Phylogeographic Pattern of *Populus cathayana* in the Southeast of Qinghai-Tibetan Plateau of China Revealed by cpSSR Markers

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The vegetation in the Qinghai-Tibetan Plateau is thought to be highly sensitive and more vulnerable to global climate change than that of other areas. The uplift of the plateau as well as the climatic oscillations during glacial periods had a profound impact on plant species distribution and genetic diversity there. In the present study, seven pairs of cpSSR (chloroplast Simple Sequence Repeat) primers were utilized to detect genetic varieties of *Populus cathayana* Rehd populations from their natural range in the southeastern areas of Qinghai-Tibetan Plateau. A total of 28 alleles and 12 different haplotypes were detected. The proportion of haplotype variation among populations ($G_{ST} = 0.794$, $N_{ST} = 0.900$) indicated high level of genetic differentiation among populations and a significant phylogeographic structure ($N_{ST} > G_{ST}$, $P < 0.05$). This appears to support the hypothesis that these populations were derived from multiple refugia areas during the Quaternary climatic oscillations. Based on the haplotype network and mismatch distribution analyses, we found no evidence of postglacial range recolonization and expansion by *P. cathayana* in this region. This might be mainly due to the complex topography of the southeastern part of the Qinghai-Tibetan Plateau. The lofty mountain ranges and deep valleys in this region might have prevented long-distance migrations of this species during the climatic amelioration.

1 Introduction

The Qinghai-Tibetan Plateau is the largest and highest plateau of the world, covering approximately 2.5×10^6 km^2 (Zheng 1996). Its uplift has profoundly impacted Chinese geomorphic patterns and climate, bringing about a series of eco-environmental consequences (Zhang et al. 2000). For example, its uplift changed the atmospheric circulation of wind systems, adjusted the transport of water vapor and heat and developed the East Asian and South Asian monsoon (An et al. 1991). The unique ecological characteristics of this plateau have lead to vegetative communities which are thought to be highly sensitive and more vulnerable to global climate change than that of other areas (Thompson et al. 1994, Zhang et al. 1996, Ni 2000).

The large-scale uplift of the Qinghai-Tibetan Plateau was an important geological event in the Quaternary period. The uplift formed a natural division between the temperate and subtropical zones of mainland China. Consequently, Southwest China was less affected by cold air from Siberia during the glaciations. According to pollen fossil records and biological evidence, the uplift and climatic oscillations of Quaternary period profoundly impacted biome shifts (Zhang et al. 2000, Sun 2002). This event was characterized by migration of the northerly biome from north to south into the glacial region and the southern biome northward into the interglacial region. In the Qinghai-Tibetan Plateau, especially in its southern and eastern areas, many huge mountains run south to north, with basins, rivers and other geomorphic units on the surface. During the Quaternary glaciations, when the northerly biome was gradually receding from north to south, the southeast region of the Qinghai-Tibetan Plateau provided refugia for this biome. Fossil records of many species (i.e., *Picea*, *Abies*, *Populus*, *Ginkgos* and *Metasequoia*) also revealed that this region was an important refugium for species that survived the Quaternary glaciations (Wang and Liu 1994). Because of its role as an important refugium during the glacial periods, the southeastern region of the Qinghai-Tibetan Plateau of China is extremely rich in species (Tang and Shen 1996). This species richness had led the plateau

to be regarded as one of the largest "biodiversity hotspots" in the world (Myers et al. 2000).

Glaciations had important effects on the patterns of spatial distribution and genetic structure of species (Avise 1998, Hewitt 2000, 2004). Analyzing glacial refugia and postglacial recolonization patterns can help to reveal historic events and improve understanding the phylogeographic pattern of species (Walter and Epperson 2001, Richardson et al. 2002, Cuenca et al. 2003, Gómez et al. 2005). Although in the southeast of Qinghai-Tibetan Plateau, specific geological factors (especially the Quaternary glaciations) have significantly shaped the present-day distribution and diversity of many species. Using chloroplast DNA sequence variation, Zhang et al. (2005) found that the northeast edge of the Qinghai-Tibetan Plateau was likely a large refugium for the endemic *Juniperus przewalskii* (Cupressaceae) during the last glacial period and that *J. przewalskii* of the plateau platform is probably derived from a recent colonization. Studies on *Ginkgo biloba* using chloroplast DNA (cpDNA) also found that refugia of this species were located in the southwest China, but detected no recent long-distance dispersal and population expansion (Shen et al. 2005). Few other studies about glacial refugia and postglacial recolonization have been carried out with a focus on the species in this region.

The species of the genus *Populus* L. (Salicaceae), collectively known as poplars, are widely distributed in the forests of the Qinghai-Tibetan Plateau. The distribution and differentiation of this genus appear to have been profoundly influenced by the glacial periods (Yu et al. 2003). In the present study, we employed molecular markers to analyze the genetic relationships and genetic structure of *Populus cathayana* Rehd populations from the southeast of Qinghai-Tibetan Plateau.

Populus cathayana is a native species of China and it belongs to Sect. *Tacamahaca* Spach. It is found along a large geographic range, but mainly occurs in the northern, southwestern and central parts of China (Wu and Raven 1994). In the southern and eastern areas of Qinghai-Tibetan Plateau, many *P. cathayana* occur in the mountains and canyon belts between the plateau and plain at altitudes varying between 1500–3900 m above sea level (Zhao and Gong 1991). A large amount

Table 1. The ecological and geographical parameters of the *P. cathayana* populations sampled.

Population	Landform feature	Water system	Longitude (E)	Latitude (N)	Altitude (m)	Annual rainfall (mm)	Annual mean temp (°C)	Warmest month (°C)	Sample size
SHY	Riparian and basin	Dadu river	102°40'	29°25'	1500	750	17.7	22.0	29
JZ	Alpine and canyon	Bailong river	103°57'	32°13'	1600	553	12.7	16.8	21
PW	Canyon and basin	Peijing river	104°25'	32°01'	1620	566	14.7	18.1	18
QHY	Plateau and mountain	Huangshui river	100°23'	37°54'	3100	412	3.3	11.5	28
LD	Plateau and mountain	Huangshui river	102°28'	36°31'	3160	335	6.9	13.4	28
TS	Canyon and basin	Wei river	105°40'	34°37'	1650	531	11.5	22.6	17

of genetic diversity has been revealed by previous studies on this species (Peng et al. 2005, Lu et al. 2006). This diversity could be exploited for conservation, breeding programs and afforestation schemes.

Molecular markers, in particular organelle genome (cpDNA and mtDNA) markers have proven powerful in identifying refugia and tracking colonization routes, because organelle genomes are inherited without recombination (Newton et al. 1999, Caron et al. 2000, Petit et al. 2003). In general, chloroplast genomes show low levels of nucleotide variation below the species level (Schaal et al. 1998). Chloroplast Simple Sequence Repeats (cpSSR) is most suitable for use in intraspecific studies because this technique enables detection more variation than possible using other molecular markers (e.g., RFLPs) (Powell et al. 1995, Provan et al. 1996, 1998, Mengoni et al. 2000). In this study, we used seven paired cpSSR universal primers described for dicotyledonous angiosperms (Weising and Gardner 1999, Lian et al. 2003) to detect genetic variation within six populations of *P. cathayana* collected from their natural range in the southeastern areas of Qinghai-Tibetan Plateau. The objectives of the present study were: 1) to determine the genetic relationships and differentiation and analyze the phylogeographic pattern of *P. cathayana* populations; 2) increase understanding of the cpDNA haplotypes' diversity of *P. cathayana* in order to infer the potential refugia.

2 Materials and Methods

2.1 Plant Materials

A total of 144 individuals from six natural populations of *P. cathayana* were collected from the southern and eastern areas of Qinghai-Tibetan Plateau in China. Three (SHY, JZ, PW) of the six sampled populations were from the Sichuan province. Two of the populations (QHY, LD) were from the Qinghai province and one (TS) was from the Gansu province. These populations were chose to represent the various climates and topographies over which *P. cathayana* of the southeastern areas of Qinghai-Tibetan Plateau is naturally distributed (Table 1) (Fig. 1). For each of the natural populations, individual cuttings separated by a distance of more than 50 m were collected from winter 2003 to spring 2004 and planted in the nursery garden located at the Maoxian Field Ecological Station.

2.2 DNA Extraction

DNA was extracted from 0.5g of fresh, young leaf material from each individual following a protocol modified from Castiglione et al. (1993). DNA concentrations were determined by comparison with a serial dilution of standard lambda DNA and the quality of DNA was checked with a DNA-Protein instrument (Bio-Rad).

2.3 cpSSR Analysis

Ten universal primer pairs (Weising and Gardner 1999) and 6 primer pairs originally developed
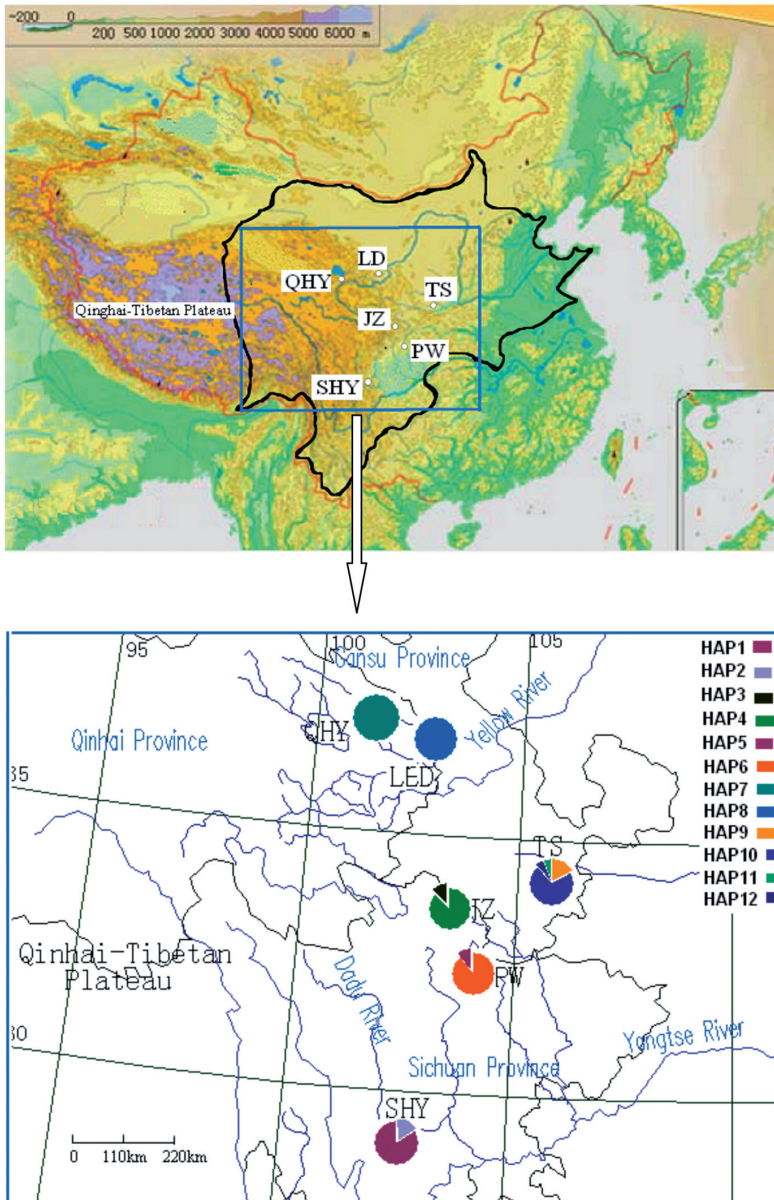
Fig. 1. The locations of the natural populations sampled and the distribution of cpDNA haplotypes. Population abbreviations are given in Table 1.

for *Salix reinii* (Lian et al. 2003) were tested for amplification of *Populus* total DNA. Out of the 16 primer pairs tested, seven primer pairs (CCMP02, CCMP05, CCMP07, CSU01, CSU03, CSU05 and CSU07) (Table 2) produced a single PCR product with a size in the expected range and were used to analyze all of the *Populus* populations.

The amplification reactions were performed in a volume of 20 μl containing 2.0 μl of the

10×reaction buffer (TaKaRa, Dalian), 1.8 mM Mg^{2+} (TaKaRa), 150 mM dNTPs (Promega), 0.2 mM of both primers specified for each microsatellite locus, 1.0 U Taq polymerase (TaKaRa) and 20–50 ng of DNA. PCR amplifications were performed using the following protocol: 5 min at 94℃; followed by 35 cycles of denaturation (45 s at 94℃); annealing (30 s at 55 or 58℃; see Table 2); extension (1 min at 72℃), followed

Table 2. The cpSSR primer pair sequences used in this study.

Locus	Region	Sequence(5"–3)	Repeat	T_a (℃)	Number of alleles	Size range of alleles(bp)
CCMP2	5'– trnS	5'-GATCCCGGACGTAATCCTG-3' 5'-ATCGTACCGAGGGTTCGAAT-3'	A_{11}	58	5	194–225
CCMP5	3'–rps2	5'-TGTTCCAATATCTTCTTGTCATTT-3' 5'-AGGTTCCATCGGAACAATTAT-3'	$(C)_7 (T)_{10}$ $(T)_5 C(A)_{11}$	55	4	108–113
CCMP7	atpB–rbcL	5'-CAACATATACCACTGTCAAG-3' 5'-ACATCATTATTGTATACTCTTTC-3	A_{13}	58	1	134
CSU01	trnC–trnD	5'-TTCCCGATTCTACTAGCACTC-3' 5'-ATTATTATCGCTGGTGCAGAG-3'	$A_4 TCT_{10}$	55	4	142–151
CSU03	trnC–trnD	5'-AAAGTATTCCTGACCCAATCG-3' 5'-ACTAGGACTTATCTTTATCGC-3'	$A_3 CA_3 CA_8$	55	8	279–319
CSU05	trnV2–rbcL	5'-TGTTCGATAGCAAGTTGATTG-3' 5'-GAGTTAGTTGAACTTATCACTC-3'	T_{12}	55	1	147
CSU07	trnD–trnT	5'-GACTTTCTACTTACAAATCCTG-3' 5'-ATAATTCACTGATCCACCATG-3'	A_{14}	58	5	184–192

T_a: annealing temperature

by a final step for 10 min at 72℃. The amplified PCR products were separated on 8% (w/v) non-denaturing polyacrylamide gels containing 1×TBE buffer. The electrophoresed gels were silver-stained to visualize the produced bands using the procedure of Panaud et al. (1996). In all cases, PCR reactions were performed at least twice in order to ensure the reproducibility. According to the method of Bonin et al. (2004), the genotyping error rate was found to be relatively low (0.3%) in this experiment.

2.4 Data Analysis

In the present study, chloroplast haplotype was defined as the combination of the alleles obtained at each locus. Based on the resulting data, Nei's haplotype diversity (H) was estimated as:

$$H = [n/(n – 1)] \, (1 – \sum pi^2)$$

where n is the number of individuals analyzed in the population and pi the frequency of the ith haplotype in the population (Nei 1987).

The CPSSR program (http://www.pierroton.inra.fr/genetics/labo/Software) as described in Pons and Petit (1995, 1996) was employed to analyze population cpDNA diversity. In order to reveal the genetic differentiation among populations, total genetic diversity (H_T), mean genetic diversity within populations (H_S) and the level of population subdivision of diversity (G_{ST}, $G_{ST} = (H_T – H_S) / H_T$) were estimated using unordered alleles (based on allelic frequencies). The level of population subdivision for ordered alleles (based on DNA sequences) (N_{ST}, $N_{ST} = (V_T – V_S) / V_T$), V_T and V_S (the analogues of H_T and H_S) were also estimated based on DNA sequences. Phylogeographic pattern was viewed through G_{ST}/N_{ST} comparison. Here, G_{ST} makes use only of haplotype frequencies while N_{ST} also takes into account differences between haplotypes. A higher N_{ST} than the estimated G_{ST} indicates the presence of a phylogeographical structure.

A haplotype network was obtained by using the program TCS version 1.18 (Clement et al. 2000). The maximum parsimony tree (the phylogenetic tree drawn with the maximum parsimony method) was constructed with the cpSSR data set using PHYLIP Version 3.5c (Felsenstein 1993). This program was used to obtain a 95% confidence limit for parsimony (Templeton et al. 1995). In order to further estimate population expansion, mismatch analysis and Tajima's D (Tajima 1989) neutrality tests were also performed with the program Arlequin Version 3.11 (Excoffier et al. 2005).

Table 3. Distribution of the 12 haplotypes of the *P.cathayana* populations.

Haplotype	SHY	JZ	PW	QHY	LD	TS	Haplotype frequency
Hap1	5	0	0	0	0	0	0.1724
Hap2	24	0	0	0	0	0	0.8276
Hap3	0	19	0	0	0	0	0.8696
Hap4	0	3	0	0	0	0	0.1304
Hap5	0	0	16	0	0	0	0.8889
Hap6	0	0	2	0	0	0	0.1111
Hap7	0	0	0	28	0	0	1.0000
Hap8	0	0	0	0	29	0	1.0000
Hap9	0	0	0	0	0	2	0.1768
Hap10	0	0	0	0	0	13	0.7059
Hap11	0	0	0	0	0	1	0.0588
Hap12	0	0	0	0	0	1	0.0588
Haplotype diversity (h)	0.2956	0.2372	0.2092	0	0		0.4926

Hap (1...12) were the abbreviations of the 12 type haplotypes.

3 Results

## 3.1 The Analysis of Haplotype Polymorphisms

In the present study, cpSSR revealed a wide range of diversity within the *P. cathayana* genome. The number of amplified alleles per cpSSR loci ranged from 1 to 8. Overall, a total of 28 alleles were detected and combined to produce 12 different haplotypes (Hap1 to Hap12) among the six *P. cathayana* populations (Table 3). Each of the 6 populations had their own unique haplotype and none of them shared same haplotype with each other. The distribution of haplotypes across populations was uneven. The TS population had the largest number of haplotypes (4). The SHY, JZ and PW populations had two haplotypes each and the QHY and LD populations had only one haplotype each.

The level of haplotype diversity (H) varied amongst the six populations. Over all populations, the value of the haplotype diversity (H) was 0.8591 (SE = 0.1364). The highest haplotype diversity was in the TS population (0.4926) (SE = 0.0202). The diversity of the SHY, JZ and PW populations was 0.2956 (SE = 0.0091), 0.2372 (SE = 0.0128) and 0.2092 (SE = 0.0185), respectively. Haplotype variation was lowest (0) for the QHY and LD populations.

3.2 Distribution and Relationships of the Haplotypes

The level of haplotype diversity of *P. cathayana* was apparently related to geographical regions with most haplotypes detected in populations found in the center and south zone of the region (Fig. 1). It was apparent that QHY and LD from the northern part of the plateau each had only one fixed haplotype. On the other hand, the populations from the center and southern part of the plateau (TS, PW, JZ and SHY) contained at least two haplotypes each and all of their haplotypes were unique to the particular population.

Phylogenetic relationships among the 12 cpDNA haplotypes were investigated using a maximum parsimonious tree (Fig. 2). Haplotypes
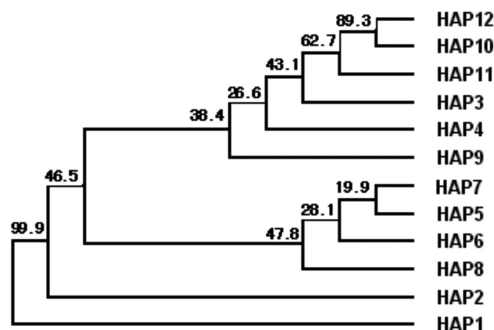
Fig. 2. A Single maximum parsimonius tree showing the phylogenetic relationships among the 12 cpDNA haplotypes resolved in *P. cathayana*. Bootstrap values are denoted above branches.

were divided into three major clades. The first clades consisted of Hap3, Hap4, Hap9, Hap10, Hap11 and Hap12 (detected in JZ and TS populations). The second clades consisted of Hap5, Hap6, Hap7 and Hap8 (detected in PW, QHY and LD populations). However, relationships within the two clades were not resolved because of low bootstrap values among haplotypes. Hap1 and Hap2 (detected in SHY population) were distinct from others and were clustered alone with high bootstrap support (99%).

The relationship among cpSSR haplotypes was investigated with a haplotype network (Fig. 3). In the network, haplotypes coming from the same population were grouped together, whereas haplotypes coming from different populations were separated by many genetic divergences, probably caused by a number of evolutionary events. Surprisingly, the network contained a large number of ambiguous connections and did not show the clear 'star-like' pattern characteristic of postglacial expansion.

In order to test the hypothesis of population expansion, we computed the distribution of pairwise differences from the segregating sites of cpDNA haplotypes of *P. cathayana*. The mismatch analysis showed a multimodal distribution for all samples, suggesting there is no expanding population of this species. There was also no clear evidence of population expansion using the Tajima'D neutrality test. With a D of 1.601, the positive values were not significantly different from zero, $P > 0.05$.

3.3 Differentiation and Relationships among Populations

Diversity analysis showed the total diversity among populations to be high ($H_T = 1.000$) and the genetic variation to be distributed more among ($G_{ST} = 0.794$, SE = 0.0768) than within populations ($H_S = 0.206$, SE = 0.0766) (Table 4). The proportion of genetic variation among popula-

Table 4. Results of the diversity analysis for *P. cathayana* populations. Standard errors of the estimates are in parentheses.

Total genetic diversity (H_T)	1.000 (0.0241)
Genetic diversity within populations (H_S)	0.206 (0.0766)
The level of population subdivision of diversity (G_{ST})	0.794 (0.0768)
Total genetic diversity (V_T)	1.020 (0.0538)
Genetic diversity within populations (V_S)	0.102 (0.0515)
The level of population subdivision of diversity (V_{ST})	0.900 (0.0537)
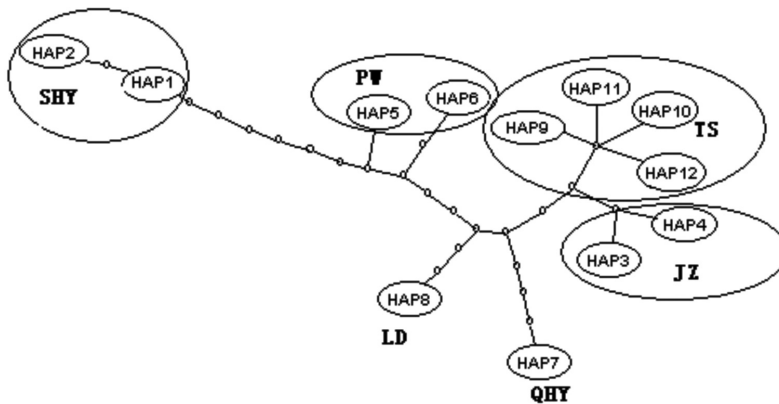
Fig. 3. The haplotype network of the 12 cpSSR haplotypes detected in *P. cathayana*. Haplotypes are represented in circles and lines between haplotypes represent a one-step mutational change.

tions accounted for 79.4% of all genetic diversity, indicating a relatively high degree of genetic differentiation among populations. The difference between N_{ST} and G_{ST} was significant (N_{ST} = 0.900, SE = 0.0537; $G_{ST} < N_{ST}$, $P < 0.01$), showing that there was significant correlation between the phylogeny of haplotypes and their geographic locations.

4 Discussion

The southeastern part of the Qinghai-Tibetan Plateau is regarded as the natural distribution and variation center of the genus *Populus* in China (Fang and Zhao 1981, Wang et al. 1984, Zhao and Gong 1991, Yu et al. 2003). Seventeen species, fifteen varieties and ten natural hybrids of native poplar are recognized and most species are endemic to this area or neighboring areas (Zhao and Gong 1991, Yu et al. 2003). The abundant genetic resource diversity of poplar is mainly attributed to the specific geological factors and ecological conditions in this area and the biological properties of the genus (Ding 1995).

In the present study, based on an investigation of chloroplast markers (cpSSR), we found that natural populations of *P. cathayana* possess moderate level haplotype diversity (H = 0.8591, SE = 0.1364, 12 haplotypes in 144 individuals) when compared to other poplar species using the same markers (Salvini et al. 2001, Cottrell et al. 2005). However, it should be noted that *P. cathayana* showed a significantly high level of inter-population differentiation as reflected by the high values of G_{ST} and N_{ST} obtained in the present study ($G_{ST} = 0.794$, $N_{ST} = 0.900$). Furthermore, no haplotype was found to be particularly common to all populations, which suggests genetic isolation among populations. The high inter-population differentiation among poplar populations of the Qinghai-Tibetan Plateau might be attributed to the fact that the natural populations occur in disjunctive mountain areas including plateaus and valleys with high degrees of geographical isolation (at least 100km between populations). Physical obstacles and variable climate conditions throughout the region could block gene flow even for a species with generally good seed dispersal ability.

Therefore, the high inter-population differentiation of *P. cathayana* populations was likely to be due to infrequent gene migration caused by long distance isolation. In the present study, strong phylogeographic structure was apparent, because all of the haplotypes were unique to a particular refugium. This is further supported through the significant difference between G_{ST} and N_{ST} ($G_{ST} < N_{ST}$, $P < 0.01$), which showed strong correlation between the phylogeny of haplotypes and their geographic locations.

The southeastern part of Qinghai-Tibetan Plateau served as an important refugium during the Quaternary glaciations, abundant species were preserved in this region (Wang and Liu 1994). According to fossil records and geological events, the poplar originated in the northeast part of East-Asia (Fang 1987). When temperature decreased during the Quaternary, poplar was forced to migrate southward to the Himalayan-Hengduan Mountains (located in the southeastern part of Qinghai-Tibetan Plateau), where abundant poplar genetic diversity could survive the severity of the climate and physical conditions during glaciations (Ding 1995, Sun 2002). The glacial climatic oscillations as well as complex topography further promoted intraspecific divergences and consequently formed distribution and variation centers for poplar (Yu et al. 2003).

In the present study, the ample genetic variation and high level inter-population differentiation of *P. cathayana* in this region supports the hypothesis that these populations were derived from refugia areas during the Quaternary climatic oscillations. Most importantly, the observed genetic isolation and lack of sharing of haplotypes among populations suggests that multiple refugia must have existed and each of these fragmented refugia must have experienced considerable genetic differentiation. Our hypothesis was also supported by the isolated distribution of clades in the haplotype network analysis which showed the haplotypes separated into at least three clades based on numerous genetic differences (perhaps caused by mutations, introgression or genetic drift).

In addition to showing all haplotypes to be unique, the cpDNA variation pattern suggested that most of *P. cathayana* populations in the southeastern part of Qinghai-Tibetan Plateau must have experienced *in situ* shrink-expansion

cycles during the Quaternary climatic oscillations. When temperature decreased during glacial periods, poplar moved into the southeastern part of Qinghai-Tibetan Plateau in the Quaternary period (Ding 1995). However, because of complex topography, such as plateaus and deep valleys, it was difficult for the surviving populations to interbreed in a common refugium. Similarly, the topography also blocked interglacial range expansion of *P. cathayana* populations. The complex topography might have accelerated inter-population differentiation and retained multiple refugia of *P. cathayana* during glacial stages.

In the present study, based on the haplotype network analysis and mismatched distribution analyses of the cpDNA haplotypes, we found no evidence of postglacial range recolonization or expansion in the Qinghai-Tibetan Plateau. In the case of this species, the populations in each glacial refugium spread only within their refugium during the climatic amelioration. The populations could not migrate among refugia areas, because of barriers like lofty mountain ranges and deep valleys. The present study's findings agreed with previous studies in that modern *P. cathayana* populations originated from Quaternary period refugia areas and that inter-population differentiation was high. For example, Zhang et al. (2005) studied phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* using chloroplast DNA sequence variation. They found the plateau edge was likely a large refugium during the last glacial period and that the marked population differentiation exhibited by the species in its refugium was likely, due to high mountain barriers. Recent studies conducted by organelle DNA also came to similar conclusions, that plant populations from the southeastern part of the Qinghai-Tibetan Plateau had higher genetic diversity and higher population differentiation than those from other areas and that there were multiple refugia for plant species during the Quaternary period glaciations (Meng et al. 2007, Li et al. 2010, Wang et al. 2010).

In summary, the high levels of genetic diversity and inter-population differentiation of *P. cathayana* in the Qinghai-Tibetan Plateau is likely due to that the southeastern part of the plateau's role as an important refugium during glaciations. The glacial climatic oscillations and complex topography in the southeastern part of the plateau further promoted intraspecific and population divergences. According to previous reports, most tree species that survived in refugia had experienced postglacial range recolonization and expansion during climatic amelioration (Hampe et al. 2003, Cuenca et al. 2003, Zhang et al. 2005, Bucci et al. 2007). These species exhibited marked population differentiation in their refuge area and almost complete genetic uniformity in regions it had recolonized. Previous studies showed that *P. cathayana* coming from central and northern parts of China showed low genetic diversity and little population differentiation (Li et al. 1997), which might suggest the phylogeographic patterns and historical dynamics of these populations differed. However, considering the lack of information on cpDNA variation of this species from the other part of China resulted, it is difficult to draw decisive conclusions about the recolonization of the postglacial range and population expansion of *P. cathayana* in other regions (i.e., the central and northern parts of China). More studies are required to improve the understanding of the phylogeographical pattern of *P. cathayana*.

Acknowledgements

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