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RESEARCH ARTICLE

Genetic structure of the endangered *Leucomeris decora* (Asteraceae) in China inferred from chloroplast and nuclear DNA markers

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Abstract The genetic variation and structure of *Leucomeris decora*, an endangered species in China were investigated. Analyses of three chloroplast DNA (cpDNA) regions (the *rpl16* intron, *trnQ-5′rps16* intergenic spacer and *rpl32-trnL* intergenic spacer) and one nuclear gene (*GAPDH*: encoding glyceraldehyde 3-phosphate dehydrogenase) were conducted on 11 *L. decora* populations. Low levels of cpDNA genetic diversity were found in this species and within populations, with the identification of 2 haplotypes in a total of 2,745 bp, while the level of genetic diversity revealed by the nuclear gene *GAPDH* was relatively high, indicating that random losses of genetic polymorphisms from populations may have occurred recently. High levels of genetic differentiation among populations for both markers were detected in *L. decora*, which could be a consequence of the limited gene flow caused by geographic isolation among populations. An analysis of molecular variance revealed at the nuclear locus suggested the presence of geographic structure within the haplotype distribution possibly due to geographical barriers among populations. The haplotype network and mismatch distribution analyses did not detect the signal for a recent population expansion in *L. decora*. *L. decora* may persist in situ during climatic oscillations. Based on the genetic diversity and uniqueness of the populations, conservation strategies are discussed for this endangered species.

Keywords *Leucomeris decora* · Chloroplast DNA · *GAPDH* · Genetic structure · Phylogeography · Conservation

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Introduction

The central goal of conservation genetics is to understand the levels and partitioning of genetic variation across populations and geographical regions of endangered species. Especially, detailed analyses of the levels and spatial distribution of genetic diversity are important for the development of effective conservation strategies and management practices for endangered species (Hedrick and Miller 1992). For example, knowledge of the distribution of genetic variation can be valuable in determining how many or which populations to protect as well as in guiding policies for seed collection and the establishment of new populations. A particular population genetic structure and the levels of genetic diversity within populations/species are usually results of various evolutionary forces acting in concert through time and space, and may therefore reflect not only historical events such as population bottlenecks, expansion over wide geographical areas and gene flow between lineages but contemporary evolutionary events (Cruzan and Templeton 2000). Phylogeographical methods provide useful tools for inferring the demographic and historical events that have shaped the evolution of populations and species. Determination of phylogeographic structure can also provide useful information for identification of evolutionary significant units and management units for endangered species, aiming to preserve maximum genetic diversity within the target gene pool (Avise 2000).

Leucomeris decora Kurz (Hyalideae: Asteraceae) (Funk et al. 2009; Panero and Funk 2008), is a large shrub or

small tree, distributed in Yunnan Province in China, Myanmar, Thailand and Vietnam. It grows to 3–5 m and is usually found in dry valleys, at the edge of the forests and on isolated mountaintops at elevations of 1,100–1,900 m, where there is sufficient sunlight and the temperature is relatively high. It is monoecious and has white flowers that open between March and April. Its fruits have pappi. At present, the natural populations are located in anthropogenically slightly affected areas like the top of the mountains or severely affected areas where is easily reached by people. *L. decora* has become locally rare with limited number of individuals and small population sizes due to rapid habitat destruction and fragmentation, together with unrestricted collection for medicinal use. Thus, it has been listed in the Red book of Chinese plants (Fu and Jin 1992) and the IUCN Red List of Threatened Species since 1992 and 1998, respectively. Currently, there is little knowledge of the status of *L. decora* populations throughout its distribution in China. The continuous exploitation of this species requires an accurate description of genetic structure to identify populations of special conservation concern.

In population genetics and phylogeographic studies, chloroplast (cp) DNA has been extensively used (Bain and Golden 2003; Ikuyo and Noriaki 2009; Li et al. 2008; Mousadik and Petit 1996; Yang et al. 2008), for it is maternally inherited and non-recombining in most angiosperms (Reboud and Zeyl 1994). Furthermore, chloroplast genomes are particularly sensitive to the effects of fragmentation as a result of their small effective population sizes and restricted seed-mediated gene dispersal. Thus, chloroplast-specific markers are particularly useful for identifying genetic bottlenecks and founder effects, and for measuring genetic drift (Petit et al. 1997). However, cpDNA is considered to evolve more slowly than nuclear DNA (nDNA) (Wolfe et al. 1987) and in some cases, the level of cpDNA variation is too low to elucidate the population relationships, especially for species with limited ranges. Therefore, the combination of data acquired using markers from different inheritance systems has been proposed to elucidate more thoroughly the genetic structure of investigated species (Eidesen et al. 2007; Ikeda et al. 2008; Petit et al. 2005; Wang and Ge 2006).

In this study, we selected three cpDNA sequences from *rpl16*, *trnQ*$^{(UUG)}$*-5′rps16* and *rpl32- trnL*$^{(UAG)}$, and one nuclear DNA locus *GAPDH* to explore the genetic structure and phylogeography of 11 *L. decora* populations sampled throughout the species' entire geographical range in China. We addressed the following questions. Firstly, do cpDNA and nDNA markers both display low levels of genetic polymorphism in *L. decora* as generally expected for a rare species? Secondly, given the complex topology of this region, are there relatively high levels of genetic differentiation among populations or between geographical regions? Thirdly, what factors are involved in shaping its population genetic structure? This information not only provides insight into levels of DNA polymorphism but may also provide the ability to identify genetically distinct populations across the species range for conservation.

Materials and methods

Population sampling

Eleven natural *L. decora* populations in Yunnan Province, China, were sampled. We tried to collect the samples in other countries, but unfortunately we did not obtain any. The collection sites covered almost the entire geographical distribution of the species except for some highly cultivated or urbanized locations, where *L. decora* was not found. Each population was represented by 10–20 individuals, with a few exceptions, such as ML (only two individuals) and JD (seven individuals) (Table 1). For each population, leaf materials were collected from individuals separated by at least 50 m except for the populations ML and JD. The population sizes of most of the populations are less than 50 individuals except the populations YB, YD and SP. A total of 159 individuals were collected and fresh leaves were dried in silica gel immediately after collection and stored at room temperature until DNA extraction.

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted from the silica-dried leaves using the CTAB method (Doyle 1991) with some modifications. Three chloroplast DNA regions were amplified and sequenced: the *rpl16* intron (Small et al. 1998), the *trnQ*$^{(UUG)}$*-5′rps16* intergenetic region (Shaw et al. 2007) and the *rpl32-trnL*$^{(UAG)}$ intergenetic region (Shaw et al. 2007), using the primers reported in the references above. These regions had been successfully amplified and shown to exhibit some variations in preliminary experiments.

To amplify the nuclear gene, we selected the key gene *GAPDH* encoding glyceraldehyde 3-phosphate dehydrogenase, since it is a low-copy nuclear locus (Strand et al. 1997; Vaezi and Brouillet 2009) that has been widely used as a gene marker for inferring population-level variation and phylogenetic relationships at various taxonomic levels (Aguilar-Melendez et al. 2009; Johansson and Ericson 2005; Olsen and Schaal 1999; Rønsted et al. 2008; Tani et al. 2003; Yue et al. 2009). One pair of primers, *GAPDHx4F* and *GAPDHx6R* (Vaezi and Brouillet 2009), were used for the amplification (Peralta and Spooner 2001) and sequencing in a preliminary study. The sequence homology was then checked and a new pair of specific primers for *L. decora* (GAPDHF, 5′-ATC ATT CCC AGC AGY ACT-3′;

Table 1 Details of sample locations, the estimates of population size (*N*), sample sizes (*n*), haplotype diversity (Hd) and nucleotide diversity (π) surveyed for combined cpDNA sequences and *GAPDH* of the 11 *Leucomeris decora* populations investigated in Yunnan Province

Population code	Location	Latitude (°N)	Longitude (°E)	Elevation (*m*)	*N*	*n*	cpDNA haplotypes	Hd	$\pi \times 10^{-3}$	*GAPDH* haplotypes (no. alleles)	Hd	$\pi \times 10^{-3}$
ML	Mengla	21.990	101.220	1,440	5	2	H1 (2)	0	0	H11 (4)	0.000	0
NE	Ninger	23.065	101.026	1,800	20	10	H1 (10)	0	0	H1 (10), H9 (8), H12 (2)	0.611	3.25
YJ	Yuanjiang	23.490	102.011	1,450	10	10	H1 (10)	0	0	H1 (8), H3 (1), H4 (8), H7 (3)	0.689	1.18
SP	Shiping	23.886	102.267	1,600	200	10	H1 (4), H2 (6)	0.533	1.17	H1 (11), H2 (2), H3 (1), H4 (2), H7 (3), H8 (1)	0.684	1.15
ES	Eshan	24.252	102.177	1,470	30	10	H1 (10)	0	0	H1 (11), H3 (5), H6 (1), H7 (3)	0.642	1.33
YD-1	Yongde-1	24.021	99.284	1,680	800	10	H1 (10)	0	0	H1 (1), H3 (12), H7 (7)	0.542	0.94
YD-2	Yongde-2	24.230	99.738	1,480	150	10	H1 (10)	0	0	H1 (3), H3 (13), H7 (4)	0.542	0.76
JD	Jingdong	24.478	100.875	1,580	50	7	H1 (7)	0	0	H1 (3), H3 (8), H7 (2), H10 (1)	0.648	1.83
BS	Baoshan	24.943	98.868	1,510	45	10	H2 (10)	0	0	H1 (2), H3 (9), H7 (9)	0.616	0.89
YB	Yangbi	25.476	99.902	1,390	180	10	H2 (10)	0	0	H1 (9), H3 (3), H4 (4), H5 (3), H6 (1)	0.747	1.66
YL	Yunlong	25.675	99.065	1,790	40	10	H2 (10)	0	0	H1 (1), H3 (15), H7 (4)	0.416	0.62
Total						99		0.468	1.02		0.773	2.37

and GAPDHR, 5′-GCA TCA GCA GAA TTA GAA GG-3′) designed based on the obtained sequences and used in subsequent amplification and sequencing.

Cloning of nuclear gene

For the nuclear gene region, products from some individuals that had a single heterozygous site were directly split into two alleles through haplotype subtraction (Clark 1990), which was used in other population genetics studies (Léotard et al. 2009; Olsen 2002; Olsen and Schaal 1999). The products from individuals with more than two heterozygous sites (according to the first sequencing round) were re-sequenced to eliminate sequencing errors. Products from any other individuals that could not be directly sequenced or had more than two heterozygous sites according to the second sequencing round were cloned. The pMD-19T Simple Vector (TaKaRa) was used for cloning. The purified PCR products were ligated at 4°C overnight and transformed into competent *Escherichia coli* DH5-α at 42°C. The transformed bacteria were screened on a selective and solid LB Petri dish media containing 100 mg/ml ampicillin at 37°C overnight. Five to ten positive colonies per individual were selected after screening in 1.5 ml Eppendorf tubes containing LB liquid as well as ampicillin. Prior to sequencing, positive cultures were amplified following the same protocol as for direct sequencing to determine whether they contained the inserts of putative length. Finally, the recombinant clones containing the desirable inserts were sequenced using vector-specific universal primers ($M_{13}F/R$) in both directions.

Data analysis

Sequences were aligned by Clustal X 1.81 (Thompson et al. 1997) and manually corrected using BioEdit 7.0.5 (Hall 1999). The length variations of mononucleotide repeats (poly A or T stretches) were excluded for the haplotype analysis, given their proneness to homoplasy. DnaSP version 3.95 (Rozas and Rozas 1999) was used to calculate: (1) nucleotide diversity per site (π) (Nei 1987); (2) haplotype diversity (Hd) (Nei and Tajima 1983); (3) Tajima's *D* (Tajima 1989) and Fu and Li's F^* (Fu and Li 1993) statistics to assess the likelihood that the DNA sequences have evolved in a neutral manner with significance tests (1,000 simulations) (significant, $P < 0.05$).

Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was applied to assess the genetic differentiation

among populations, with significance tests (1,000 permutations). To assess possible isolation by distance patterns, we evaluated the significance of the correlation coefficient between the matrix of pairwise *F*st values and the matrix of geographic distances between population-pairs using Mantel tests (Mantel 1967) with 1,000 random permutations. All these analyses were conducted by ARLEQUIN version 3.0 (Excoffier et al. 2005). PERMUT (Pons and Petit 1996) was used to calculate within-population diversity (H_S), total diversity (H_T) and the level of population differentiation (G_{ST}, N_{ST}) at the species level. A permutation approach (1,000 permutations) was used to test whether N_{ST} was significantly larger than G_{ST}, i.e., whether there was a correspondence between haplotype similarities and their geographic distribution.

Genealogical haplotype networks (with 95% most parsimonious connection limits) were constructed using TCS version 1.21 (Clement et al. 2000). To infer possible demographic expansion of *L. decora*, mismatch distribution analyses, based on the sudden population expansion model (Rogers and Harpending 1992) using the observed number of differences between pairs of haplotypes, were conducted with DnaSP.

Results

Chloroplast haplotypes and distribution patterns

The aligned sequences of *trnQ*$^{(UUG)}$-*5′rps16*, *rpl16* and *rpl32-trnL*$^{(UAG)}$ were 2,749 bp long with six substitutions and two indels. The nucleotide diversity (π) at the species level was 0.00102 and analysis of the variation over all the three regions identified two haplotypes (H1 and H2) with the two indels removed. All haplotype sequences were deposited in GenBank databases under the accession numbers: *rpl16* HQ671082-HQ671083, *trnQ-5′rps16* HQ671096-HQ671097 and *rpl32-trnL* HQ671098-HQ671099. The total haplotype diversity (Hd) was 0.468 (Table 1). All populations had only one haplotype except the population SP, in which both haplotypes were present. H2 was fixed in populations BS, YB and YL, while H1 was fixed in the remaining populations (Table 1; Fig. 1). Therefore, the SP population possessed the maximum nucleotide diversity ($\pi = 0.00117$) and haplotype diversity (Hd = 0.533), while the remaining populations displayed genetic homogeneity (Table 1).

Sequence characters and variation of *GAPDH*

The aligned *GAPDH* sequence was 797 bp long, including one exonic region (691–792 bp). From 10 polymorphisms (Table 2) all in noncoding regions, a total of 12 haplotypes (designated from H1 to H12) were identified, with sequences deposited into GenBank under the accession numbers HQ671084-HQ671095. The nuclear locus had more allele variation than the chloroplast DNA fragments; many individuals were heterozygous at the examined locus and contained more than twice much nucleotide diversity ($\pi_{species} = 0.00237$) as the chloroplast sequences ($\pi_{species} = 0.00102$) (Table 1). Haplotype diversity (Hd) across the *GAPDH* locus within the species and for each population are shown in Table 1.

Neutrality tests and genealogical haplotype relationships

Significantly positive Tajima's *D* ($D = 3.18660$, $P < 0.01$) and Fu and Li's *F** values ($F^* = 2.13430$, $P < 0.05$) were obtained from the chloroplast sequence analysis (Table 3). Neither Tajima's *D* nor Fu and Li's *F** (Table 3) for the entire *GAPDH* locus dataset deviated from the expectation of neutrality. In the unrooted TCS network of cpDNA haplotypes, five haplotypes inferred by TCS were not found in the analyzed individuals and occurred as missing intermediates (Fig. 2a). In the *GAPDH* network (Fig. 2b), most haplotypes differed from each other by only one mutational step and some (e.g., H1, H3 and H7) were shared by more than six populations. In contrast, H9 and H12 were private in the NE population, and H5 only occurred in the YB population (Table 1; Fig. 1).

Population genetic structure and mismatch distribution analyses

For the cpDNA data, there was a significant association between genetic and geographic distances ($r = 0.40$, $P < 0.05$), supporting isolation by distance between *L. decora* populations. On the population level, total diversity (H_T) was estimated to be 0.480 and within-population diversity (*Hs*) was 0.048 (Table 3). Significant population differentiation was observed, with $G_{ST} = N_{ST} = 0.899$ ($G_{ST} = N_{ST}$, $P < 0.05$). For the nDNA *GAPDH* locus, there was a marked phylogeographic structure across all populations in which the N_{ST} value was significantly larger than the G_{ST} value ($G_{ST} = 0.309$, $N_{ST} = 0.567$, $N_{ST} > G_{ST}$, $P < 0.001$). The isolation-by-distance analysis showed an increase of divergence among populations with geographical distance ($r = 0.478$, $P < 0.05$). The AMOVA analysis revealed that most variance resided within populations (Table 4) for nDNA, whereas more than 88% ($P < 0.001$) of the variance was attributable to differences among populations for cpDNA. The mismatch distributions based on the cpDNA and *GAPDH* haplotype datasets for the total sample were both multimodal (Fig. 3) and inconsistent with the bell-shape curve expected for an expanding population, suggesting a recent population expansion is unlikely in this species.

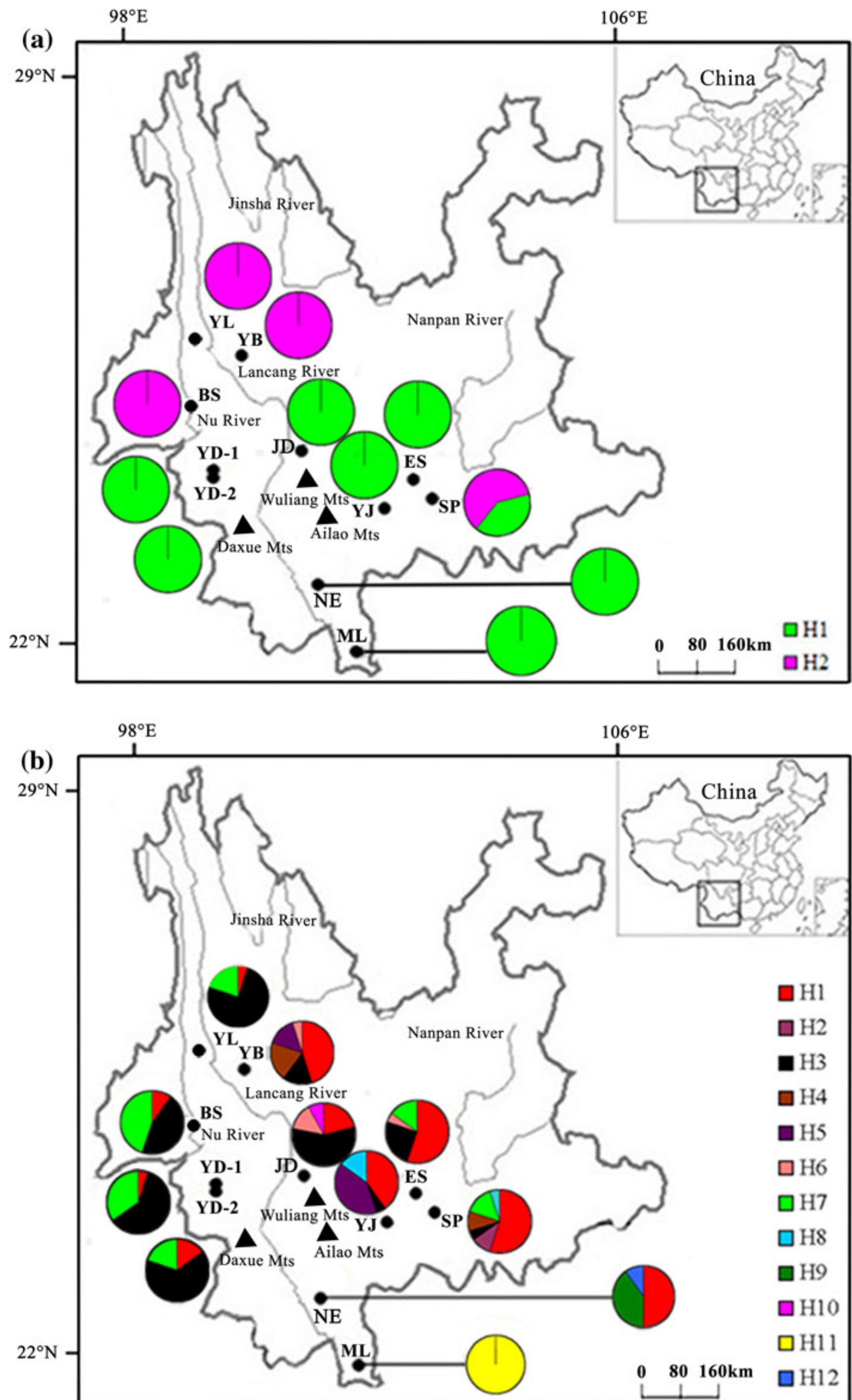

Fig. 1 Distribution of chloroplast DNA haplotypes (**a**) and *GAPDH* haplotypes (**b**) detected among 11 populations of *Leucomeris decora* in Yunnan Province. Full names of the abbreviations for the populations are shown in Table 1. *Lines* represent main rivers and *black triangles* represent main mountains in the sampling area in Yunnan Province, respectively

Discussion

Genetic diversity and population differentiation

We observed low levels of genetic diversity based on the cpDNA sequences for *L. decora*. In the three cpDNA sequences, only six substitutions were detected and two haplotypes (H1 and H2) were found in 11 populations for *L. decora*, although we used three fragments with a total length of 2,749 bp. On the species level, the nucleotide diversity ($\pi = 0.00102$) was lower than in other endangered plants for which one or several cpDNA regions have

Table 2 Polymorphisms detected in *Leucomeris decora GAPDH* nuclear DNA sequence that distinguished 12 haplotypes

Haplotype	Nucleotide position									
	3	147	210	220	245	279	364	494	529	698
H1	C	A	G	T	C	T	T	A	A	G
H2	.	.	.	.	.	.	.	.	.	A
H3	.	.	T	.	.	.	.	G	.	.
H4	T	.	.	.	.	.	.	.	.	.
H5	T	.	.	.	.	.	.	G	.	.
H6	T	.	T	.	.	.	.	G	.	.
H7	.	.	.	.	.	.	.	G	.	.
H8	.	G	.	.	.	.	.	.	.	.
H9	.	G	.	G	.	C	C	.	C	.
H10	.	G	.	G	.	C	C	G	.	.
H11	.	G	.	G	A	C	.	.	.	.
H12	.	G	.	G	.	C	.	.	.	.

Table 3 Genetic diversity, differentiation parameters and the results of neutrality tests for chloroplast DNA (cpDNA) and nDNA (*GAPDH*) in all sampled *Leucomeris decora* populations

Locus	H_T	H_S	G_{ST}	N_{ST}	Tajima's *D*	Fu and Li's *F**
cpDNA	0.480 (0.0973)	0.048 (0.0485)	0.899 (0.1003)	0.899 (0.1003)	3.18660**	2.13430*
GAPDH	0.807 (0.0375)	0.558 (0.0620)	0.309 (0.1051)	0.567 (0.1102)	−0.52959	0.42468

Standard errors are shown in parentheses; * $P < 0.05$, ** $P < 0.01$

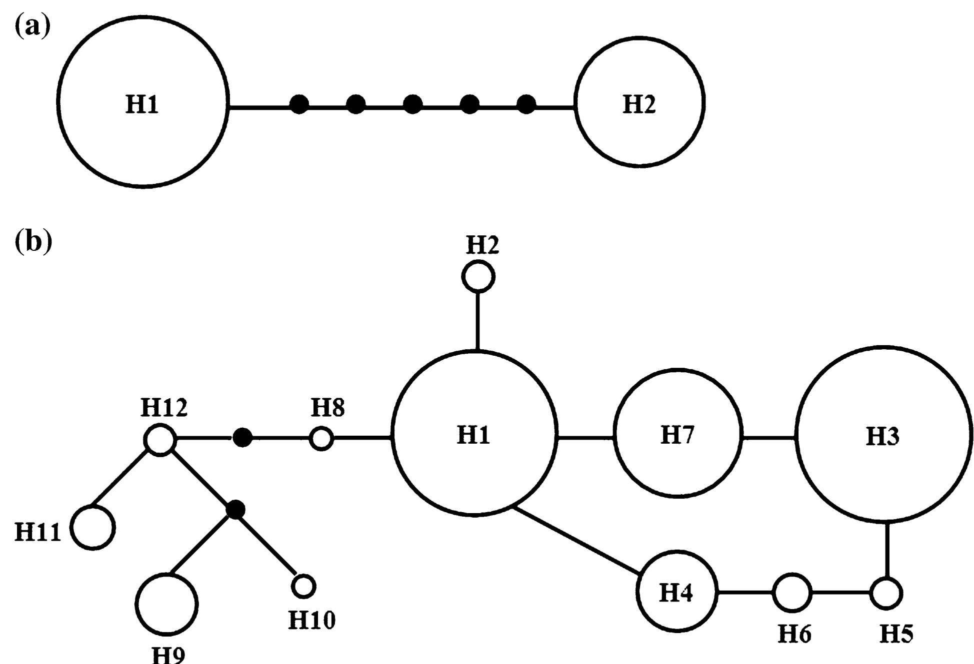

Fig. 2 Statistical parsimony network of cpDNA (**a**) and *GAPDH* (**b**) haplotypes detected in *Leucomeris decora*. *Letters* in *circles* represent haplotypes at each locus. The size of the *circles* corresponds to the frequency of each haplotype, each *solid line* represents one mutational step and the *small black circles* indicate hypothetical missing haplotypes

been sequenced, e.g., $\pi = 0.00140$ of *Dysosma versipellis* (Berberidaceae) (Qiu et al. 2009) and $\pi = 0.0035$ of *Sagittaria potamogetifolia* (Alismataceae) (Tan et al. 2008). The haplotypes diversity value (Hd = 0.468) obtained for *L. decora* was also lower than other endangered species, e.g., Hd = 0.924 for *trnL-trnF*, *trnF-ndhJ* and *trnS-trnfM* of *Dysosma versipellis* (Berberidaceae) (Qiu et al. 2009), Hd = 0.7525 for cpDNA *trnV-trnM* and *trnL* for

Table 4 Results of analysis of molecular variance (AMOVA) for cpDNA and nuclear locus sequence data from the *Leucomeris decora* populations

Markers	Source of variation	*df*	Sum of squares	Variance components	Percentage of variation (%)
cpDNA	Among populations	10	123.055	1.35854	89.3*
	Within populations	88	14.400	0.16364	10.7
GAPDH	Among populations	10	77.123	0.40194	43.2*
	Within populations	187	98.700	0.52781	56.8

df degree of freedom; * $P < 0.001$

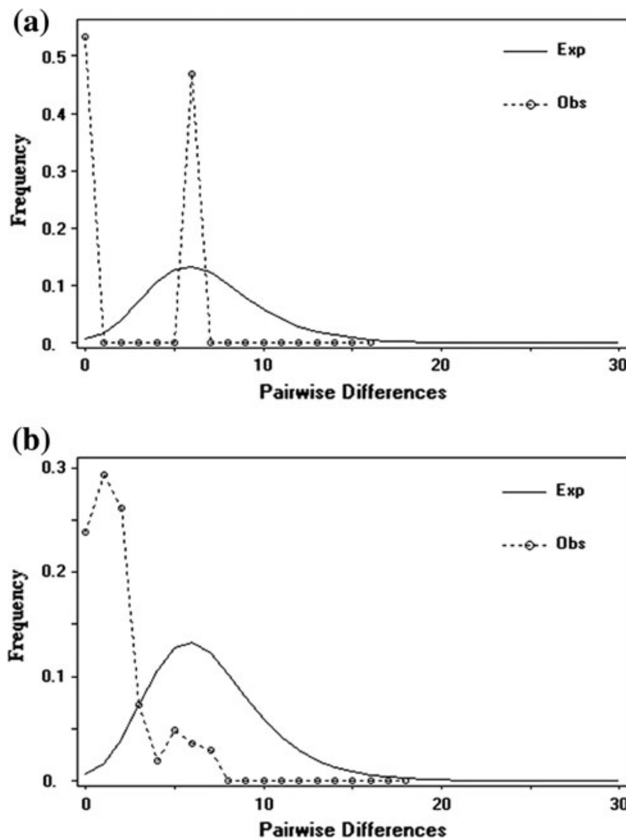

Fig. 3 Mismatch distribution for *Leucomeris decora* populations for cpDNA data (**a**) and *GAPDH* data (**b**) showing observed (*dotted line*) and expected (*solid line*) pairwise nucleotide site divergences obtained with DnaSP

Dalbergia nigra (Papilionoideae) (Ribeiro et al. 2011) and Hd = 0.870 for cpDNA *atpB-rbcL* of *Hygrophila pogonocalyx* (Acanthaceae) individuals representing eight populations (Huang et al. 2005). Likewise, the variability estimates for *L. decora* are somewhat low compared with sequencing studies of other plant species of Asteraceae in terms of the nucleotide diversity or haplotypes diversity values (Collevatti et al. 2009; Gong et al. 2011; Molins et al. 2009; Wang et al. 2011).

The low genetic diversity in *L. decora* may be ascribed to its small population sizes. For the present, recent habitat loss has reduced the number and size of *L. decora* populations, resulting in limited geographical range, with small and isolated populations. Rare and endangered species with limited distributions are expected to exhibit low levels of genetic variation due to stochastic events like genetic drift and inbreeding (Hamrick and Godt 1989; Spielman et al. 2004). Within the populations, no variation was detected except the population SP. In our field observations, the number of adult individuals in the population SP was relatively large perhaps due to little disturbance. The presence of two haplotypes in this population indicates that the population has probably been relatively large until recently.

However, in contrast to the results of cpDNA sequences, nDNA data revealed relatively high levels of genetic diversity (Hd = 0.773, $H_T = 0.807$), when compared with those of other studies from analyses of the *GAPDH* gene (Banu et al. 2010; Shih et al. 2007). These results contrast with our initial expectation that this endangered, uncommon species would have low genetic diversity, but this incongruence between these two markers could be explained by their different effective population size. In theory, the phylogeographical structure is expected to be less pronounced at diploid nuclear loci than cytoplasmic loci because of their different effective population size (McCauley 1995; Moore 1995). That means the smaller effective population size of haploid genomes makes maternally inherited organelle markers more likely to record the effects like genetic bottlenecks of population history in present-day genetic patterns than nuclear markers (Schaal et al. 1998).

The relatively high nDNA genetic diversity, on the other hand, may indicate a large effective population size of the species (Ellstrand and Elam 1993). It is likely that many individuals of this long-life perennial species analyzed in this study were alive before most of the habitat fragmentation and destruction occurred. Therefore, factors such as genetic drift and inbreeding would not have had enough time to have changed genetic diversity at the nuclear locus. The haplotype distribution patterns also indicate that population bottlenecks might have happened relatively recently. If a major ancient population bottleneck event had

happened for the species, a single and common haplotype with high frequency would be observed among all populations (Echt et al. 1998).

In addition to low genetic diversity, another important feature of the genetic structure of *L. decora* we detected is the high level of genetic differentiation at the range-wide scale, as indicated by F_{ST}, G_{ST} and N_{ST} estimates both from cpDNA and nDNA (Tables 2, 3). These values, especially estimated from cpDNA ($F_{ST} = 0.893$, $G_{ST} = 0.899$ and $N_{ST} = 0.899$), are high by comparison with other endangered plants like *Dysosma versipellis* ($F_{ST} = 0.868$, $G_{ST} = 0.600$ and $N_{ST} = 0.887$) (Qiu et al. 2009), *Dalbergia nigra* ($F_{ST} = 0.467$) (Ribeiro et al. 2011) and *Hymenaea courbaril* ($F_{ST} = 0.604$) (Ramos et al. 2009). High genetic differentiation among populations may mainly be ascribed to the limited amount of gene flow via both seeds and pollen among populations. Yunnan is a province with several mountain ranges and rivers. The big rivers (Lancang River, Nu River) and mountain ranges like Ailao Mountains and Wuliang Mountains run parallel from northwest to southeast (Fig. 1) (Wang 2002). The present populations of *L. decora* are separated by geographic and anthropogenic barriers, which largely hinders gene flow via seed and pollen dispersal among populations. For instance, the southern ML and NE populations are isolated from western YD-1 and YD-2 populations by Lancang River and Daxue Mountains; YL population is isolated from YB population by Lancang River. Mantel tests based on cpDNA and nDNA data both revealed significant correlations between genetic and geographic distances, and thus supported the isolation-by-distance (IBD) model for this species (Wright 1943). In addition, it is known that the breeding system also plays a fundamental role in the genetic structure and selfing and asexual reproduction can result in low genetic diversity within populations and high genetic differentiation between populations. In our field investigation, clonal growth was not observed. However, for the present, no comprehensive studies have been conducted concerning the reproductive strategies of *L. decora*. Detailed studies, therefore, are needed to elucidate the reproduction systems in the future, using both experimental techniques and genetic methods.

Demographic history

The tremendous global climatic oscillations together with local climatic changes caused by the uplift of QTP (Qinghai-Tibetan Plateau) particularly during Quaternary glaciations resulted in several glacial-interglacial cycles and caused expansion and contraction of habitats (Abbott and Brochmann 2003; Axelrod et al. 1996; Harrison et al. 2001; Hewitt 2004). Especially, climate changes during the last glacial maximum (LGM) had dramatic effects on the distribution ranges and genetic structure of many plants (Harrison et al. 2001). Although the Yunnan Plateau in Southwest China was less affected by cold air from Siberia during glacial periods than other regions in China due to extremely complex topography and climate, the cycles of cooler and drier climate in the glacial ages and warmer and wetter climate in the post- (inter-) glacial ages have also lead to extinction and influenced the distribution and evolution of many plants (Winkler and Wang 1993; Zheng 2000). Palynological investigation revealed that, during the LGM the abundance of cold tolerant plants like *Picea* and *Abies* increased while that of subtropical plants like *Dacrydium* and *Podocarpus* decreased or disappeared in Yunnan Plateau (Li 1998).

With the decrease of temperature, plants tend to migrate toward lower altitudes or latitudes and they migrate toward higher altitudes or latitudes when the temperature increases. However, our results from the patterns of haplotype distribution (Fig. 1) suggest that significant population expansion, which was revealed in many other Chinese plants (Chen et al. 2008; Yang et al. 2008) is not obvious in *L. decora*. As is shown in Fig. 2, the widespread haplotypes (H1, H3, H7) represent internal nodes of the network and their distribution across most of the regions owes more to the persistence of ancient polymorphism than to recent gene flow (Schaal et al. 1998). Furthermore, the haplotypes network (Fig. 2) is not a star-like phylogeny which usually indicates population expansion as evidenced in many species (Hwang et al. 2003; Novaes et al. 2010; Yang et al. 2008). The lack of population expansion was also supported by mismatch distribution analyses (Fig. 3) and neutrality tests (Table 2) of the cpDNA and nDNA datasets.

Together, these results suggest that populations of *L. decora* might have survived climatic changes in situ during the glaciations, rather than migrating long distances to the suitable habitat and backwards in the interglacial age. This scenario is highly likely when the complex topology of the Southwest China region is taken into account. Yunnan is characterized by physical environmental heterogeneity, with mountains and rivers compressed within a narrow geographical mosaic. This environmental complexity could have resulted in more geographical barriers between populations and it is difficult for different populations to retreat to common refugia and during the interglacial or postglacial periods, so they still remained separate due to the complex topology. As a result of the climatic oscillations, the population size might be shrinking and some populations even go extinct in some places. As is shown in the cpDNA network, a larger number of missing haplotypes indicates that extinction of *L. decora* populations at some locations must have taken place, resulting in the loss of chloroplast haplotypes (Jakob and Blattner 2006). Moreover, owing to the habitat destruction and degradation from agriculture and urbanization, as well as human over-exploitation, *L. decora*

was gradually restricted to small isolated areas as small-sized populations. Based on our field observations, for the present, adult individuals represent the large proportions in most populations, while seedlings and juveniles just account for small parts. These results indicate that most populations may have weak ability to regenerate naturally.

Conservation of *L. decora*

Small populations of rare and endangered species have higher risks of extinction than larger more stable ones, especially when gene flow between populations is restricted (Frankham et al. 2002). Small populations are more susceptible to the loss of genetic diversity caused by genetic drift and inbreeding, which reduces heterozygosity and the performance of various fitness related traits. Almost all extant *L. decora* populations are seriously threatened. The low genetic diversity, the limited number of individuals, and high divergence, identify the need to focus conservation activities on increasing the genetic diversity and population size of *L. decora*.

The criteria for the selection of priority populations to be conserved must include both the uniqueness of a population and its diversity level, especially in terms of its allelic composition (Petit et al. 1998). Among the extant populations, populations SP, YB, NE and JD not only possess the common haplotypes but private haplotypes, and should therefore be targeted for in situ conservation with high priority to enlarge the population sizes. Meanwhile, artificial translocating the individuals in the same populations is also necessary as it could help to increase the gene flow between the individuals with long-distances and prevent inbreeding depression. Infusion of genetic diversity by the introduction of plants or propagules into genetically depauperate populations is an alternative strategy. However, lacking knowledge of adaptive differences between populations of *L. decora*, it is better that these transplantations are carried out between populations with low genetic differentiations and similar ecological traits, such as YD-1 and YD-2. Under climate changes, marginal populations are disproportionately important for the long-term conservation of genetic diversity, phylogenetic history and evolutionary potential, and they are the most prone to extinction (Hampe and Petit 2005). Populations NE and ML located at the edge of the distribution range showed relatively high genetic divergence based on the nDNA from the remaining populations and harbor several unique haplotypes (H9, H11 and H12) and therefore should be given more attention to.

Additionally, given the current severe habitat loss and fragmentation from human disturbance, habitat conservation that allows a large number of individuals to survive will be very important for the conservation of *L. decora*. Despite the critical conservation status of *L. decora*, none of the populations except YD-1 are located inside of reserves. Therefore, nature reserves need to be established locally, especially for the populations of Yuanjiang and Eshan, which are seriously disturbed by extensive plantation of sugarcanes (pers. observation). Ex situ conservation measures, including transplantations (or seed collection) to nearby reserves, botanic gardens (Kunming, Xishuangbanna) or reintroductions at appropriate sites with similar habitats near the wild populations, is also necessary to aid the recovery and support of self-sustaining wild populations and in order to sustain the diversity in this biodiversity hotspot. Seed collection strategies for the establishment of ex situ seed banks in China Germplasm Bank of Wild Species could be taken for these populations. When ex situ conservation is put in practice, great importance should be placed on protecting as many populations as possible, given a large portion of genetic diversity exists among rather than within populations. Populations in SP, YB, NE, JD and ML should have high priority since they contain private polymorphisms (e.g., H2, H5 and H8).

Genetic diversity and phylogeography data can contribute to the development of effective conservation strategies. Although the genetic data obtained here for *L. decora* based on differentially inherited genetic markers of cpDNA and nDNA are neutral (nearly neutral) and may not reflect adaptive genetic diversity directly it provides useful data to inform the unique genetic information of each population so that the greatest amount of genetic variation in *L. decora* is preserved. Low levels of genetic diversity at neutral loci may reflect losses of genetic diversity at loci that influence fitness and preserving such variation may help to inhibit the dissolution of locally well-adapted phenotypes (Godt et al. 1996; Jones and Gibson 2011; Ueno et al. 2005). While it is worth to remark that a species should be definitely maintained within its ecological community, therefore, more genetic diversity work related to adaptive traits and knowledge on ecosystem functions and species interactions will be helpful for understanding how the species adapt to the changing conditions.

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References

Abbott RJ, Brochmann C (2003) History and evolution of the arctic flora: in the footsteps of Eric Hultén. Mol Ecol 12:299–313

Aguilar-Melendez A, Morrell PL, Roose ML, Kim S-C (2009) Genetic diversity and structure in semiwild and domesticated chiles (*Capsicum annuum*; Solanaceae) from Mexico. Am J Bot 96:1190–1202

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge

Axelrod DI, Al Shehbaz I, Raven PH (1996) Floristic characteristics and the modern flora of China. In: Zhang A, Wu S (eds) History of the modern flora of China. Springer, New York, pp 43–55

Bain JF, Golden JL (2003) Phylogeographic relationships within *Packera sanguisorboides* (Asteraceae), a narrow endemic species that straddles a major biogeographic boundary. Am J Bot 90:1087–1094

Banu S, Bhagwat R, Kadoo N, Lagu M, Gupta V (2010) Understanding the genetic structure of *Symplocos laurina* Wall. Populations using nuclear gene markers. Genetica 138:197–210

Chen KM, Abbott RJ, Milne RI, Tian XM, Liu JQ (2008) Phylogeography of *Pinus tabulaeformis* Carr. (Pinaceae), a dominant species of coniferous forest in northern China. Mol Ecol 17:4276–4288

Clark AG (1990) Inference of haplotypes from PCR-amplified samples of diploid populations. Mol Biol Evol 7:111–122

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Collevatti RG, Rabelo SG, Vieira RF (2009) Phylogeography and disjunct distribution in *Lychnophora ericoides* (Asteraceae), an endangered cerrado shrub species. Ann Bot 104:655–664

Cruzan MB, Templeton AR (2000) Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. Trends Ecol Evol 15:491–496

Doyle J (1991) DNA protocols for plants—CTAB total DNA isolation. In: Hewitt GM, Johnston A (eds) Molecular techniques in taxonomy. Springer, Berlin

Echt CS, Deverno LL, Anzidei M, Vendramin GG (1998) Chloroplast microsatellites reveal population genetic diversity in red pine, *Pinus resinosa* Ait. Mol Ecol 7:307–316

Eidesen PB, Alsos IG, Popp M, Stensrud Ø, Suda J, Brochmann C (2007) Nuclear vs. plastid data: complex Pleistocene history of a circumpolar key species. Mol Ecol 16:3902–3925

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Syst 24:217–242

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Frankham R, Briscoe DA, Ballou JD (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Fu LG, Jin JM (1992) The red book of Chinese plants: rare and endangered plants. Science Press, Beijing

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709

Funk VA, Susanna A, Steussy TF, Bayer RJ (2009) Systematics, evolution, and biogeography of Compositae. International Association for Plant Taxonomy, Vienna

Godt MJW, Johnson BR, Hamrick JL (1996) Genetic diversity and population size in four rare Southern Appalachian plant species. Conserv Biol 10:796–805

Gong X, Luan SS, Hung KH, Hwang CC, Lin CJ, Chiang YC, Chiang TY (2011) Population structure of *Nouelia insignis* (Asteraceae), an endangered species in southwestern China, based on chloroplast DNA sequences: recent demographic shrinking. J Plant Res 124:221–230

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. Ecol Lett 8:461–467

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer Associates, Sunderland

Harrison SP, Yu G, Takahara H, Prentice IC (2001) Diversity of temperate plants in East Asia. Nature 413:129–130

Hedrick PW, Miller PS (1992) Conservation genetics: techniques and fundamentals. Ecol Appl 2:30–46

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos Trans R Soc Lond Ser B Biol Sci 359:183–195

Huang JC, Wang WK, Peng CI, Chiang TY (2005) Phylogeography and conservation genetics of *Hygrophila pogonocalyx* (Acanthaceae) based on atpB-rbcL noncoding spacer cpDNA. J Plant Res 118:1–11

Hwang SY, Lin TP, Ma CS, Lin CL, Chung JD, Yang JC (2003) Postglacial population growth of *Cunninghamia konishii* (Cupressaceae) inferred from phylogeographical and mismatch analysis of chloroplast DNA variation. Mol Ecol 12:2689–2695

Ikeda H, Senni K, Fujii N, Setoguchi H (2008) Consistent geographic structure among multiple nuclear sequences and cpDNA polymorphisms of *Cardamine nipponica* Franch. et Savat. (Brassicaceae). Mol Ecol 17:3178–3188

Ikuyo S, Noriaki M (2009) Chloroplast DNA phylogeography of the endangered Japanese red maple (*Acer pycnanthum*): the spatial configuration of wetlands shapes genetic diversity. Divers Distrib 15:917–927

Jakob SS, Blattner FR (2006) A chloroplast genealogy of *Hordeum* (Poaceae): long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. Mol Biol Evol 23:1602–1612

Johansson US, Ericson PGP (2005) A re-evaluation of basal phylogenetic relationships within trogons (Aves: Trogonidae) based on nuclear DNA sequences. J Zool Syst Evol Res 43:166–173

Jones J, Gibson J (2011) Population genetic diversity and structure within and among disjunct populations of *Alnus maritima* (seaside alder) using microsatellites. Conserv Genet 12:1003–1013

Léotard G, Duputi A, Kjellberg F, Douzery EJP, Debain C, de Granville J-J, McKey D (2009) Phylogeography and the origin of cassava: new insights from the northern rim of the Amazonian basin. Mol Phylogenet Evol 53:329–334

Li WY (1998) Vegetation and climate in the Quaternary in China. Science Press, Beijing

Li EX, Yi S, Qiu YX, Guo JT, Comes HP, Fu CX (2008) Phylogeography of two East Asian species in *Croomia* (Stemonaceae) inferred from chloroplast DNA and ISSR fingerprinting variation. Mol Phylogenet Evol 49:702–714

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends Ecol Evol 10:198–202

Molins A, Mayol M, Rosselló JA (2009) Phylogeographical structure in the coastal species *Senecio rodriguezii* (Asteraceae), a narrowly distributed endemic Mediterranean plant. J Biogeogr 36:1372–1383

Moore WS (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726

Mousadik AE, Petit RJ (1996) Chloroplast DNA phylogeography of the argan tree of Morocco. Mol Ecol 5:547–555

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nei M, Tajima F (1983) Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105:205–217

Novaes RML, Filho JPDL, Ribeiro RA, Lovato MB (2010) Phylogeography of *Plathymenia reticulata* (Leguminosae) reveals patterns of recent range expansion towards northeastern Brazil and southern Cerrados in Eastern Tropical South America. Mol Ecol 19:985–998

Olsen KM (2002) Population history of *Manihot esculenta* (Euphorbiaceae) inferred from nuclear DNA sequences. Mol Ecol 11:901–911

Olsen KM, Schaal BA (1999) Evidence on the origin of cassava: phylogeography of *Manihot esculenta*. Proc Natl Acad Sci USA 96:5586–5591

Panero JL, Funk VA (2008) The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. Mol Phylogenet Evol 47:757–782

Peralta IE, Spooner DM (2001) Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. subsection *Lycopersicon*). Am J Bot 88:1888–1902

Petit RJ, Pineau E, Demesure B, Bacilieri R, Ducousso A, Kremer A (1997) Chloroplast DNA footprints of postglacial recolonization of oaks. Proc Natl Acad Sci USA 94:9996–10001

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Conserv Biol 12:844–855

Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Mol Ecol 14:689–701

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144:1237–1245

Qiu YX, Guan BC, Fu CX, Comes HP (2009) Did glacials and/or interglacials promote allopatric incipient speciation in East Asian temperate plants? Phylogeographic and coalescent analyses on refugial isolation and divergence in *Dysosma versipellis*. Mol Phylogenet Evol 51:281–293

Ramos ACS, De Lemos-Filho JP, Lovato MB (2009) Phylogeographical structure of the neotropical forest tree *Hymenaea courbaril* (Leguminosae: Caesalpinioideae) and its relationship with the vicariant *Hymenaea stigonocarpa* from Cerrado. J Hered 100:206–216

Reboud X, Zeyl C (1994) Organelle inheritance in plants. Heredity 72:132–140

Ribeiro RA, Lemos JP, Ramos ACS, Lovato MB (2011) Phylogeography of the endangered rosewood *Dalbergia nigra* (Fabaceae): insights into the evolutionary history and conservation of the Brazilian Atlantic Forest. Heredity 106:46–57

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Rønsted N, Weiblen GD, Savolainen V, Cook JM (2008) Phylogeny, biogeography, and ecology of *Ficus* section *Malvanthera* (Moraceae). Mol Phylogenet Evol 48:12–22

Rozas J, Rozas R (1999) DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175

Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. Mol Ecol 7:465–474

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94:275–288

Shih FL, Hwang SY, Cheng YP, Lee PF, Lin TP (2007) Uniform genetic diversity, low differentiation, and neutral evolution characterize contemporary refuge populations of Taiwan fir (*Abies kawakamii*, Pinaceae). Am J Bot 94:194–202

Small RL, Ryburn JA, Cronn RC, Seelanan T, Wendel JF (1998) The tortoise and the hare: choosing between noncoding plastome and nuclear Adh sequences for phylogeny reconstruction in a recently diverged plant group. Am J Bot 85:1301–1315

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci USA 101:15261–15264

Strand AE, Leebens-Mack J, Milligan BG (1997) Nuclear DNA-based markers for plant evolutionary biology. Mol Ecol 6:113–118

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Tan B, Liu K, Yue XL, Liu F, Chen JM, Wang QF (2008) Chloroplast DNA variation and phylogeographic patterns in the Chinese endemic marsh herb *Sagittaria potamogetifolia*. Aquat Bot 89:372–378

Tani N, Tsumura Y, Sato H (2003) Nuclear gene sequences and DNA variation of *Cryptomeria japonica* samples from the postglacial period. Mol Ecol 12:859–868

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Ueno S, Setsuko S, Kawahara T, Yoshimaru H (2005) Genetic diversity and differentiation of the endangered Japanese endemic tree *Magnolia stellata* using nuclear and chloroplast microsatellite markers. Conserv Genet 6:563–574

Vaezi J, Brouillet L (2009) Phylogenetic relationships among diploid species of *Symphyotrichum* (Asteraceae: Astereae) based on two nuclear markers, ITS and GAPDH. Mol Phylogenet Evol 51:540–553

Wang SY (2002) Yunnan geography. Yunnan Nation Press, Kunming

Wang HW, Ge S (2006) Phylogeography of the endangered *Cathaya argyrophylla* (Pinaceae) inferred from sequence variation of mitochondrial and nuclear DNA. Mol Ecol 15:4109–4122

Wang JF, Pan YZ, Gong X, Chiang YC, Kuroda C (2011) Chloroplast DNA variation and phylogeography of *Ligularia tongolensis* (Asteraceae), a species endemic to the Hengduan Mountains region of China. J Syst Evol 49:108–119

Winkler MG, Wang PK (1993) The late-Quaternary vegetation and climate of China. In: Wright HE Jr, Kutzbach JE, Webb T III, Ruddiman WF, Street-Perrott FA, Bartlein PJ (eds) Global climates since the last glacial maximum. University of Minnesota, Minneapolis

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

Wright S (1943) Isolation by distance. Genetics 28:114

Yang FS, Li YF, Ding X, Wang XQ (2008) Extensive population expansion of *Pedicularis longiflora* (Orobanchaceae) on the Qinghai-Tibetan Plateau and its correlation with the Quaternary climate change. Mol Ecol 17:5135–5145

Yue JP, Sun H, David AB, Li JH, Ihsan AA-S, Richard REE (2009) Molecular phylogeny of *Solms-laubachia* (Brassicaceae) s.l., based on multiple nuclear and plastid DNA sequences, and its biogeographic implications. J Syst Evol 47:402–415

Zheng Z (2000) Late Quaternary vegetational and climatic changes in the tropical and subtropical areas of China. Acta Micropalaeontol Sin 17:125–146