



Letter to the editor

## Maternal genetic structure of a neolithic population of the Yangshao culture

The Yangshao culture is one of the most influential Neolithic cultures in East Asia. The populations associated with the Yangshao culture were widely distributed along the Yellow River basin and were suggested to play a profound role in the present-day East Asians. To date, the population of the Yangshao culture is largely investigated through archaeological studies. Archaeological evidence, especially the characteristic buildings and potteries, suggests the population of the Yangshao culture had wide connections with surrounding areas, including the Majiayao culture in the Ganqing region, the Laohushan culture in the Hetao Plain region, and the Dawenkou culture in the Shandong region (Liu and Chen, 2012). Both archaeological and paleoenvironmental studies suggest that during the Miaodigou Phase I (~6000 to 5500 years before present [BP]) of the Yangshao culture, the Yangshao culture spread to present-day eastern Henan Province, Ganqing region, the Hetao Plain, and Huaihe-Hanshui River basin (Liu and Chen, 2012; Hou et al., 2019). However, despite the great efforts put into the recovery of genetic records of ancient East Asians of different cultures (Ning et al., 2020; Yang et al., 2020), the population of the Yangshao culture has yet been thoroughly characterized regarding their maternal genetic structure so far. Consequently, the genetic connections between the population of the Yangshao culture and other East Asian populations remain to be further investigated.

Ancient mitochondrial genomes obtained using ancient DNA (aDNA) techniques have advanced our understanding of human maternal genetic history and offered better insights into complex human evolutionary questions (Lippold et al., 2014; Prüfer et al., 2014). Mitochondrial DNA (mtDNA) contains maternal genetic information and offers a distinctive perspective on the matrilocality aspect of population history (Petr et al., 2020). Because of the degradation and fragmentation of aDNA, the short hypervariable regions of mtDNA are frequently adopted for the reconstruction of the maternal genetic structure of ancient populations, whereas the resolution of these regions is limited by the short fragment lengths.

Here, we obtained the whole mitochondrial genomes of 60 individuals from the Qingtai site (~5500 to 5000 BP) located in Qingtai Village, Guangwu Township, Xingyang City, Henan Province, China (Fig. 1A). This site was considered as a Yangshao culture site because pottery characteristics of the Yangshao culture were identified from here (Zhang and Zhao, 1987). Specifically, DNA was extracted from ~100 mg bone powder and double-stranded DNA libraries were prepared, and then mtDNA fragments were captured and sequenced as previously described (Dabney et al., 2013; Fu et al., 2013). Following the established criteria (Fu et al., 2016), we excluded two individuals showing a relatively high level of contamination (>5%, 95% confidence interval [CI] >15%), two individuals with

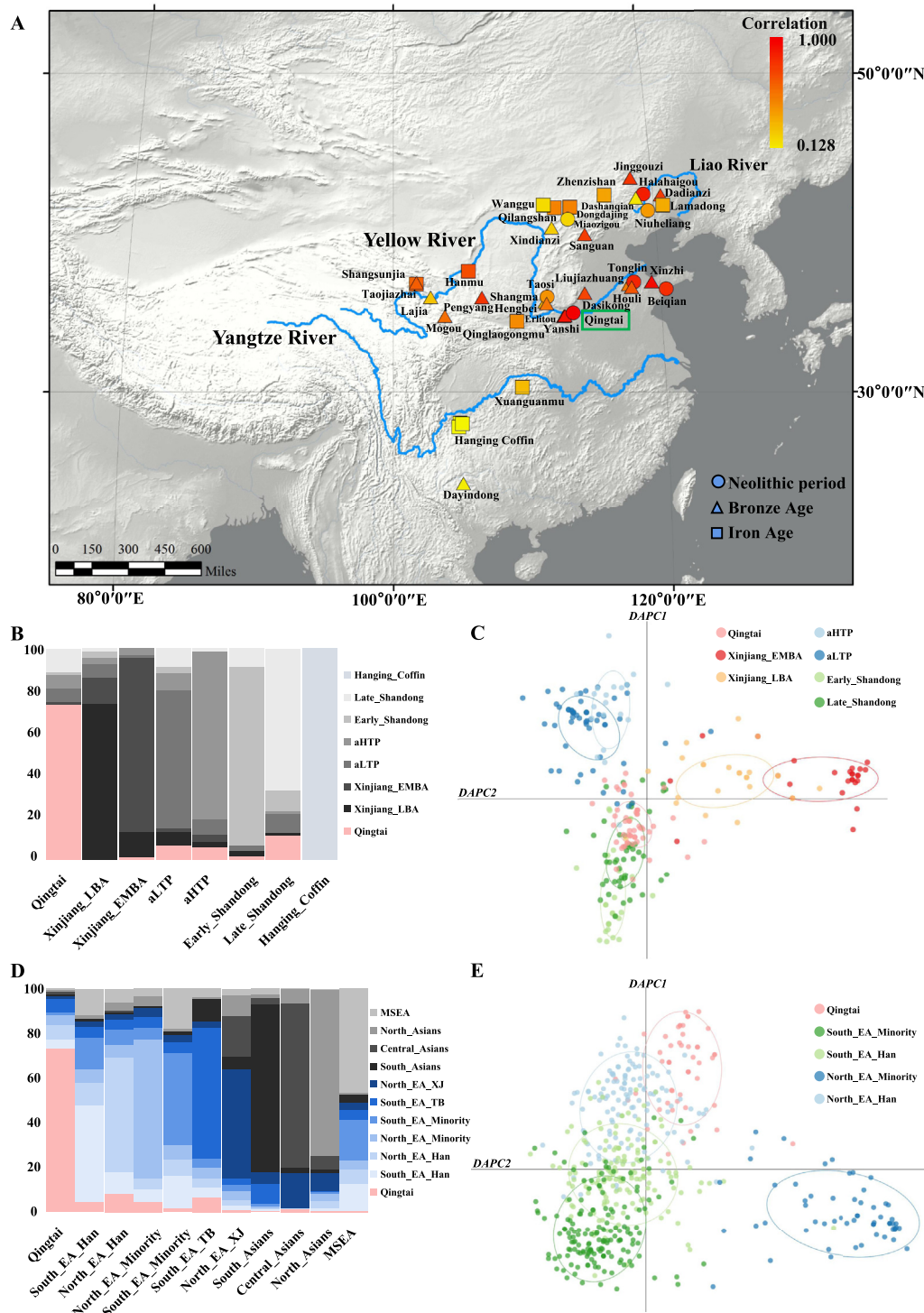
high proportions of ambiguous bases ("N"), and five individuals with probable matrilineal kinship (individuals share an identical mitochondrial genomic sequence and from the same tomb were considered to be related to each other by matrilineal relationship; a total of five pairs of matrilineal-related individuals were identified, and only one individual from each pair were kept for follow-up analyses; Table S1). In total, we obtained high-quality complete mitochondrial sequences (coverage, 21.47- to 418.21- fold; contamination estimate, 0.0–2.6%, and upper bound of 95% CI 1.9–3.4%) from 51 individuals for further analyses (Table S1). All the newly reported mtDNA genome sequences have been deposited in the Genome Warehouse in National Genomics Data Center (National Genomics Data Center Members and Partners, 2020), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences under accession number PRJCA004284.

Complete mitochondrial genome results showed that the Qingtai individuals can be unambiguously allocated into haplogroups **A, B, D, F, G, M9, and M10**, which can be further assigned into 35 haplotypes. The mtDNA haplogroups of the Qingtai individuals are dominated by haplogroup D (D4, 27.45% and D5, 13.73%), followed by haplogroup B (B4, 17.65% and B5, 1.96%) and haplogroup A (13.73%). We then examined the frequencies of the identified haplogroups in present-day populations in northern and southern East Asia (here, northern and southern East Asia is divided by the Qinling-Huaihe line between the Qinling Mountains and the Huaihe River). Haplogroup D (D4 and D5) has high frequencies in present-day populations in northern East Asia (range from 1.90% to 25.00%), although it is less frequent in present-day populations in southern East Asia (<16.67%; Table S2). Haplogroup B (B4 and B5) is more common in present-day populations in southern East Asia (<34.80%) than that of present-day populations in northern East Asia (< 25.30%; Table S2). Haplogroup A is slightly more frequently present in present-day populations in northern East Asia (range from 2.20% to 17.80%) than that of present-day populations in southern East Asia (<16.70%). Haplogroup M9 is frequently observed in present-day populations in the Tibetan plateau (29.90%) (Ding et al., 2020). Overall, the haplogroups that mainly distributed in both northern (haplogroup A, D4, D5, F2, and G) and southern East Asia (haplogroup B4, B5, F1, and M9a) were observed in our samples, and the proportion of the former (64.71%) is about two times of the latter (31.37%), suggesting a stronger connection between the Qingtai population and northern East Asia-associated populations. These genetic results are consistent with the archaeological evidence that the population of the Yangshao culture was extensively connected with populations located in surrounding areas. Although our data highlight a close relationship between the population of the Yangshao culture (represented by the

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**Fig. 1.** Maternal genetic relationships between the Qingtai population and ancient/present-day populations in East Asia. **A:** The location of the Qingtai site and the correlation of haplogroup frequencies between the Qingtai population and ancient populations (Table S3). The correlations are reflected by colors (red indicates a higher correlation and yellow indicates a lower correlation); the shape of the symbol indicates the age of the samples (square, the Neolithic period; triangle, the Bronze Age; circle, the Iron Age). The identifications of the sites are showed on the side of the symbols: “Hanging Coffin” including three sites (Doushaguan, Changan, and Longma) located in Zhaotong City, Yunnan Province. The Qingtai site was marked with a green rectangle. **B:** The haplotype sharing between the Qingtai population and ancient populations (Tables S4 and S5). Xinjiang\_LBA: Xinjiang\_Late Bronze Age, Quanergou site and Haiziyuan site (eastern Xinjiang); Xinjiang\_EMBA: Xinjiang\_Early and Middle Bronze Age, Chagangole site (northern Xinjiang); aLTP: ancient low-altitude Tibetan plateau; aHTP: ancient high-altitude Tibetan plateau. **C:** The discriminant analysis of principal components (DAPC) plot of the Qingtai population and ancient populations (Table S4). **D:** The haplotype sharing between the Qingtai population and present-day populations (Tables S4 and S5). MSEA: present-day populations in mainland of Southeast Asian; North\_Asians: North Asians; Central\_Asians: Central Asians; South\_Asians: South Asians; North\_EA\_XJ: North East Asians in Xinjiang, China, includes Uygur, Kyrgyz, Tajik in present-day China; South\_EA\_TB: South East Asians in the Tibetan plateau, China, includes Chamdo, Lhasa, Ngari, Shannan, Nagqu, Shigatse, Nyingchi. Deng, Nyingchi. Lhoba, Nyingchi. Monpa, Sherpa, Shigatse. Sherpa, and Shigatse. Tingri in present-day China; South\_EA\_Minority: Minority of present-day South East Asians; North\_EA\_Minority: Minority of present-day North East Asians; North\_EA\_Han: Han of present-day North East Asians; South\_EA\_Han: Han of present-day South East Asians. **E:** The DAPC plot of the Qingtai population and present-day populations (Table S4).

Qingtai population) and the northern East Asians, the maternal genetic components in the Qingtai population that related to the southern East Asians are also not negligible. Indeed, archaeological and physical anthropological evidence suggests a close tie between the populations of southern China and Southeast Asia (Blench et al., 2005). Future genetic and archaeological research on additional Asian populations, especially prehistoric populations in South China and Southeast Asia, could shed further light on their demographic dynamics.

We also looked into the haplogroup composition (reflected by correlation of haplogroup frequencies) between the Qingtai population and ancient populations in East Asia. We incorporated mtDNA haplogroup data of additional 37 ancient populations in East Asia for the analysis (Table S3). To avoid artificially low genetic diversity introduced from insufficient sampling, an empirical threshold of a minimum of six individuals in a population (Liu et al., 2021) was applied to our data, and three populations were excluded from follow-up analyses. We observed that several ancient populations (Erlitou, Yanshi, Xinzi, etc.) in the Yellow River region and several ancient populations in the West Liao River region (Halahaigou, Jinggouzi, etc.) are more similar to the Qingtai population compared with other ancient populations in East Asia (Fig. 1A).

To further investigate the maternal genetic relationships between the Qingtai population and ancient populations in East Asia, we used complete mtDNA genome sequences of another 226 ancient individuals for analyses (Table S4). These individuals were grouped the same way as previously described for the following analyses, including the early (before 4600 BP) and late (after 4600 BP) Shandong populations that were defined based on the Analysis of Molecular Variance results (Liu et al., 2021). In the haplotype sharing analysis (Ko et al., 2014; Ding et al., 2020), the Qingtai population shared a higher level of haplotypes with the late Shandong populations (11.00–11.70%) than the early Shandong populations (1.43–1.61%) or other ancient populations (0.00–6.81%; Fig. 1B; Table S5). Simultaneously, except Hanging Coffin and Xinjiang\_LBA did not share any haplotype with the Qingtai population, other populations had different degrees of haplotype sharing with the Qingtai population (Xinjiang\_EMBA, 1.40–1.60%; aLTP, 6.30–6.80%; and aHTP, 5.70–6.10%; Table S5). In addition, many sub-haplogroups of haplogroup B5b were distributed in ancient Shandong populations (Liu et al., 2021), and haplogroup D5a2a1 were also identified in the Longshan period population (Cui et al., 2019). Therefore, the network analyses of haplogroup B5b and D5a2a1 were performed to explore the relationships between these populations and the Qingtai population. The result of haplogroup B5b and D5a2a1 network analyses showed that the Qingtai population and the late Shandong populations were in the same branch, and these two populations are only 3–4 mutations away (Fig. S1A). The phylogenetic analysis of haplogroup D5a2a1 also showed that these two populations belong to the same mega branch, and the posterior values of many sub-branches are very low (~0.13–0.44; Fig. S1B), indicating a fast expansion of this haplogroup in a short time. Furthermore, we used discriminant analysis of principal components (DAPC) (Jombart et al., 2010) to maximize between-population differences and minimize within-cluster variation based on alleles. We observed that the Qingtai population was clustered with the late Shandong populations in the DAPC plot (Fig. 1C). This observation is consistent with genetic distance results. The Qingtai population had a closer genetic distance ( $\phi_{ST} = 0.007$ ,  $P > 0.05$ ) with the late Shandong populations compared with that of the early Shandong populations ( $\phi_{ST} = 0.030$ ,  $P < 0.05$ ) and other ancient populations (Table S6). These results indicate a close maternal relationship between the Qingtai population and the late Shandong populations. It is known from archaeological studies that the Yangshao culture was closely related to the Longshan culture in the Central Plain (Liu and Chen, 2012). Our results here may suggest a potential connection between

the population of the Yangshao culture, at least the sampled Qingtai population, and the population of the Shandong Longshan culture (a proportion of the late Shandong population are Longshan culture). However, this connection needs to be further explored and verified in the future. In addition, from the haplogroup composition, we found that the Qingtai population have fewer proportions of haplogroups that mainly distributed in southern East Asians than that of the late Shandong populations (haplogroups B4, B5, F1, F4, N9a, M9a, and R; 40.8%), which imply that the Qingtai population have fewer southern East Asia components than that of the late Shandong populations.

Finally, we examined the connections between the Qingtai population and the present-day populations in East Asia. The Qingtai population has higher levels of haplotype sharing with the present-day Han (3.45–8.51%) than other populations (0.45–1.36%; Fig. 1D; Table S5). In a DAPC analysis including populations from Table S4, the Qingtai population was close to the present-day Han in northern East Asia (Fig. 1E). This observation is consistent with the minor genetic distance between the Qingtai population and present-day Han (Han in northern East Asia,  $\phi_{ST} = 0.019$ ,  $P < 0.05$ ; Han in southern East Asia,  $\phi_{ST} = 0.026$ ,  $P < 0.05$ ; Fig. S2; Table S6). Besides, the haplogroup D4h1c network analysis showed that the Qingtai population and several present-day populations in East Asia were in the same branch, and these populations were separated by only 1–5 mutations (Fig. S3A), and the phylogenetic analysis of haplogroup D4h1c also showed that these populations belong to the same mega branch (Fig. S3B). Furthermore, we observed a high maternal genetic diversity (35 haplotypes identified from 51 individuals) in the Qingtai population, and ~40% of these haplotypes (Table S1) were maintained in the present-day Han. The present-day Han is also highly diverse regarding maternal genetics (Yao et al., 2002; Li et al., 2019). Considering the close connections and high levels of haplotypes shared between the Qingtai population and present-day Han, it is very likely that the Qingtai population played a role in the maternal diversity of the present-day Han.

In summary, our study explores the connections between a population of the Yangshao culture and the related populations using whole mitochondrial genomes reconstructed from 60 Neolithic individuals. For the first time, the maternal genetic structure of a population of the Yangshao culture was profiled in detail. We found that the Qingtai population was a population with high maternal genetic diversity and possessed dynamic and close relationships with multiple ancient and present-day East Asia populations. Specifically, we observed close connections between the Qingtai population and ancient populations distributed around the Qingtai site, and a similar genetic association was observed between the Qingtai population and multiple present-day populations in China. By obtaining comprehensive maternal genetic information of the Qingtai population, we provide genetic evidence for further exploration of both the genetic history of the Yangshao culture and its impact on present-day East Asians.

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## Supplementary data

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