

ORIGINAL ARTICLE

Range shifts of *Potentilla fruticosa* on the Qinghai-Tibetan Plateau during glacial and interglacial periods revealed by chloroplast DNA sequence variationA Shimono¹, S Ueno², S Gu³, X Zhao³, Y Tsumura² and Y Tang¹¹Environmental Biology Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan; ²Tree Genetics Laboratory, Department of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan and ³Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, Qinghai, PR China

The Qinghai-Tibetan Plateau is one of the most extensive habitats for alpine plants in the world. Therefore, the patterns of genetic variation in populations on the Plateau can reveal the detailed demographic history of alpine plants. We analysed the geographical structure of chloroplast *matK* sequence variation in *Potentilla fruticosa* L. (Rosaceae), a shrub currently found across the entire Plateau. We obtained sequence data from 508 individuals from 23 populations at sites ranging from the high-altitude interior to the relatively low-altitude northeastern Plateau. In the interior region, genetic diversity was high and included

ancestral haplotypes. In contrast, northeastern populations were characterized by relatively low genetic diversity and recently derived haplotypes. The estimated expansion time in the interior population was 17 times that in the northeastern population. These data suggest that *P. fruticosa* expanded its range on the Plateau during periods of climatic cooling and contracted to the interior region during warmer periods. Thus, the interior region acted as a refugium and greatly contributed to the diversification of *P. fruticosa*. *Heredity* (2010) **104**, 534–542; doi:10.1038/hdy.2009.158; published online 18 November 2009

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Introduction

Global climate fluctuated greatly during the Pleistocene, causing the distributions of most species to change markedly, including range expansion and contraction and by founder events. Such range and demographic changes undoubtedly affect the geographical patterns of genetic variation within and among populations (Hewitt, 2004). Extensive molecular-based research has helped to identify areas where species survived and also to elucidate their subsequent spread, which resulted in their present-day distributions (Avise, 2000; Petit *et al.*, 2003; Soltis *et al.*, 2006).

Molecular studies have clearly shown, for example, the demographic histories of arctic and alpine plants in high-latitude regions (Hewitt, 2000; Abbott and Brochmann, 2003; Schonswetter *et al.*, 2005). These plant species survived in unglaciated peripheral or nunatak refugia during glacial periods, when massive ice sheets covered these regions, and alpine species expanded from these glacial refugia after the last glacial period.

Contrasting demographic patterns to those in high-latitude regions have been suggested for alpine plants in

both temperate and tropical regions, where the cooler periods of the Pleistocene had a crucial role in their expansion (Ikeda *et al.*, 2006; Assefa *et al.*, 2007). During warm periods, alpine species probably retreated to a number of refugia, and they probably experienced several cycles of range contraction and expansion in response to the climatic fluctuations of the Pleistocene (Koch *et al.*, 2006). However, genetic patterns of alpine plants in temperate and tropical regions are complex, and it is difficult to detect clear range shifts because alpine habitats are highly fragmented, having never been connected during glacial or interglacial periods. High-elevation populations may have mainly migrated upslope and downslope, thus remaining isolated throughout the palaeoclimatic cycles, with the result that there was little opportunity for inter-population gene flow (DeChaine and Martin, 2005). Moreover, stochastic demographic events, including extinction, population isolation and long-distance dispersal, can have more noticeable effects in fragmented habitats than in connected habitats, further obscuring geographic genetic patterns.

The Qinghai-Tibetan Plateau occupies an area of 2.5 million km², with an average altitude of 4500 m. Although it is the plateau with highest altitude in the world, it was not covered by massive ice sheets during the last glacial period (Shi, 2002). Limited ice coverage during glaciations resulted in less habitat fragmentation, enabling the Plateau to serve as a refuge for many alpine

Correspondence: Dr A Shimono. Current address: Department of Ecology and Environmental Science, Umeå University, Umeå 901-87, Sweden.
E-mail: ayako@tibetplant.com.

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plants. More than 12 000 species of plants grow on the Plateau, and it is especially rich in endemic species and genera (Lopez-Pujol *et al.*, 2006). Therefore, it is one of the most biologically important and outstanding examples of alpine habitat. The patterns of genetic variation in present-day plant populations on the Plateau are expected to exhibit deep divergence and outstanding diversification, which should allow us to reconstruct their demographic history in more detail.

A rough demographic history of alpine plants can be deduced from palaeoenvironmental changes. Fossil pollen records suggest that the typical vegetation of the Plateau during the last glacial period was adapted to permafrost-steppe, tundra or desert habitats (Yu *et al.*, 2000), although the temperature was 6–9 °C lower than at present (Shi, 2002). These plant communities also developed in the interior of the Plateau during the postglacial period, when forests became widespread on the eastern Plateau (Herzschuh *et al.*, 2006). After the forest retreat, populations may have then expanded from the interior of the Plateau towards its eastern margin. Therefore, results of palaeovegetation studies indicate that the interior Plateau, with its relatively high altitudes, may have provided habitats for alpine plants throughout the glacial and interglacial periods. However, fossil pollen data from the Plateau before the last glacial period are extremely rare and insufficient for the construction of palaeovegetation maps covering the entire region, and the extent of the glacial advance on the Plateau is still under debate (Zheng *et al.*, 2002; Lehmkuhl and Owen, 2005).

The geographical pattern of genetic variation within species of the Qinghai-Tibetan Plateau should provide insight into the above-mentioned glacial and interglacial demographic changes. Recent phylogeographic studies have examined the demographic history of alpine plants across the Plateau (Chen *et al.*, 2008; Yang *et al.*, 2008; Wang *et al.*, 2009). Wang *et al.* (2009) suggested that *Aconitum gymnandrum* has survived on high-altitude parts of the central Plateau throughout the Quaternary. In contrast, *Metagentiana striata* and *Pedicularis longiflora* probably expanded from refugia on the eastern edge to the central Plateau during the interglacial and postglacial periods (Chen *et al.*, 2008; Yang *et al.*, 2008). Therefore, these three investigated alpine species do not share a common phylogeographical history. To understand climatic changes and past distributions of alpine plant species on the vast Plateau, further study of the extensive high-elevation area is required, for which sampling is difficult even today.

For phylogeographic study, we selected the alpine shrub *Potentilla fruticosa* L. (Rosaceae) as a representative of the alpine plants that currently grow throughout the Qinghai-Tibetan Plateau. *P. fruticosa* is a small, long-lived, deciduous shrub species that is widely distributed in alpine meadows, grasslands and forest margins. Its altitude ranges from 400 to 5000 m, according to specimen records in China (Li *et al.*, 2003). Its flowers are self-incompatible and are pollinated not only by Hymenoptera, mainly wild bees, but also by Diptera, Lepidoptera and occasionally by Hemiptera (Guillen *et al.*, 1995). The mature achenes are covered with long hairs, which presumably aid in their dispersal by wind (Elkington and Woodell, 1963). We examined the spatial pattern of chloroplast DNA variation in *P. fruticosa* populations in

habitats across the northeastern and interior Plateau. Our objective was to test the following two hypotheses: (1) that the interior of the Plateau served as a refugium for *P. fruticosa* during interglacial periods and (2) that the range of this species expanded eastwards from the interior of the Plateau.

Materials and methods

Plant material and study sites

Leaves from *P. fruticosa* were collected in 2006 and 2007 from 23 sites distributed across the Qinghai-Tibetan Plateau, from the interior to the northeastern region (Figure 1). At each site, leaves were collected from spatially separated individuals; 508 samples were collected in total (Table 1). The leaf material was dried in silica gel and stored at room temperature.

DNA extraction and sequencing

Total DNA was extracted from dried leaf material using a modified version of the hexadecyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980).

Part of the *matK* gene of the chloroplast DNA was amplified by PCR using the AF and 8R primers (Ooi *et al.*, 1995). PCR was performed in a total volume of 15 µl, containing 1 × PCR buffer, 2.5 mM MgCl₂, 0.2 µM of each primer, 0.2 mM of each dNTP, 0.25 U Ampli Taq Gold (Applied Biosystems, Foster City, CA, USA) and ~20 ng of template DNA. PCR products were cleaned using the QIAquick PCR purification Kit (Qiagen, Tokyo, Japan).

Forward and reverse strands were cycle-sequenced with the same PCR primers, using a BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). For the cycle-sequencing reaction, the thermal profile was 25 cycles at 96 °C for 10 s, 50 °C for 5 s and at 60 °C for 4 min. Products were analysed on an ABI 3730 automated sequencer (Applied Biosystems). Forward and reverse sequences were assembled using Vector NTI software (Invitrogen, Carlsbad, CA, USA). Sequences were compared visually with the original chromatograms to avoid reading errors. All sequences are accessible at GenBank (accession nos. AB458562–AB458594).

Sequence analysis

The DNA sequences were aligned using the CLUSTAL_X 1.81 software (Thompson *et al.*, 1997) and refined manually. Indels were treated as a single character resulting from one mutation event.

Relationships among haplotypes were resolved by constructing a statistical parsimony network and a maximum parsimony tree. The statistical parsimony network was produced using the TCS version 1.13 software (Clement *et al.*, 2000) with a 95% statistical confidence limit, as outlined by Templeton *et al.* (1992). Maximum parsimony trees were constructed using the PAUP 4.0b10 software (Swofford, 2003), applying a tree bisection and reconnection branch-swapping algorithm. The consensus tree was estimated using a bootstrap approach with 1000 replicates. To determine the ancestral haplotype, *Fragaria vesca* was selected as an outgroup by referring to the phylogeny of the Rosaceae (Eriksson *et al.*, 2003). Eriksson *et al.* (2003) reported that the clade including *P. fruticosa* comprises a number of *Potentilla* species along with several other

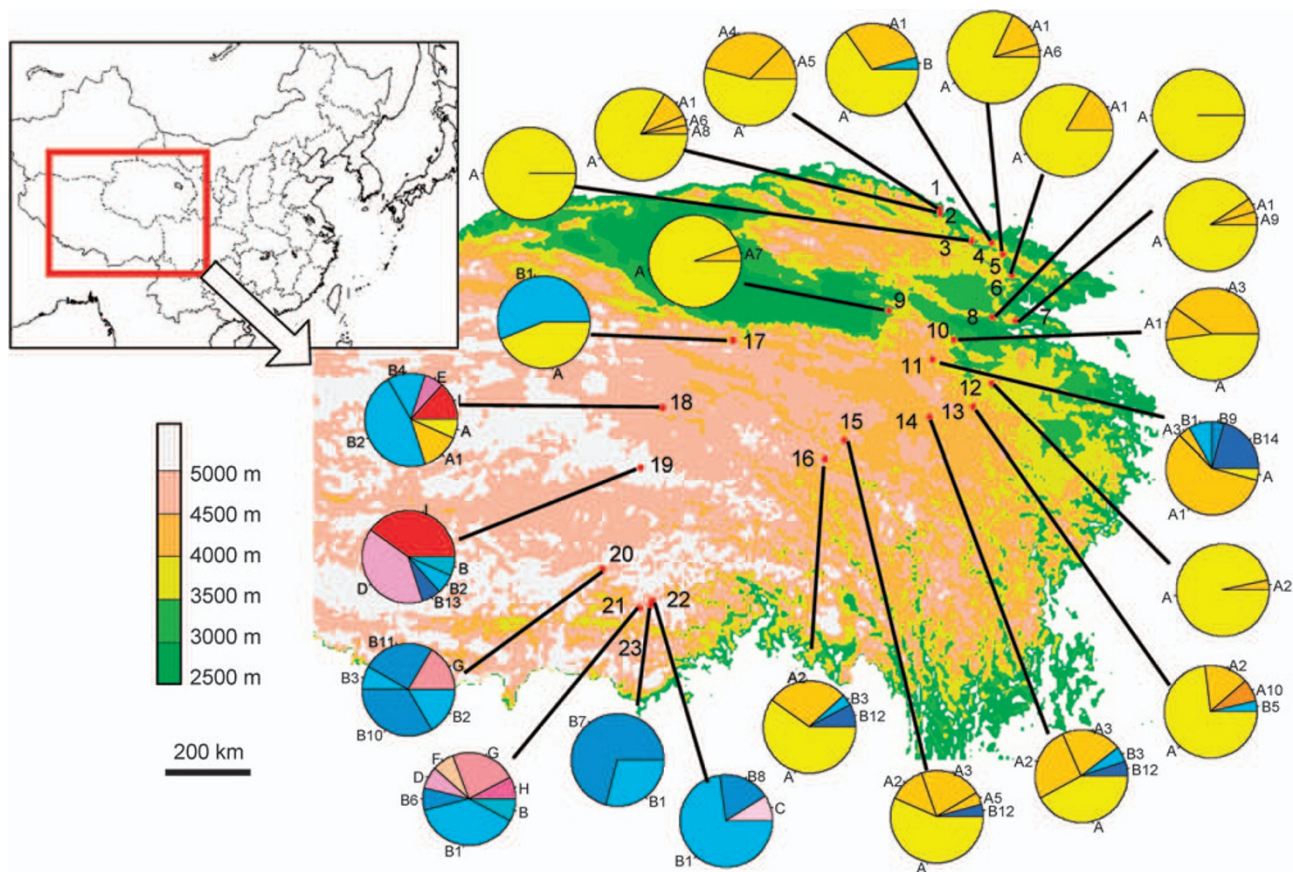


Figure 1 Map of the locations of the populations sampled in this study and the geographical distribution of cpDNA haplotypes detected in *Potentilla fruticosa*. cpDNA, chloroplast DNA.

Table 1 Population number, location, coordinates, altitude, number of samples (*N*), number of haplotypes (*nh*), nucleotide diversity (π) and haplotype diversity (*h*) with s.d. of the 23 populations of *Potentilla fruticosa*

No.	Latitude	Longitude	Alt. (m)	N	nh	π (s.d.) ($\times 10^{-3}$)	h (s.d.)
1	38°47'	99°29'	2720	24	3	0.633 (0.554)	0.605 (0.066)
2	38°40'	99°30'	3820	30	4	0.292 (0.340)	0.303 (0.104)
3	38°05'	100°22'	2910	23	1	0	0
4	38°03'	100°53'	3570	23	3	0.564 (0.515)	0.502 (0.083)
5	37°47'	101°11'	3420	22	3	0.309 (0.356)	0.325 (0.117)
6	37°20'	101°24'	3690	30	2	0.263 (0.320)	0.287 (0.092)
7	36°19'	101°29'	3560	21	3	0.174 (0.257)	0.186 (0.110)
8	36°22'	100°54'	3560	21	1	0	0
9	36°28'	98°13'	3840	18	2	0.102 (0.193)	0.111 (0.096)
10	35°51'	99°55'	3770	25	3	0.659 (0.568)	0.620 (0.052)
11	35°25'	99°22'	4050	24	6	2.619 (1.592)	0.630 (0.095)
12	34°54'	100°53'	3940	28	2	0.065 (0.150)	0.071 (0.065)
13	34°24'	100°25'	3950	26	4	0.684 (0.581)	0.452 (0.109)
14	34°08'	99°19'	4420	19	5	1.336 (0.947)	0.743 (0.064)
15	33°33'	97°13'	4410	23	5	0.939 (0.726)	0.640 (0.090)
16	33°06'	96°45'	4280	25	4	1.127 (0.824)	0.577 (0.083)
17	34°12'	92°26'	4350	25	2	1.409 (0.972)	0.513 (0.037)
18	29°54'	92°21'	4580	15	6	5.241 (2.981)	0.771 (0.098)
19	32°50'	91°55'	5150	15	5	8.183 (4.480)	0.714 (0.081)
20	30°32'	91°03'	4930	12	5	4.561 (2.685)	0.833 (0.069)
21	29°43'	92°02'	4280	13	7	5.935 (3.376)	0.833 (0.086)
22	29°54'	92°21'	4520	22	3	1.869 (1.217)	0.450 (0.112)
23	29°48'	92°20'	4850	24	2	0.394 (0.411)	0.431 (0.081)

groups (namely *Fragaria*, *Alchemilla*, *Chamaerhodos* and *Sibbaldia*) and that *F. vesca* is the sister group of *P. fruticosa*. The out-group sequence for *matK* (Potter et al., 2002) was downloaded from GenBank (accession no. AF288102). We also tested another related species (*Potentilla anserine*, accession no. AF288113) as an out-group, to confirm whether we would obtain the same topology.

To measure levels of genetic variation, nucleotide and haplotype diversities (Nei, 1987) were calculated for each population using ARLEQUIN version 2.0 software (Schneider et al., 2000).

An AMOVA (analysis of molecular variance) (Excoffier et al., 1992) was also conducted using ARLEQUIN version 2.0 (Schneider et al., 2000). The *F*-statistic (F_{st}) was calculated, and significance was tested by using 10 000 permutations.

Spatial genetic analyses

We visualized detailed patterns of spatial genetic structure among populations by genetic landscape shape interpolation using the AIS (Alleles in Space) software (Miller, 2005). This procedure is initiated by constructing a Delaunay triangulation-based connectivity network of sampling areas, and it creates three-dimensional genetic landscapes by interpolation of measured genetic distances between sampled individuals. As AIS interpolates

using residuals from an isolation-by-distance regression across individuals, unusually high genetic distances between individuals within an area result in high elevations on the structural map. We selected a grid size of 50×46 to best represent the rectangular shape of the study area and a distance-weighting parameter of 1. We also tested other grid sizes and a range of distance-weighting parameters (0.5–2) to confirm that qualitatively similar results would be obtained.

Demographic analyses

To test the hypothesis that the range of *P. fruticosa* expanded eastwards, we divided the populations into two regions, namely the northeast (sites 1–17) and the interior (sites 18–23), and analysed the demographic change according to a sudden expansion model by examining the mismatch distribution (the distribution of pairwise differences between haplotypes). This model assumes a sudden population growth from N_0 to N_1 that occurred t generations ago, after which the population reaches demographic equilibrium (Rogers and Harpending, 1992). The mismatch distribution is usually multimodal in samples collected from populations at demographic equilibrium that have been of relatively stable size over time, but it is unimodal in populations that have experienced a recent demographic expansion (Rogers and Harpending, 1992). We used ARLEQUIN version 2.0 software for this implementation (Schneider *et al.*, 2000). This software estimates three demographic parameters by a generalized nonlinear least-squares approach (Schneider and Excoffier, 1999), $\theta_0 = 2uN_0$, $\theta_1 = 2uN_1$ and $\tau = 2ut$, where u is the mutation rate. The validity of this stepwise expansion model is tested by a parametric bootstrap approach simulating sum of squared deviations, which is also used to estimate confidence interval values of the estimated parameters.

Results

Geographical distribution and phylogenetic analysis of haplotypes

We analysed a total of 508 individuals from 23 populations of *P. fruticosa* on the Qinghai-Tibetan Plateau and detected 33 haplotypes by examining ~ 1120 bp of chloroplast DNA from the *matK* region. Variable sites among the 33 haplotypes were observed as 56 nucleotide substitutions and 2 indels, of which 18 were parsimony informative (Table 2). Haplotype A was dominant and widely distributed over the northeastern Plateau (Figure 1). Haplotypes A1 and B1 were also detected frequently in the northeastern and interior Plateau, respectively, whereas the other haplotypes tended to be restricted to local areas. Haplotype B especially occurred with low frequency in two disjunct areas, that is, in site 4 in the northeast and in sites 19 and 21 in the interior region.

Parsimony analysis produced 12 most-parsimonious trees, which required 80 steps (consistency index = 0.950; retention index = 0.944). The topology of the strict consensus tree of the 12 most-parsimonious trees is shown in Figure 2. The same topology was obtained using another outgroup, *P. anserine*. The haplotype network drawn by the statistical parsimony method also clearly supports this topology. Three haplogroups are

apparent in both the parsimony tree and the haplotype network. The relationships among the haplotypes within each haplogroup are unresolved polytomies; that is, each haplotype network exhibits a star-shaped phylogeny. Geographically, the basal haplotype I and haplogroup 1, which consists of haplotypes C–H, were confined to the interior region (sites 18–22). Haplogroup 2, consisting of haplotype B and haplotypes derived from haplotype B, was widespread throughout the Plateau, but occurred with low frequency in the northeastern region. Haplogroup 3, consisting of haplotype A and the haplotypes derived from haplotype A, was dominant and widely distributed over the northeastern region.

Genetic diversity and spatial genetic structure

Most interior populations (sites 18–23), which had widely divergent haplotypes (haplotypes C–I), exhibited high levels of nucleotide diversity (Table 1). In contrast, northeastern populations (sites 1–17) exhibited relatively low levels of nucleotide diversity, a tendency that was particularly noteworthy in populations occurring at altitudes below 4000 m (Table 1).

The AMOVA showed that $\sim 48.7\%$ of the genetic variation occurred among populations, whereas $\sim 51.3\%$ of the variation occurred within populations in the study area. The F_{st} value was 0.487 ($P < 0.0001$).

Genetic landscape shape interpolation analysis produced a clear surface plot, showing that genetic distances increased towards the interior region (Figure 3a). Four high ridges were observed between site 19 and the adjacent sampling sites (Figure 3b).

Demographic analyses

The distribution of pairwise differences between plants from the northeastern populations (sites 1–17) was unimodal, indicating that the sudden expansion model is applicable (Figure 4a). The main expansion event was estimated to have occurred at $\tau = 0.8$ (95% confidence interval, 0–2.1). The newly derived haplotypes (haplogroup 3) were mainly found in these populations.

The pairwise differences in the interior populations (sites 18–23), in contrast, exhibited a bimodal distribution, although this result did not lead us to reject the sudden expansion model (Figure 4b). The main expansion event was estimated to have occurred at $\tau = 13.2$ (95% confidence interval, 6.3–23.7). The estimated expansion time in the interior region was thus 17 times that in the northeastern region. These estimates suggest that interior populations existed for a long period of time before they expanded towards the northeast.

Discussion

The interior as a refugium on the Qinghai-Tibetan Plateau Refugia, where species are able to survive for a long time, generally represent long-term reservoirs of the genetic variation of a species. In these locations, evolution often produces unique genotypes and high levels of diversity (Comes and Kadereit, 1998; Hewitt, 2000). We identified the interior as one such location on the Plateau for *P. fruticosa*. The following three pieces of evidence led to this conclusion: (1) the interior populations (sites 18–22) had widely divergent haplotypes (Figure 2); (2) these populations also exhibited high

Table 2 Variable sites of the aligned sequences of the chloroplast DNA fragment matK region in the haplotypes of *Potentilla fruticosa*

Haplo- type	16	23	43	49	98	110	120	149	154	160	172	216	223	233	258	317	318	346	361	398	419	446	478	596	598	611	612	643	647	674
A	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A1	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A2	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A3	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A4	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A5	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A6	G	C	T	T	C	T	—	G	A	A	C	G	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A7	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A8	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A9	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	A	A	A	C	T	C	T	A	G
A10	G	C	T	T	C	T	—	G	C	A	C	T	T	A	G	T	C	T	T	C	A	A	A	A	C	T	C	T	A	G
B	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B1	G	C	T	T	C	T	—	A	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B2	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B3	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	C	A	C	T	C	T	A	G
B4	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B5	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B6	G	C	T	T	C	T	—	A	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B7	G	C	T	T	C	T	—	A	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B8	G	C	T	T	C	T	—	A	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B9	G	C	T	T	C	T	—	A	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B10	G	C	A	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B11	G	C	T	G	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B12	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	C	A	C	T	C	T	A	G
B13	G	C	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
B14	G	C	T	T	T	T	—	G	A	A	C	T	T	A	G	T	C	T	T	C	G	G	A	A	C	T	C	T	A	G
C	T	T	T	T	C	T	—	G	A	A	C	T	C	A	G	T	C	T	T	C	G	G	A	A	C	C	C	T	A	G
D	T	T	T	T	C	G	—	G	A	A	C	T	T	G	G	C	C	T	T	C	G	G	C	A	C	C	C	T	A	G
E	T	T	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	A	T	C	G	G	A	A	C	C	C	T	A	G
F	T	T	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	T	G	G	A	A	T	C	C	A	A	G
G	T	T	T	T	C	T	—	G	A	A	C	T	T	A	G	T	C	T	T	T	G	G	A	A	T	C	C	A	A	G
H	T	T	T	T	C	T	—	G	A	A	C	T	T	A	C	T	C	T	T	T	G	G	A	A	T	C	C	T	A	G
I	T	T	T	T	C	T	^a	G	A	A	A	T	T	A	G	T	C	T	T	T	G	G	A	T	C	C	T	T	G	G
Haplo- type	719	732	748	750	759	760	781	805	828	835	860	885	909	912	949	960	1004	1016	1019	1028	1069	1085	1090	1105	1106	1112	1114	1121		
A	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A1	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A2	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A3	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A4	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A5	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A6	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A7	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A8	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A9	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
A10	T	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B1	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B2	C	T	A	G	C	T	A	G	C	G	G	—	T	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B3	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B4	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B5	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B6	C	T	A	G	A	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B7	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	T	G	G	G	G	T		
B8	C	T	A	G	C	T	A	G	C	G	A	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B9	C	T	A	G	C	T	A	T	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B10	C	T	G	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B11	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	^G	G	A	C	G	G	A	G	T		
B12	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B13	C	T	A	G	C	T	A	G	T	G	G	—	T	A	C	T	A	G	G	G	G	A	C	G	G	G	G	T		
B14	C	T	A	G	C	T	A	G	C	A	G	—	C	A	C	T	A	G	G	G	G	A	C	T	G	G	G	T		
C	C	A	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	A	G	G	G	A	C	G	G	A	C	T		
D	C	T	A	A	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	G	A	C	T		
E	C	T	A	G	C	G	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	A	C	G	A	G	A	C		
F	C	T	A	G	C	T	A	G	C	G	G	—	C	A	A	T	A	G	G	G	G	A	C	G	G	A	C			
G	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	A	G	G	A	C	G	G	A	C			
H	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	T	A	G	G	G	G	C	C	^G	G	G	A	C		
I	C	T	A	G	C	T	A	G	C	G	G	—	C	A	C	C	A	G	G	T	G	A	C	G	G	G	C			

^aGTTAATGATTCTAATCAAAAT.^bTTGGGTTAT.

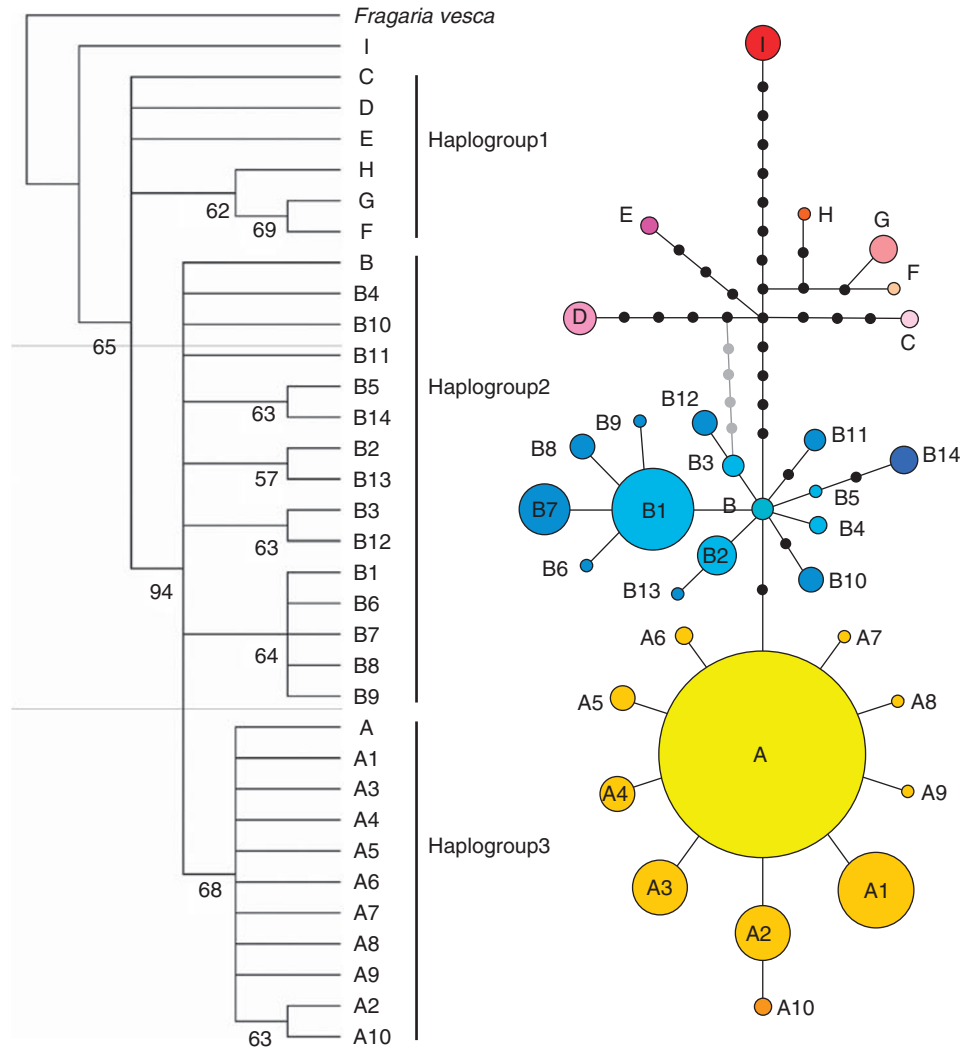


Figure 2 Phylogenetic relationships inferred among the cpDNA haplotypes of *Potentilla fruticosa* based on the sequences of the cpDNA *matK* region. Left: strict consensus tree of the 12 most-parsimonious trees. The numbers on the branches are bootstrap support values for 1000 replicates. Branches corresponding to partitions reproduced in <50% of the bootstrap replicates are collapsed. Right: the haplotype networks. The sizes of circles correspond to the frequency of each haplotype. The small black circles on the branches represent the inferred intermediate haplotypes not observed in the samples. Grey line and dots indicate the network that was not supported by the phylogenetic tree. cpDNA, chloroplast DNA.

levels of genetic diversity (Table 1) and (3) within these populations, unique haplotypes were generally dominant and restricted to small areas (Figure 1). The high levels of diversity and unique genotypes in the interior region may also explain the clear tendency of genetic distances to increase towards the interior in the genetic landscape shape interpolation analysis (Figure 3). In particular, population 19 seems to be the longest surviving population in the sampling area of this study, because (1) it shows the highest genetic diversity among all populations (Table 1), (2) it harbours the ancestral haplotype I (Figures 1 and 2), and (3) clear genetic barriers exist between it and adjacent populations (Figure 3).

Palaeoenvironmental evidence also supports our view that the interior region may have served as a refugium for alpine species. The fossil pollen record shows that, during the mid-Holocene, when temperatures exceeded present-day temperatures by at least 1–2 °C (Yu *et al.*,

2000), forests were located at markedly higher elevations than they are today (Herzschuh *et al.*, 2006; Ren, 2007). This phenomenon had probably occurred during each interglacial period. Therefore, at these times, alpine species in the eastern region must have withdrawn to unforested areas of relatively high altitude, such as in the interior region, where altitudes exceed 4500 m.

The interior region probably provided a suitable habitat for *P. fruticosa* during glacial periods as well, because the Plateau was apparently not covered by massive ice sheets even during the last glacial maximum (Zheng *et al.*, 2002). During the last glacial maximum, glaciers are estimated to have descended to ~3500 m in the northeast and to 5000 m in the interior of the Plateau, reflecting the decreasing precipitation from east to west (Shi, 2002). These altitudes are more than 1000 m higher than those in the other regions at the same latitude (for example, Japan), partly as a result of the low precipitation in winter on the Plateau.

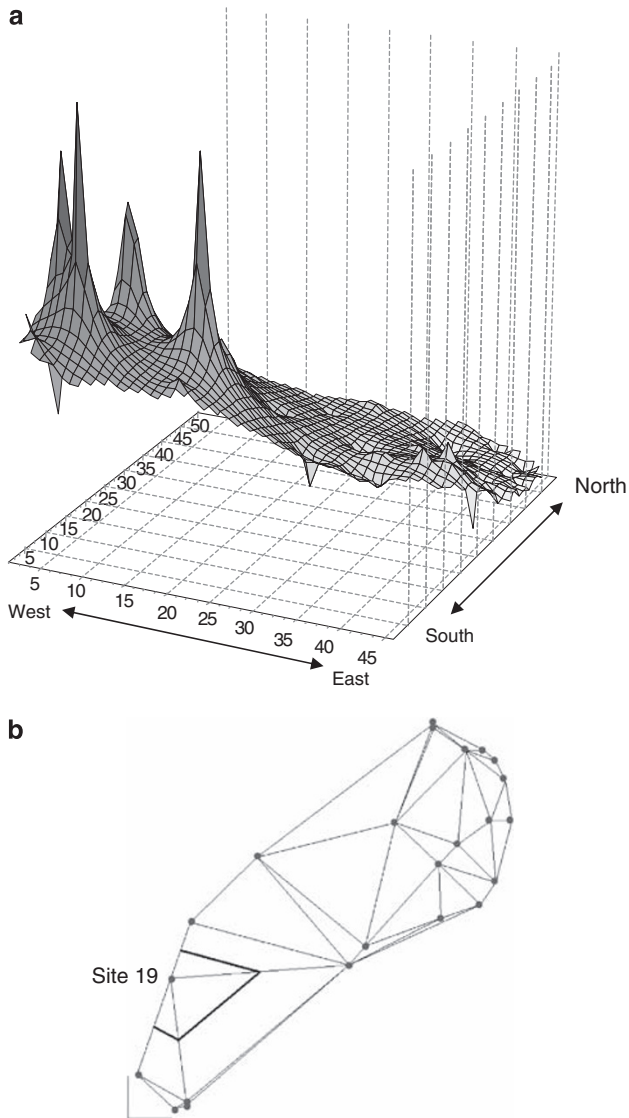


Figure 3 (a) Genetic landscape shape interpolation analysis using a 46×50 grid. X and Y axes correspond to geographical locations within the overall physical landscape examined in this study (Figure 1). Surface plot height (Z axis) reflects residual genetic distance. (b) Delaunay triangulation (fine lines) and the primary barriers (thick line). Dots indicate the sampling points. The greatest genetic barrier was detected between site 19 and its neighbours.

Demographic history of *P. fruticosa*

Past demographic and distributional changes of the alpine species are expected to be reflected in the genetic variations within and between populations. Our data strongly suggest that populations of *P. fruticosa* experienced drastic range expansions in response to past climatic fluctuations. The current northeastern populations apparently underwent a recent expansion from a restricted source area. This conclusion is supported by the unimodal mismatch distribution of the northeastern populations (Figure 4a), suggesting historical population expansion, and by the star-shaped phylogeny of haplotypes derived from haplotype A (Figure 2), the reduced levels of haplotypic and nucleotide diversity (Table 1), and the prevalence of haplotype A in the northeastern populations (Figure 1). The homogeneous genetic pattern

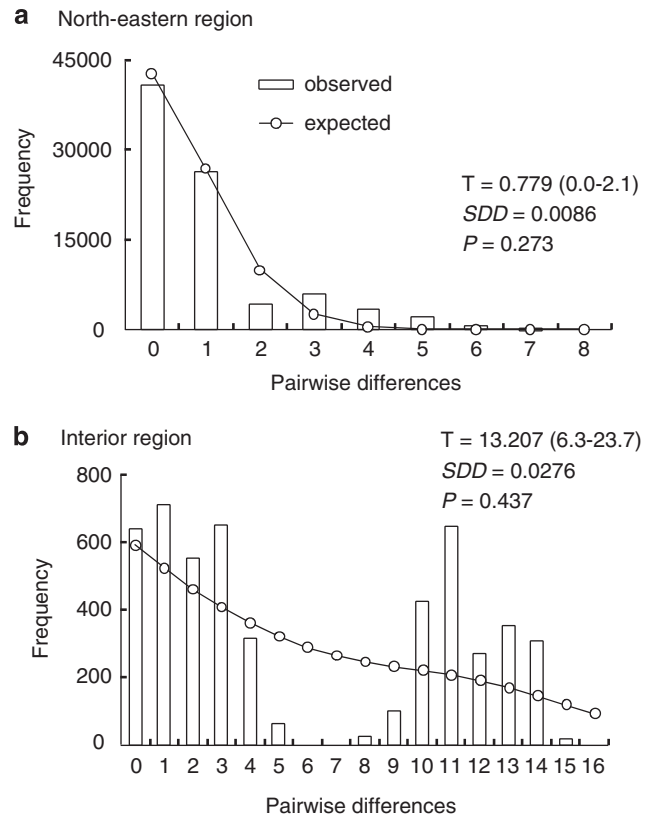


Figure 4 Mismatch distribution analysis for the (a) northeastern and (b) interior populations. Histograms correspond to observed frequencies of pairwise nucleotide differences, and lines represent the expected frequencies under the sudden expansion model. Goodness-of-fit of the observed versus the theoretical mismatch distributions under a sudden expansion model was tested using the sum of squared deviations (SDD). Upper and lower 95% confidence limits around estimates of τ are given in parentheses.

in the northeastern populations is consistent with the AMOVA result, which showed relatively low genetic variation among populations (48.7%) in comparison with other alpine plant species on the Plateau (Chen *et al.*, 2008; Yang *et al.*, 2008; Wang *et al.*, 2009). These patterns are consistent with the results of a simulation study in which sudden expansion from a few populations produced reduced allele diversity and areas of genetic homogeneity (Avise, 2000).

This recent expansion most likely occurred after the beginning of the Holocene, as suggested by the fossil pollen record for the region. For example, a site at 3400 m on the eastern margin was covered by a cool mixed forest at 6 ka B.P. (Yu *et al.*, 2000). After 6 ka B.P., the forest cover decreased and alpine meadow became widely distributed in this region (Ren, 2007). At present, coniferous forest is located between 2000 and 2900 m.

Phylogeographic studies suggest a similar history of coniferous trees (Zhang *et al.*, 2005; Meng *et al.*, 2007), the dominant species in the forested zone and which occasionally grow within the alpine zone on the northeastern Plateau. These scattered conifer populations probably reflect colonization from a refugial population that existed in the past along the Plateau's edge. In addition, results of a phylogeographic study of the alpine herb *A. gymnanthrum*, which is widely

distributed in alpine meadows across the Plateau, suggest the existence of refugia at high altitudes on the central Plateau (Wang *et al.*, 2009). Conversely, refugia of another two alpine species were inferred to have been located at the Plateau's edge (Chen *et al.*, 2008; Yang *et al.*, 2008).

Further comparative studies with other regions and other species are required to confirm the history of alpine species on the Qinghai-Tibetan Plateau. Extensive studies conducted in high-latitude regions, especially in Europe, allow us to extract shared phenomena among species, although each species has its own unique history during glacial and interglacial periods (Schonswetter *et al.*, 2005).

We also inferred that *P. fruticosa* populations probably experienced a demographic expansion before the recent expansion described above, and haplotype B prevailed across the Plateau. This scenario is supported by the star-shaped phylogeny exhibited by haplotypes derived from haplotype B (Figure 2) and by the occurrence of haplotype B in two disjunct areas, that is, in site 4 in the northeast and in sites 19 and 21 in the interior region (Figure 1). After the expansion, the *P. fruticosa* population would have contracted to the interior of the Plateau in response to climatic fluctuations, with the result that lineages derived from haplotype B would have been distributed mainly in interior regions, with only a few examples left in the northeast region (Figure 1). However, further studies are required to confirm this early range shift of the *P. fruticosa* population.

Conclusions

We reconstructed the demographic history of *P. fruticosa* on the Plateau by using genetic variation data obtained from spatially distributed populations. Our results suggest that periods of population expansion were associated with climatic cooling, and that during climatic warming, populations contracted to the interior of the Qinghai-Tibetan Plateau. Thus, the interior region acted as a refugium during interglacial periods, and greatly contributed to the diversification of *P. fruticosa*.

Conflict of interest

The authors declare no conflict of interest.

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