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Article

Maternal genetic structure in ancient Shandong between 9500 and 1800 years ago

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ABSTRACT

Archaeological and ancient DNA studies revealed that Shandong, a multi-culture center in northern coastal China, was home to ancient populations having ancestry related to both northern and southern East Asian populations. However, the limited temporal and geographical range of previous studies have been insufficient to describe the population history of this region in greater detail. Here, we report the analysis of 86 complete mitochondrial genomes from the remains of 9500 to 1800-year-old humans from 12 archaeological sites across Shandong. For samples older than 4600 years before present (BP), we found haplogroups D4, D5, B4c1, and B5b2, which are observed in present-day northern and southern East Asians. For samples younger than 4600 BP, haplogroups C (C7a1 and C7b), M9 (M9a1), and F (F1a1, F2a, and F4a1) begin to appear, indicating changes in the Shandong maternal genetic structure starting from the beginning of the Longshan cultural period. Within Shandong, the genetic exchange is possible between the coastal and inland regions after 3100 BP. We also discovered the B5b2 lineage in Shandong populations, with the oldest Bianbian individual likely related to the ancestors of some East Asians and North Asians. By reconstructing a maternal genetic structure of Shandong populations, we provide greater resolution of the population dynamics of the northern coastal East Asia over the past nine thousand years.

1. Introduction

Ancient DNA (aDNA) studies have helped clarify human population history and evolution in East Eurasia [1–9], such as revealing a genetic distinction between ancient populations in northern and southern East Asia. Shandong province is situated in the northern coastal region of China, and is geographically connected to both northern and southern China. As a multi-culture center, Shandong was home to the Dawenkou culture (6000–4600 years before present (BP), distributed in the lower Yellow River [10]) and the Shandong Longshan culture (4600–4000 BP, a local manifestation of the Longshan Culture [10]). Previous aDNA studies focused on the short hypervariable region I of the mitochondrial DNA (mtDNA) of several Shandong remains [10–13], but these had limited power identifying haplogroups. Recently, a genome-wide study [6] and a review of the human population history of Eastern Eurasia [9] revealed that ancient Shandong individuals played an important role in defining the population genetic history and past migrations of East Asia: six ancient Shandong individuals (~9500–7700 BP) from four Early Neolithic archaeological sites were shown to have ancestry related to northern East Asian populations, an ancestral component that later spread into southern East Asia [6]. However,

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the above studies were limited by a scarcity of samples, leaving large gaps in our understanding of the changing genetic relationships over time across Shandong and its neighboring regions.

In this study, we present the analyses of 86 high-quality complete mtDNA sequences of ancient individuals (~9500–1800 BP) collected from the northern Chinese province of Shandong (12 archaeological sites, Fig. 1a). Our study provides a wider and finer temporal and geographical resolution than previous work in describing the population dynamics regarding Shandong's maternal genetic structure, and how these populations connected with those outside of Shandong.

2. Materials and methods

2.1. Ancient DNA extraction and library preparation

DNA was extracted from 88 samples (aged between 9500 and 1800 BP from 12 sites) including temporal bones, limb bones, phalanges, and molars (Table S1 online) using a method described previously [14]. A total of 88 double-stranded libraries were constructed according to previously published protocols [15,16] and all were partially treated with uracil DNA glycosylase ("UDG. half") [17].

2.2. In-solution capture and analysis of mitochondrial DNA

Libraries were hybridized with oligonucleotide probes that overlapped with the mitochondrial genome as previously described [18]. From the enriched mitochondrial DNA libraries, we used the Illumina Miseq platform to generate 2 × 76 bp paired-end reads. After sequencing, forward and reverse reads that overlapped by at least 11 bp were merged into a single sequence, with one mismatch allowed [18]. The merged sequences (≥30 bp in length) were mapped to the mtDNA revised Cambridge Reference Sequence using BWA (version 0.5.10evan.91g44db244, https://bitbucket.org/ustenzel/networkawarebwa) [19], after which duplicate reads and those with mapping qualities lower than 30 were removed. The subset of libraries that included the characteristic ancient DNA damage at the first position of the 5′ end and the last position of the 3′ end are noted as "DS.half" [16].

The contamination rate was estimated by calculating the fraction of ancient mtDNA fragments compared to 311 published modern human mitochondrial genomes using ContamMix [20,21]. After manually excluding two individuals with large numbers of missing positions, our final dataset included 86 complete mtDNA genomes (16,569 bp) with high coverage (101- to 591-fold) and low contamination rates (0.01% to 4.52%) (Table S1 online).

2.3. Genetic analysis

To compare with present-day East Asian populations, we prepared mtDNA haplogroup frequencies from published studies [10,22] (Table S3 online) and those of 528 complete mitochondrial sequences (Table S4 online) from populations across East Asia. For our newly sampled individuals, HaploGrep2 and PhyloTree build 17 were used to call the haplogroup of each mtDNA sequence [23,24]. Certain haplotypes were found to be shared by individuals (ranging from 2 to 5) buried in the same graves at the Beiqian site, which may have been due to matrilineal kinship among these individuals (Table S1 online). In these cases, only one individual was used for the analyses. Principal component analysis (PCA) of haplogroup frequencies was carried out to investigate the general population structure. The sequences of the mitochondrial genomes from the ancient samples were aligned using MUSCLE v3.8.3 [25]. Genetic distances (Φst) and corresponding P-values were calculated by Arlequin 3.518 [26]. We also used Arlequin 3.518 [26] to carry out the analysis of molecular variance (AMOVA). To avoid artificially low genetic diversity at certain sites, sites covered by fewer than five individuals were excluded from the PCA and correlation coefficient analyses of haplogroup frequency. The correlation coefficients (R) of the frequencies of different haplogroups in ancient and modern individuals were calculated using the following equation:

$$R(x,y) = \frac{\sum(x-\bar{x})(\hat{y}-\bar{y})}{\sqrt{\sum(x-\bar{x})^2(\hat{y}-\bar{y})^2}},$$

where $0 < R < 1$ means a positive correlation and $-1 < R < 0$ means a negative correlation, x and y are the frequencies of a given haplogroup of the two populations [27]. The network was constructed with complete mtDNA sequences using the median-joining method in the Popart software [28,29]. The Time of the Most Recent Common Ancestor (TMRCA) was estimated by BEAST [30] with the HYK + G + I model because it resulted in the best fit for our data according to jModelTest 2 [31]; we also investigated a model with a Constant Population Size and Strict Clock [20]. For this model, the Markov-Chain Monte Carlo (MCMC) ran for 100,000,000 iterations sampling every 10,000 steps.

3. Results and discussion

3.1. Genetic structure separated by 4600 BP

Complete mtDNA genomes (16,569 bp) were obtained from bone and tooth samples of 86 out of 88 ancient individuals aged between 9500 and 1800 BP from 12 sites in Shandong. From these 86 individuals, we identified a total of 56 haplotypes (Table S1 online). Certain haplotypes (e.g., A5a and B5b2a2) were found to be shared by two to five individuals buried in the same graves at the Beiqian site, which may indicate a matrilineal kinship among them (Table S1 online). For samples having possible matrilineal kinship, only one was kept for the subsequent genetic analyses (10 individuals were removed). The 56 haplotypes could be assigned to 13 basal mtDNA haplogroups [32–37]: A, B, C, D4, D5, F, G, M8, M9, M11, N9a, R, and Z. We used AMOVA to test which population groupings, based on the age of samples, would best represent the genetic structure of all Shandong sites (Table S2 online). We found that the highest variance (variance among groups = 0.17%) was observed when populations were divided into groups older and younger than 4600 BP (group sizes were 27 and 49), which coincides with the interval between the Dawenkou (6000–4600 BP) and Longshan cultural (4600–4000 BP) periods.

3.2. Maternal genetic structure in Shandong before 4600 BP

Twenty-eight individuals were dated to before 4600 BP: a ~9500-year-old individual from Bianbian, an ~8200-year-old Xiaogao individual, an ~8200-year-old Boshan individual [20], three 8000–7700-year-old Xiaojingshan individuals, three 6000 to 4600-year-old individuals from Fujia site, and nineteen 5500 to 5300 BP individuals from Beiqian site (Fig. 1a and Table S1 online). Among them, the samples older than 4600 BP show a high frequency of haplogroups B (B4c1 and B5b2, 24.14%) and D (D4 and D5, 37.93%). The haplogroup B4 is mainly distributed in present-day central-eastern Asians [34], while haplogroup B4c1 is found predominantly in southern East Asians (61.70%), as well as populations from Southeast Asia (Mueang, Tay, PhuLa, Kinh, and LaHu; 25.53%) that are closely related to southern East Asians (Table S6 online). Haplogroup B5b is found widely across East Asia, with its highest diversity in present-day Koreans [34]. B5b2 represents a

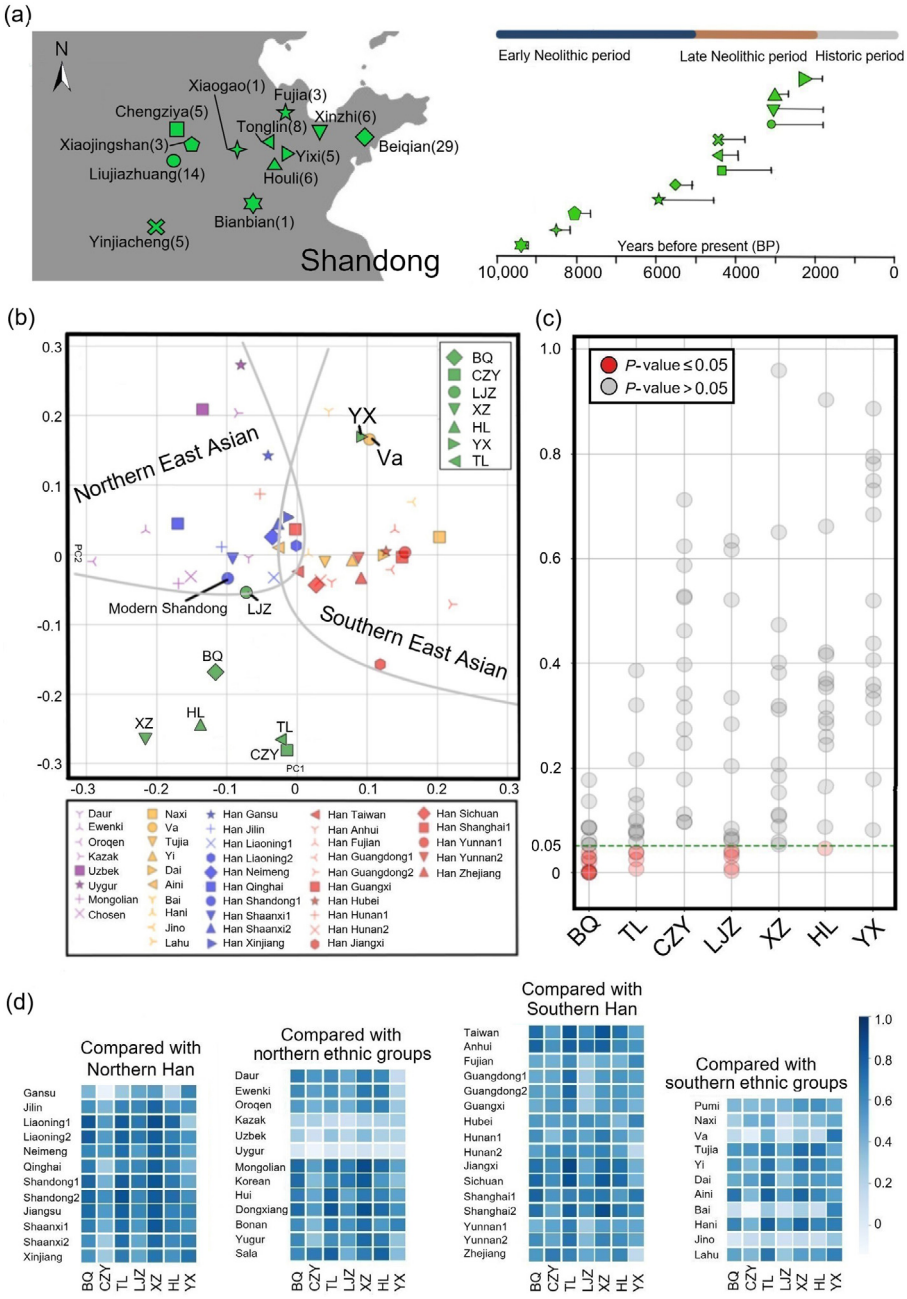

Fig. 1. Genetic analysis of ancient Shandong populations. (a) Geographical and temporal distribution of the ancient humans from 12 archaeological sites across the Shandong Province; (b) PCA plot of the shared mtDNA haplogroup frequencies between the ancient Shandong populations and present-day East Asian populations, showing changes in the maternal genetic structure over time (BQ = Beiqian, CZY = Chengziya, TL = Tonglin, LJZ = Liujiazhuang, XZ = Xinzhi, HL = Houli, and YX = Yixi). Present-day East Asian populations form two major clines, denoted as "northern East Asian" and "southern East Asian"; (c) The *P*-values of genetic distances (Φst) between the ancient Shandong populations and various present-day East Asian populations (Table S4 online) (dots above green dash line have *P*-values > 0.05, representing no significant differentiation, red color means *P*-value $\leq$ 0.05 and color gray means *P*-value > 0.05); (d) Heatmap of mtDNA haplogroup frequency correlations between the ancient Shandong populations and present-day East Asian populations, where values toward 1 indicate a positive correlation (blue), meaning that the two populations share a similar maternal genetic structure, otherwise indicating a negative correlation (white).

more derived haplogroup of B5b that is mainly found in Han, Hezhen, Minnan, and Makatao (63.64%), but is also found in other East Asians, including Japanese, Kinh, Buryat, Khamnigan, and Kizhi (Table S6 online). For haplogroup D, both D4 and D5 have high frequencies in ancient populations of northern East Asia (17.60%–43.75%), but are less frequent in ancient populations of southern East Asia (0–20%) [22,34]. Additionally, N9a was found in one individual aged ~8200 BP from Xiaogao. N9a belongs to the Eastern Eurasian lineage [10], and it is distributed predominantly in East Asia [38]. These results indicate that Shandong populations contained both northern and southern East Asian-associated haplogroups prior to 4600 BP (Fig. 3a).

3.3. Maternal genetic structure in Shandong after 4600 BP

For most of the Shandong sites, haplogroups B and D maintain their high frequencies (>20%) throughout our sampling period (9500–1800 BP) (Table S1 online), while haplogroups C (6.00%, C7a1 and C7b), M9 (6.00%, M9a1), and F (2.00%, F1a1, F2a, and F4a1) are only observed in samples younger than 4600 BP

(Fig. 3b). These newly introduced haplogroups indicate that the maternal genetic structure of Shandong became more diverse beginning with the onset of the Longshan cultural period (4600–4000 BP). In examining the possible sources of these newly introduced haplogroups (Table S3 online), we find haplogroup C is prevalent in northern ethnic groups in East Asia, e.g., the Oroqen population (29.55%, Table S3 online), which is consistent with previous observations that this haplogroup is more diverse among the northern ethnic groups [34]. Haplogroup M9a was suggested to have spread from Southeast Asia northward into the East Asian mainland about 15,000 BP [38], and has a limited distribution among the present-day northern East Asians (0–4.20%) [33]. Haplogroup F1 and F4 are more common in southern East Asia and Southeast Asia than in northern East Asia [34,35], while haplogroup F2 is more frequently distributed among the northern East Asians [10]. As these more recent haplogroups have been found in both southern and northern East Asians, their appearance in Shandong, without replacing those observed before 4600 BP, may represent an influx of populations from outside Shandong after this period.

We further investigated populations from each site between 4600 and 1800 BP using PCA of haplogroup frequencies (Fig. 1b). Present-day northern and southern East Asians can be distinguished along the PC1 axis, driven mainly by the different proportions of haplogroup D, B, and F. Present-day northern East Asians have a higher proportion of haplogroup D (e.g., Mongolian 39.58%, Han-Jilin 35.29%, and Han-Shandong1 36.00%), while haplogroup B and F are more frequent among southern East Asians (e.g., B: Han-Jiangxi 34.78%, Han-Guangdong2 30.43%, F: Va 31.82%, Lahu 33.33%, and Bai 25.00%) (Table S3 online). The individuals from the Chengziya site (CZY, 4400–3300 BP, during and after the Longshan culture) and Tonglin site (TL, 4600–4000 BP, belonging to the Longshan culture) are close to the center of PC1 (Fig. 1b) mainly due to these two sites having high proportions of haplogroups elevated in either southern or northern East Asians. Specifically, the frequencies of haplogroup B of the two sites are higher than 37.50%, and the frequencies of haplogroup D are 20.00% (CZY) and 37.50% (TL), respectively. Haplogroup F was also found in the Tonglin site with a frequency of 12.50%. The Xinzhi (XZ, 3050–1800 BP) and Houli (HL, 3050–2750 BP) are closer to northern East Asians on PC1 (Fig. 1b), which can be explained by their higher proportion of haplogroup D (50.00% in XZ and 33.30% in HL). The population from the Liujiazhuang site (LJZ, 3100–1800 BP) clusters with present-day northern East Asians (Fig. 1b), likely owing to the Liujiazhuang population sharing 59.40% of their haplogroups (A, B, D, G, M11, M8, and N9a) with the present-day Shandong population (Han-Shandong1). On the other hand, the Yixi (YX, 2300–1800 BP) site is closer to southern East Asians on PC1 with a haplogroup F frequency of 40.00% (Fig. 1b).

3.4. Relationship between ancient Shandong populations and present-day East Asians

We then investigated how the ancient Shandong populations are related to present-day East Asians. First, we calculated the Φst between the ancient Shandong populations and present-day East Asians (15 selected populations from Han and other ethnic groups) (Fig. 1c and Table S5 online). Beqian (5500–5300 BP) is found to be more genetically different from present-day East Asians (eight populations show significant Φst with Beiqian), compared with younger Shandong populations which are more similar to present-day East Asians (0–4 populations show significant Φst). We further looked into the haplotype composition (reflected by correlation of haplotype frequencies) between the ancient Shandong populations and present-day East Asians. We observe that ancient Shandong populations are less similar to several northern ethnic groups (Kazak, Uzbek, and Uygur) and southern ethnic groups (Bai and Jino) compared with other present-day East Asians (Fig. 1d).

3.5. Possible genetic exchange between coastal and inland Shandong

We also investigated the population dynamics between the coastal site (Beiqian, 5500–5300 BP) and inland regions across time. In the coastal region, the site contained haplogroups M8 (10.50%) and A (5.30%) (Fig. 3b). However, in the inland sites, we observed these two haplogroups (M8 10% and A 16%) only in sites younger than 3100 BP (Fig. 3b). Furthermore, we found that populations from the coastal site and older inland sites (4600–3100 BP) had a larger value of genetic distance (Φst = 0.037, P = 0.06), compared with that between the coastal site and younger inland sites (3100 to 1800 BP) (Φst = 0.005, P = 0.30). We observed a similar pattern in the AMOVA analysis, where the coastal site and older inland sites had a larger value of variance (5.19%) than that between the coastal site and younger inland sites (1.39%). These results suggest that exchange between populations from the coastal and inland regions was less frequent before 3100 BP, which is consistent with archaeological findings that there were cultural differences between coastal and inland regions before and during the Longshan culture [39–41]. An alternative explanation could be due to the influx of people with ancestry from other parts of East Asia that introduced haplogroups M8 and A [6,13,33].

3.6. Discovery of the haplogroup B5b2 lineage in Shandong

A previous study speculated that populations carrying haplogroup B5b migrated from northwestern mainland East Asia into other East Asian areas, bypassing Shandong, as this haplogroup had not been previously observed in ancient Shandong based on ~3000-year-old individuals from northwestern mainland East Asia [10,34]. Here, we identified nine ancient individuals belonging to haplogroup B5b from six sites in Shandong, (9500 to 1800 BP): Bianbian (n = 1), Beiqian (n = 4), Houli (n = 1), Tonglin (n = 1), Xinzhi (n = 1), and Chengziya (n = 1). To investigate this haplogroup in more detail, we obtained 20 complete B5b mitochondrial sequences from present-day individuals (Table S6 online) representing present-day East Asians, including the Northern Han (n = 2), Southern Han (n = 3), Hezhen (n = 1), Minnan (n = 1), Makatao (n = 1), Hakka (n = 1), Kyrgyz (n = 2), and Tingri (n = 1), Buryat (n = 1), Khamnigan (n = 1), Kizhi (n = 1), Japanese (n = 1), Suay (n = 1), Shan (n = 1), and Kinh (n = 2). Mitogenome coalescence times were estimated from the complete data using BEAST [30]. Table S7 (online) shows the B5b lineage after calibrating with the radiocarbon dates of our samples. Under the B5b2 lineage, the B5b2a2 sublineage from the 5500 to 5300-year-old Beiqian (BQ-M2*) shares a common ancestor with the B5b2a2 in present-day Hezhen (HGDP01238), Buryat (JN857016), and Khamnigan (JN857039) (Fig. 2a–c). The estimated Time to the Most Recent Common Ancestor (TMRCA) of B5b2a2 is 8056 BP (95% highest probability density (HPD), 5400–12,192, Table S7 online) with a posterior value of 1.00. Furthermore, two Beiqian individuals (BQ-M31-A and BQ-M24*) are likely to share a common B5b2a ancestor with the Japanese (HGDP00767) around 13,040 BP (95% HPD, 7595–20,329, Table S7 online) with a posterior value of 0.99. Beiqian (BQ-M139-D) and Xinzhi (XZ-M59) individuals are likely to share a common B5b2b ancestor with Han individuals from the Southern Han (HG00690) and Northern Han (NA18643) around 11,513 BP (95% HPD, 6368–18102, Table S7 online) with a posterior value of 0.98. Accordingly, all of them share a common B5b2 ancestor related to the ~9500-year-old Bianbian individual

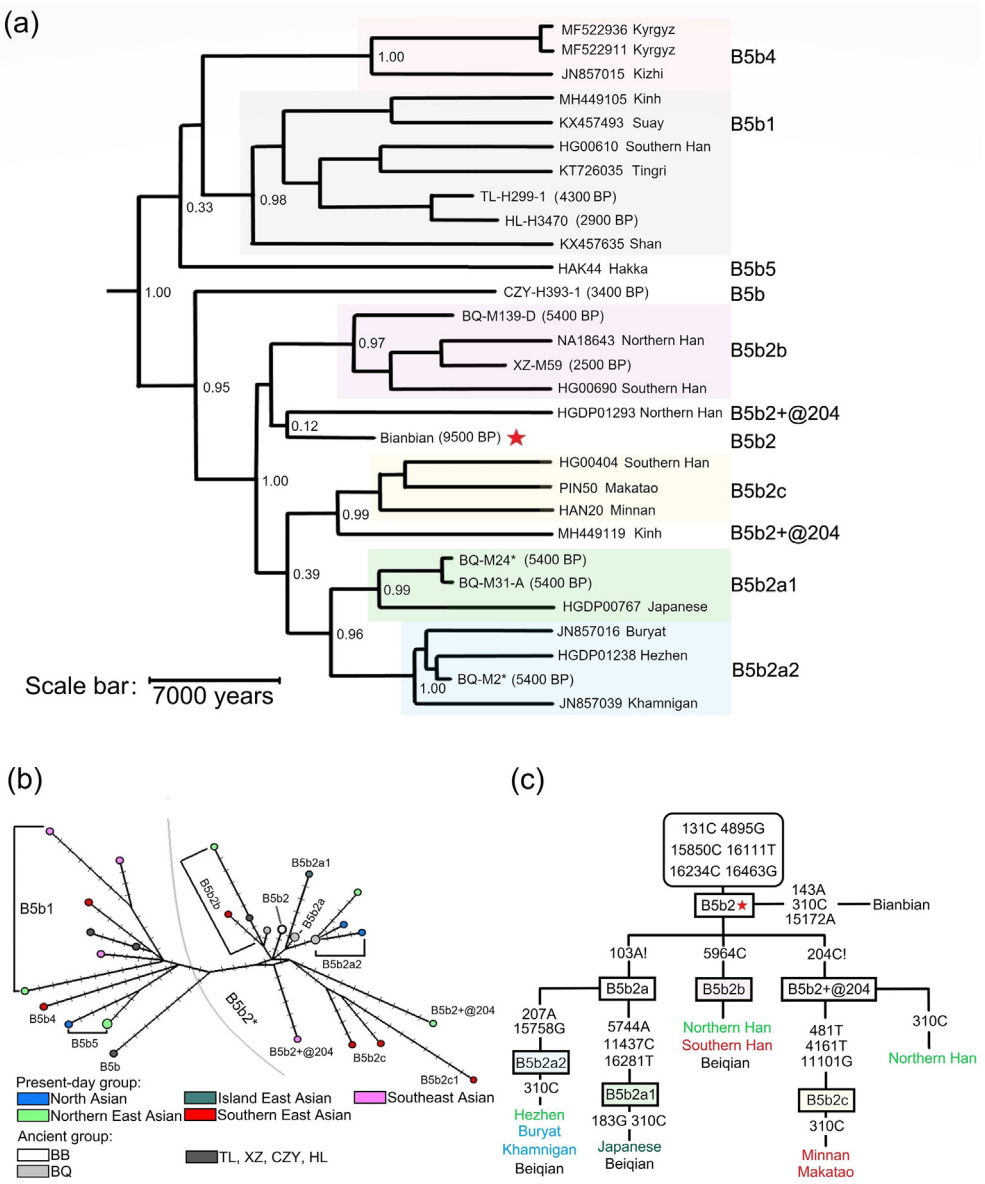

Fig. 2. Genetic analysis of populations in the B5b lineage. (a) Bayesian coalescence tree of the B5b lineage with the basal position of Bianbian indicated (red star) (BQ-M2-B, BQ-M2-E, BQ-M2-H, BQ-M2-L, and BQ-M2-J, these 5 individuals in one matrilineal kinship were regarded as BQ-M2*, BQ-M24-4 and BQ-M24-23 in one matrilineal kinship were regarded as BQ-M24*), all the individuals labeled with the sample ID and dates (without a population name) are from ancient Shandong sites, the whole tree can be seen in Fig. S3 (online); (b) Network of the B5b lineage (B5b2 lineage is in the right part of the grey line) using complete mitochondrial genomes of ancient Shandong and present-day Asians showing that the Bianbian individual and Beiqian population are closest to the root of haplogroup B5b2, and in particular, some Beiqian sequences are ancestral to present-day East Asians and North Asians; (c) Private mutations under the B5b2 lineage showing that Bianbian is three mutations (143A, 310C, and 15172A) away from the root of the haplogroup B5b2, and that Bianbian and Beiqian are closely related to their common ancestor with present-day East Asians and North Asians (@ = assumed back mutation or missing mutation).

around 17,293 years ago (95% HPD, 10,503–26,726 BP, Table S7 online). These results suggest that the B5b2 lineage found in the Bianbian individual (9500 BP) is likely ancestral to East Asians and North Asians (Fig. 2a–c and Fig. 3a). This coincides with the population dynamics in Shandong proposed in previous studies, with an outward spreading to the west and south of East Asia and interactions with other cultures (e.g., the Yangshao culture) [41–43].

4. Conclusion

In this study, we reconstructed the maternal genetic structure of Shandong populations over the past nine thousand years (Fig. 3). Since 9500 BP, the main haplogroups of present-day northern and southern East Asia could be observed in the ancient populations of Shandong. After 4600 BP, additional connections were identified between populations inside and outside of Shandong. After 3100 BP, a possible genetic exchange between the coastal and inland regions within Shandong was observed. Additionally, in the B5b2 lineage newly discovered in Shandong populations, we observed (1) the Bianbian individual likely belongs to a lineage ancestral to East Asians and North Asians; (2) a continuous maternal genetic structure from the ~9500-year-old Bianbian individual to the 5500–5300-year-old Beiqian population. A wider range of temporal and geographical Y-chromosome and nuclear genomic data could further build on these insights and provide a more comprehensive picture of the genetic history and population movements of the people of ancient Shandong.

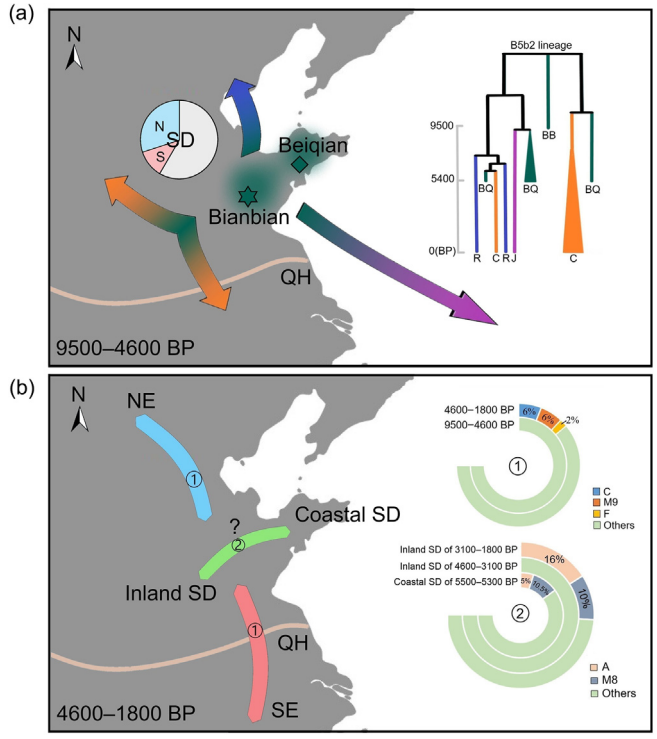

Fig. 3. The maternal genetic history of ancient Shandong population from 9500 to 1800 BP. (a) The pie chart shows that Shandong populations from 9500 to 4600 BP contained both northern and southern East Asian associated haplogroups (N = northern East Asian associated haplogroups; S = southern East Asian associated haplogroups); arrows with different colors indicate that the populations with B5b2 lineage in the Shandong region (the ~9500-year-old Bianbian individual and the 5500- to 5300-year-old Beiqian population) were ancestral to present-day East Asians and North Asians. (SD = Shandong populations in color green; C = Han, Hezhen, Minnan, and Makatao in color orange; R = Buryat and Khamnigan (located in North Asia) in color blue; J = Japanese in color purple; QH and the brown line = Qinling-Huaihe); (b) The maternal genetic sources in Shandong became more diverse since the Longshan cultural period (4600–4000 BP). New haplogroups (C, M9, and F) appeared since 4600 BP in the ancient Shandong population, indicating the appearance of northern (blue arrow) and southern (red arrow) maternal lineages in Shandong; The possible exchange with haplogroups M8 and A between the populations from the coastal and inland regions was less frequent before 3100 BP but frequent after 3100 BP (green arrow with the question mark) (NE = northern East Asian, SE = southern East Asian).

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Author contributions

Qiaomei Fu designed the research project. Qiaomei Fu, Juncen Liu, Wen Zeng, Bo Sun, and Xiaowei Mao managed the project. Wen Zeng, Bo Sun, Yongsheng Zhao, Fen Wang, Zhenguang Li, Fengshi Luan, Junfeng Guo, Chao Zhu, Zimeng Wang, Chengmin Wei, Ming Zhang, Weihong Hou, Wanjing Ping, and Xiaohong Wu collected archaeological samples and dating. Qiaomei Fu, Peng Cao, Ruowei Yang, Feng Liu, Xiaotian Feng, and Qinyan Dai, performed or supervised wet laboratory work. Qiaomei Fu and Xiaotian Feng did the data processing and quality control. Qiaomei Fu, Ming Zhang, Juncen Liu, and Xiaowei Mao supervised or performed the analyses. Juncen Liu, Wen Zeng, Xiaowei Mao, and Qiaomei Fu wrote the manuscript. E. Andrew Bennett, Yichen Liu, and all other authors discussed, critically revised, and approved the final version of the manuscript.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2021.01.029.

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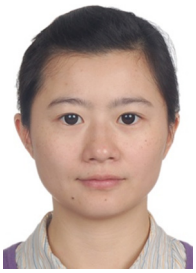

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