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Phylogeography of *Prunus armeniaca* L. by Chloroplast DNA and Nuclear Ribosomal Sequences

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8 Abstract

9 To clarify the phylogeography of *Prunus armeniaca* L., two chloroplast DNA
10 fragments (*trnL-trnF* and *ycf1*) and the nuclear ribosomal DNA internal transcribed
11 spacer (ITS) were employed to assess the genetic variation across 12 *P. armeniaca*
12 populations. The results of cpDNA and ITS sequence data analysis showed that the
13 level of genetic diversity in *P. armeniaca* was high (cpDNA: $H_T=0.499$; ITS:
14 $H_T=0.876$), and the level of genetic differentiation was low (cpDNA: $F_{ST}=0.1628$; ITS:
15 $F_{ST}=0.0297$). An analysis of molecular variance (AMOVA) revealed that most of the
16 genetic variation in *P. armeniaca* occurred among individuals within populations. The
17 value of interpopulation differentiation (N_{ST}) was significantly higher than the number
18 of substitution types (G_{ST}), indicating a genealogical structure in *P. armeniaca*. *P.*
19 *armeniaca* shared the same genotypes with related species and may be associated with
20 them through continuous and extensive gene flow. The haplotypes/genotypes of
21 cultivated apricot populations in Xinjiang, North China, and foreign apricot
22 populations were mixed with large numbers of haplotypes/genotypes of wild apricot
23 populations from the Ili River Valley. The wild apricot populations in the Ili River
24 Valley contained the ancestral haplotypes/genotypes with the highest genetic diversity
25 and were located in an area considered a potential glacial refugium for *P. armeniaca*.
26 Since population expansion occurred 16.53 kyr ago, the area has provided a suitable
27 climate for the population and protected the genetic diversity of *P. armeniaca*.

28 Introduction

29 The evolutionary history of organisms, including their genetic diversity, population
30 structure and historical dynamics, is critical to species conservation ¹. Understanding
31 the effects of climate change on the spatial genetic patterns of species, particularly
32 endangered species, can help reveal not only the evolutionary history of species but
33 also conservation strategies ²⁻³. The origins of mountain biodiversity are complex and

may include the immigration of preadapted lineages⁴⁻⁵, in situ diversification⁶, or the continuation of ancestral lineages⁷. Compared with those of other mountains, such as the Hengduan Mountains, the evolution and diversity of the Tianshan Mountains are still poorly understood. The Ili River Valley is located in the western part of the Tianshan Mountains in China and is surrounded by mountains on three sides. This valley was the main northern crossing of the ancient Silk Road. In the late Tertiary period, a large number of species, including wild apricot, wild apple and wild hawthorn, remained in the Ili River Valley and were important components of the deciduous broad-leaved forest at elevations below the coniferous forest and above the mountain grassland belt in the Xinjiang Uygur Autonomous Region, China⁸. However, there are few studies on the phylogeography of plant species in the arid region of Northwest China⁹⁻¹⁰.

In recent decades, the method of combining molecular data with paleoclimatic and geographical evidence has been effective in the study of system geography¹¹⁻¹². Many systematic geographic studies have shown that the Last Glacial Maximum (LGM) of the Pleistocene strongly influenced the genetic variation and biodiversity of plants throughout the Northern Hemisphere¹³⁻¹⁴. Especially during the Quaternary glacial period, species in the ice-free zone were mainly affected by the cold and dry climate¹⁵. Climatic fluctuations cause the distribution of species to shrink and expand¹⁶, and cold and dry climates prompted species to retreat to refugia, providing shelter for plants and animals¹⁷. As temperatures rose after the glacial period, species underwent population expansion, changes that left a genetic signature in their current populations¹⁸. Although no unified glacial sheet had occurred in Asia, paleodata suggested that the distribution ranges of woody species in this region were similarly changed by the Quaternary climatic oscillations¹⁹. Based on the geographical distribution of genetic variation, the glacial refuges and postglacial revegetation routes of most woody plants are roughly consistent with fossil evidence²⁰. However, due to the lack of fossil evidence, genetic evidence has become an important means of providing information on the distribution range of some woody species and the history of glacial refugia²¹⁻²². Volkova²³ interpret the observed genetic structure [nuclear (ITS) and plastid DNA] of the Eurasian populations of *Prunus padus* as plausibly resulted from at least two cycles of glacial survivals in refugia followed by post-glacial colonization events.¹⁷The complex geographic history may have provided a refuge for species from the last glacial period¹⁷. Xu et al.²⁴ proposed that there were two possible independent glacial refugia in Northwest China: the Ili River Valley and the Northern Junggar Basin. Su et al.⁹ speculate that aggravation of the dry and cold climate during the early Quaternary, combined with plant physiological features, were determining factors contributing to the lineage split, and climate oscillations most likely led to the Yili range expansion. Therefore, it is of great interest to study the systematic geography of endemic plants in Northwest China against the background of Quaternary climatic fluctuations.

Apricot is a fruit of temperate and subtropical regions. Turkey, Uzbekistan, Iran, Algeria, Italy, Spain, China, Pakistan, France and Japan are the main producers of

apricots (<http://www.fao.org/home/zh>). Apricot belongs to the section *Armeniaca* (Lam.) Koch, subgenus *Prunophora* Focke and genus *Prunus* (Rosaceae)²⁵. Almost all cultivated apricots originated from *P. armeniaca*²⁶. In China, wild apricot is distributed in the Ili River Valley (Tianshan Mountain area). The species is also distributed along the Tianshan mountains and westward to Kazakhstan, Kyrgyzstan and Uzbekistan. It is a relic of a broad-leaved forest from the late Tertiary period, which played a decisive role in the domestication of cultivated apricots worldwide²⁷. As the world experienced extreme weather events, especially glacial periods, and most species went extinct, some of the more complex valleys may have become refugia for surviving forests and locally present species. As a result, traces of the species may be gradually shrinking in such areas. So, is Ili River Valley a glacial refugia for wild apricot?

Generally, seed dispersal distance is much less than that of pollen, and population divergence due to genetic drift will be more marked for chloroplast DNA (cpDNA) than for nuclear DNA. Indeed, cpDNA is considered to evolve very slowly, with low recombination and mutation rates²⁸. Organelle markers could provide powerful tools for studying the phylogeography and migratory footprints of species²⁹. Parental genetic markers are often combined with single-parent organelle markers for population genetics studies³⁰. cpDNA and ITS sequence variations have been very effective in revealing the glacial refuges of plants²⁹. cpDNA lineages usually show the unique geographical distribution and evolutionary history of natural populations and are therefore widely used in studies of systematic geography³¹. Li et al.³² successfully used cpDNA and ITS markers to evaluate the diversity and phylogenetic relationships among populations of *Saxifraga sinomontana*, indicating that the species had microrefugia during the Quaternary glacial period. In this study, we employed cpDNA (*trnL-trnF* and *ycf1*) and nuclear ribosomal DNA (nrDNA) sequences to (1) reveal the haplotype/genotypic diversity and population genetic structure of the species; (2) test for potential glacial refugia for the species in the Ili River Valley; and (3) assess whether the refugia show signs of recent expansion.

Materials and methods

Sample collection

The samples used for cpDNA analysis included 123 individuals from 20 populations. A total of 171 individuals from 19 populations was used for the ITS analysis, of which 38 samples were obtained from the NCBI database (Table S1). The samples studied were from *P. armeniaca* and related species (*P. sibirica*, NAG; *P. mandshurica*, LX; *P. dasycarpa*, ZX; *P. mume*, ECG; *Prunus zhengheensis*, ZHX³³; *Prunus limeixing*, LMX³³ and *Prunus brigantina*, FGX). *Prunus davidiana* (T) was regarded as the outgroup.

Our collection of wild apricot (*P. armeniaca*) populations covered most of the natural distribution in China, including Huocheng County (DZGhcmd, DZGhcy and DZGhem populations), Yining County (DZGyn population), Gongliu County

(DZGglb and DZGgld populations) and Xinyuan County (DZGxyt, DZGxya and DZGxyz populations). The distance between individuals sampled in each population was at least 100 m. Young leaves were collected and dried immediately with silica gel.

The cultivated populations of *P. armeniaca* included the Xinjiang apricot group (CAG, cultivated apricots in Xinjiang), the North China apricot group (NCG, cultivated apricots in Shandong, Shaanxi, Gansu, Liaoning and Ningxia) and the foreign apricot group (EG, cultivated apricots in the USA, France, Italy and Australia). Detailed sample information was provided in Table 1 and Table S2. The main characteristics of different populations can be found in Zhang et al.⁸.

DNA sequencing

The total genomic DNA was extracted from the silica gel-dried leaf materials using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China)³⁴. The quality and concentration of the extracted DNA were determined by 1% agarose gel electrophoresis and ultraviolet spectrophotometry, respectively.

cpDNA and nrDNA sequences were initially screened from 15 samples using universal primers. The sequencing results showed that the sequences of cpDNA (*trnL-trnF* and *ycf1*) and nrDNA (ITS) were polymorphic. cpDNA and ITS fragments were amplified by polymerase chain reaction (PCR), and the details of their primers are provided in Table S3³⁵⁻³⁷. PCR was performed in a total volume of 25 μ L that contained 1 μ L DNA, 5.5 μ L PCR mix, 16.5 μ L double-distilled water and 1 μ L each forward or reverse primer. PCR amplifications were performed under the following conditions: 5 min of initial denaturation at 94°C; 35 cycles of 0.5 min at 94°C, 0.5 min of annealing at 58°, and 0.5 min of extension at 72°C, with 10 min of final extension at 72°C. A CASpure PCR Purification Kit (CASarray, Shanghai, China)³² was used for purification. The purified PCR products were sequenced on an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA)³⁸.

Data analysis

We used BioEdit³⁹ to view and manually correct the sequencing results. We first used CLUSTAL W⁴⁰ to align the sequences and coded indels following the method of Simmons and Ochoterena⁴¹. Then manual adjustments were made in MEGA ver. 7.0.26⁴² to remove the overhanging tails to ensure a uniform sequence length. We concatenated two chloroplast fragments (*trnL-trnF* and *ycf1*) to a separate matrix for subsequent analysis. We used DnaSP ver. 5.10 to identify different haplotypes (cpDNA sequences) or genotypes (ITS sequences). A haplotype network was constructed using TCS ver. 1.2.1⁴³. ArcGIS ver. 10.2 (<http://desktop.arcgis.com>) software was used to draw a haplotype geographical distribution map.

The haplotype/genotype diversity (H_d) and nucleotide diversity (π) were calculated using DnaSP ver. 5.10⁴⁴ software. The within- population gene diversity (H_s), gene diversity in all populations (H_T), interpopulation differentiation (G_{ST}) and

number of substitution types (N_{ST}) were calculated using PERMUT ver. 2.0⁴⁵. The last two indexes (G_{ST} and N_{ST}) were analyzed via permutation tests including 1,000 permutations. When N_{ST} is greater than G_{ST} , it indicates the existence of genealogical geographic structure⁴⁵. Analysis of molecular variance (AMOVA) was performed using Arlequin ver. 3.5.2.2⁴⁶ to partition the genetic variation at different levels, with statistical significance determined by 1,000 permutations.

To investigate the historical dynamics of *P. armeniaca*, mismatch distribution analysis was conducted using DnaSP ver. 5.10. The sum of squared deviations (SSDs), Harpending's raggedness index (HRI)⁴⁵ and corresponding P values were calculated using Arlequin ver. 3.5.2.2⁴⁶. Neutrality tests based on Tajima's D and Fu's F_S were conducted to detect departures from the population equilibrium by Arlequin ver. 3.5.2.2⁴⁶. According to the formula of Rogers and Harpending⁴⁷, $T=\tau/2u$, where " τ " is the parameter value from the mismatch distribution model. In the formula $u=\mu kg$, " μ " is the base substitution rate (chloroplast angiosperms⁴⁸: 1.1×10^{-9}), " k " is the fragment length (cpDNA length after combination: 2,062 bp), and " g " is the generation time (20 years)⁴⁹.

The outgroup was *P. davidiana*, and the time of peach-apricot differentiation was used as the calibration point⁵⁰. The BEAUti interface was used to create an input file for BEAST⁵¹, to which the GTR+I+G nucleotide substitution model. The data was analyzed using a relaxed log-normal clock model and a Yule Process speciation model for the tree priors. Prior settings for calibrating node were: offset of 55.1 Ma, a log mean of 1.0 (log stdev of 0.5). The Bayesian Markov chain Monte Carlo simulation was run for 100 million generations with a sample frequency of 1,000, and the first 20% of generations were discarded as burn-in. Three independent analyses were conducted and combined by LogCombiner ver. 1.8.4. Finally, annotation and visualization of the maximum clade credibility tree were performed in TreeAnnotator 1.8.4 and FigTree ver. 1.4.3, respectively.

Results

Haplotype/genotype phylogenetics and distribution

Based on the concatenated cpDNA sequences (*trnL-trnF* and *ycf1*), 33 haplotypes (H1-H33) were identified in 123 individuals from 20 populations of *P. armeniaca* and related species (Figure 1, Table 2). The alignment lengths of the two chloroplast fragments were 746 bp and 1,316 bp, respectively, and the combined length was 2,062 bp. Variable sites among the 33 haplotypes are shown in Table S4. Species *P. armeniaca* harboured haplotype H1-H17 and H19-H20; species *P. sibirica* harboured haplotype H28-H29; species *P. mandshurica* harboured haplotype H27; species *P. dasycarpa* harboured haplotype H33; species *P. mume* harboured haplotype H20; species *P. zhengheensis* harboured haplotype H32; species *P. limeixing* harboured haplotype H23-H26; species *P. brigantina* harboured haplotype H21-H22; and species *P. davidiana* harboured haplotype H30-H31. The results showed that populations of different species did not share haplotypes. The results showed that, except for the EG

accessions, the diversity of wild *P. armeniaca* (DZG accessions) (nucleotide diversity and haplotype diversity: 0.0013, 0.444, respectively) was higher than that of the CAG (0.0006, 0.239) and NCG (0.0002, 0.400) accessions.

In the ITS dataset, a total of 57 ITS genotypes were discovered in 171 individuals from 19 populations of *P. armeniaca* and its related species (Table 2, Figure S1), and the alignment length was 545 bp. The variable sites among the 57 genotypes are shown in Table S5. Species *P. armeniaca* harboured haplotype T1-T30, T42 and T48-T50; species *P. sibirica* harboured haplotype T2-T3, T8-T9, T12, T25 and T44-T47; species *P. mandshurica* harboured haplotype T3 and T9; species *P. dasycarpa* harboured haplotype T2 and T55-T57; species *P. mume* harboured haplotype T11 and T31-T41; species *P. zhengheensis* harboured haplotype T52-T54; species *P. limeixing* harboured haplotype T3 and T43; and species *P. davidiana* harboured haplotype T51. The results showed that, except for the EG accessions, the diversity of wild *P. armeniaca* (DZG accessions) (nucleotide diversity and haplotype diversity: 0.0055, 0.878, respectively) was higher than that of the CAG (0.0053, 0.832) and NCG (0.0047, 0.818) accessions.

Based on the concatenated cpDNA sequences (*trnL-trnF* and *ycf1*), 19 haplotypes (G1-G19) were identified in 107 individuals from 12 populations of *P. armeniaca* (Figure 2). Variable sites among the 19 haplotypes are shown in Table S6. The *Hd* and π values detected at the cpDNA sequence level in *P. armeniaca* were 0.548 and 1.9×10^{-3} , respectively. The geographic distribution of the 19 haplotypes is shown in Figure 2A, showing that G1 haplotypes were distributed in all populations. The cpDNA haplotype network (Figure 2B) showed that 7 haplotypes were differentiated from haplotype G1 by a one-step mutation with G1 at the center. Three haplotypes were differentiated from G11 by a one-step mutation. The overall network map showed a "star-like" distribution pattern. Based on the ITS sequences, 34 haplotypes (P1-P34) were identified in 124 individuals from 12 populations of *P. armeniaca* (Figure S2). The *Hd* and π values detected at the ITS sequence level in *P. armeniaca* were 0.865 and 5.4×10^{-3} , respectively.

Population structure and genealogical geography

In *P. armeniaca*, the gene diversity in all populations (cpDNA: $H_T=0.499$; ITS: $H_T=0.876$) was higher than that within populations (cpDNA: $H_S=0.490$; ITS: $H_S=0.0.794$) (Table 3). A permutation test showed that N_{ST} was significantly higher than G_{ST} (cpDNA: $N_{ST}=0.227 > G_{ST}=0.020$; ITS: $N_{ST}=0.126 > G_{ST}=0.094$; $P < 0.05$), indicating that *P. armeniaca* has a significant geographical structure (Table 3).

AMOVA revealed significant genetic differentiation among all populations of *P. armeniaca* (cpDNA: $F_{ST}=0.1628$, $P < 0.001$; ITS: $F_{ST}=0.0297$, $P < 0.001$), with most of the genetic diversity occurring within the populations and relatively little occurring among them (Table 4).

Demographic history and estimation of divergence times

The mismatch distribution based on cpDNA and ITS dataset analysis, in which multimodal data were drawn from the cultivated populations or all populations, indicated a demographic equilibrium (Figure 3, Figure S3). Both the neutrality tests based on Tajima's D (cpDNA: -2.272, $P < 0.05$; ITS: -0.966, $P < 0.05$) and Fu's F_S (cpDNA: -5.8253, $P < 0.05$; ITS: -12.223, $P < 0.05$) and the mismatch distribution analysis (Figure 3) based on the cpDNA and ITS dataset suggested recent range or demographic expansion in wild populations of *P. armeniaca*. In addition, neither the SSDs (cpDNA: 0.037, $P > 0.05$; ITS: 0.002, $P > 0.05$) nor the HRI (cpDNA: 0.179, $P > 0.05$; ITS: 0.017, $P > 0.05$) showed significant positive values (Table 5), indicating no deviation of the observed mismatch distribution from that obtained via model simulation under sudden demographic expansion. Thus, we concluded that the demographic expansion time of the wild populations of *P. armeniaca* was 16.53 kyr ago.

The cpDNA dataset was employed to estimate the time of the onset of divergence between *P. armeniaca* and its related species (Figure 4, Table S7). The results showed that 33 haplotypes were divided into two groups: *P. armeniaca* (blue) and related species (green) (Figure 4). The divergence time revealed that the differentiation of *P. armeniaca* from its related species occurred during the middle Eocene, at approximately 45.68 Ma (95% HPD = 28.47-61.87). The onset of intraspecific divergence in *P. armeniaca* was estimated to be 25.55 (95% HPD = 12.93-39.63) Ma.

Discussion

Based on the cpDNA sequences, the total genetic diversity in *P. armeniaca* was relatively low but sufficient to reflect the long evolutionary history and wide distribution of the species⁴⁹. Many scholars⁵²⁻⁵⁵ believed that the diversity of wild apricot was richest in the Ili River Valley, with low levels of genetic differentiation and genetic variation mainly occurring within populations. Hu et al.⁵⁴ used simple sequence repeat markers to analyze the diversity of 212 apricot germplasm from 14 populations in Ili River Valley. Among them, the Tuergen township in Xinyuan County had the highest genetic diversity, and the genetic distance between populations was significantly correlated with the geographical distance. The self-incompatible pattern, wide distribution, long-distance transmission of pollen through insects and strong winds are the main factors affecting the genetic variation structure⁵⁴. The haplotype/genotypic diversity of the wild populations of *P. armeniaca* in the Ili River Valley obtained from cpDNA and ITS data was relatively high (Table 2). The results of AMOVA (Table 4) showed that the genetic diversity in *P. armeniaca* mainly occurs within populations (cpDNA: 83.72%; ITS: 97.03%), but there were also significant differences among populations (cpDNA: 16.28%; ITS: 2.97%), which was consistent with previous results based on simple repeat markers⁵⁴. The relatively high genetic diversity also confirmed the status of Tianshan wild apricot as the origin center of cultivated apricot⁵⁴. The limited informative mutation sites among ITS genotypes led to an almost complete lack of resolution for the construction of genotype relationships (Figure S2), suggesting rapid intraspecific

differentiation in the most recent species of *P. armeniaca*, and similar results were found in *Saxifraga sinomontana*³².

The genetic backgrounds of the NAG, LX, and ZX populations had the same genotypes (T2, T7-8 and T12) as *P. armeniaca*, indicating that they are associated with *P. armeniaca* through continuous and extensive gene flows⁵⁶. Based on microsatellite markers, Liu et al.⁵⁶ believed that *P. sibirica* was divided into two groups, one of which may have under gene exchange with *P. armeniaca*, further verifying our results. In addition, the authors found an extensively mixed genetic background in the germplasm of cultivated apricots in China. This study indicated that the cultivated and wild populations of *P. armeniaca* had the same ancestral haplotype, which was G1. The haplotypes of the CAG, EG and NCG populations were mixed with the haplotypes/genotypes of the large wild populations (*P. armeniaca*). According to coalescent theory⁵⁷, chloroplast haplotype G1, which was widely distributed and located in the center of the chloroplast network (Figure 2), should be considered the oldest haplotype. The Kashgar, Hotan and Aksu oasis areas around the Tarim Basin in the southern part of the Xinjiang Uygur Autonomous Region of China are the main apricot-producing areas and contain the greatest abundance of apricot cultivars. There is only one mountain between southern Xinjiang and the Ili Valley, and there are several corridors between the northern and southern Tianshan Mountains. Therefore, the apricots cultivated in Xinjiang, southern Tianshan Mountains (CAG), most likely evolved from the spread of wild apricots in the Ili River Valley.

Liu et al.⁵⁶ believed that apricots have experienced at least three domestication events, which formed apricots in Europe (the United States and continental Europe), southern Central Asia (Turkmenistan, Afghanistan, India) and China, respectively, in which both ancient gene flows and recent gene mixing phenomena exist. Central Asia was the most diverse center of wild apricots, with genetically differentiated populations that may have resulted from population isolation in glacial shelters⁵⁶. In this study, the nucleotide diversity and haplotype/genotype diversity of wild apricots (DZG accessions) were higher than that of CAG and NCG accessions. These findings are reasonable from a historical perspective, as there was extensive cultural contact along the Silk Road from 207 BCE to 220 CE⁵⁸. Therefore, historical and commercial influences may have contributed to the development of this unique species of cultivated apricot.

The theory and method of phylogeography can reveal the historical dynamics of species or populations, such as expansion, differentiation, isolation, migration and extinction²⁹. Phylogeographic studies have shown that ancient haplotypes and high genetic diversity can be used to identify refuges^{1, 59}. Many scholars^{17, 19, 24} have suggested that the complex geographic history of Northwest China may have provided refuges for species during glacial periods. By combining two markers, we showed that the wild populations distributed in the Ili River Valley all contained ancestral haplotypes/genotypes and had high genetic diversity (Table 2). These populations were located in areas considered glacial refugia for *P. armeniaca*, which

appears to be a relic of Quaternary glaciation. The region provides a suitable climate for the biological community and protects the genetic diversity of *P. armeniaca*. Both neutrality tests and mismatch distribution analysis based on the cpDNA and ITS dataset suggested recent range or demographic expansion of wild populations of *P. armeniaca*. We estimated that the recent demographic expansion time of the wild populations of *P. armeniaca* was 16.53 kyr ago, that is, at the end of the LGM⁴⁹.

The taxa-sampling and the fossil calibration strategy will influence the age estimation⁶⁰⁻⁶¹. Due to the lack of fossil evidence of *P. armeniaca*, we used the peach-apricot divergence time as the calibration point. In this study, we tried to use cpDNA data set to construct the divergence time, and the effect was relatively ideal. However, compared with the median ages estimated by Chin et al.⁵⁰ (mean age of 31.1 Ma), the divergence time estimates in this study should be treated with caution, because the limited coverage and a low number of calibration points may skew divergence time estimation of *P. sibirica* (mean age of 33.43 Ma) too high.

Conclusion

Based on the cpDNA and ITS datasets, the genetic diversity of *P. armeniaca* remains high. Haplotypes/genotypes shared with close relatives tend to occur in geographically similar populations, and *P. armeniaca* exhibits a genealogical structure. The haplotypes/genotypes of cultivated apricot populations in Xinjiang, North China, and foreign apricot populations were mixed with large numbers of haplotypes/genotypes of wild apricot populations from the Ili River Valley. We argue that *P. armeniaca* originated in Northwest China (Ili Valley). Affected by the Quaternary glaciation of the Pleistocene, the Ili River Valley in Northwest China served as a glacial refugium for *P. armeniaca*, providing the species with a suitable climate and preserving its genetic diversity. During the interglacial period, the species underwent a recent expansion in the face of favorable climatic and environmental conditions. Apricots originated during the middle Eocene, and the cultivated apricot in Xinjiang originated from the Ili River Valley in Northwest China.

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Author contribution

W.W.L., and K.L. conceived the study. L.Q.L., Q.P.Z., W.Q.Z. and G.Q.F. contributed to the sampling. W-WL and Adam collected and analyzed the data. W.W.L. and K.L. wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

567
568 **Figure 1.** The haplotype network generated from the haplotypes of *P. armeniaca* and
569 related species based on cpDNA dataset. The small black circles shown an
570 intermediate haplotype not detected in this study. A, *P. armeniaca*; B, *P. zhengheensis*;
571 C, *P. davidiana*; D, *P. sibirica*; E, *P. dasycarpa*; F, *P. limeixing*; G, *P. mandshurica*;
572 H, *P. mume*; I, *P. brigantina*.

Figure 2. Geographic location and haplogroup distribution patterns of the 12 populations of *P. armeniaca* based on cpDNA dataset. (A) Geographic distribution of haplotypes of *P. armeniaca*; Pie chart size corresponds to the sample size of each population. (B) The haplotype network generated from the haplotypes of *P. armeniaca*. The haplotype network generated from the 19 haplotypes of *P. armeniaca*. The small black circles shown an intermediate haplotype not detected in this study.

Figure 3. Mismatch distribution analysis of *P. armeniaca* based on overall gene pool of cpDNA dataset.

Figure 4. Bayesian phylogenetic tree based on cpDNA dataset. Gray bars represent 95% highest posterior density intervals. Plio, Pliocene; Pl, Pleistocene; numbers above the branches indicate Bayesian posterior probabilities.

Population	Origin/location	Type	Latitude (N°)	Longitude (E°)	Altitude (m)
<i>P. armeniaca</i>					
DZGhcmd	Huocheng,Xinjiang	W	44.43	80.79	1187.6
DZGhcy	Huocheng,Xinjiang	W	44.44	80.79	1245.7
DZGhcm	Huocheng,Xinjiang	W	44.40	80.71	1244.1
DZGyn	Yining,Xinjiang	W	44.12	81.62	1983.6
DZGglb	Gongliu,Xinjiang	W	43.25	82.86	1371.5
DZGgld	Gongliu,Xinjiang	W	43.23	82.75	1269.4
DZGxvt	Xinyuan,Xinjiang	W	43.54	83.44	1138.8
DZGxya	Xinyuan,Xinjiang	W	43.50	83.70	1275.3
DZGxyz	Xinyuan,Xinjiang	W	43.38	83.61	1374.1
CAG	Luntai,Xinjiang	C	41.78	84.23	972.0
NCG	Luntai,Xinjiang	C	41.78	84.23	972.0
EG	Xiongyue,Liaoning	C	40.17	122.16	20.4
<i>P. sibirica</i>					
NAG	Xiongyue,Liaoning	W	40.17	122.16	20.4
<i>P. mandshurica</i>					
LX	Xiongyue,Liaoning	C	40.17	122.16	20.4
<i>P. dasycarpa</i>					
ZX	Luntai,Xinjiang	C	41.78	84.23	972.0
<i>P. mume</i>					
ECG	Xiongyue,Liaoning	C	40.17	122.16	20.4
<i>P. zhengheensis</i>					
ZHX	Xiongyue,Liaoning	C	40.17	122.16	20.4
<i>P. limeixing</i>					
LMX	Xiongyue,Liaoning	C	40.17	122.16	20.4
<i>P. brigantina</i>					
FGX	Xiongyue,Liaoning	C	40.17	122.16	20.4
<i>P. davidiana</i>					
T	Luntai, Xinjiang	O	41.78	84.23	972.0

Table 1. Population code, sampling location, coordinates, altitude and types of *P. armeniaca* and relative species. W, wild; C, cultivars; O, out group.

Population	cpDNA				ITS			
	Haplotype composition	N	Hd	π	Genotype composition	N	Hd	π
DZGhcmd	H1(6), H10(1), H11(1), H12(1)	9	0.583	0.0016	T2(2), T9(1), T14(1), T17(2), T19(1), T20(1), T21(1), T22(1)	10	0.956	0.0082
DZGhcy	H1(9)	9	0.000	0.0000	T3(2), T8(1), T9(3), T23(1),	7	0.810	0.0067
DZGhcm	H1(7), H8(1), H9(1)	9	0.417	0.0002	T2(1), T3(4), T8(1), T9(1), T18(1)	8	0.786	0.0000
DZGyn	H1(6), H7(1), H15(1), H16(1), H17(1)	10	0.667	0.0011	T1(1), T3(2), T8(2), T9(1), T14(2), T30(1)	9	0.917	0.0087
DZGglb	H1(6), H5(1), H6(1), H7(2)	10	0.644	0.0012	T2(1), T3(3), T8(2), T13(1), T14(1), T15(1)	9	0.889	0.0043
DZGgld	H1(6), H3(1)	7	0.286	0.0010	T3(4), T8(1), T16(1), T17(1)	7	0.714	0.0027
DZGxyt	H1(8), H2(1)	9	0.222	0.0001	T3(4), T8(2), T9(2), T12(1), T25(1)	10	0.822	0.0032
DZGxya	H1(1), H4(1), H13(1)	3	1.000	0.0039	T2(2), T24(1)	3	0.667	0.0988
DZGxyz	H1(7), H3(1), H14(1)	9	0.417	0.0031	T2(2), T3(2), T26(1), T27(1), T28(1), T29(2)	9	0.917	0.0057
Within population		75	0.444	0.0013		72	0.878	0.0055
CAG	H1(21), H2(1), H3(1), H4(1)	24	0.239	0.0006	T1(5), T2(11), T3(8), T4(1), T5(1), T6(1), T7(1), T8(1), T9(1), T10(1), T11(2), T12(1)	34	0.832	0.0053
NCG	H1(4), H2(1)	5	0.400	0.0002	T1(1), T3(5), T9(2), T48(1), T49(1), T50(2)	12	0.818	0.0047
EG	H1(1), H18(1), H19(1)	3	1.000	0.0045	T2(1), T3(4), T42(1)	6	0.600	0.0053
Within population		32	0.343	0.0012		52	0.833	0.0052
Among population		107	0.548	0.0019		12	0.865	0.0054
NAG	H28(1), H29(2)	3	0.667	0.0023	T2(1), T3(6), T8(1), T9(1), T12(1), T25(1), T44(1), T45(1), T46(1), T47(1)	15	0.857	0.0104
LX	H27(1)	1	0.000	0.0000	T3(4), T9(1)	5	0.400	0.0014
ZX	H33(1)	1	0.000	0.0000	T2(2), T55(1), T56(2), T57(2)	7	0.857	0.0229
ECG	H20(1)	1	0.000	0.0000	T11(1), T31(1), T32(1), T33(1), T34(1), T35(1), T36(1), T37(1), T38(1), T39(1), T40(1), T41(1)	12	1.000	0.017
ZHX	H32(1)	1	0.000	0.0000	T52(1), T53(1), T54(1)	3	1.000	0.0047
LMX	H23(1), H24(1), H25(1), H26(1)	4	1.000	0.0051	T3(3), T43(1)	4	0.500	0.0170
FGX	H21(2), H22(1)	3	0.667	0.0003				

T	H30(1), H31(1)	2	1.000	0.0005	T51(1)	1	0.000	0.0000
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599 **Table 2.** Sample information and summary of haplotype/genotype distribution, genetic diversity for each population. N, sample size; *Hd*,
600 haplotype/genotype diversity; π , nucleotide diversity.
601

Populations	cpDNA				ITS			
	H_S	H_T	G_{ST}	N_{ST}	H_S	H_T	G_{ST}	N_{ST}
<i>P. armeniaca</i> (Cultivated & Wild)	0.490	0.499	0.020	0.227*	0.794	0.876	0.094	0.126*

Table 3. Estimates of average gene diversity within populations (H_S), total gene diversity (H_T), interpopulation differentiation (G_{ST}) and number of substitution types (N_{ST}) for cpDNA haplotypes and ITS genotypes of *P. armeniaca*. Ns, not significant; *, $P < 0.05$.

Source of variation	cpDNA					ITS				
	df	SS	VC	PV (%)	F_{ST}	df	SS	VC	PV (%)	F_{ST}
Among populations	11	31.699	0.209	16.28		11	23.777	0.068	4.38	
Within populations	95	102.086	1.075	83.72		112	166.844	1.490	95.62	
Total	106	133.785	1.284		0.1628*	123	190.621	1.558		0.0297*

Table 4. Analyses of molecular variance (AMOVA) of cpDNA haplotypes and ITS genotypes for populations of *P. armeniaca*. df , degrees of freedom; SS , sum of squares; VC , variance components; PV , percentage of variation; F_{ST} , fixation index; * $P < 0.001$.

Populations		Tajima's D -test		Fu's F_S -test		Mismatch distribution			
		D	P	F_S	P	SSD	P	HRI	P
cpDNA	Overall	-2.417	0.000*	-6.350	0.025*	0.232	0.150	0.218	0.600
	<i>P. arminiaca</i> (cultivated)	-1.933	0.003*	1.289	0.772	0.038	0.250	0.356	0.500
	<i>P. arminiaca</i> (wild)	-2.272	0.001*	-5.825	0.021*	0.037	0.070	0.179	0.880
ITS	Overall	-1.193	0.106	-8.587	0.010*	0.002	0.660	0.023	0.700
	<i>P. arminiaca</i> (cultivated)	-1.420	0.155	-4.951	0.024*	0.015	0.020	0.063	0.310
	<i>P. arminiaca</i> (wild)	-0.966	0.041*	-12.223	0.000*	0.002	0.810	0.017	0.880

Table 5. Neutrality tests (Tajima's D and Fu's F_S) and mismatch distribution analysis for the *P. armeniaca* based on the cpDNA and ITS dataset. * $P < 0.05$.

Figures

Figure 1

The haplotype network generated from the haplotypes of *P. armeniaca* and related species based on cp DNA dataset. The small black circles shown an intermediate haplotype not detected in this study. A, *P. armeniaca* ; B, *P. zhengheensis*; C, *P. davidiana*; D, *P. sibirica*; E, *P. dasycarpa*; F, *P. limeixing* G, *P. mandshurica* H, *P. mume*; I, *P. brigantina*.

Figure 3

Mismatch distribution analysis of *P. armeniaca* based on overall gene pool of cpDNA dataset.

Figure 4

Bayesian phylogenetic tree based on cpDNA dataset. Gray bars represent 95% highest posterior density intervals. Pli o, Pliocene; PI, Pleistocene; numbers above the branches indicate Bayesian posterior probabilities.

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