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Phylogeography of an alpine species *Primula secundiflora* inferred from the chloroplast DNA sequence variation

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Abstract The Hengduan Mountains (HM) and adjacent regions have been suggested as the important refugia of the temperate plants during the glacial stages. However, it remains unknown how the HM endemic species can respond to the climatic oscillations. In this study, we examined the chloroplast *trn*L-*trn*F and *rps*16 sequence variation of *Primula secundiflora*, a relatively common alpine perennial endemic to this region. Sequence data were obtained from 109 individuals of 11 populations covering the entire distribution range of the species. A total of 15 haplotypes were recovered and only one of them is commonly shared by three populations while the others are respectively fixed in the single population. The total diversity (H_T=0.966) is high while the within-population diversity (H_S=0.178) is low. Despite the high uniformity of the intraspecific morphology, an analysis of molecular variance (AMOVA) revealed a high level of genetic differentiation (97.65%) among populations. The higher N_{ST} (0.982) than G_{ST} (0.816) (P<0.05) suggested a distinctly phylogeographical pattern. Phylogenetic analyses of haplotypes identified four major clusters of the recovered haplotypes: three clades in the north, and the other one in the south. The isolated distribution of clades suggested multiple refugia of this species during the glacial stages. We failed to detect the interglacial or postglacial range expansion of this species as revealed for the other temperate plants. However, the low intra-population diversity suggested that most of the populations should have experienced the *in situ* shrink-expansion cycles during the climatic oscillations. This inference was further supported by the nested clade analysis, which indicated that restricted gene flow with isolation by distance and allopatric fragmentation were likely the major processes that shaped the present-day spatial distribution of haplotypes in this species. Such a special phylogeographic pattern may have resulted from a combination of both climatic oscillation and complex topology of HM.

Key words Phylogeography, *Primula secundiflora*, cpDNA, *trn*L-*trn*F, *rps*16, Hengduan Mountains.

Hengduan Mountains (HM) in the southeast Qinghai-Tibetan Plateau (QTP) comprise the major component of the south-central "biodiversity hotspot": one of the 25 areas recognized globally as featuring exceptional concentrations of endemic species (Myers et al., 2000). This region consists of a series of spectacular north-south trending ridges alternating with deep valleys, with altitudes ranging from 2000 to 6000 m a.s.l. (Shi et al., 1998) and contains more than 12000 species of plants and is especially rich in endemic species and genera (Ying & Zhang, 1984, 1993; Li & Li, 1993; Li, 1994; Hao, 1997; Wang, 2000). The production of such high diversity was suggested to be due to two major factors: (i) this region served as an important refugium that harbored the ancient species; and (ii) the uplifts of the QTP as well as the Quaternary climatic oscillations further promoted the divergences of intraspecific lineages and consequently leaded to adaptive diversification of plants (Wang & Liu, 1994; Sun, 2002; Wang et al., 2005; Liu et al., 2006). These hypotheses were partly confirmed in a few species-rich genera at the species level (Li & Li, 1993; Wang et al., 2004, 2005, 2007; Liu et al., 2002, 2006). However, very few population genetic analyses have been conducted on the species occurring in HM and therefore the phylogeographic patterns, intraspecific divergence and glacial refugia remains largely unknown in this region. The few phylogeographic studies focused on the species distributed in the north of HM, mostly from the QTP platform, and these studies suggested that these species responded extensively to the past climatic oscillations (e.g. Zhang et al., 2005; Meng et al., 2007). A few of them (Zhang et al., 2005) had experienced a similar glacial retreat and postglacial range shift as those temperate organisms in Europe and North America during the past climatic oscillations (Avise,

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1998; Comes & Kadereit, 1998; Hewitt, 1996, 2000, 2004; Newton et al., 1999; Widmer & Lexer, 2001; Abbott & Brochmann, 2003). However, this scenario might not have occurred in the southeast QTP because the high mountains and deep valleys may have blocked the interglacial or interglacial range expansion of plants. This topological effect might have accelerated inter-population differentiation but retained multiple refugia of plants during glacial climatic oscillations. In order to test this prediction, in the present study, we aimed to recover the phylogeographic pattern of another alpine perennial that is endemic to HM.

Primula L. is one of the species-rich genera in the HM and adjacent regions of the QTP with more than 75% of the total number of species (ca. 425) distributed in this region (Hu, 1994; Richards, 2002). Despite the lack of the detailed research, the diversification of this genus may have similar causes as those revealed for other species-rich genera (Wang et al., 2004, 2005, 2007; Liu et al. 2002, 2006). *Primula secundiflora* Franch. is endemic to HM and sparely distributed in alpine habitats of this region. In addition, this perennial species has a wide geographical coverage from west Sichuan to northwest Yunnan and southeast Tibet (Hu, 1990; Hu & Kelso, 1996). This species displays the high uniformity in morphology (Hu, 1990) and our field investigations failed to find any intraspecific variations. This species provides a good model to investigate the intraspecific genetic divergence and phylogeographic structure of alpine species in HM. We used the chloroplast (cp) *trn*L-*trn*F and *rps*16 sequence variations to explore the genetic structure of this species. The phylogeographic structures constructed by cpDNA may accurately reflect range shifts and population dynamics of plants because this genome is maternally inherited in most angiosperms (Schaal et al., 1998; Newton et al., 1999; Soltis & Gitzendanner, 1999). The phylogeographic patterns of plants in other regions based on cpDNA sequence variations have been revealed to correspond well with the past range shifts and population dynamics inferred from pollen and glacial or climatic signatures (Fujii et al., 2002; Okaura & Harada, 2002; Newton et al., 1999; Petit et al., 2003; Bartish et al., 2006). Overall, our objectives in this study were: (i) to establish the phylogeographic structure of *P. secundiflora* based on the sequence variations of two cpDNA fragments; and (ii) to test whether this species has a high inter-population differentiation and had possibly retained multiple separate refugia during the glacial stages.

1 Material and methods

1.1 Plant materials

Leaf materials of *Primula secundiflora* were sampled from almost the entire range of species, including western Sichuan, north-western Yunnan and south-eastern Xizang (Tibet) in HM (Table 1; Fig. 1). Nine or 10 individuals from each population were collected, with samples at least 10 m apart. In total, 109 individuals from 11 populations were used in the present study. Leaf material was dried in silica gel and stored at room temperature.

1.2 DNA extraction, PCR amplification and sequencing

Total DNA was extracted using the CTAB method (Doyle, 1991). PCR amplification and DNA sequencing were performed with universal primers for *trn*L-*trn*F (5′-CGAAATCGGTAGACGCTACG-3′ and 5′-ATTTGAACTGGTGACACGAG-3′; Taberlet et al., 1991; Zhang et al., 2006) and *rps*16 (5′-GTGGTAGAAAGCAACGTGCGACTT-3′ and 5′-TCGGGATCGAACATCAATTGCAAC-3′; Oxelman et al., 1997). Polymerase chain reaction (PCR) was conducted in a total volume of 50 μL containing 20 ng template DNA, 5 μL 10×reaction buffer, 5 μL $MgCl_2$ (25 mmol/L), 1 μL dNTP mix (10 mmol/L), 10 μmol/L of each primer, and 1.5 unit of Taq polymerase. PCR reaction was run in a DNA Programmable Thermal Cycler (PTC-200, MJ Research) with initial denaturation at 94 ℃ for 5 min, followed by 34 cycles of 1 min at 94 ℃, 1 min of annealing at 56 ℃ and 59 ℃, respectively, for *trn*L-*trn*F and *rps*16, 1 min at 72 ℃, and a subsequent 7 min of final extension at 72 ℃. Sequencing reactions were performed by using the dye-terminator cycle-sequencing ready-reaction kit following the manufacturer's protocol, and analyzed on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA 94404, USA).

1.3 Data analysis

The DNA sequences were aligned using the program Clustal X 1.81 (Thompson et al., 1997). Estimates of average gene diversity within populations (H_S), total gene diversity (H_T) and the proportion of total diversity due to differences between populations (G_{ST} and N_{ST}) were calculated using the program PERMUT (Pons & Petit, 1996, available at http://www.pierrton.intra.fr/genetics/labo/software/permut). G_{ST} is calculated solely based on haplotype frequencies, whereas N_{ST} takes into account the genetic relation among haplotypes. When N_{ST} value is higher than the G_{ST} estimated, it indicates the presence of a phylogeographical structure (Petit et al., 2005).

Table 1 Sample locations of *Primula secundiflora* and genetic estimates of diversity within the studied populations

Pop. No.	Location	Sample number	Longitude (E)	Latitude (N)	Altitude (m)	Haplotype number	H_d	π
KD	Kangding, Sichuan, China (四川康定)	10	101°57′	30°02′	3500	H4 (10)	0.000±0.000	0.00000±0.00000
YJ	Yajiang, Sichuan, China (四川雅江)	10	101°00′	30°02′	4400	H5 (9), H6 (1)	0.000±0.000	0.00000±0.00000
ZD-1	Zhongdian, Yunnan, China (云南中甸)	9	99°43′	27°47′	3500	H1 (9)	0.000±0.000	0.00000±0.00000
ZD-2	Zhongdian, Yunnan, China (云南中甸)	10	99°45′	27°27′	4100	H2 (10)	0.000±0.000	0.00000±0.00000
XC	Xiangcheng, Sichuan, China (四川乡城)	10	99°47′	28°56′	3600	H10 (9), H11 (1)	0.000±0.000	0.00000±0.00000
DQ	Dêqên, Yunnan, China (云南德钦)	10	99°02′	28°13′	4200	H8 (10)	0.000±0.000	0.00000±0.00000
ML	Muli, Sichuan, China (四川木里)	10	101°15′	27°54′	3200	H15 (10)	0.000±0.000	0.00000±0.00000
LJ	Lijiang, Yunnan, China (云南丽江)	10	100°15′	26°52′	na	H3 (10)	0.000±0.000	0.00000±0.00000
ZG	Zogang, Xizang, China (西藏左贡)	10	97°54′	29°41′	na	H7 (3), H8 (7)	0.467±0.132	0.00029±0.00008
MK-1	Markam, Xizang, China (西藏芒康)	10	98°41′	29°38′	na	H8 (7), H9 (3)	0.467±0.132	0.00029±0.00008
MK-2	Markam, Xizang, China (西藏芒康)	10	98°41′	29°32′	na	H12 (2), H13 (6) H14 (2)	0.356±0.159	0.00043±0.00019
Total		109					0.895±0.012	0.00495±0.00018

H_d, haplotype diversity; π, nucleotide diversity; na, not available.

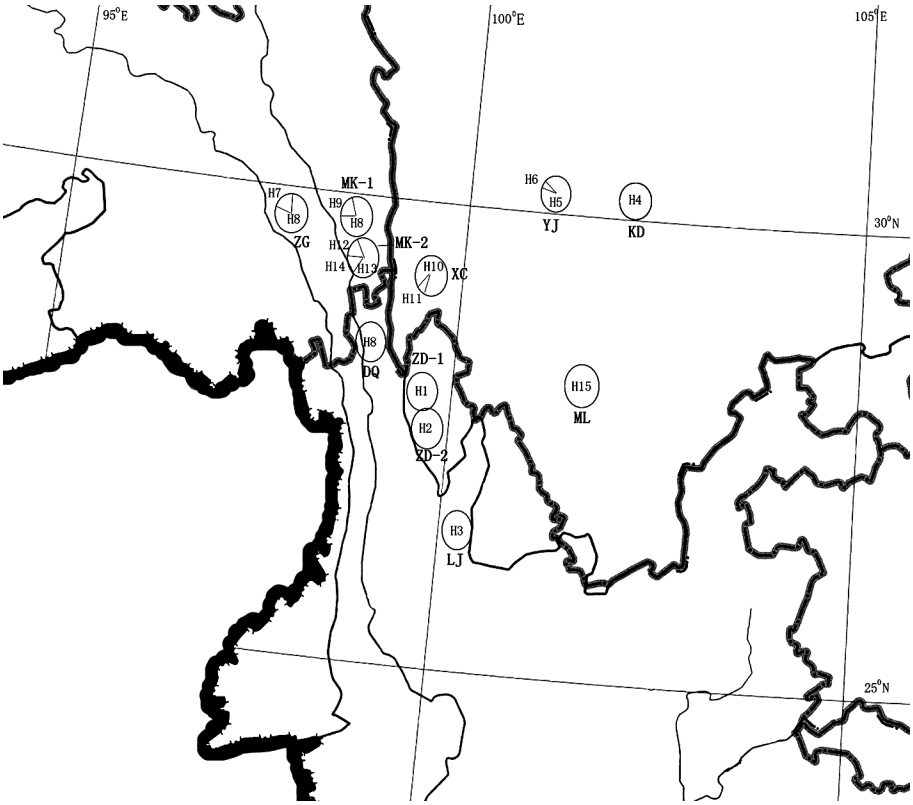

Fig. 1. Sample locations and distribution of cpDNA haplotypes of *Primula secundiflora*. Frequency of cpDNA haplotypes in each population is indicated in pie charts.

A hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed using ARLEQUIN software version 3.0 (Excoffier et al., 2005) with significance tested by 1000 permutations. Nucleotide diversity (π) and haplotype diversity (*H*d) were calculated with DnaSP version 4.0 for each population (Rozas et al., 2003).

Phylogenetic relationships between the cpDNA haplotypes were reconstructed by neighbour-joining (NJ) and maximum-parsimony (MP) analyses in PAUP 4.0b10 (Swofford, 2000). A closely related species, *P. helodoxa* Balf. f., was used as outgroup. In the MP analyses, gaps were treated as missing and indels were scored as binary characters. NJ analyses were performed based on Kimura's two-parameter model. MP tree was also constructed with the heuristic search algorithm with tree-bisection-reconnection. Bootstrap values were estimated (with 1000 replicates) to assess the relative support for relationships between haplotypes (Felsenstein, 1985).

To reveal the phylogenetic relationships among haplotypes, a minimum spanning haplotype tree was constructed by linking the haplotypes in a hierarchical manner based on the single step mutations, with the aid of MINSPNET (Excoffier & Smouse, 1994). We used the nested clade analysis (NCA) to infer the patterns of population history. The NCA nesting design was constructed by hand on the haplotype network following the rules given in Templeton et al. (1987) and Templeton and Sing (1993). The program GeoDis 2.2 (Posada et al., 2000) was used to calculate the various NCA distance measures and their statistical significance levels. All statistical analyses in GeoDis were performed using 1000 permutations. Two major clade distance statistics were calculated, viz., the clade distance (Dc), which measures the average distance of all clade members from the geographical center of distribution, and the nested clade distance (Dn), which measures the geographical distribution of a clade relevant to other clades in the same nested group and the interior-tip. These measures of geographical distribution were used to infer historical processes following the methods of Templeton et al. (1995). The results were interpreted using the latest inference key of Templeton provided at http://darwin.uvigo.es (updated November 2005).

2 Results

In this study, *trn*L-*trn*F and *rps*16 regions of cpDNA in *P. secundiflora* were PCR amplified and sequenced for 109 individuals of 11 populations. Sequences of *trn*L-F and *rps*16 were deposited in the GenBank database under the accession numbers EF595526–EF595536 and EF595537–EF595550, respectively. For the *trn*L-*trn*F region (including *trn*L intron, exon and *trn*L-*trn*F spacer), length polymorphism ranges from 855 bp to 877 bp. Difference between *trn*L-*trn*F sequences was mainly ascribed to 16 point mutations and six indels. For the *rps*16 region, high levels of length polymorphism, ranging from 752 bp to 789 bp, were detected. Six indels were found, while 15 sites belong to point mutations after alignment (Table 2). Fifteen haplotypes were recovered from the combined data of *trn*L-*trn*F and *rps*16 data sets. Haplotype composition, haplotype number and total cpDNA diversity in each population are listed in Table 1 with geographical distributions illustrated in Fig. 1. Four (H1, H2, H3 and H15) of the 15 haplotypes were fixed in the south distribution range, while the other haplotypes were fixed in the north distribution range.

The overall haplotype diversity (*H*d) is 0.895 ± 0.012 and nucleotide diversity (π) is 0.00495± 0.00018 (Table 1). The average gene diversity within population (H_S) is 0.178±0.0710 and the total gene diversity (H_T) is 0.966±0.0308. Interpopulation differentiation across the total distribution of the species was very high (G_{ST}=0.816), and AMOVA revealed that 97.65% of the total genetic variation are partitioned among populations (Table 3). A test for phylogeographic structure of haplotype variation across the distribution of the species showed that N_{ST} (0.982) was significantly higher than G_{ST} (0.816) (P<0.05), indicating the distinct correlation between distribution and fixture of haplotypes (Pons & Petit, 1996).

The total alignment of *trn*L-*trn*F and *rps*16 sequences that included indels covered 1677 characters, of which 1626 were constant, and 29 were parsimony-informative characters. The strict consensus tree of the six most parsimonious trees (Tree length=55, *CI*=0.95, *RI*=0.95) was shown in Fig. 2. Four major clades were identified and tentatively supported by the bootstrap statistics. The first clade (I) consisted of haplotypes H7, H8, H9, H12, H13 and H14 that were respectively fixed in populations DQ, ZG, MK-1 and MK-2. The second one (II) included H4, H5 and H6, presenting in populations KD and YJ while the third one (III) comprised H1, H2, H3 and H15 in ZD-1, ZD-2, ML and LJ. The last clade (IV) consisted of H10 and H11, both fixed in XC.

The minimum spanning tree was shown in Fig. 3. Five one-step clades (clade 1-1, 1-2, 1-3, 1-4, 1-5)

Table 2 Variable sites of the aligned sequences of two chloroplast DNA fragments in 15 haplotypes of *Primula secundiflora*

Haplotype	Variable sites																																								
	184	201	205	210	256	262	270	295	321	408	418	541	549	576	647	662	696	733	773	791	833	946	947	961	969	979	1003	1015	1090	1167	1201	1206	1228	1349	1398	1447	1501	1542	1548	1589	1596	1598
H1	–	A	G	–	C	–	A	–	C	G	G	G	G	G	&	–	A	T	C	T	T	C	T	C	A	G	T	G	+	–	–	–	A	C	☆	C	C	G	C	–	C	C
H2	–	G	G	–	C	–	A	–	C	G	G	G	G	G	&	–	A	C	C	C	T	C	T	C	A	G	T	G	+	–	–	–	A	C	☆	C	C	G	C	–	C	C
H3	–	G	A	–	C	–	A	–	T	G	G	G	G	G	&	–	A	C	C	C	T	C	T	C	A	G	T	G	+	–	–	–	A	C	☆	T	C	G	C	–	C	C
H4	G	G	G	–	C	–	A	–	C	G	G	A	G	T	&	–	G	C	T	C	T	T	C	T	G	G	C	G	+	–	–	A	A	C	☆	C	C	G	A	–	C	A
H5	G	G	G	–	C	–	A	–	C	G	G	A	G	T	&	–	G	C	T	C	T	T	C	T	G	G	C	G	+	–	–	A	A	C	☆	C	G	G	A	–	C	A
H6	G	G	G	–	C	–	A	–	C	G	G	A	G	T	&	–	G	C	T	C	T	T	C	T	G	G	C	G	+	–	–	A	A	C	–	C	G	G	A	–	C	A
H7	G	G	G	T	T	–	T	–	C	G	G	G	G	T	&	*	A	C	C	C	T	C	C	C	G	G	C	G	+	△	–	–	A	C	☆	C	T	G	A	–	C	A
H8	G	G	G	T	T	–	T	–	C	G	G	G	G	T	&	*	A	C	C	C	T	C	C	C	G	G	C	G	+	△	–	–	A	C	☆	C	C	G	A	–	C	A
H9	G	G	G	T	T	–	T	–	C	G	A	G	G	T	&	*	A	C	C	C	T	C	C	C	G	G	C	G	+	△	–	–	A	C	☆	C	C	G	A	–	C	A
H10	G	G	G	–	C	–	A	T	C	G	G	G	G	G	&	–	A	C	C	C	T	C	C	C	G	G	C	G	+	–	–	–	A	T	☆	C	C	A	A	–	C	A
H11	G	G	G	–	C	–	A	T	C	G	G	G	G	G	&	–	A	C	C	C	T	C	C	C	G	G	C	G	+	–	–	–	A	T	☆	C	C	A	A	T	C	A
H12	G	G	G	–	T	–	A	–	C	G	G	G	A	T	&	–	A	C	C	C	G	C	C	C	G	G	C	G	–	△	–	–	A	C	☆	C	C	G	A	–	C	A
H13	G	G	G	–	T	#	A	–	C	G	G	G	A	T	&	–	A	C	C	C	G	C	C	C	G	G	C	G	–	△	–	–	A	C	☆	C	C	G	A	–	C	A
H14	G	G	G	–	T	#	A	–	C	G	G	G	G	T	&	–	A	C	C	C	G	C	C	C	G	G	C	G	–	△	–	–	C	C	☆	C	C	G	A	–	C	A
H15	–	G	G	–	C	–	A	–	C	T	G	G	G	G	–	–	A	C	C	C	T	C	C	C	G	A	T	A	+	–	※	A	A	C	☆	C	C	G	C	–	T	C

#, TAAAAT; &, TCTTATT; *, GTGGTAT; +, TGAAATTTGAATCTAA; △, AAGACA; ※, CTATG; ☆, TAAAAGAAA; –, delete.

Table 3 Analysis of molecular variance (AMOVA) for populations of *Primula secundiflora* based on sequences of cpDNA *trn*L-*trn*F and *rps*16 regions

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	10	1093.780	11.01230	97.65
Within populations	98	26.000	0.26531	2.35
Total	108	1119.780	11.27761	

d.f., degrees of freedom.

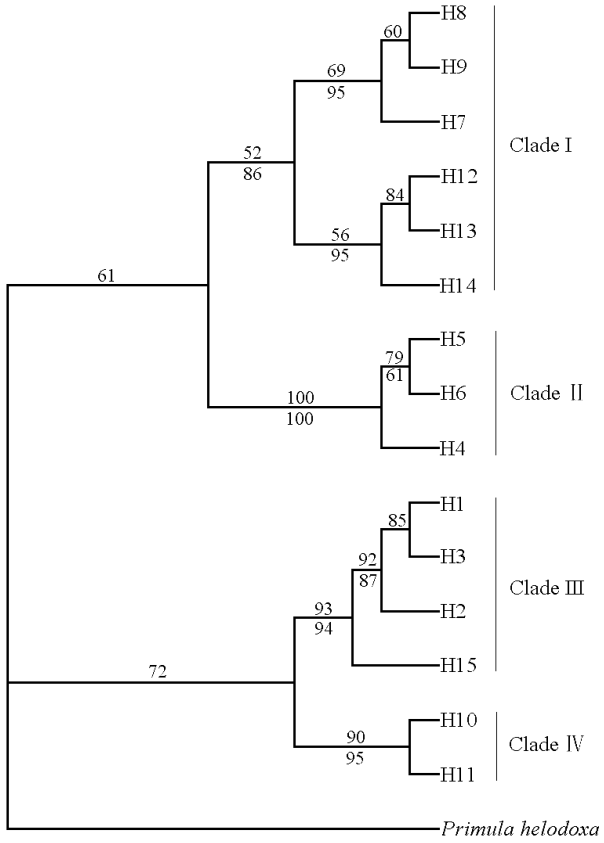

Fig. 2. The neighbour-joining tree of cpDNA haplotypes. Bootstrap support values >50% are shown in the NJ (above branches) and in MP analyses (below branches).

were identified in the cpDNA haplotype tree. Clade 1-1 and clade 1-2 were grouped into a higher-level clade 2-1. The most widespread clade 1-5 was distributed in four populations, and clade 1-2 occurred in three populations. The demographic inferences suggested that the restricted gene flow with isolation by distance is the primary process within clade 1-2 and clade 2-1, allopatric fragmentation within clade 1-3, 1-5 and total cladogram (Table 4) according to the statistic significance between the clade distance (Dc) and the nested clade distance (Dn).

3 Discussion

In this study, we recovered 15 cpDNA haplotypes based on a combination of two cpDNA sequence variations. These haplotypes clustered into four distinct clades (Figs. 2, 3). The haplotypes in these four clades were found in geographically different regions (Fig. 2), with haplotypes within clades I, II and IV in the northern populations, while those of the clade III in the southern populations. H8 is commonly shared by three adjacent populations (DQ, ZG and MK-1 in the northwest Yunnan and southeast Xizang; Table 1 and Fig. 1). Minimum spanning tree suggested that H8 might be the ancestral haplotype in the clade 2-1 and two haplotypes (H7 and H9) that have the same distribution were derived from it recently due to the short mutations between them (Fig. 3). The other haplotypes in this clade (H12, H13 and H14) have longer mutational steps, suggesting the later origins. However, their distributions are close to H8 in the north range of the species, similarly indicating that they may have derived from this hypothesized ancestor. Clade 1-3 consisted of three haplotypes (H4, H5 and H6) that were only recovered in west Sichuan (KD and YJ). Clade 1-4 included two haplotypes (H10 and H11) fixed in XC and this clade seems to have a ancient origin and isolated position (Figs. 2, 3). Clade 1-5 consisted of four haplotypes (H1, H2, H3 and H15) centered in the northwest Yunnan and southwest Sichuan (ZD-1, ZD-2, LJ and ML; Table 1 and Fig. 1). H2 from Zhongdian was inferred as ancestral in this clade (Fig. 3) and the other haplotypes radiated from this haplotype in their sympatric distributions. Because of the low mutation rate in the cpDNA (Newton et al., 1999; Petit et al., 2003), the divergences between four clades obviously predated the Quaternary stages. The isolated distribution of these clades therefore suggested that multiple refugia (at least four) must have existed for this species during the glacial stages. It is interesting to note that another alpine shrub *Hippophae neurocarpa* S. W. Liu & T. N. He on the QTP was also revealed to have independent refugia in the high altitude regions (Meng et al., 2007).

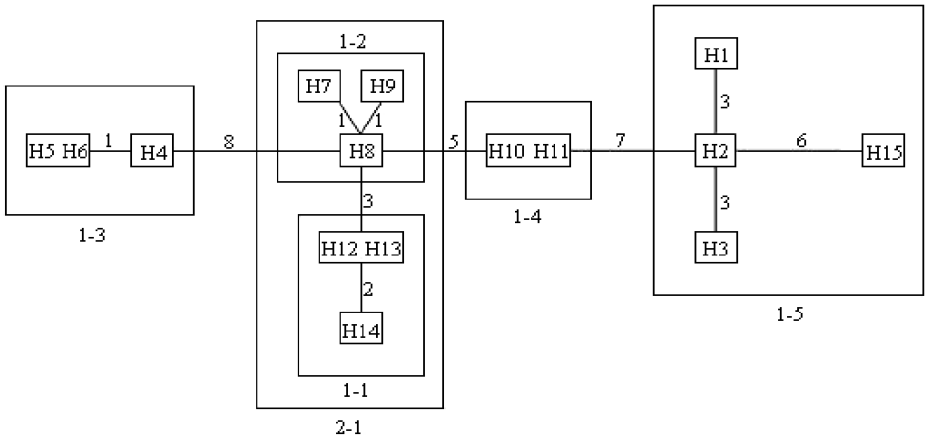

Fig. 3. Minimum spanning tree of cpDNA haplotypes used for the nested clade analysis. The numbers above each connection represent mutational steps.

Table 4 Inference chains based on results of geographical dispersion analysis (GEODIS)

Clade	Clade key	Inferences
1-2	1-2-3-4-NO	Restricted gene flow with isolation by distance
1-3	1-19-NO	Allopatric fragmentation
1-5	1-19-NO	Allopatric fragmentation
2-1	1-2-3-4-NO	Restricted gene flow with isolation by distance
Total cladogram	1-19-NO	Allopatric fragmentation

In addition, the genetic measures suggested a high inter-population differentiation in *P. secundiflora* (G_{ST}=0.816, F_{ST}=0.976). Similarly, the AMOVA analyses also indicated that 97.65% of the total genetic variations are partitioned among populations. However, it should be noted that the average gene diversity within populations (H_S=0.178) was extremely low. Obviously, the current distribution range of *P. secundiflora* is characterized by low within-population diversity, but high diversity between populations due to genetic drift favouring/fixing different haplotypes in different populations. Furthermore, the higher value of N_{ST} (0.982) than G_{ST} (0.816) (P<0.05) suggested a distinct phylogeographic structure of haplotype distributions (Pons & Petit, 1996). These data together suggested that the common interglacial or postglacial range expansion as revealed for most of the temperate plants (e.g. Newton et al., 1999; Petit et al., 2003; Zhang et al., 2005) obviously did not occur in this species. Under this alternative assumption, most of the current populations should fix a common haplotype and contain low levels of genetic diversity both within and between populations if they shared or were derived from a common refugium. These inferences were also supported by the nested clade analysis (NCA) although this method is open to question (Petit et al., 2005). The restricted gene flow/dispersal with isolation by distance is likely the major process in clade 1-2 and 2-1. In addition, allopatric fragmentation was inferred as the major process influencing the present-day spatial distribution of haplotypes within clades 1-3 and 1-5 (Table 4).

However, our results suggest that most of the current populations of *P. secundiflora* must have experienced the *in situ* shrink-expansion cycles during the Quaternary climatic oscillations. This scenario is highly likely if the complex topology of HM and QTP are taken into account. In the QTP, especially in its southeast part, no extensive ice had developed in the Quaternary stages (Li, 1995; Shi et al., 1998). This allows the persistence of plant species in the *in situ* distributions during the glacial stages. In addition, the alpine species like *P. secundiflora* might retreat to the low altitude region during this arid stage. However, because of the deep valleys, it is difficult for different populations to mix together in a common refugium during such a retreat. Similarly, during the interglacial or postglacial expansions, they still retained separate due to the complex topology. Therefore, the climatic oscillations only resulted in the repeated

expansion-contraction cycles of the existent populations. The subsequent genetic drifts reduced the intra-population diversity, but further promoted inter-population differentiation (Powell et al., 1995; Petit et al., 1997; Cruzan & Templeton, 2000). It should be noted that the long-distance dispersal of seeds or pollen grains may disrupt these genetic drifts as revealed in a few species (Birky et al., 1983). However, to our knowledge, seeds of *P. secundiflora* scatter from the ripened capsules within the limited vicinity of the parental plants, rather than being dispersed over long distances by the unexpected mechanism.

In conclusion, our results suggest that the phylogeographic pattern of *P. secundiflora* is different from those of the other temperate plants in which most of the current populations had usually experienced a common recolonization during the interglacial or postglacial range shifts (for an example, Zhang et al., 2005). This is mainly due to the complex topology of HM where deep valleys and high mountains might have prevented migrations of plants during the climatic oscillations. However, more phylogeographic studies are now required on a wide range of different species endemic to this region to obtain a better understanding of the factors that have influenced the evolutionary history of this region's flora.

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基于叶绿体 DNA 变异研究高山植物偏花报春的种内谱系地理结构

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摘要 横断山地区是许多温带植物的冰期避难所。为揭示该地区分布物种的亲缘地理结构, 检测了该地区特有、分布相对较为普遍的偏花报春*Primula secundiflora*的叶绿体*trn*L-*trn*F和*rps*16区序列变异。研究了11个居群109个个体, 一共发现了15种单倍型。只有一种单倍型为3个居群所共有, 其他单倍型都只存在于单个居群内。总的遗传多样性较高(H_T=0.966), 但居群内遗传多样性较低(H_S=0.178)。尽管种内形态十分一致, 居群间却存在高水平的遗传分化(F_{ST}=0.976)。N_{ST} (0.982)显著高于G_{ST} (0.816), 表明偏花报春在居群间存在明显的亲缘地理结构。单倍型聚成四个主要的分支: 三个分支的单倍型分布在北部, 而另一分支的单倍型分布在南部。四个分支的隔离分布表明该物种在冰期存在多个避难所。未发现在其他温带物种中广泛存在的间冰期或者冰期后物种分布范围的统一扩张现象。但是, 在气候变迁过程中由于居群增长-缩小反复发生, 多数居群的遗传多样性降低。这些推断也被巢式分支分析所证实, 距离隔离而导致的限制性基因流以及异域片断化被认为是该物种现有单倍型分布格局形成的主要原因。这种独特的谱系地理结构主要是由于气候变迁与该地区复杂的地质环境相结合造成的。

关键词 亲缘地理学; 偏花报春; 叶绿体DNA; *trn*L-*trn*F; *rps*16; 横断山