unearthed, including 42 rectangular earthen pit tombs and 3 circular ash pit tombs. Archaeological features of house foundations, tombs, and ash pits belonging to the late Dawenkou to early Longshan transition period were recognised, and it seemed that during that time, Wutai was a regional centre in the eastern part of the Shandong Peninsula based on its large size. The funerary objects were quite wealthy and made of pottery, stone and bone. Two elite-exclusive black eggshell-thin potteries, including one high-handle cup and one small pot, were very delicate among these objects. In addition, this site contained obvious functional zones, such as living, burial and handicraft workshop zones. By investigating faunal remains at Wutai,[73] scholars observed that while main livestock such as pigs, dogs and cattle were domesticated in this region, wild boars, deer and other wildlife were still harvested. Moreover, an archaeobotanical study[74] showed that ancient people living at the Wutai site exploited plant resources by gathering wild plants and practising agriculture. Based on the dominant proportion of non-agricultural crops, the people could mainly gather wild plants and cultivate crops as a supplement. Dry farming agriculture in the settlement was dominated by millet (*Setariaitalica*) and broomcorn (*Panicummiliaceum*), possibly with small-scale rice cultivation. The wheat remains were also discovered. Rice and wheat remains recovered from the Wutai site offer substantial proof for understanding the spread of agriculture in the Haidai Region and further to Northeast Asia. The Wutai could be classified into late Dawenkou and Shandong Longshan based on the archaeological stratigraphy and pottery typological seriation. For example, the unearthed objects of Dawenkou mainly contained pottery *ding*-tripods and pottery pots with red-brown coarse sand. In contrast, the Longshan includes more types of black pottery, such as *ding*-tripods, pots, *gui*-tripod pitchers, *dou*-stemmed bowls and cups. In our study, one individual of Dawenkou obtained sufficient genome data for analysis and AMS dated.

2013M40: 2578-2463 cal BCE (4000±30 BP, Beta-671269)

Longshan Culture

The Longshan culture,[26] known for almost 100 years, was first identified when Wu Jinding discovered the Chengziya site in 1928. Eight types of sites were selected to be analysed from all the excavated sites, including Yinjiacheng, Xiwusi, Shangzhuang, Dinggong, Sanlihe, Chengzi, Yaowangcheng, and Wangyoufang. Furthermore, the special characteristics of all areas were synthesised, and Shandong Longshan was divided into six stages and nine periods. Based on ^{14}C dates, Shandong Longshan culture dates to 4600-4000 BP. Longshan culture can be divided into at least six regional types based on different features. These types include Chengziya, Yaoguanzhuang, Yinjiacheng, Yaowangcheng, Yangjiaquan, and Wangyoufang. Three Longshan Culture sites were used in this study:

Wutai (午台) site

The introduction of the Wutai site was described in "Dawenkou Culture, Wutai (午台) site" section. In our study, six Longshan individuals were successfully sequenced, and two were directly radiocarbon-dated.

2013M30: 2871-2581 cal BCE (4130±30 BP, Beta-668465)
2011M9: 2561-2299 cal BCE (3930±30 BP, LZU231182)

Sanlihe (三里河) site

The Sanlihe site[68] was excavated by the Shandong Archaeological Team, Institute of Archaeology, Academy of Social Sciences in 1974 and 1975. A total of 66 tombs belonging to the late Dawenkou and 98 tombs to Shandong Lonshan were unearthed. So many Longshan tombs were first discovered in Shandong then, and it also provided empirical evidence for the time sequence and inheritance relationship between Dawenkou and Shandong Longshan culture. As for the inheritance relationship, we briefly describe the following four aspects[68]: 1) artefacts unearthed, including the stone and pottery objects. Scholars, especially regarding pottery objects, observed an obvious succession of the production process and decorative style. These objects mainly comprised the *ding*-tripod, *dou*-stemmed bowl, *gui*-tripod pitcher and black pottery goblet; 2) burial customs. The dental extraction and occipital deformity customs prevailed in both Dawenkou and Longshan. In addition, the Longshan habitants continued to use the vertical earthen pit tomb with a rectangular floor plan and headed in the northwest direction with extended supine. The deceased people still held the roe deer canines and strip-shaped mussel products, and a jade *han* (玉琀, a flat disc or teardrop shape with central perforation and cicadas of all types found in the mouth of some buried skeletons) was also found in the mouths of the some of the dead; 3) cellar style. The habitants are all accustomed to building cylindrical or conical cellars; 4) features of house foundations. Similar house styles continued from Dawenkou to Longshan, with some improvements. Overall, the Longshan culture succeeded from the Dawenkou culture in the Sanlihe site. Moreover, the Sanlihe was a small central regional type area and subject to the famous “Yaoguanzhuang type”.[26] Five individuals were successfully sequenced in our study, and three were directly radiocarbon-dated.

M203: 2284-2037 cal BCE (3750±30 BP, Beta-668464)
M258: 2201-1984 cal BCE (3710±30 BP, Beta-668467)
M278: 2466-2299 cal BCE (3900±20 BP, LZU231185)

Chengzi (呈子) site

The Chengzi site[75] was in the Chengzi village, Zhucheng County (now Zhucheng City), Weifang City, southeastern Shandong Province. It was excavated twice from 1976 to 1977 and included two phases. The first phase was dated to the middle Dawenkou culture based on the archaeological contexts. In contrast, the second phase mainly dates to Shandong Longshan culture and some Shang to Zhou period. There were a total of 87 tombs subjected to the second phase. Next, we will briefly introduce the archaeological features of the Longshan tombs. 1) agricultural cultivation. Rich farm tools unearthed, such as stone axes, stone spades, stone knives and mussel sickles, pointed to the development of agriculture; 2) stock breeding. Numerous pig lower jawbones were found in large burials; 3) handicraft industry development, especially pottery-making. The fast wheel was developed to speed up the whole pottery-making process. The delicate elite-exclusive black eggshell-thin pottery represented the highest level of pottery-making at that time; 4) social differentiation and private ownership development. According to the burial scale, burial receptacle, and the quantity and quality of unearthed artefacts, the 87 tombs could be classified into four groups. In the first group, the large tomb pit comprised receptacles, which accounted for 5.7% of all the tombs. Substantial pig lower jawbones and delicate eggshell-thin pottery were discovered, symbolising wealth and status. The second and third groups occupied the middle ground between The first and fourth. As for the fourth group, the tombs contained fewer burial goods, and there were no observable wealth differences among individuals. This group accounted for 62% of all over the site. Finally, the Chengzi was one of the five typical sites in southeastern Shandong and was subject to the famous “Yaoguanzhuang type”.[26] In our study, two individuals were successfully sequenced, and one was directly radiocarbon-dated.

M42: 2453-2148 cal BCE (3830±30 BP, Beta-679458).

Historical period sites

Wucun (五村) site

The introduction of the Wucun site was described in “Dawenkou Culture, Wucun (五村) site” section. Based on the archaeological context, we collected two individuals from the Late Shang to Zhou Dynasty (around 1300-256 BC) during this period.

Chengzi (呈子) site

The introduction of the Chengzi site was described in “Longshan Culture, Chengzi (呈子) site” section. In historical times, we dated one individual to Late Shang and Early Zhou and successfully sequenced them.

M53: 1211-1012 cal BCE (2910±30 BP, Beta-670618)

Liangchun (两醇) site

The Liangsun cemetery[41] is located to the north of Sunjiazhuang village, Dawu township, Linzi district of Linzi city, Shandong Province, about 12 km northeast of the ancient Linzi (临淄) city (the capital of Qi State) site of Qi State. In 1984, in cooperation with the construction of ethanol and propanol (Liangchun for short) project of China Qilu Petrochemical Corporation, Shandong Provincial Institute of Cultural Relics and Archaeology and other institutions carried out a comprehensive excavation at this cemetery. 321 tombs and one pit with a sacrificial horse were unearthed from Liangchun cemetery. This cemetery measures about 280 meters from east to west and covers an area of more than 32000 square meters. Mostly in medium and small scales, tombs at this cemetery are densely arranged, which was used to bury the commoners.

The 228 from the total 321 tombs were buried with pottery. A clear evolution clue can be observed among ten categories of potteries. Based on the characteristics, style, and assembly of the buried pottery, those tombs can be divided into seven phases, from the late West Zhou period to the Warring States period. From the early to late spring and autumn periods, the basic assembly of pottery included *li*-tripods, *dou*-stemmed bowls, *guan*-jars, and *yu*-basins. From the end of Spring and Autumn, the assembly began to change and among the newly appeared vessels were *ding*-tripods, lipped *dou*-stemmed bowls, *dui*-containers and so forth. In the Warring States period, the complete assembly is represented by *ding*-tripod, *dou*-stemmed bowl, lipped *dou*-stemmed bowl, *li*-tripod, *yu*-basin, *hu*-pot, *pan*-plate and *yi*-pourers.

By sorting and researching the excavation artefacts, the archaeologist deems this cemetery existed from the late West Zhou to the end of the Warring States period. In the Warring States period, some elements of the Qi culture emerged, such as a set of ritual pottery, *yaokeng*-wrist pits, niches, stone chambers, catacombs, and sacrificial dogs. In general, this cemetery will provide key empirical data for studying funeral customs and ritual systems in Qi State.

In this study, we selected one individual (M3179) from the late Western Zhou, one individual (M2080) from the middle of Spring and Autumn, and one individual (M2074) for sequencing.

*Dongkangliu (*东康留*) site*

Dongkangliu site[40] is near Dongkangliu village, Tengzhou county, Zaozhuang city, southern Shandong, about 3 km northeast of the ancient Xuecheng (薛城) city (the capital of Xue State) site of Xue State. This site was excavated in 1999, and 124 burial sites were discovered. All the tombs were small rectangular vertical earthen pits, with the tomb pit 2.1-2.7m long, 0.9-1.3 wide, 1.6-2.9 deep. Based on archaeological culture sequencing, the site existed from the late West Zhou to the early Warring States period, mainly dated to Eastern Zhou, and less than ten tombs belonged to Western Zhou. The pottery combination in the late Western Zhou includes *li*-tripod, *gui*-tureen, *dou*-stemmed bowl, *guan*-jar and *pen*-basin. From the early Spring and Autumn, the *yu*-basin replaced the *gui*-tureen. In the middle of Spring and Autumn, the small-pot-shaped single-handle cup appeared apart from the *li*-tripod, *yu*-basin, *dou*-stemmed bowl and *guan*-jar. From the late Spring and Autumn to the early Warring States period, the *ding*-tripod replaced *li*-tripod.

This cemetery is only 3km from the ancient Xuecheng city and mainly consists of small-scale tombs. Therefore, scholars infer that the Dongkangliu region may have served as the suburb of Xuecheng. This will provide a significant comparative study of the relationship between the *du* (都, the capital of a state) and *bi* (鄙, the suburb of the capital) within a state. Moreover, the assembly of funeral potteries exhibits some resemblances with contemporary pottery from the ancient capitals of Xue State, Lu State, and Ju Stat (莒国), which will assist in studying the relations within states in Shandong during the Eastern Zhou.

Five individuals in the Eastern Zhou (770-221 BC) were screened, and three individuals produced enough genomic data for analysis.

*Xisanjia (*西三甲*) site*

The Xisanjia cemetery[42] was located around the Xisanjia village, Longkou city of Yantai city, Shandong Province, about 14 km northwest of the ancient Guicheng (归城) city (the capital of Lai State) site of Lai State. From May to July 2013, the Yantai Municipal Museum conducted a salvage archaeology project at the site. This cemetery covers nearly 1200 square meters and comprises 91 tombs and one water well.

According to burial forms and excavation artefacts, this cemetery could be divided into three phases: Warring States, Western Han and Eastern Han to Wei and Jin. In the Warring States period, the basic pottery assembly included a *ding*-tripod, *dou*-stemmed bowl, and *hu*-pot. Some high-ranking tombs had pottery vessels (imitating the bronze vessels) for ritual purposes, including *pan*-plate, *yi*-pourer and *he*-box. The tombs were all the vertical earthen pits, some with *yaokeng*-wrist pits, fewer with niches, no with side compartment. The burials mainly were facing north and south. In Western Han, the number of burial objects was significantly reduced, and the pottery assembly was represented by *guan*-jars and *hu*-pots. Some bronze mirrors and bronze seals were also unearthed. The tombs were all the vertical earthen pits, some with niches and side compartment, no with *yaokeng*-wrist pits. The burials mostly headed in east-west directions. From Eastern Han to Wei and Jin, the funerary objects mainly consisted of glazed and white potteries, combining *hu*-pot, *pan*-plate, and cup with handle. The tombs were all brick tombs with long entrance ramps and oriented in east-west directions.

The Xisanjia cemetery was a significant archaeological finding in the Yantai region, spanning from the Warring States to Wei and Jin. With its clear burial time, distinct funerary objects, and burial type, this site will provide critical material data for studying funeral customs, ritual systems, settlement patterns, and social structures in the Jiaodong Peninsula region.

In this study, we collected one late Spring and Autumn individual (2022M100), one Warring States individual (2022M214), one Han individual (2022M171 middle), and one Northern Qi to Sui individual (2022M126 east) for sequencing. Two individuals were directly radiocarbon-dated.

2022M100: 775-486 cal BCE (2490±30 BP, LZU231189)

2022M126 east: 569-643 cal CE (1470±20 BP, LZU231190)

*Fengshan (*封山*) site*

Fengshan Site is in Xigongqiao village, Tengzhou County, Zaozhuang City, southern Shandong. 109 Han tombs[76] were excavated in 1999, and about 200 funerary objects were found in these burials, including potteries, copper mirrors, copper coins, jade walls, iron swords and so on. According to superposition among the burials, an association of grave goods, and typological patterns of some artefacts, archaeologists could also infer the dates of the tombs. Finally, the archaeological culture sequencing was verified using copper coins in tombs. The Han tombs could be divided into five phases, from the early Western Han Dynasty to the late Eastern Han Dynasty (around 202 BCE to 220 CE). In this study, we sequenced one individual from couples in M2, the third phase in the late Western Han Dynasty (around 48 BCE to 8 CE).

*Xujiaying (*徐家营*) site*

Xujiaying Site[76] is from Xujiaying village, Yanzhou County, Jining City, south-central Shandong. The site was discovered in 2000, and 350 tombs were found, including 347 tombs from the Han Dynasty, 1 tomb from the Song Dynasty and 2 from the Qing Dynasty.

Regarding archaeological culture sequencing, this study is mainly based on superposition among the burials, an association of grave goods, and typological patterns of some artefacts. The summarised characteristics are compared with some unearthed copper coins, which helped archaeologists to infer the dates of the burials. At last, the Han tombs were divided into five phases, spanning from the early Western Han Dynasty to the late Eastern Han Dynasty (around 202 BCE to 220 CE). In this study, we sequenced three individuals from M261, M301 and M311, which belonged to the second and third phases from the middle to late Western Han Dynasty (around 118 BCE to 5CE).

Dongxiaogong (东小宫) site

The Dongxiaogong cemetery[76] was in Dongxiaogong village, Tengzhou City, Shandong Province. From 1998 to 1999, the Shandong Provincial Institute of Cultural Relics and Archaeology and the Tengzhou Museum jointly conducted a salvage archaeology project at the site. A total of 369 tombs, 312 of which belong to the Han dynasty, were excavated during this joint archaeological campaign. Most tombs headed in north-south directions, while the remainder headed in east-west. The burial types comprised stone chamber tombs, earthen pit tombs and other tomb types. The dead mainly took the form of an extended supine position. As for the funeral objects, there were obvious differences among these tombs, fluctuating from 20-30 artefacts to 1-2 artefacts, even with no burial object. Apart from the pottery, other burial contents included iron, bronze, stone, and jade artefacts. In particular, funeral bronze coins prevailed all over the cemetery, at most about 100 coins in one tomb. Finally, by sorting and researching the excavation artefacts and burial forms, the archaeologist deems that this cemetery existed from the middle of Western Han to the middle and end of the Eastern Han period. In this study, we collected two individuals for DNA sequencing.

Xiyanchi (洗砚池) site

Xiyanchi cemetery[77] is in Lanshan District, Linyi City, southern Shandong. During the extended construction of Wang Xizhi (王羲之)s former residence, two large-scale brick tombs (coded M1 and M2) were excavated in April 2003. M1 is a vaulting tomb with two juxtaposing chambers. Many burial objects were discovered in the western chamber of M1, including 273 goods such as pottery, porcelain, bronze, iron, gold, jade, and lacquerware. Among those relics, the most exquisite objects are the celadon *shuizhu*-ewer in the shape of a hu (barbarian), people riding a lion and a bronze lamp pedestal in the shape of a celestial being riding a lion. A batch of lacquer was inscribed with words such as “the 7th year of Taikang (太康)” and “the 8th year of Taikang”. M2 is a joint couple burial consisting of a corridor and a vaulting top chamber. Unfortunately, this tomb has been stolen and disturbed.

This site could be dated between the late Western Jin and the early Eastern Jin Dynasty, based on funerary objects and tomb form systems, especially Chinese characters in lacquerware that could determine the time.[78] There are Chinese characters “Taikang Qinian Lici Zuolao(太康七年李次作牢)” on the back of the lacquered wooden cup. That means a craftsman named Lici produced this cup in the seventh year of Taikang (a title of an emperor’s reign in the Western Jin Dynasty, in 287CE). Furthermore, judging from the valuable articles, the tomb occupants are subjected to rich and powerful families.

In our study, we sequenced two individuals from the M2.

Jianyaomiao (煎药庙) site

The Jianyaomiao cemetery[79–82] was located at Jianyaomiao in Pizhou City, northern Jiangsu Province. From 2015 to 2016, the Nanjing Museum and other institutions carried out a rescue excavation on the cemetery. Because this cemetery had not been looted or disturbed before excavation, the burial structures and grave goods were well preserved, providing important archaeological material for the Western Jin period in northern Jiangsu. The tombs were arranged into two rows from north to south, and all of them were tandem double-chamber tombs on large scales, some of which had black screen walls in the antechamber and stone reliefs. From these tombs, precious burial contents were unearthed, including a nautilus cup, glass bowl, and eared cups made of shell and golden earrings, showing the high-ranking aristocratic statuses of tomb occupants. The celadon *hu*-jar with the dish-shaped mouth, *hu*-jar with cock-shaped spout, grain barn *guan*-jar, and water pitcher in the shape of *bixie*-guardian beast unearthed from these tombs were all typical artefacts of the Western Jin period. The stone reliefs in these tombs were reused from the Eastern Han Dynasty. The inscription “Xiapi Guo Xian Jianzhong Li Mou Xianbo Zhongbo Xiaobo (Xianbo, Zhongbo and Xiaobo of the Jianzhong Ward of Xiapi District, Xiapi State)” seen on the bricks of the retaining wall of tomb M8 showed that this cemetery was subjected to a high-ranking aristocrat family of the Xiapi State during the Western Jin Dynasty. The assemblage of grave goods and the placement of these goods within the tombs are significantly meaningful for the studies on the funeral and burial system of the Western Jin Dynasty.

The nautilus cup and glass bowl unearthed from these tombs reflected the maritime trade and cultural communication between the East and West then. Meanwhile, the coexistence of the celadon wares made in southern China and brown glazed wares in northern China coincided with the background of population southward migration and the cultural communication between the north and south. Moreover, the excavation of this cemetery is important for the study of the history of the Xiapi State during the Western Jin Dynasty.

M1 is a double-passaged earthen pit joint burial with brick chambers. Ceramics, gold and silverware, bronzeware, ironware, and other artefacts were unearthed. The nautilus cups and glass bowls are particularly precious as they provide invaluable materials and clues for the study of the Silk Road at that time. In our study, we successfully sequenced one individual for M1.

Dongguandongnan (东关东南) site

The Dongguandongnan cemetery[83] was located southeast of Dongguan village, Laizhou City, Yantai City, Shandong Province. From April to June 2023, the Yantai Municipal Museum conducted a salvage archaeology project at the site. This cemetery comprised 88 tombs, 6 wells, 4 ash pits and one kiln site. There was a wealth of funerary articles, including ceramic, stoneware, bronze, and talc objects. By sorting and researching the unearthed artefacts and burial styles, the scholars deemed this cemetery existed from the

Tang Dynasty to the Ming and Qing periods. Up to ten burial forms were found, including vertical earthen pit tombs, earthen cave tombs, boat-shaped brick tombs, brick tombs with simulated wooden structures, and rectangular brick tombs. In particular, the discovery of 34 earthen cave tombs from the Tang and Song dynasties was the first in the Yantai area. In addition, several elegant stonewares of Tang and Song were also found in tombs.

In burial M32, one bluish-white glaze furnace with a chrysanthemum petal pattern was discovered. This type of furnace was first found in Shandong and had great significance. Compared with similar artefacts unearthed from published kiln sites and tombs, this furnace could be produced from a Hutian kiln at Jingdezhen in the Late Northern Song Dynasty. Combined with ancient paintings, historical documents, and the special position in the tomb, the burial occupant may be a Buddhist or Taoist, and the furnace could be a travelling furnace containing incense. The discovery of this furnace provided new data for studying the religion and the spread of Buddhism and Taoism among civilians in the Song Dynasty.

In this study, we collected one individual (M32-1) of Northern Song, two individuals (M47-1 and M52-1) of Yuan, and one individual (M31-1) of Ming, and we successfully sequenced these individuals.

Background for modern populations

In this study, 325 individuals were sampled from sixteen cities across Shandong. All samples were newly genotyped using genome-wide arrays named R Global Screening Array (GSA) version 2. 310 individuals were used for subsequent analysis after quality control and filtering out first- and second-degree relatives. Details are as follows: Han_Binzhou (n=19), Han_Dezhou (n=20), Han_Dongying (n=19), Han_Heze (n=19), Han_Jining (n=19), Han_Laiwu (n=20, Laiwu has been under the jurisdiction of Jinan since 2018), Han_Liaocheng (n=20), Han_Linyi (n=18), Han_Qingdao (n=20), Han_Rizhao (n=19), Han_Taian (n=20), Han_Weifang (n=19), Han_Weihai (n=20), Han_Yantai (n=19), Han_Zaozhuang (n=20), and Han_Zibo (n=19). We performed this study in strict accordance with regulations contained in the Human Genetic Resources Administration of China (HGRAC) and the recommendations of the Helsinki Declaration of 2000 (Association, 2001). The research protocol was approved by the Medical Ethics Committee of Xiamen University (XDYX201909). Informed consent was obtained from each participating volunteer. To keep a high representative of our included samples, test subjects were required to be indigenous to Shandong Province and to have lived in the sample collection area for at least three generations.

Shandong Province, located on the eastern coast of China, consists of sixteen subordinate cities and covers an area of 157,900 square kilometres. The Shandong people, who all share the same culture and language, exceed 100 million (2020 census). The population history of the Shandong people is thus of great interest to researchers in many files. The language of Shandong is Mandarin, which is classified into three major dialects[84]: Jiaoliao, Jilu and Zhongyuan. The Jiaoliao Mandarin is mainly distributed over the peninsula region of eastern Shandong. The Jilu Mandarin covers the north-central Shandong Province. Zhongyuan Mandarin is primarily used in south-central Shandong. On the other hand, according to physical geography, Shandong is divided into five geographic areas[85] (Figure S3B): Eastern Shandong, South-central Shandong, Southwestern Shandong, Northwestern Shandong and the Yellow River Delta. The terrain is mainly mountainous and hilly in Eastern Shandong and South-central Shandong. Meanwhile, the terrain in Southwestern Shandong, Northwestern Shandong, and the Yellow River Delta is primarily low-lying and plain.

METHOD DETAILS

Radiocarbon dating of sample materials

24 human bone samples were analyzed by Accelerator Mass Spectrometry (AMS) at Beta Analytic, Miami, USA, and Lanzhou University in Lanzhou, China.[62] Uncalibrated direct carbon date was successfully obtained (Data S1B). The resulting ^{14}C dates were calibrated using the program OxCal v4.4.4[86] and the IntCal20 calibration curve.[87]

Ancient DNA extraction and library preparation

We extracted DNA from 69 samples in a dedicated aDNA facility at Fudan University, following established precautions for working with ancient human DNA.[88,89] Human remains were surface-cleaned and ground to a fine powder. We used 50 mg of bone powder to extract DNA.[90] The lysis step included the addition of 1 ml extraction buffer to each 50 mg sample, containing 0.45 M EDTA (PH 8.0), 0.25 mg/ml Proteinase K (Merck, Germany), 0.05% (vol/vol) Tween-20 (Sigma Aldrich, Germany). After suspending the sample powder by vortexing, the sample was incubated overnight (15–24 h) at 37 °C. After centrifugation, we transfer the lysate supernatant to a fresh tube. We mixed 17.5 μl magnetic beads (G-Biosciences, USA) with 2.5 ml binding buffer containing 5 M GuHCl, 40% (vol/vol) Isopropanol, 0.12 M sodium acetate, 0.05% (vol/vol) Tween-20 (Sigma Aldrich, Germany). Then, we transferred the supernatant (500μl) to a binding buffer/bead mixture, followed by an extraction using a robot (Enlighten Biotech, China) procedure.[88] Finally, the DNA was eluted with 50 μl TET buffer (QIAGEN, Germany). We prepared initial double-stranded and double-indexing libraries following Meyer's protocols[91] but with minor modifications[92,93] outlined below. The end-repair step was performed in 25 μl reactions using 20 μl DNA extract. This was incubated for 20 mins at 12°C and 15 mins at 37°C, purified using a standard MinElute (Qiagen, Germany) purification step, and eluted in 15 μl TET (Qiagen, Germany). Next, Illumina-specific adapters were ligated to the end-repaired DNA in 25 μl reactions. The reaction was incubated for 15 mins at 20°C and purified with another MinElute purification step before being eluted in 20 μl EB Buffer (Qiagen, Germany). The adapter fill-in reaction was performed in a final volume of 25 μl and incubated for 20 mins at 37°C followed by 20 mins at 80°C to inactivate the Bst enzyme (NEB, USA). Libraries were amplified with indexing primers in two parallel PCRs using Q5 High-Fidelity DNA Polymerase (NEB, USA). Single-stranded libraries using 30µl of purified extract were also prepared to improve endogenous DNA conten.[94,95] We purified indexed products using the MinElute PCR Purification Kit (Qiagen, Germany). We qualified the clean-up libraries using Qubit 2.0 (Thermo Fisher, USA). Finally, the shotgun libraries were sequenced on an Illumina HiSeq X10 instrument at the Annoroad Company, China, in the 150-bp paired-end sequencing design. In the meantime, some libraries were converted into circular single-strand libraries adapted to the DNBSEQ-T7 instrument using 2×100-bp chemistry at the Geneplus Company, China.[88,96]

Nuclear SNPs capture and sequencing

The double-stranded and single-stranded shotgun libraries were enriched using the Twist Ancient DNA panel, following the protocol developed by David Reich's lab (short for twist 1240K).[97] The sequencing of the double-stranded library was performed using the Illumina Nova6000 platform at the Mingma Technologies Company, China, in the 150-bp paired-end sequencing design. The single-stranded library was sequenced using the Element AVITI platform at the Mingma Technologies Company in the 75-bp paired-end sequencing design.

QUANTIFICATION AND STATISTICAL ANALYSIS

Sequence data processing

We used AdapterRemoval v2.3.1[47] to trim the sequencing adapters and merged the paired-end reads into a single sequence, and only those overlapped by at least 11 bp were retained. The merged reads were then mapped onto the human reference genome (hs37d5; GRCh37 with decoy sequences) using BWA v0.7.17[48] samse, with parameters -l 1024 and -n 0.01. Dedup v0.12.3[49] was used to remove the PCR duplicates. Considering that our samples are from different time periods and varies in degree of post-mortem transition, we estimated the C>T and G>A misincorporation rate at each position at the ends of sequences for each sample[50] (https://github.com/pontussk/PMDtools), and then trimmed bases to ensure that the transition rates at the ends of reads <3%. 2-11 bp were trimmed (Data S1C) according to the PMDtools results using trimBam implemented in BamUtil v1.0.14 (https://github.com/statgen/bamUtil). We filtered alignment quality using mpileup implemented in samtools with parameters -q30 and -Q30. Pseudo-haploid calls for out samples were generated using the 1240k dataset as reference and parameter –RandomHaploid in pileupCaller (https://github.com/stschiff/sequenceTools).

Authentication of Ancient DNA

We used PMDtools to calculate the deamination patterns of ancient DNA (5' C>T and 3' G>A misincorporation).[50] Schmutzi v1.5.5.5 was used to estimate mtDNA contamination rates.[51] ANGSD v0.910 was used to estimate the nuclear genome contamination rate for all male individuals.[52]

Genetic Sexing and uniparental haplogroup assignment

Three methods were used to assign the genetic sexing for our samples. Rx compared the coverage of the X chromosome to the autosome.[98] Ry compared the coverage of the X chromosome to sex chromosomes.[99] We also compared the genome coverage of the X and Y chromosomes to autosomes.[100] The log2fasta program implemented in Schmutzi[51] was used to call the mtDNA consensus sequences, and the mitochondrial haplogroups were assigned using Haplogrep2.[53] Y chromosome haplogroups were examined by aligning a set of positions in ISOGG (http://isogg.org/) and Y-full (https://www.yfull.com/tree/) databases, in which only base and mapping quality higher than 30 were used for analysis. Haplogroup determination was performed with the script Yleaf.py in Yleaf software,[54] which provides outputs for allele counts of ancestral and derived SNPs along a path of branches of the Y-chromosome tree. Finally, we re-checked the SNPs by visual inspection with IGV software.[55]

Data merging

We merged our data with the Allen Ancient DNA Resource (AADR) datasets,[101] using mergeit implemented in EIGENSOFT.[56] The "Human Origin" dataset contained 597,573 SNPs, involving various modern populations and was used in smartpca and ADMIXTURE analyses. The "1240k" dataset containing 1,233,013 SNPs, including a number of representative modern populations and a large number of ancient populations, was used for *f*-statistics-based analyses.

Kinship detection

We used READ[25] software to detect the genetic kinship between ancient individuals. Default normalization settings (median) were used for populations, including sample sizes of 4 or more. We used the mean value (0.221) of the normalization values calculated from the last step for populations, including sample sizes of less than 3 (Data S1D). If two samples were second or under-second-degree relatives, we only retained one high-coverage individual in the downstream analysis.

Principal Components Analysis

We conducted PCA analysis on the Human Origin dataset using smartpca v16000 implemented in EIGENSOFT.[56] The default parameters were used, and lsqproject was set to YES. The modern populations were used to calculate the principal components (PCs), and then the ancient samples were projected onto the top two components. A total of 470,607 SNPs were used to calculate the PCs. Due to the large number of modern Shandong populations included in the PCA analyses (310 individuals from 16 cities), we only plotted the mean value for each group.

Pairwise F_{ST} Genetic Distance

We calculated the pairwise F_{ST} genetic distance on the 1240k dataset using smartpca v16000 implemented in EIGENSOFT.[56] We used parameters inbreed: YES and fstonly: YES to estimate F_{ST} for our ancient Shandong populations.

f-statistics

We used qp3Pop v651 to calculate the outgroup-f_3 using parameter inbreed: YES and qpDstat v980 to calculate the f_4-statistics using parameter f_4-mode: YES.[57,102] Both software are implemented in ADMIXTOOLS.[57] The f4-statistics statistics were performed in the format of f_4(Mbuti, References; Shandong, YR_MN). We used various East Asian populations as References to distinguish the ancient Shandong population from YR_MN.

Admixture modelling

We performed the admixture modelling using *qpAdm* v810 implemented in ADMIXTOOLS to estimate the ancestries proportion of our individual as the combination of the source populations.[57] We set the parameters allsnps: YES and inbreed: YES, and used the rotation strategy described in Harney et al.[103] Specifically, we used a set of fixed outgroups consisting of distantly related individuals to model our samples as the combination of one to three potential sources, and a population was added to the outgroup whenever it was not chosen as a potential source.