Conserv Genet
DOI 10.1007/s10592-011-0250-9

RESEARCH ARTICLE

Chloroplast phylogeography of *Helianthemum songaricum* (Cistaceae) from northwestern China: implications for preservation of genetic diversity

Zhihao Su · Mingli Zhang · Stewart C. Sanderson

Received: 22 May 2011 / Accepted: 12 July 2011
© Springer Science+Business Media B.V. 2011

Abstract Two chloroplast intergenic spacers (*trn*D-*trn*T and *rps*16-*trn*K) were used to study the phylogeographical structure of *Helianthemum songaricum* in northwestern China, with 12 haplotypes detected. Phylogenetic analysis showed that *H. songaricum* comprised two lineages, one distributed in the Yili Valley and the other in the western Ordos Plateau. Nested clade phylogeographic analysis likewise indicated that haplotypes in the Yili Valley and those in the western Ordos Plateau formed sister monophyletic clades. This lineage split was also supported by AMOVA analysis and the Mantel test. AMOVA showed that 99.41% of variance in *H. songaricum* occurred between the two distribution regions, and correlation between genetic differentiation and geographical distances was significant ($r^2 = 0.877, P < 0.0001$). All populations in the Yili Valley shared haplotype A with high frequency, and range expansion was detected by nested clade analysis and strongly supported by negative Fu's F_S-values, Tajima's D-values, and mismatch distribution analysis. We 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. The high drift load ($F_{ST} = 0.9923$, $G_{ST} = 0.663$) and inbreeding load ($H_S = 0.219$) of *H. songaricum* indicate a significant extinction risk, and protective measures should be taken immediately.

Keywords Chloroplast DNA · *Helianthemum* · Phylogeography · Yili Valley · Western Ordos Plateau

Z. Su · M. Zhang (✉)
Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China
e-mail: zhangml@ibcas.ac.cn

Z. Su
Graduate University, Chinese Academy of Sciences, Beijing 100049, China

M. Zhang
Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

S. C. Sanderson
Shrub Sciences Laboratory, Intermountain Research Station, Forest Service, U.S. Department of Agriculture, Utah 84601, USA

Introduction

Phylogeography can make a valuable contribution to conservation biology. Adequate biological information for declining species, such as genetic diversity, population structure, and evolutionary history, are essential to the development of broad-scale conservation strategies and planning of management actions (Avise et al. 1987; Moritz 1994; Pope et al. 1998; Osborne et al. 2000; Browning et al. 2001). For example, phylogeographic studies can help in identification of a phylogenetic unit that is reciprocally monophyletic with its sister clade at a cytoplasmic locus and shows significant divergence of allele frequencies. Such an entity is defined as an Evolutionarily Significant Unit (ESU) (Ryder 1986; Fraser and Bernatchez 2001). Usually the ESU is a top-priority protection target below taxonomic levels (Ryder 1986). Phylogeography studies can also identify haplotypes that are significantly divergent from other alleles. These haplotypes can be used to assist the development of plans for the conservation and sustainable use of plant species, especially for informing the transfer of germplasm within and between regions (Newton et al. 1999).

Published online: 31 July 2011

Natural processes, such as vicariance caused by geological changes or climatic oscillations, can profoundly impact the genetic diversity and population structure of plant species, leading to genetic divergence among populations in the species. For example, Liao et al. (2007) found that the land barrier of the Malay Peninsula has caused population differentiation between the Indian Ocean and South China Sea in *Ceriops tagal*; Rowden et al. (2004) concluded that *Fagus* populations in Mexico retreated northward and expanded southward repeatedly during alternate glacial and interglacial periods in the Pliocene and Pleistocene, resulting in population fragmentation and a high degree of genetic variation within the species. In addition, many studies have also indicated that within different allopatric regions, patterns of range shift have been distinct; in some regions, range expansion might have occurred. For example, in the eastern Qinghai-Tibetan Plateau (QTP), most populations of *Allium przewalskianum* contained multiple haplotypes and showed significant genetic divergence from each other, but in the western QTP, this species experienced an earlier range expansion at the end of the last glacial maximum (LGM) (Wu et al. 2010); two distinct lineages of *Hippophae tibetana* were found distributed in eastern and western regions of the QTP, but within each, populations had experienced a recent postglacial expansion (Jia et al. 2011). For those species which are in decline, the identified differentiated populations should be taken into consideration for conservation measures.

China has some distinctive biogeographic features. There are many studies concerning phylogeography in the context of the Tibetan plateau (Zhang et al. 2005; Yuan et al. 2008), because of its complex topography and the climatic effects of plateau uplift. However, investigations on the phylogeography of plant species in the arid northwestern part of China are scarce, including only genetic diversity measurements on a few plant species (Chen et al. 2009). The arid zone in northwestern China comprises the entire Xinjiang Autonomous Region, the Hexi Corridor in Gansu Province, the Caidamu Basin in Qinghai Province, and western parts of the Helan Mountains in the Inner Mongolia Autonomous Region (Alxa Dersert) (Zhu et al. 2004). Species in this combined area are often sparsely distributed and ancient. Many of them may be survivors from the ancient Tethys region, such as *Gymnocarpos przewalskii*, *Cynomorium songaricum*, *Helianthemum songaricum* (Tang 2000). This illustrates the close relationship among floras of the Mediterranean, Xinjiang, and Inner Mongolia regions.

Helianthemum is mostly distributed in the Mediterranean region and extends to Central Asia. There is usually only considered to be one species of *Helianthemum* which occurs in China, *H. songaricum*, which is mainly distributed in the Yili Valley of Xinjiang, and in the western Ordos Plateau of Inner Mongolia (Yang and Michael 2003). However, based on flower characteristics and chromosome number, Zhao et al. (2000) has proposed segregation of the populations in the western Ordos as a new taxon, *H. ordosicum*.

Helianthemum songaricum in the wider sense has declined drastically over the past several decades and is classified as endangered in the China Species Red List (Fu 1992). This phylogeographic study aims to provide useful information for its conservation. Furthermore, because of its rather typical disjunctive distribution in China, this study provides insight into the origin, evolvement, and migratory routes of the flora of northwestern China (Fu 1992). *H. songaricum* is a deciduous shrub and primarily occurs on lithoid hillsides (about 1000–1300 m) with an arid climate. It has leathery leaves, yellow flowers, and ovoid capsules with short pubescence, and chiefly reproduces sexually by way of insect pollination. Previous research, employing horizontal starch gel electrophoresis, dealt with genetic diversity and population differentiation of this species, but was limited to Inner Mongolia (Duan et al. 2000), and therefore did not provide information concerning genetic structure and phylogeography across the whole distribution range, or use modern molecular techniques.

In this study, we used two chloroplast DNA regions, *trn*D-*trn*T, and *rps*16-*trn*K, to examine the phylogeographical pattern of 15 populations of *H. songaricum* across the entire distribution range in China. Specific questions addressed were: (1) Do populations of the two allopatric regions represent different evolutionary units? (2) Were there similar range shifts within each of the two regions?

Materials and methods

Sampling

A total of 154 individuals from 15 populations of this species were sampled, covering almost the entire geographical range of distribution in the Yili Valley and western Ordos Plateau. We were unable to find the species at locations in Gansu (in the Hexi Corridor), probably because it has become extinct there due to deforestation and other human activities in recent decades. Across all of the species range, nine populations were sampled in the Yili Valley and six in the western Ordos Plateau; 6 to 15 individuals were collected per population. Fresh leaves were gathered from each individual and dried in silica gel. We also selected two species, *Helianthemum nummularium* and *Crocanthemum canadense* as outgroups for the phylogenetic analysis (Guzmá and Vargas 2009).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica-gel-dried leaf tissue using a modified 2× CTAB method (Rogers and Bendich 1985; Doyle and Doyle 1987). The intergenic spacer *trn*D-*trn*T was amplified and sequenced using the primers and protocols of Demesure et al. (1995), and the *rps*16-*trn*K region was amplified and sequenced using the primers and protocols of Takahashi et al. (2005). Amplification products were purified using the PCR product purification kit (Shanghai SBS, Biotech Ltd., China), following directions provided by the manufacturer. Sequencing reactions were conducted with the forward or reverse primers of the amplification reactions, using the DYEnamic ET Terminator Kit (Amersham Pharmacia Biotech), with an ABI-PRISM3730 automatic DNA sequencer from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). Electropherograms were edited and assembled using SEQUENCHER 4.1 (Gene Codes, Ann Arbor, MI, USA). Sequences were aligned using the CLUSTAL W program (Thompson et al. 1994).

Phylogenetic analysis

Phylogenetic analysis of the resulting alignments was carried out by maximum-parsimony (MP) and maximum-likelihood (ML) in PAUP* version 4.0b10 (Swofford 2002). We constructed MP trees using a heuristic search, 100 random additions of sequences, with the tree-bisection-reconnection (TBR) branch swapping, and MULTREES, COLLAPSE, and STEEPEST DESCENT options switched on. Characters were weighted equally and their state changes were treated as unordered. Gaps in sequences were treated as the fifth character state. We used Modeltest 3.7 (Posada and Crandall 1998) to search for the best nucleotide substitution model. The reliability of the trees obtained with the ML and MP methods was tested using bootstrapping with 1000 replicates.

Haplotype network and nested clade phylogeographic analysis

For this analysis, indels were coded as single binary characters following Simmons and Ochoterena (2000). The analysis was performed following the approach of Templeton et al. (2005) using the program ANeCA (Panchal 2007). A statistical parsimony network of all sequences was constructed with the program TCS (Clement et al. 2000), and the resulting network was nested hierarchically following the rules described by Templeton et al. (1987) and Templeton and Sing (1993). Based on the geographical coordinates of each population, clade and nested clade distances were estimated to assess the geographical association of the clades and nested clades (Templeton et al. 1995). The null hypothesis of no geographical associations for the tip and interior clades was tested using GEODIS 2.5 (Posada et al. 2000), with 10,000 random permutations and a 95% confidence level. The inference key given by Templeton (2004) was then used to infer the probable historical processes that shaped the observed patterns of clade structure.

Population genetic analyses

For population genetic analyses, population subdivision was first performed using program SAMOVA v.1.0 to define groups of locations that are geographically homogeneous and genetically differentiated from each other (Dupanloup et al. 2002). Parameters of within-population diversity (h_S), total gene diversity (h_T), and genetic differentiation (G_{ST}) at species and group levels, as well as those of population subdivision for phylogenetically ordered alleles (N_{ST}), and the fixation index (F_{ST}) were then estimated. These parameters were calculated using the programs HAPLONST and PERMUT, which were available at WWW.pierroton.inra.fr/genetics/labo/Software/index.html. Analysis of genetic structure was performed using AMOVA to estimate differentiation within and among populations and among the subdivisions which had been detected. These analyses were performed using the program ARLEQUIN v.3.01 (Excoffier et al. 2005), with significance tests by 10,000 permutations. To evaluate the relative historical influences of gene flow and drift on regional population structure, the Mantel test with 10,000 random permutations was performed using the program TFPGA v. 1.3 (Miller 1997). Pairwise population differentiation statistics (F_{ST}) and the fixation index (F_{ST}) were calculated using DNASP 4.0 (Rozas et al. 2003), and the logarithm of geographical distance was calculated using GEODIS 2.5 (Posada et al. 2000). A correlation coefficient describing the relationship between the two matrices was calculated, to determine the significance of genetic differentiation by distance.

Demographic analyses

Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) statistics were calculated using ARLEQUIN to test for evidence of range expansion (Hurtado et al. 2004; Jaeger et al. 2005; Smith and Farrell 2005). These parameters are highly sensitive to departures from population equilibrium. A significant value for D may be due to factors such as population expansion or bottlenecks (Aris-Brosou and Excoffier 1996; Tajima 1996), and a significantly large negative value for F_S may be due to population expansion (Fu 1997). Mismatch distribution analysis was also calculated using ARLEQUIN with 1000 parametric bootstrap replicates. A significant sum of squared differences (SSD;

$P \leq 0.05$) and a unimodal distribution of pairwise differences were taken as evidence for departure from a model of population expansion (Schneider and Excoffier 1999). Demographic analyses were performed for the clades and the region in which contiguous range expansion had been detected by the nested clade phylogeographic analysis. If the sudden expansion model was not rejected, the corresponding value τ was used to infer the expansion time scale. We used the relationship $\tau = 2ut$ (Rogers and Harpending 1992) to estimate the expansion time (t), where u is the mutation rate per generation for the whole analyzed sequence. The value of u was calculated as $u = 2\mu kg$, where μ is the substitution rate per nucleotide site per year, k is the average sequence length of the analyzed DNA region, and g is the generation time in years. According to our initial observations, we assumed the generation time of this desert species to be 3 years.

Divergence time estimate

Using PAUP*, version 4.0b10 (Swofford 2002), we first performed a likelihood ratio test of the hypothesis of equal substitution rates for the cpDNA sequences (Posada and Crandall 1998), which led to acceptance of the molecular clock hypothesis ($P > 0.01$). We used Bayesian analysis to estimate divergence time between the two lineages with the software BEAST (Drummond et al. 2002; Drummond and Rambaut 2007), based on substitution rates of the chloroplast sequence recovered for most angiosperm species ($\mu = 1.0$–3.0×10^{-9} substitutions per site per year; Wolfe et al. 1987). Following a burn-in of 500,000 steps, all parameters were sampled once every 100 steps from 5,000,000 MCMC steps. Using the software Tracer (Drummond and Rambaut 2007), we checked convergence of the stationary distribution by visual inspection of plotted posterior estimates, and the effective sample size for each parameter sampled was found to exceed 200.

Results

Sequence analysis

The cpDNA region *trn*H-*psb*A is acknowledged as one of the most variable intergenic spacers, however, we could not obtain the middle portion because of a large A/T polymorphism, and the same situation occurred in *trn*L-*trn*F; therefore we discarded these regions. *ycf*6-*psb*M, *trn*Q-*rps*16, *rpl*32-*trn*L, and *ndh*F-*rpl*32, were difficult to amplify and so they were discarded as well. At last, we chose the two regions *trn*D-*trn*T and *rps*16-*trn*K for this study. After the sequences were aligned, the length of the *rps*16-*trn*K region was 640 bp, and the *trn*D-*trn*T region 1107 bp. For both sequences combined, 18 substitutions were detected, at positions 11, 127, 297, 383, 441, 465, 605, 606, 685, 686, 698, 813, 838, 861, 1162, 1597, 1604, and 1706. Also, there were 21 indels, which occurred at positions 15–16, 120–126, 129–140, 213, 397–399, 624, 645–651, 659, 667–670, 683, 685, 701–705, 707, 860, 926, 989–996, 1362–1381, 1413–1414, 1417–1433, 1533, and 1743. In all, a total of 12 haplotypes (A–L) were identified (Table 1). GenBank accession numbers of the cpDNA sequences are JN315829–JN315848, JN381194.

Phylogenetic analysis

The best nucleotide substitution model selected by AIC was TVM+I. Two phylogenetic analyses by MP and ML methods produced a consistent topology (Fig. 1). The 12 haplotypes of *H. songaricum* clustered into a monophyletic group, and two highly supported clades (clade I, II) were identified. Clade I included six haplotypes (A–F) distributed in the Yili Valley with a 'star-like' phylogeny. Clade II consisted of six haplotypes (G–L) from the western Ordos populations of the species.

Interestingly, the results from the MP and ML were congruent with the nested haplotype network (this will be shown in the following section).

Haplotype geographical distribution and relationships

Haplotype geographical distribution and frequencies in each of the populations are presented in Fig. 2 and Table 2. With regard to the Yili Valley in Xinjiang, six populations had one haplotype [Kebo (A), Junmachang (A), Kalabula (A), Zhongyangchang (A), Heishantou (A), Baishidun (A)], one population had two haplotypes [Longkou (A, D)], and two populations had three haplotypes [Kekesu (A, B, C), Bole (A, E, F)]. In the western Ordos Plateau, except for Qianligou which had three haplotypes (G, K, L), all the other populations had two [Kabuqi (G, H), Qipanjing (G, I), Hainan (G, J), Mengxi (G, K), Qianlishan (G, K)]. Thus, haplotype A was shared by all the populations in the Yili Valley, and haplotype G was shared by all the populations in the western Ordos Plateau. No haplotypes were shared between the two regions.

The network, constructed using parsimony analysis, indicated phylogeny among the haplotypes. As shown in Fig. 3, haplotype D was connected to A by five hypothetical haplotypes, haplotypes C, E, and F were each connected to A by one substitution, and haplotype B was connected to A through one hypothetical haplotype. Haplotype J was connected to G by two hypothetical haplotypes, and haplotype H and I were each connected to G by one substitution. Haplotype L was connected to K by one substitution, which in turn was connected to G by one substitution.

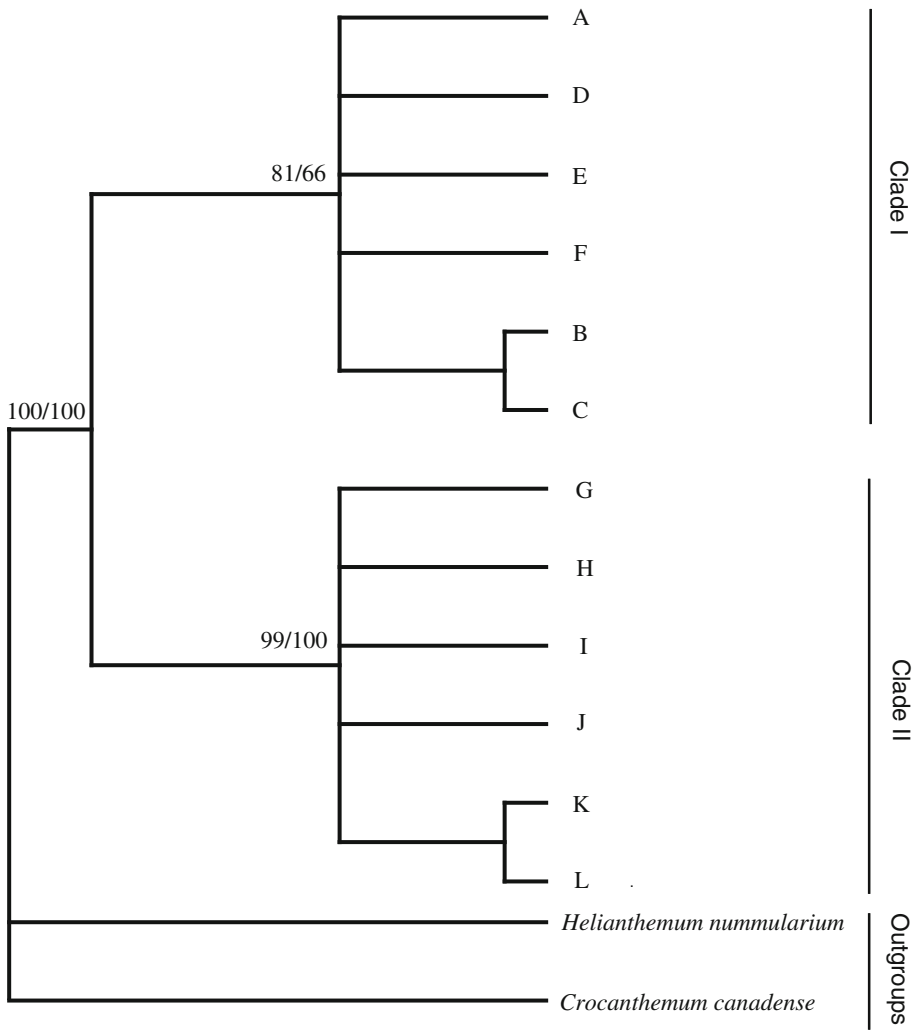

Fig. 1 Phylogenetic relationships of 12 haplotypes in *H. songaricum*. Support values (maximum parsimony bootstrap/maximum likelihood bootstrap) greater than 50% are shown at nodes

Nested clade phylogeographic analysis

After converting the statistical parsimony network into a hierarchically nested design, we obtained eight 1-step, four 2-step, and two 3-step clades (Fig. 3). Two clades, for which the null hypothesis of no geographic structuring of haplotypes could not be rejected, were identified by nested clade phylogeographic analysis. As shown by the nested clade phylogeographic analysis results in Table 3 and Fig. 3, the relationship between clades and geographical distribution was significant in *H. songaricum*. Haplotypes included in clade 3-1 were all from the western Ordos Plateau, while haplotypes in clade 3-2 were all from the Yili Valley. There are 95 hypothetical haplotypes between the two clades. Contiguous range expansion was detected within clade 1-4. Clade 1-4 included haplotype A, which was spread across the Yili Valley, and also haplotypes E and F, found only in Bole, located in the northern part of the valley, and haplotype C, only fixed in Kekesu. Restricted gene flow, with isolation by distance (restricted dispersal by distance in non-sexual species), was detected in clade 2-1. Clade 2-1 contained haplotypes H–L; haplotype G was shared by all populations in the western Ordos Plateau. The frequency of haplotypes H, I, and J was low; these three only appeared in the populations Kabuqi, Hannan, and Qipanjing, all located in the south of the Plateau. In the northern populations (Mengxi, Qianlishan, and Qianligou), haplotype K appeared at high frequency, and haplotype L was also distinct from those observed in the south of the Plateau. Allopatric fragmentation was diagnosed over the total cladogram.

Genetic diversity and genetic structure

SAMOVA results revealed the presence of two groups of populations with a Φ_{CT} value of 0.9940 ($P < 0.001$), a Φ_{SC} value of 0.3100 ($P < 0.001$), and a Φ_{ST} value of 0.9959 ($P < 0.001$). The groups were as follows: populations 1–9

Table 1 Twelve haplotypes of *H. songaricum* were recognized based on two chloroplast DNA sequences, *trn*D-*trn*T and *rps*16-*trn*K

Haplotype	Sequence position																																				
	11	15	120	127	129	213	297	383	397	441	465	605	606	624	645	659	667	683	685	686	698	701	707	813	838	860	861	926	989	1162	1362	1413	1417	1533	1597	1604	1706	1743
A	A	▲	☆	T	●	–	T	C	○	G	G	G	T	T	–	A	–	T	G	A	G	◆	C	G	A	A	C	A	–	C	□	▼	▽	A	C	A	C	–
B	A	▲	☆	T	●	–	T	C	○	G	G	G	T	T	–	A	–	–	A	A	G	◆	C	G	A	A	C	A	–	C	□	▼	▽	A	C	A	C	–
C	A	▲	☆	T	●	–	T	C	○	G	G	G	T	T	–	A	–	T	–	A	G	◆	C	G	A	A	C	A	–	C	□	▼	▽	A	C	A	C	–
D	A	▲	☆	T	●	–	T	C	○	G	G	G	T	T	–	A	–	T	G	A	G	–	–	G	A	A	C	A	–	C	□	▼	▽	A	C	A	C	–
E	A	▲	☆	T	●	–	T	C	○	G	G	G	T	T	–	A	–	T	G	A	G	◆	C	G	A	A	C	–	–	C	□	▼	▽	A	C	A	C	–
F	A	▲	☆	T	●	–	T	C	○	G	G	G	T	T	–	–	–	T	G	A	G	◆	C	G	A	A	C	A	–	C	□	▼	▽	A	C	A	C	–
G	A	▲	–	C	–	A	T	G	–	C	A	G	G	–	★	A	◇	T	G	C	C	◆	C	G	C	–	T	A	△	A	–	–	–	–	G	C	C	A
H	A	▲	–	C	–	A	T	G	–	C	A	G	G	–	★	A	◇	T	G	C	C	◆	C	G	C	–	T	A	△	A	–	–	–	–	G	C	G	A
I	A	▲	–	C	–	A	A	G	–	C	A	G	G	–	★	A	◇	T	G	C	C	◆	C	G	C	–	T	A	△	A	–	–	–	–	G	C	C	A
J	C	–	–	C	–	A	T	G	–	C	A	G	G	–	★	A	◇	T	G	C	C	◆	C	G	C	–	T	A	△	A	–	–	–	–	G	C	C	A
K	A	▲	–	C	–	A	T	G	–	C	A	T	G	–	★	A	◇	T	G	C	C	◆	C	G	C	–	T	A	△	A	–	–	–	–	G	C	C	A
L	A	▲	–	C	–	A	T	G	–	C	A	T	G	–	★	A	◇	T	G	C	C	◆	C	T	C	–	T	A	△	A	–	–	–	–	G	C	C	A

▲: TT, ☆: ATTAAC, ●: AAGAAAATTCCA, ○: TTT, ★: TGATTCA, ◇: AGAT, ◆: ACAAA, △: CCCCATTT, □: TTCTACATACATTCTTATCT, ▼: AA, ▽: CAACTAACAAATCAAAA

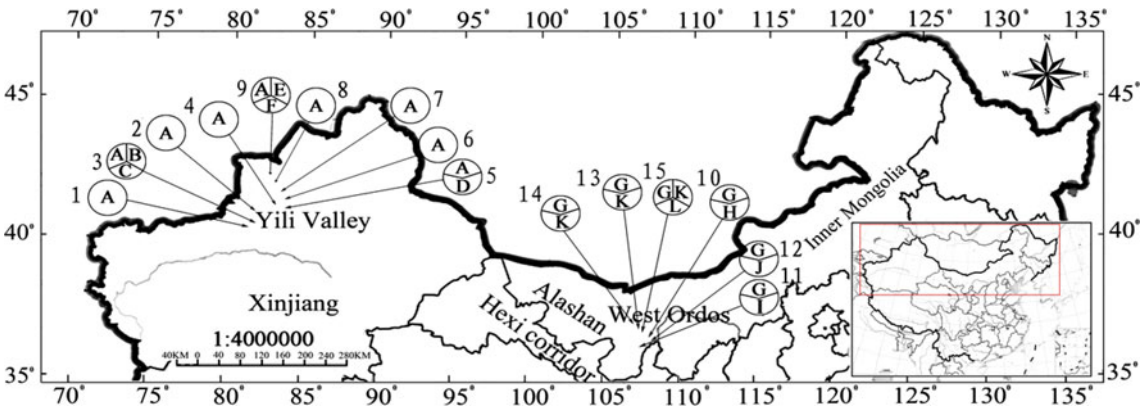

Fig. 2 The geographical distribution of *H. songaricum* in China. Population numbers correspond to those in Table 2; haplotypes to those in Table 1

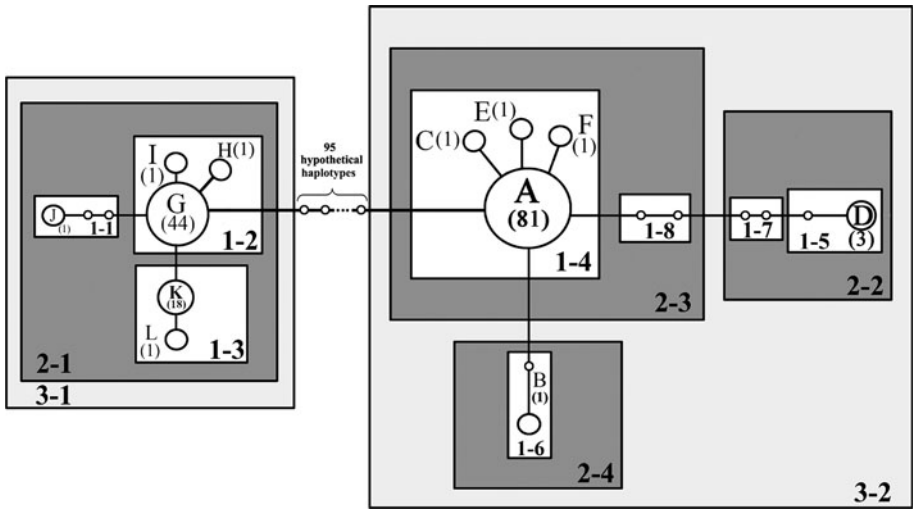

Fig. 3 Nested cladogram of the 12 cpDNA haplotypes found in *H. songaricum*. A–L: sampled haplotypes. *Blank dot*: hypothetical haplotypes. The number of individuals is given in parentheses

belonging to the Yili Valley, and populations 10–15 belonging to the western Ordos Plateau. Because most variation was found between the two groups, and because significant relationships were found in each of the geographic regions and nested clades (clade 3-1, clade 3-2), the large-scale structure of genetic variation as indicated by SAMOVA was very similar to that revealed by nested clade analysis.

Differentiation among populations was high (G_{ST} = 0.663, SE 0.0654), indicating population structure in *H. songaricum*. Within-population gene diversity (h_S) was 0.219 (SE 0.0575), and total gene diversity (h_T) was 0.651 (SE 0.0869). N_{ST} was 0.905 (SE 0.0394), significantly higher than G_{ST} as shown by the *U*-test ($U = 4.56$, $P < 0.01$), indicating phylogeographical structure. The parameter V_S was 0.063 (SE 0.0270), and V_T was 0.661 (SE 0.0835), almost identical to h_S and h_T. Genetic differentiation within the two regions, the Yili Valley (G_{ST} = 0.100, $h_S = 0.145$, $h_T = 0.162$; $N_{ST} = 0.181$, $V_S = 0.119$, V_T = 0.145) and western Ordos Plateau ($G_{ST} = 0.418$, $h_S = 0.330$, $h_T = 0.566$; $N_{ST} = 0.461$, $V_S = 0.308$, $V_T = 0.570$) was similarly low in both. AMOVA results showed that most of the total variation (99.23%, $P < 0.001$) was explained by differences among populations, and the fixation index F_{ST} was 0.9923. When populations were grouped according to geographical region, AMOVA results showed that 99.41% ($P < 0.001$) of the total variation occurred between the two regions. Considering the two regions separately, 46% of the total variation occurred among populations in the western Ordos Plateau, and 17.88% in the Yili Valley (Table 4).

Correlation between genetic and geographical distances

The scatterplot for all regions (Fig. 4) shows a significant positive linear relationship between log-transformed

Table 2 Details of sample locations and sample size and haplotype frequencies in 15 populations of *H. songaricum*

Population	Location	Latitude (N)	Longitude (E)	Altitude (m)	Haplotype
Yili Valley					
1	Kebo	43°10′	81°45′	1246	A(10)
2	Junmachang	43°14′	81°59′	1318	A(10)
3	Kekesu	43°12′	81°56′	1166	A(8), B(1), C(1)
4	Kalabula	43°27′	82°37′	829	A(10)
5	Longkou	43°25′	82°28′	855	A(7), D(3)
6	Zhongyangchang	43°34′	82°34′	952	A(10)
7	Heishantou	43°35′	82°28′	810	A(10)
8	Baishidun	43°41′	82°03′	1220	A(10)
9	Bole	44°50′	82°03′	566	A(6), E(1), F(1)
Western Ordos					
10	Kabuqi	39°35′	106°55′	1248	G(14), H(1)
11	Qipanjing	39°21′	107°04′	1328	G(14), I(1)
12	Hainan	39°27′	106°59′	1230	G(9), J(1)
13	Mengxi	39°48′	106°53′	1260	G(3), K(7)
14	Qianlishan	39°51′	106°56′	1359	G(2), K(4)
15	Qianligou	39°46′	106°55′	1450	G(2), K(7), L(1)

pairwise linearised F_{ST} values and the logarithm of the geographical distance between populations ($r^2 = 0.877$, $P < 0.0001$), indicating that populations become less genetically similar as the geographical distance between them increases.

Demographic analyses

Demographic analyses showed evidence for range expansions in the clade 1-4 and the Yili Valley. For the clade 1-4, the value for Fu's F_S was significantly negative (Table 5), and for the whole Yili Valley, the negative values for Tajima's D and Fu's F_S were both significant. The mismatch analyses indicated that the distribution of pairwise differences was unimodal for both clade 1-4 and the whole Yili Valley (Fig. 5).

The time of this expansion event was estimated to be 50–140 kya, based on the cpDNA substitution rate ranges, a haplotype sequence length of 1747 bp, and 3 years per generation.

Divergence time estimate

Using the fast and slow substitution rates, the two intraspecific lineages of *H. songaricum* were estimated to have diverged between 0.65 (95% HPD: 0.29–1.27) and 1.94 (95% HPD: 0.86–3.84) Mya, that is, from about early-Pleistocene to early-Middle Pleistocene, according to Shi et al. (2005).

Discussion

Allopatric divergence between the Yili Valley and western Ordos Plateau

Phylogenetic analyses showed that *H. songaricum* comprised two lineages, distributed respectively in the Yili Valley and the western Ordos Plateau (Figs. 1, 2; Table 2). Nested clade phylogeographic analysis also indicated a similar result, that haplotypes in the Yili Valley form a

Table 3 Results of the nested clade phylogeographical analysis and interpretations according to the revised inference key given by Templeton et al. (2005) for 15 populations of *H. songaricum* from arid northwestern China

Nested clade	χ^2	*P*-value	D_C	D_n	Inference chain	Inferred pattern
1-4	27.856	0.0619	43.7477*s	−64.3083*s	1-2-11-12 NO	Contiguous range expansion
2-1	46.6027	0.0000	13.5081*l	0.8313	1-2-3-4 NO	Restricted gene flow with isolation by distance (restricted dispersal by distance in non-sexual species)
3-2	24.2259	0.0091	46.3925*s	826.5134*s	1-2 IO	Inconclusive outcome
Total cladogram	153.0	0.0000	NO interior clades		1-19 NO	Allopatric fragmentation

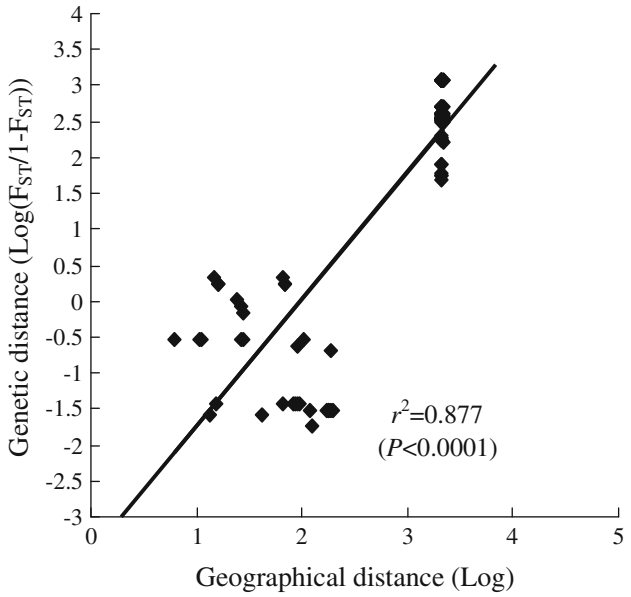

Fig. 4 The figure shows a significant relationship between geographic and genetic distance ($r^2 = 0.877$, $P < 0.0001$)

monophyletic clade, 3-1, and those in the western Ordos Plateau form a monophyletic clade, 3-2, as well. AMOVA analysis indicated that almost all of the variance (99.41%) occurred between the two lineages/regions, and the Mantel test showed that correlation between the genetic and geographical distance matrices was significant ($r^2 = 0.877$, $P < 0.0001$). All the above demonstrated significant genetic divergence between the two regions, giving further support to the suggestion that populations of *Helianthemum* in the western Ordos Plateau should be given species rank, as proposed by Zhao et al. (2000).

The western Ordos plateau is a part of the Alxa desert, whose flora formed during the Tertiary (Liu 1995). The Hexi Corridor in the eastern Junggar Basin was a passage through which floristic elements of Central Asia diffused into the Alxa desert, so we can infer that the species diffused into the western Ordos Plateau through this passage during the Tertiary period (Liu 1995). At about 2 Ma, the temperature declined markedly and glaciation developed on a large scale in the Northern Hemisphere (Williams et al. 1993); also many mountains of the Tianshan Belt became elevated to the height of the cryosphere by the early-Middle Pleistocene (Shi et al. 2005). The developing cold climate would have destroyed a part of the xerophil flora (Liu 1995), but it appears that *H. songaricum* could have survived in some valleys of the Tianshan Mountains. Likewise, the western Ordos resides greatly inland, and thus in spite of a high topography, it was not covered by glaciers. As a result, it suffered relatively little damage from the cold (Liu 1995), and is therefore another habitat in which this species would have survived during the climatic extremes. The northwest region of China had already begun aridification by early Pleistocene; desert and gobi terrain further expanded and dry climate continued to develop during the middle Pleistocene (Williams et al. 1993). The worsening of the dry, cold climate restricted the distribution range, and acceptable habitats for the species

Table 4 Results of the analysis of molecular variance for 15 populations of *H. songaricum* grouped in two geographical regions (the Yili Valley and western Ordos Plateau) based on cpDNA sequence data

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation (%)
Among populations	14	3633.860	25.34671	99.23*
Within populations	139	20.250	0.14568	0.77*
(1, 2, 3, 4, 5, 6, 7, 8, 9) vs. (10, 11, 12, 13, 14, 15)				
Among groups	1	3623.807	48.03196	99.41*
Among populations within groups	13	10.053	0.06168	0.18*
Within populations	139	20.250	0.14568	0.41*
Among (western Ordos) populations	5	8.939	0.14909	46.00*
Within populations	60	10.500	0.17500	54.00*
Among (Yili) populations	8	2.709	0.01658	17.88*
Within populations	79	13.950	0.17658	82.12*

* $P < 0.001$

Table 5 Results of neutrality tests and mismatch distribution analysis for clade 1-4 and all samples in the Yili Valley

Group	τ	SSD (*P* value)	Hrag (*P* value)	Fu's *Fs* (*P* value)	Tajiam's *D* (*P* value)
Clade 1-4	3.00	0.0000 (0.30)	0.7438 (0.72)	−5.3461 (0.000)	0.00 (1.000)
Yili Valley	3.00	0.0069 (0.12)	0.6241 (0.80)	−2.5967 (0.057)	−1.0436 (0.042)

τ time in number of generations elapsed since the sudden expansion episode, *Hrag* the Harpending's Raggedness index, *SSD* sum of squared deviations

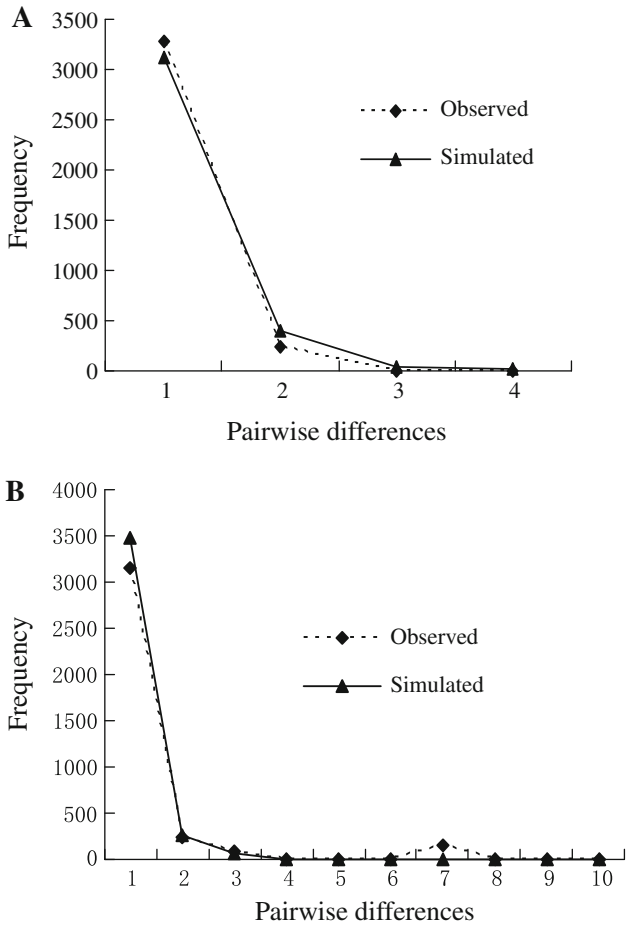

Fig. 5 Mismatch distribution analysis for cpDNA data for all samples in the clade 1-4 ((**a**) SSD = 0.0000, $P = 0.30$) and for all samples in the Yili Valley ((**b**) SSD = 0.0069, $P = 0.12$)

gradually became reduced and isolated. In addition, the species has a low rate of seed production, and germination is low as well because of a hard testa and poor water translocation (Cao et al. 2000), so that dispersability is very poor. In addition, due to the long distance between the western Ordos Plateau and the Yili Valley (about 2000 km), gene flow between the two regions was interrupted easily. Divergence time was estimated at about 0.65–1.94 Mya (from early-Pleistocene to early-Middle Pleistocene), also consistent with the period during which the climate became colder and dryer in the northwest zone.

The difference of climate between the two regions is large. The climate in the Tianshan Mountains is peculiar; mountains surround the Yili Valley on the north, east, and south, so that the shape of the valley resembles a bugle with the peristome towards the west, in contact with Central Asia. In the Yili Valley, it is droughty in summer, but wet in the spring and winter, similar to the ancient Tethys climate (Institute of Botany, Chinese Academy of Sciences 1978). This region has been described as a wet island in an arid zone (Shi et al. 2005). In contrast, the climate in the western Ordos Plateau is typically dry (Walker 1974). Populations of the species have been exposed to these geographically separated and distinct climates for a lengthy period of time, resulting in the potential for genetic divergence between the two regions.

Regional expansion in the Yili Valley

Contiguous range expansion was detected within clade 1-4 by nested clade analysis. Whenever large-scale range expansion occurs, it usually causes two distinct genetic signatures: wide distribution of a single genotype, and a star-phylogeny pattern (a dominant haplotype with multiple rare alleles) (Comes and Kadereit 1998; Hewitt 2000). The distribution of genetic variation in the Yili Valley was consistent with these expected signatures. We found that haplotype A was widespread across every sampled location, and rare haplotypes such as B, C, D, E, F, were limited to random single population of the valley. Genetic diversity and differentiation within the Yili Valley ($h_S = 0.145$, $h_T = 0.162$; $G_{ST} = 0.100$, $N_{ST} = 0.181$) were very low, and the amount of total variation occurring among populations was also small (17.88%). Both of these illustrate the inconspicuous genetic structure of populations in this region. This signature of low genetic diversity is in agreement with the leading-edge model: colonization results in loss of diversity in the colonized regions (Comes and Kadereit 1998). Such an expansion is also supported by strongly negative Fu's F_S-values, Tajima's D-values, and unimodal mismatch distributions (Fig. 5).

Climate oscillation since the late Quaternary is usually considered an important factor influencing the current geographical distribution and genetic structure of plants (Hewitt 2004). There was a great deal of glaciation in the Tianshan Mountains, and the extent of glacial area varied in response to the alternation of ice ages and interglacials (Shi et al. 2005). As mentioned, the time of the expansion event in *H. songaricum* was estimated at 50–140 kya, largely consistent with the last interglacial period (75–130 kya). During the last interglacial period, the climate in the Tianshan Mountains was warmer and wetter than that during the penultimate glacial period, and with the increase of temperature, glaciers retreated continuously (Shi et al. 2005). The changing climate and glacial area caused corresponding range shifts of the populations. Cold and dry climate would have reduced the distribution area and damaged some individuals during the glacial period, but once environmental condition became more suitable for survival, that is, warmer and wetter, the species would have thrived and expanded outwards. So we speculate that wetness associated with increasing temperature after the penultimate glacial period resulted in expansion and consequently reduced the level of genetic diversity among

populations in the valley (Hewitt 1996). Similar patterns of genetic variation have been reported in species in Europe, North America, and north-east Asian (Broyles 1998; Hewitt 2000; Aizawa et al. 2009).

Compared with the Yili Valley, there was more genetic structure in the populations in the west Ordos Plateau. Restricted gene flow with isolation by distance was detected for clade 2-1. It included individuals of six populations, all from the western Ordos Plateau. In addition, the genetic diversity and differentiation within the western Ordos Plateau ($h_S = 0.330$, $h_T = 0.566$; $G_{ST} = 0.418$, $N_{ST} = 0.461$) were both higher than that within the Yili Valley ($h_S = 0.145$, $h_T = 0.162$; $G_{ST} = 0.100$, $N_{ST} = 0.181$). This greater genetic structure might have been mainly caused by the seriously fragmented habitat of the species, due to the further development of dry climate in the Quaternary (Liu 1995), as well as the poor dispersability already discussed above.

Implications of conservation

Genetic drift and inbreeding are the most important genetic factors pushing species with small population sizes and fragmented distribution patterns into "extinction vortices" (Lande 1998). The fixation index F_{ST} can be used as a susceptive index of a population to the deleterious effects of drift load (Keller and Waller 2002), and the parameter of within-population diversity (H_S), on the other hand, more likely relates to the deleterious effect of inbreeding load (Jaquiéry et al. 2009); a high F_{ST} value signifies high drift load, whereas a low H_S value signifies high inbreeding load (Jaquiéry et al. 2009). For *H. songaricum*, the pattern over the total cladogram inferred by nested clade analysis was allopatric fragmentation, and the drift load and inbreeding load of *Helianthemum* were both high ($F_{ST} = 0.9923$, $H_S = 0.219$), indicating a significant extinction risk. Therefore, protective measures should be taken immediately. Besides the parameters F_{ST} and H_S, G_{ST} is another frequently used parameter employed to estimate the proportion of genetic diversity residing among populations. The higher the G_{ST} value, the lower the level of inter-population gene flow, which therefore may suggests a risk of genetic drift and/or inbreeding (Caujapé-Castells 2010). In *H. songaricum*, differentiation among populations across the whole distribution range ($G_{ST} = 0.663$) was high, and in addition, restricted gene flow with isolation by distance was detected among the populations in the western Ordos Plateau ($G_{ST} = 0.418$), also indicating a need for protection.

Conservation management decisions should be made to relieve both drift load and inbreeding load. First, the two regional units should be highly important to protect. Although a nature reserves has been set up near Wuhai city, Inner Mongolia, for *H. songaricum* and other rare and endangered plants, such nature reserves should likewise be set up in Xinjiang as well. Second, efforts for recovery should be implemented at the scale of population. Actions such as transplantation of seedlings in natural habitats and/or ex-situ conservation should be taken in order to create augmented space for genetic exchange and recombination. In addition, rare haplotypes in a few populations of each region should be considered, although for most populations, conservation of a few individuals is enough for preservation of cpDNA diversity.

In conclusion, physiological features and climate change that increased deterioration of the dry and cold climate during early Quaternary climate oscillations may have had fundamental influences on the evolution of this species. Investigations on the genetic structure of plant species in arid northwestern China have been few, and so phylogeographical studies of additional endemic taxa are now required, to obtain a better understanding of factors that have influenced the evolutionary history of species in this region.

Acknowledgments We thank Yuxiao Zhang and Dequan Zhang at the Kunming Institute of Botany, CAS, for their kindly help in molecular expertise and data processing, and thank Zhibin Wen at the Xinjiang Institute of Ecology and Geography, CAS, for her useful suggestions. Funding was provided by CAS Important Direction for Knowledge Innovation Project (No. KZCX2-EW-305), and Xinjiang Institute of Ecology and Geography, CAS.

References

Aizawa M, Yoshimaru H, Saito H, Katsuki T, Kawahara T, Kitamura K, Shi F, Sabirov R, Kaji M (2009) Range-wide genetic structure in a north-east Asian spruce (Picea jezoensis) determined using nuclear microsatellite markers. J Biogeogr 36:996–1007

Aris-Brosou S, Excoffier L (1996) The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol Biol Evol 13:494–504

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann Rev Ecol Syst 18:489–522

Browning TL, Taggart DA, Rummery C, Close RL, Eldridge MDB (2001) Multifaceted genetic analysis of the "Critically Endangered" brush-tailed rock-wallaby *Petrogale penicillata* in Victoria, Australia: Implications for management. Conserv Genet 2:145–156

Broyles SB (1998) Postglacial migration and the loss of allozyme variation in northern populations of *Asclepias exaltata* (Asclepiadaceae). Am J Bot 85:1091–1097

Cao R, Duan FZ, Ma H, Wang LQ (2000) The biodiversity and population biology of a relic species-*Helianthemum songaricum*. Chin Sci Abstr 6:220–222

Caujapé-Castells J (2010) General G_{ST} and θ inflation due to biased intra-population sampling, and its consequences for the conservation of the Canarian Flora. Conserv Genet 11:709–720

Chen GQ, Huang HW, Crawford DJ, Pan BR, Ge XJ (2009) Mating system and genetic diversity of a rare desert legume *Ammopiptanthus nanus* (Leguminosae). J Syst Evol 47:57–66

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659
Comes HP, Kadereit JW (1998) The effect of quaternary climatic changes on plant distribution and evolution. Trends Plant Sci 3:432–438
Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. Mol Ecol 4:129–131
Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem Bull 19:11–15
Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214
Drummond AJ, Nicholls GK, Rodrigo AG, Solomon W (2002) Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. Genetics 161:1307–1320
Duan FZ, Cao R, Ma H, Zhao YZ, Hou X (2000) Genetic divertisty and differentiation of endangered species—*Helianthemum songaricum*. Chin Sci Abstr 6:217–219
Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. Mol Ecol 11:2571–2581
Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50
Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741–2752
Fu LG (1992) Rare and endangered plants in China. Science Press, Beijing, pp 218–219
Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147:915–925
Guzmá B, Vargas P (2009) Historical biogeography and character volution of Cistaceae (Malvales) based on analysis of plastid rbcL and trnL-trnF sequences. Org Divers Evol 9:83–99
Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc 58:247–276
Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913
Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Phil Trans R Soc Lond B Biol Sci 359:183–195
Hurtado LA, Erez T, Castrezana S, Markow TA (2004) Contrasting population genetic patterns and evolutionary histories among sympatric Sonoran Desert cactophilic *Drosophila*. Mol Ecol 13:1365–1375
Institute of Botany, Chinese Academy of Sciences (1978) Vegetation in Xinjiang and its use. Science Press, Beijing, pp 200–211
Jaeger JR, Riddle BR, Bradford DF (2005) Cryptic neogene vicariance and Quaternary dispersal of the red-spotted toad (*Bufo punctatus*): insights on the evolution of North American warm desert biotas. Mol Ecol 14:3033–3048
Jaquiéry J, Guillaume F, Perrin N (2009) Predicting the deleterious effects of mutation load in fragmented populations. Conserv Biol 23:207–218
Jia DR, Liu TL, Wang LY, Zhou DW, Liu JQ (2011) Evolutionary history of an alpine shrub *Hippophae tibetana* (Elaeagnaceae): allopatric divergence and regional expansion. Biol J Linn Soc 102:37–50
Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241
Lande R (1998) Anthropogenic, ecological and genetic factors in extinction and conservation. Res Popul Ecol 40:259–269
Liao PC, Havanond S, Huang S (2007) Phylogeography of *Ceriops tagal* (Rhizophoraceae) in Southeast Asia: the land barrier of the Malay Peninsula has caused population differentiation between the Indian Ocean and South China Sea. Conserv Genet 8:89–98
Liu YX (1995) A study on origin and formation of the Chinese desert flora. J Syst Evol 33:131–143
Miller M (1997) Tools for population genetics analysis (TFPGA), version 1.3. A Windows® program for the analysis of allozyme and molecular population genetic data. Department of Biological Sciences, Northern Arizona University, Flagstaff
Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. Mol Ecol 3:401–411
Newton AC, Allnutt T, Gillies ACM, Lowe A, Ennos RA (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol Evol 14:140–145
Osborne MJ, Norman JA, Christidis L, Murray ND (2000) Genetic distinctness of isolated populations of an endangered marsupial, the mountain pygmy-possum, *Burramys parvus*. Mol Ecol 9: 609–613
Panchal M (2007) The automation of nested clade phylogeographic analysis. Bioinformatics 23:509–510
Pope LC, Sharp A, Moritz C (1998) The genetic diversity and distinctiveness of the Yellow-footed rock-wallaby *Petrogale xanthopus* (Gray, 1854) in New South Wales Pacific. Conserv Biol 4:164–169
Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818
Posada D, Crandall KA, Templeton AR (2000) GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol Ecol 9:487–488
Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant-tissues. Plant Mol Biol 5:69–76
Rogers A, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569
Rowden A, Robertson A, Allnutt T, Heredia S, Williams-Linera G, Newton AC (2004) Conservation genetics of Mexican beech, *Fagus grandifolia* var. *Mexicana*. Conserv Genet 5:475–484
Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497
Ryder OA (1986) Species conservation and the dilemma of subspecies. Trends Ecol Evol 1:9–10. doi:10.1016/0169-5347(86)90059-5
Schneider S, Excoffier L (1999) Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089
Shi YF, Cui ZJ, Su Z (2005) The quaternary glaciations and environmental variations in China. Hebei Science and Technology Publishing House, Hebei, China, pp 450–499
Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381
Smith CI, Farrell BD (2005) Range expansions in the flightless longhorn cactus beetles, *Moneilema gigas* and *Moneilema armatum*, in response to Pleistocene climate changes. Mol Ecol 14:1025–1044
Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (and other methods), Version 4.0b10. Sinauer Associates, Sunderland
Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595
Tajima F (1996) The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. Genetics 143:1457–1465
Takahashi S, Furukawa T, Asano T, Terajima Y, Shimada H, Sugimoto A, Kadowaki K (2005) Very close relationship of the

chloroplast genomes among Saccharum species. Theor Appl Genet 110:1523–1529

Tang YC (2000) On the affinities and the role of the Chinese flora. Acta Bot Yunnanica 22:1–26

Templeton AR (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol Ecol 13:789–809

Templeton AR, Sing CF (1993) A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. 4. Nested analyses with cladogram uncertainty and recombination. Genetics 134:659–669

Templeton AR, Boerwinkle E, Sing CF (1987) A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. 1. Basic theory and an analysis of alcohol-dehydrogenase activity in Drosophila. Genetics 117:343–351

Templeton AR, Routman E, Phillips CA (1995) Separating population-structure from population history—a cladistic-analysis of the geographical-distribution of mitochondrial-DNA haplotypes in the tiger salamander, Ambystoma-Tigrinum. Genetics 140:767–782

Templeton AR, Maxwell T, Posada D, Stengard JH, Boerwinkle E, Sing CF (2005) Tree scanning: A method for using haplotype trees in phenotype/genotype association studies. Genetics 169:441–453

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal-W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Walker JW (1974) Evolution of exine structure in pollen of primitive angiosperms. Am J Bot 61:891–902

Williams MAJ, Dunkerley DL, De Dekker P, Kershaw AP, Stokes T (1993) Quaternary environments. Edward Arnold, London, pp 87–112

Wolfe KH, Li WH, Sharp PM (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAS. Proc Nat Acad Sci USA 84:9054–9058

Wu LL, Cui XK, Milne RI, Sun YS, Liu JQ (2010) Multiple autopolyploidizations and range expansion of *Allium przewalskianum* Regel. (Alliaceae) in the Qinghai-Tibetan Plateau. Mol Ecol 19:1691–1704

Yang QE, Michael GG (2003) Cistaceae. In: Wu ZY, Raven PH (eds) Flora of China, vol 13. Science Press, Beijing

Yuan QJ, Zhang ZY, Peng H, Ge S (2008) Chloroplast phylogeography of *Dipentodon* (Dipentodontaceae) in southwest China and northern Vietnam. Mol Ecol 17:1054–1065

Zhang Q, Chiang TY, George M, Liu JQ, Abbott RJ (2005) Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. Mol Ecol 14:3513–3524

Zhao YZ, Cao R, Zhu ZY (2000) A new species of *Helianthemum* Mill. (Cistaceae). J Syst Evol 38:294–296

Zhu YH, Wu YQ, Drake S (2004) A survey: obstacles and strategies for the development of ground-water resources in arid inland river basins of Western China. J Arid Environ 59:351–367