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REGULAR PAPER

Genetic variation and conservation assessment of Chinese populations of *Magnolia cathcartii* (Magnoliaceae), a rare evergreen tree from the South-Central China hotspot in the Eastern Himalayas

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Abstract Nine natural populations of the rare evergreen tree *Magnolia cathcartii* (Magnoliaceae) were sampled across its natural range, and amplified fragment length polymorphism (AFLP) markers were used to assess genetic variation within and among populations. Three ex situ populations were also surveyed to determine whether conservation plantings include the entire genetic diversity of the species. Genetic diversity within the natural populations was very low (0.122 for Nei's gene diversity), and the southeast populations had the highest diversity. The ex situ populations had a lower diversity than the mean diversity for all populations, and none of the ex situ populations reached the levels of diversity found in their source populations. Genetic differentiation was high among natural populations ($G_{st} = 0.247$), and an isolation-by-distance pattern was detected. Habitat fragmentation, restricted gene flow, and geitonogamy are proposed to be the primary reasons for the low genetic diversity and high genetic differentiation. More protection is needed, especially for the southeast populations, which possess the highest numbers of unique alleles according to AFLP fragment analyses. The ex situ program was a good first step towards preserving this species, but the current ex situ populations preserve only a limited portion of its genetic diversity. Future ex situ efforts should focus on enhancing the plantings with individuals from southeastern Yunnan.

Keywords Breeding system · Conservation · Fragmentation · *Magnolia cathcartii* · Gene flow · Genetic diversity

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Introduction

Genetic variation is thought to be critical to the long-term survival of a population or species (Beardmore 1983; Antonovics 1984). Understanding the genetic variation within and among populations of rare and endangered species is essential when developing management strategies for both in situ and ex situ conservation activities (Hogbin and Peakall 1999). Genetic data help to guide sampling strategies for ex situ conservation (Ceska et al. 1997; Wolf and Sinclair 1997), can be used to evaluate the conservation value of in situ and ex situ populations (Hogbin and Peakall 1999), and can be employed to monitor the reintroduction program (Robichaux et al. 1997).

The evolutionary and biogeographic histories of a species may play critical roles in determining its current genetic composition (Schaal et al. 1998). The historical patterns of gene flow and vicariance among populations determine contemporary biographic patterns of genetic variation (Hewitt 1996; Soltis et al. 1997; Avise 2000).

This history should be reflected in the genetic structure and phylogeography of extant populations, which would provide information for biogeographical scenarios that underlie patterns of genetic differentiation to be tested.

Plant reproductive traits also determine the population's genetic structure via the plant's mating system (Hamrick and Godt 1990; Schoen et al. 1996). A close relationship between mating system and the level of genetic variation and genetic structure has been demonstrated in many studies (Brown et al. 1989; Hamrick and Godt 1990). Inbreeding species are expected to harbor less genetic diversity within populations and more genetic differentiation among populations than mixed-mating or outcrossing species (Charlesworth and Charlesworth 1995; Hamrick and Godt 1996).

The South-Central China hotspot is one of the 25 world biodiversity hotspots that were defined based on species endemism and degree of threat. About 12,000 plant species and 1,141 vertebrate species have been reported in this area, and 3,500 of the world's 300,000 plant species (1.2%) and 178 of the world's 27,298 vertebrate species (0.7%) are endemic to it. This area is likewise significant in having its endemic species concentrated in exceptionally small areas. The species/area ratios per 100 km^2 for endemic plants and endemic vertebrates are 5.5 and 0.3, respectively. Moreover, in this hotspot there is species congruence (58%) insofar as high counts for endemic plants (1.2% of the world's 300,000 plant species) are matched by high counts for endemic vertebrates (0.7% of the world's 27,298 vertebrate species) (Myers et al. 2000). Undoubtedly, the South-Central China hotspot provides a natural laboratory for studying the origin and conservation of biodiversity. However, 92% of its primary vegetation has been lost due to habitat loss, and today only 25.9% of the hotspot area is protected through the establishment of parks and reserves (Myers et al. 2000). According to the well-established theory of island biogeography (MacArthur and Wilson 1967), when an area loses a large proportion of its original habitat, and especially when the remaining habitat is severely fragmented, it will eventually lose some of its species through what are technically known as "ecological equilibration" or delayed fallout effects (Brooks and Balmford 1996; Brooks et al. 1997, 1999; Laurance 1999; Gaston and Nicholls 1995; Turner 1996; Pimm and Askins 1995; Cowlinshaw 1999; Newmark 1996; Tilman et al. 1994). However, the prospect of a mass extinction can be made far less daunting and much more manageable through the hotspot strategy, with its tight targeting of conservation efforts.

Magnolia cathcartii (Hook. f. et Thomson) Noot. [=*Alcimandra cathcartii* (Hook. f. et Thoms.) Dandy] has a restricted distribution in the eastern Himalayan Mountains, ranging across Bhutan, northeastern India, northern Myanmar, southwestern China and northern Vietnam (Fig. 1). In recent decades, *M. cathcartii* has been threatened by rapid habitat destruction and overexploitation of forests for timber. Based on the IUCN criteria, *M. cathcartii* in China has been persistently threatened for the last three generations (here, the length of a generation refers to the average lifespan of the parents of the current population) and its population size has decreased by at least 50%. It has been listed in the *China Species Red List* as a category "EN A 2c" species since 2004 (see http://www.chinabiodiversity.com/redlist/search/redlist.shtm), and was assigned "First Grade" status in the first edition of the *Chinese Catalogue of Protective Plants* (Fu 1999).

Our field studies suggest that *M. cathcartii* has a sporadic distribution with little seedling recruitment in its natural habitat. Some conservation initiatives have been initiated by the national and regional governments, and these include establishing nature reserves and conducting population surveys (Li et al. 2003). However, habitat degradation and destruction continue in unmanaged areas. In these areas, the populations are dominated by secondary and immature individuals (e.g., in the population of Heizhiguo Township, Guangnan County in Yunnan Province). In one case, a population in Gongpinghe Township, Jingdong County was totally extirpated. Therefore, a detailed survey of the level of genetic diversity in the extant populations of *M. cathcartii* is urgently needed, as no such studies have been conducted for this species.

In this study, we have used AFLP markers (Vos et al. 1995) to evaluate the levels and the geographic distribution of genetic variation within and among populations of *M. cathcartii*. Our objectives were: (1) to examine genetic variation within and among populations in order to infer factors that have influenced the genetic structure; (2) to assess ex situ populations in order to evaluate if conservation efforts to date have sufficiently preserved the genetic diversity of the species; and (3) to develop conservation strategies for in situ and ex situ conservation of the species.

Materials and methods

Plant species

The generic placement of *Magnolia cathcartii* has been difficult. The species was originally described as a *Michelia* (Hooker and Thomson 1855) and later segregated as the monotypic *Alcimandra cathcartii* based on the presence of pseudolateral flowers (Lozano-Contreras 1975) rather than axillary flowers (Dandy 1927; Law 1996). Nooteboom (1985) treated *A. cathcartii* as *Magnolia cathcartii* in section *Magnolia*, and several subsequent studies (Kim et al. 2001; Figlar and Nooteboom 2004; Figlar 2006) support this change. In China, however, the

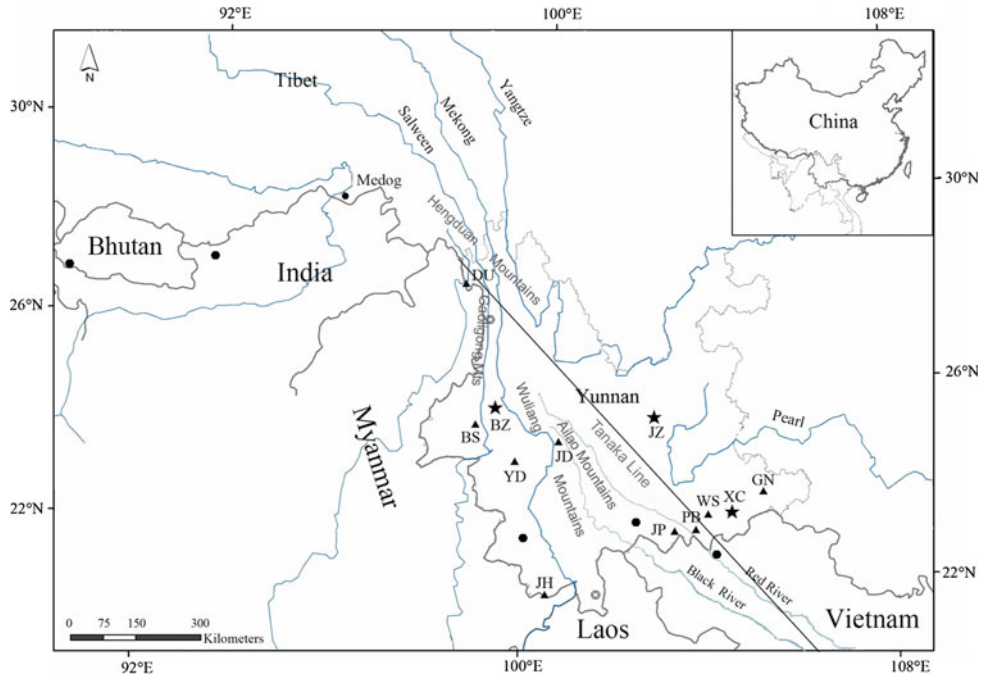

Fig. 1 A map of the natural distribution of *Magnolia cathcartii* based on descriptions in the literature and specimen records, and the locations of the sampled populations (see Table 1 for population abbreviations and more detailed locality information). The *black line* represents the Tanaka Line, which is a boundary between the Sino-Japanese plate/biogeographic region in the east and the Sino-Himalayan plate/biogeographic region in the west. It starts approximately at the intersection of 28°N, 98°E and proceeds southward to approximately 18°45′N or 19°N, 108°E. *Filled circles* indicate recorded or described populations that have not been surveyed. *Double walled circles* indicate populations that have been surveyed but could not be found. *Filled triangles* indicate natural populations sampled in the present study. *Asterisks* indicate cultivated populations that were sampled in the present study

name *Alcimandra cathcartii* is still widely used in floras and in the conservation literature (e.g., Li 1994; Law 1996; Fu 1999; Li et al. 2003; Law and Xia 2006).

Magnolia cathcartii is an occasional tree in humid, evergreen, broad-leaved forests in the region (Li and Mao 1990). The habitat of this species is sunny mountain tops between 1,600 and 2,700 m asl; it occasionally also occurs in valleys and on hillsides. In China, *Magnolia cathcartii* occurs in northwestern to southeastern Yunnan Province and southeastern Tibet Municipality (Fig. 1). This region has been referred to as the South-Central China hotspot (Myers et al. 2000), the Hengduan Mountains hotspot (Boufford and van Dijk 1999), or the biodiversity center of Yunnan province (Li 1994). *Magnolia cathcartii* is a diploid ($2n = 38$), evergreen tree that reaches heights of 50 m (Chen et al. 1989; Zhang et al. 2006b). It blooms in April and May and has an androgynous breeding system. The flowers have nine petals and stamens that are usually longer than the pistils. Fruit is produced in September and October and they often bear nonviable seeds (Law 1996).

Sample collection

Young, healthy leaves were randomly sampled from 179 individuals (intervals $\geq$ 20 m) of 9 geographically and environmentally representative natural populations that covered almost the entire distribution range of the species (see Fig. 1). In addition, 54 individuals were collected from three cultivated populations to survey the genetic variation in populations under ex situ conservation (Table 1). The cultivated populations of JZ, XC and BZ came from seeds collected from Jinping County (JP), Baoshan City (BS) and Baoshan City (BS), respectively. The fresh leaf tissue collected was dried with silica gel and stored at 4°C until DNA extraction.

DNA extraction and AFLP amplification

Genomic DNA was extracted using the plant genomic DNA mini-prep kit (V-gene Biotechnology Ltd., Stonebridge, Hangzhou, China). The AFLP reactions were conducted following Vos et al. (1995) with minor modifications. All amplifications were carried out using a PTC-200 Peltier thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Sixty-four selective primer combinations (Beckman Coulter, Inc., LA, CA, USA/Invitrogen Corp., Carlsbad, CA, USA) were tested in a pilot study on a small number of representative samples. The *Eco*RI primers were fluorescently labeled with D4PA at the first base of the 5′-end. Three combinations (*Eco*RI-ACT/*Mse*I-CAA, *Eco*RI-ACC/

Table 1 Localities and the sizes of the samples taken from the twelve *Magnolia cathcartii* populations from Yunnan Province, China for AFLP analysis

Population code	Region	Locality	Sample size	Latitude (N)	Longitude (E)	Size (individuals)
Natural						
JP	SE Yunnan	Jinping County	20	22°52′23.2″	103°14′16.2″	~540
PB	SE Yunnan	Pingbian County	20	22°54′48.1″	103°41′54.6″	~260
WS	SE Yunnan	Wenshan County	20	23°13′42.2″	103°57′28.9″	~280
GN	SE Yunnan	Guangnan County	20	23°42′01.0″	105°09′41.5″	~300
BS	W Yunnan	Baoshan City	22	24°49′17.7″	98°46′01.8″	~600
YD	SW Yunnan	Yongde County	20	24°07′50.6″	99°41′12.4″	~250
JD	SW Yunnan	Jindong County	16	24°34′53.5″	100°37′59.9″	~260
JH	SW Yunnan	Jinghong City	20	21°30′33.1″	100°30′29.7″	~270
DU	NW Yunnan	Dulongjiang Township	21	27°40′33.8″	98°17′56.7″	~700
Cultivated						
JZ	C Yunnan	Kunming Botanical Garden	16	25°08′36.5″	102°44′32.3″	~400
BZ	W Yunnan	A nursery in Baoshan District	20	25°10′43.0″	99°11′21.0″	~5,000
XC	SE Yunnan	Xiangpingshan Tree Plantation, Xichou County	18	23°17′40.1″	104°27′52.7″	~2,000

*Mse*I-CTC and *Eco*RI-AAC/*Mse*I-CAT) were selected and applied to all individuals.

The amplified fragments were separated using a Beckman CEQ8000 Genetic Analysis System and analyzed using the Beckman CEQ 8000 software package. Amplified fragments of between 50 and 600 base pairs were scored by visual inspection for the presence (1) or absence (0) of bands in the output traces. A binary matrix was then generated.

Genetic analyses of AFLP data

The resulting binary matrices of AFLP bands were used for the genetic analyses. Genetic diversity parameters, percentage of polymorphic loci (*P*), Nei's (1973) gene diversity (H_{pop}) and Shannon's information index (*I*) were estimated using POPGENE v.1.31 (Yeh et al. 1999). In addition, for each population, the number of "private" fragments (f_p) was assessed.

The coefficient of gene differentiation, G_{st}, was calculated following Nei's (1987) statistics. Gene flow was estimated using the equation $Nm \approx (1 - G_{st})/4G_{st}$, as modified from Wright (1951). The genetic distance (*D*) among populations was also computed using the model presented by Nei (1972).

The distribution of genetic variation at the subpopulational and the regional levels was investigated using analysis of molecular variance (AMOVA v. 1.55; Excoffier 1993), which is essentially based on hierarchical variance of gene frequencies. The input files for AMOVA were prepared with the aid of AMOVA-PREP v. 1.01 (Miller 1998).

The UPGMA (unweighted pair group method with arithmetic mean) clustering method (Sokal and Michener 1958) was used to construct the genetic distance tree. The UPGMA tree was generated with the Tools for Population Genetic Analysis (Miller 1997), and a Mantel test was also performed to test the relationship between genetic distances (*D*) and geographic distances (in km) among the populations.

To further clarify the genetic structure of the populations and the origin of cultivated populations, we conducted an analysis using the program *structure*, version 2.3.2 (http://pritch.bsd.uchicago.edu/structure.html) with the LOCPRIOR model. Specifically, we set most of the parameters to their default values, as advised in the user manual (Pritchard et al. 2009). We chose the admixture model and the option of correlated allele frequencies between populations, as this configuration is considered best by Falush et al. (2003) and Pritchard et al. (2009) for cases of subtle population structure. We allowed the degree of admixture (alpha) to be inferred from the data. When the value of alpha is small (i.e., close to zero), most individuals are essentially from one population or another; when alpha is large (i.e., >1), most individuals have substantial ancestry from multiple clusters (Falush et al. 2003; Pritchard et al. 2009). The LOCPRIOR model was turned on to infer the population structure accurately (Hubisz et al. 2009). Lambda, the parameter for the distribution of allelic frequencies, was set to 1, as recommended in the manual. A pilot study indicated that 5,000 burn-in and 5,000 MCMC (Markov chain Monte Carlo) iterations were sufficient. Increasing the burn-in did not change the results

significantly. To estimate the appropriate number of clusters (K), we used the formal method suggested in Evanno et al. (2005), where the best K value is inferred from the modal value of Δk, a quantity based on the second-order rate of change with respect to K of the likelihood function. For each value of K, three runs were carried out in order to quantify the amount of variation of the likelihood. To create nice plots, we applied the program *distruct* (http://rosenberglab.bioinformatics.med.umich.edu/software.html) (Rosenberg 2004) to the output data derived from *structure*.

Results

Genetic diversity

The three primer pairs yielded a total of 890 scorable bands. The number of unambiguous bands amplified by different primer pairs ranged from 236 to 351 per primer pair, with an average number per pair of 297 bands. All 890 bands were polymorphic (100%). Nei's (1973) gene diversity (H_{pop}) was 0.162 and Shannon's information index (I) was 0.272 (Table 2).

Table 2 Genetic variability within natural populations of *Magnolia cathcartii* from Yunnan Province, China, as revealed by AFLP

Population	P (%)	H_{pop} (SD)	I (SD)	f_p
Southeast				
JP	62.9	0.142 (0.167)	0.229 (0.239)	12
PB	66	0.147 (0.163)	0.238 (0.235)	29
WS	61.2	0.138 (0.165)	0.222 (0.238)	8
GN	69.3	0.161 (0.168)	0.260 (0.239)	26
Mean	64.9	0.147	0.234	18.8
Western				
BS	43	0.104 (0.163)	0.164 (0.237)	6
YD	33.4	0.086 (0.152)	0.136 (0.224)	1
JD	40.3	0.099 (0.154)	0.157 (0.231)	4
JH	44.2	0.108 (0.165)	0.170 (0.239)	13
DU	48.7	0.113 (0.165)	0.180 (0.239)	15
Mean	41.9	0.102	0.161	7.8
Population average	52.1	0.122 (0.026)	0.195 (0.043)	12.7
Species total	100	0.162 (0.154)	0.272 (0.211)	–
Cultivated				
JZ	49.5	0.115 (0.162)	0.185 (0.236)	0
XC	37.8	0.094 (0.158)	0.149 (0.230)	0
BZ	41.8	0.099 (0.159)	0.158 (0.232)	0

P is the percentage of polymorphic loci; H_{pop} is Nei's (1973) gene diversity; I is Shannon's information index; f_p is the number of "private" fragments

SD standard deviation

Within populations, the mean proportion of polymorphic loci (P) was 52.1%, ranging from a low of 33.4% in Yongde County (YD) to a high of 69.3% in Guangnan County (GN) (Table 2). Assuming Hardy–Weinberg equilibrium, the mean gene diversity within populations (H_{pop}) was 0.122. Among the nine populations, the gene diversity ranged from 0.086 ± 0.152 in the YD population to 0.161 ± 0.168 in the GN population. The mean Shannon information index (I) was 0.195, and ranged from 0.136 ± 0.224 in the YD population to 0.259 ± 0.239 in the GN population. As a whole, the genetic variability measures showed that genetic diversity was higher in the southeast populations ($P = 64.9\%$, $H_{pop} = 0.147$; $I = 0.237$) than in the western ones ($P = 41.9\%$, $H_{pop} = 0.102$; $I = 0.161$) (Table 2; Fig. 5). Higher numbers of "private" fragments (f_p) were also observed within the southeast populations (mean = 18.8) as compared to the western sites (mean = 7.8) (Table 2).

When the cultivated populations were included, the gene diversities of the outplanted JZ, XC and BZ populations were 0.115 ± 0.162, 0.094 ± 0.158 and 0.099 ± 0.159, respectively (Table 2). All of the values were close to or lower than the mean diversity for all sampled populations. None of the outplantings reached the genetic diversity levels found in their source populations (JP, BS and BS).

Population genetic structure and differentiation

Nei's (1973) estimator of population substructure (G_{st}) suggested a high level of population differentiation ($G_{st} = 0.247$) among the nine natural populations of *M. cathcartii* surveyed for genetic variation. Moreover, a similarly high value was found when the cultivated populations of JZ, XC and BZ were included in the sampling ($G_{st} = 0.250$). Both of the G_{st} values translated into correspondingly low levels of gene flow ($Nm = 0.763$ and 0.752; Wright 1951). These conclusions were also supported by the nonhierarchical AMOVA results, which indicated that there was a high degree of population differentiation among the *M. cathcartii* populations. Of the total AFLP variation, 30.4% was apportioned among the natural populations, whereas 69.6% of the variation still resided within the populations. Furthermore, when the cultivated populations (JZ, BZ and XC) were included in the calculations, the corresponding values for among and within populations varied little: they were 29.7 and 70.3%, respectively.

The mean genetic distance for pairwise comparisons of the nine natural populations varied from 0.012 to 0.072 (detailed data for these distances are not given here). Genetic relationships among the populations were further examined using UPGMA and *structure*. The UPGMA dendrogram (Fig. 2) grouped all populations of *M. cathcartii* into one cluster roughly corresponding to their

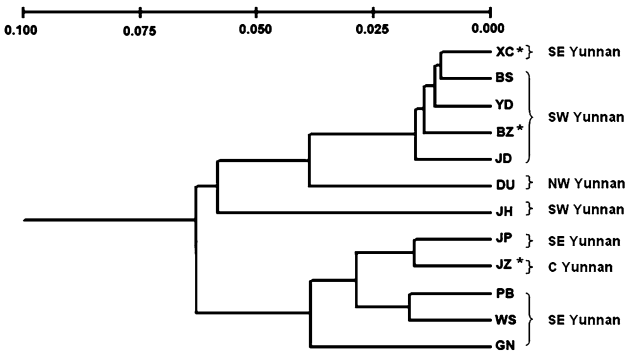

Fig. 2 The UPGMA dendrogram for nine populations of *Magnolia cathcartii* from Yunnan, China (JP, PB, WS, GN, BS, YD, JD, JH, DU) plus three ex situ conserved populations (JZ, XC, BZ), based on Nei's (1972) genetic distance. The *upper numerical scale* in the figure shows the genetic distances used to construct the UPGMA dendrogram. *Asterisks* denote ex situ populations. The corresponding source populations of *XC*, *BZ* and *JZ* were *BS*, *BS* and *JP*, respectively

geographic origins (Fig. 1). It revealed two large groups: the southeast populations (JP, PB, WS and GN) and the western populations (JH, DU, JD, BS and YD). The three ex situ populations of JZ, XC and BZ were ideally clustered with their source populations of JP, BS and BS, respectively. The *structure* plots yielded a very similar result to that obtained with UPGMA (Fig. 3). At $K = 2$, which is the best K value inferred from the modal value of Δk, the clusters were anchored by southeast and western populations. At $K = 3$, the clusters corresponded largely to the major geographic regions. However, the next cluster, at $K = 4$, did not match a major region; it showed clear substructure among the southeast populations, such that the origin of the cultivated JZ group can clearly be identified as its predefined source population, JP. Similarly, although the western populations did not group so distinctly, the cultivated XC and BZ populations always clustered with their source population of BS. Separate analyses of the southeast and western populations ($K = 3$ was the best cluster number for both analyses here) produced very similar results to the integrated analysis (Fig. 3).

The Mantel test revealed a significantly positive correlation between genetic and geographic distances among populations ($r = 0.677$, $P = 0.003$) (Fig. 4).

Discussion

Levels of genetic variation within and among populations

The AFLP survey of nine populations of *M. cathcartii* revealed a large variation in P, with values ranging from 33.4 to 69.3%, and an average of 52.1%. This implied that a large proportion of the genetic variation was partitioned among populations. In general, selfing or mixed-mating species usually possess lower genetic diversity within populations and higher genetic differentiation among populations compared to outcrossing species. Based on the above values, it is most likely that *M. cathcartii* has a mixed-mating system. Nybom (2004) reported that the mean within-population genetic diversities (H_{pop}) for self-pollinated species, mixed-mating species and outcrossers were 0.120, 0.180 and 0.250, respectively. Our results suggest that the genetic diversity within populations of *M. cathcartii* is $H_{pop} = 0.122$ (Table 2). It is conspicuously low when compared with the values of other seed plants with similar life history characteristics in Nybom (2004), and is nearly equivalent to that of self-pollinated species ($H_{pop} = 0.120$; Nybom 2004). Although the above data may suggest an inbreeding system in the self-compatible *M. cathcartii* (X. M. Zhang et al., unpublished data), this conclusion is not supported by the genetic structure among populations ($G_{st} = 0.247$), which does not correspond to that seen in self-pollinated populations ($G_{st} = 0.590$; Nybom 2004). Instead, the G_{st} value is more similar to those of mixed-mating species ($G_{st} = 0.200$) or outcrossers ($G_{st} = 0.220$) (Nybom 2004). Therefore, based on the values of H_{pop} and G_{st}, it is probable that *M. cathcartii* has a mixed-mating system, which is consistent with the inference above.

Our field controlled pollination tests suggest that *M. cathcartii* has a breeding system of simultaneous self- and cross-pollination. The fruit set, follicle set and seed set from natural pollination are significantly lower than those from either self- or cross-pollination manipulations (X. M. Zhang, unpublished data) (note that, in the present study, we refer to a ripe carpel and an aggregate of ripe carpels as a follicle and a fruit, respectively, as described by Ishida et al. 2003). Apparently, pollen shortage is the reason for this (Ishida et al. 2003). Moreover, the seed set and ovule survival rate from hand self-pollination were significantly lower than those from hand cross-pollination, while there was no significant difference in seed weight between the two (X. M. Zhang, unpublished data). These results indicate that self-pollination causes a reduction in fitness up to seed maturation for *M. cathcartii*. Furthermore, the wide range and large standard deviation of δ_e (magnitude of inbreeding depression caused by self-pollination at seed maturity) for individual *M. cathcartii* trees at seed maturity of provides evidence of inbreeding depression due to the expression of recessive deleterious alleles. The floral structure characteristics, timing of flowering and the visit behavior of pollinators could cause a high proportion of self-pollination of the same flower and geitonogamous self-pollination in *M. cathcartii* (X. M. Zhang, unpublished data). Therefore, the low natural pollination

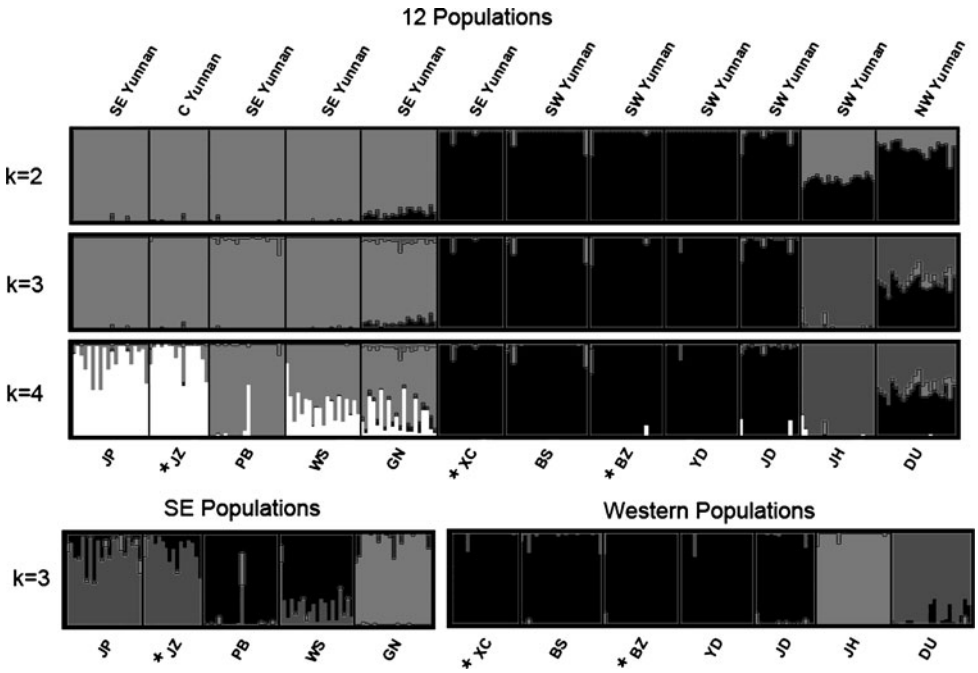

Fig. 3 *Distruct* plots for *structure* runs for 12 populations of *Magnolia cathcartii* from Yunnan, China. Each individual is represented by a *thin vertical line*, which is partitioned into *K* segments that represent the individual's estimated membership fractions in *K* clusters. *Black vertical lines* separate individuals of different populations. *Labels below the plots* provide population codes; *labels above the plots* are the populations' regional affiliations. *Asterisks* denote cultivated populations

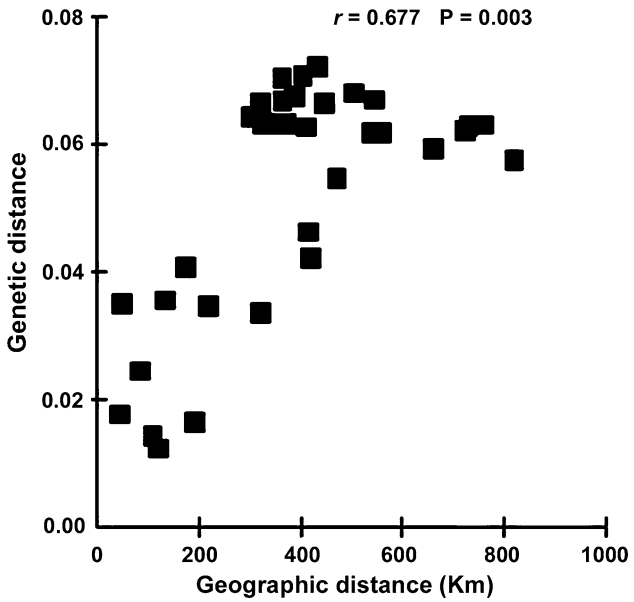

Fig. 4 Mantel test matrix of correlation between the geographic distances and the genetic distances of *Magnolia cathcartii* populations from Yunnan, China

success may be explained by both geitonogamy and pollen shortage (Ishida et al. 2003). This may be responsible for the low value of H_{pop} for *M. cathcartii*.

A clear feature of the population genetic variation of *M. cathcartii* is that it is not correlated with population size. The highest genetic variation was observed in population GN ($P = 69.3\%$, $H_{pop} = 0.161$; $I = 0.259$), which had a relatively small population size (about 300). DU had the greatest population size (about 700), but harbored only a medium level of genetic variation ($P = 48.7\%$, $H_{pop} = 0.113$; $I = 0.180$). This result indicated that current population size cannot be a criterion for population genetic variation in this species.

Genetic differentiation among populations of *M. cathcartii* and the possible cause

The AMOVA indicated that 30.4% of the total genetic variation was partitioned among populations of *M. cathcartii*. Nei's genetic diversity analysis demonstrated a similar pattern of genetic structure, with a G_{st} value of 0.247 among populations, which is higher than the average obtained for mixed-mating species (0.200) in the analysis by Nybom (2004).

A high level of population differentiation may be explained by several factors, such as geographic isolation, habitat destruction, limited gene flow, breeding system, and so on.

Parks et al. (1994) pointed out that historical factors may influence the distribution and partitioning of the genetic diversity in plant species. An important factor that may influence the geographic differentiation of *M. cathcartii* may be historical processes such as long-term isolation or habitat fragmentation (Young et al. 1996). Ongoing gene flow may be limited or absent due to physical barriers such as high, large mountain ranges and deep, broad valleys among the populations DU, BS, YD, JD, JH and the southeast populations (JP, PB, WS, GN) (Fig. 1; see also Li and Li 1993).

The family Magnoliaceae originated in the early Cretaceous (Aptian–Albian) (Azuma et al. 2001; Zhang

2001; Nie et al. 2008). The family diversified greatly in the late Cretaceous and the early Tertiary. In the Quaternary, some species suffered from extinction at high latitudes, whereas some species migrated to low-latitude regions and diversified (Zhang 2001). Reconstructions of the palaeovegetation of China achieved using ^{14}C dating of macro remains from sediment cores containing pollen showed that the northern boundary of the broadleaved-evergreen/warm-mixed forest during the last glacial maximum (LGM; 18,000 year BP) was forced to retreat southward as far as 24°N to 23°N (cf. Yu et al. 2000). The uplift of the Himalayan–Hengduan Mountains and the Quaternary glaciation within and around the distributional range of *M. cathcartii* [including northwest (Zheng 2000) and northeast Yunnan Province (Kuang et al. 1997)] may have restricted the continuous expansion of the species. The species may have subsequently migrated from the south to the north and from the east to the west, based on fossil pollen evidence of a mid-Holocene northward and westward re-expansion of subtropical forest biomes in China (ca. 6,000 BP; Yu et al. 2000). However, the Red River in southeast Yunnan Province (Fig. 1) formed in the Tertiary as a result of the India–Tibet collision (Sun et al. 2003), and the Hengduan Mountains rose quickly due to the uplift of the Himalayan Mountains during the Pleistocene epoch of the Quaternary, with the surrounding areas eroding into the deep north–south river valleys (Li and Li 1993). These geologic events led to the fragmentation of uplands in western Yunnan, with high mountains in the northeast and richly calcareous soils in the southeast, both of which were isolated from western Yunnan (Li and Li 1992). High mountains and broad gorges formed and persisted between the southeast and western populations of *M. cathcartii* (and among the populations within the western regions) in Yunnan Province. These geologic/geographic barriers may have limited the expansion of the species and may have played an important role in forming a rough "Tanaka Line" (Tanaka 1954, Fig. 1) (a boundary between the Sino-Japanese plate/biogeographic region in the east and the Sino-Himalayan plate/biogeographic region in the west, approximately corresponding to a straight line starting at 28°N, 98°E and progressing southward to approximately 18°45′ or 19°N, 108°E) distribution pattern for *M. cathcartii*; a distribution pattern that also occurs for many other Sino-Himalayan taxa (e.g., *Caryota urens* Linn, *Taiwania cryptomerioides* Hayata, *Dipentodon sinicus* Dunn and *Tacca chantrieri* André) in Yunnan Province (Li and Li 1992; Zhang et al. 2006a; Yuan et al. 2008).

These historical events may have fostered the isolation and fragmentation of the populations of *M. cathcartii*, increasing differentiation among populations. Random losses of AFLP alleles of *M. cathcartii* may have also occurred during the geologic transition and habitat fragmentation processes, which may have been an important factor that was responsible for the high genetic differentiation among populations of *M. cathcartii.*

Aside from these historical reasons, current events (i.e., ecological factors of *M. cathcartii*), including gene flow/seed dispersal, pollinator activities, breeding system and ongoing habitat destruction, are also significant factors that have determined the distinct genetic structure of *M. cathcartii*.

Magnolia cathcartii has a restricted gene flow due to limited pollen and/or seed dispersal. Based on our field observations, the pollinators of *M. cathcartii* are bees (*Andrena*, *Apis* and *Sphecodes*), beetles and syrphid flies. These pollinators all have limited abilities to fly long distances. Among the pollinators, *Apis* has a relatively strong long-range foraging ability, but the largest distance that it has been found to travel when foraging so far is 16 km (Kamm et al. 2009). Therefore, pollen transfer among populations of *M. cathcartii*, which are separated by distances ranging from 44 to hundreds of kilometers (detailed data for distances between populations are not provided here) is an unlikely or rare event.

Furthermore, the value for gene flow ($Nm = 0.7626$ with cultivated populations excluded) is lower than the criterion value ($Nm \approx 1$) needed to overcome genetic drift (Slatkin 1987), indicating relatively restricted gene flow among natural populations. The significantly positive r value obtained in the isolation-by-distance analysis also suggests that gene exchange is largely restricted to nearest-neighbor populations. Furthermore, although the pollens used for cross-pollination in the present study were only from 350 m-distant individuals, the natural and selfed seed production was significantly lower than that from cross-pollination (X. M. Zhang, unpublished data). It was reported that flower beetles fly frequently between plants (Englund 1993) and can transport a certain amount of outcross pollen (Matsuki et al. 2008); however, bees and small coleopteran species, which make up a large proportion of the pollinators in this study (X. M. Zhang, unpublished data), move mostly within and rather infrequently between trees, transferring a large proportion of the self-pollen (Matsuki et al. 2008). Therefore, we suggest that frequent geitonogamy occurs in *M. cathcartii*, just as it does in *Liriodendron tulipifera* and *M. obovata* (Magnoliaceae) (Brotoschol et al. 1986; Ishida et al. 2003; Matsuki et al. 2008). Geitonogamy restricts the gene flow among individuals within and among populations, reducing genetic recombination. Field investigations revealed that seed production in natural populations of *M. cathcartii* is generally high, whereas seedling establishment is usually low. We also observed that some of the seeds are eaten or destroyed by squirrels and birds. Even though these animals may carry some seeds to a new site away from the

source tree (for example, the seed dispersal distance of *Sorbus domestica* can extend to 12 km with the aid of seed vectors such as birds and large mammals; Kamm et al. 2009), the dispersal benefit appears limited.

Forest-clearing activities of humans in recent years may have also led to population extinction (see observations noted in the "Introduction") and/or population disruption, which contributed to the observed high G_{st} value.

In summary, the observed low level of genetic diversity and high level of genetic differentiation of *M. cathcartii* may be primarily due to habitat fragmentation resulting from geologic and subsequent climatic changes, loss of alleles during the geologic past and in present day China, restricted gene flow due to physical barriers and limited pollinator motility, and a breeding system that does not restrict self-pollination or geitonogamy.

Conservation implications

The center for the genetic diversity of *Magnolia cathcartii* occurs in the southeastern Yunnan Province (see Fig. 5), which is also the main center of biodiversity in China. The comparatively high levels of genetic diversity exhibited in the southeast populations, together with the high numbers of "private" AFLP fragments (Table 2), indicate the long-term isolation of these populations in this region, rather than the involvement of recent founder events. Long-term conservation of endangered species requires strategies that maintain their genetic diversity (Barrett and Kohn 1991). Rare alleles may be important for adapting to unusual environmental conditions (Holsinger and Gottlieb 1991). However, Marshall and Brown (1975) proposed that *common alleles* in the target population with frequencies of greater than 0.050 merit priority, and at least one copy of each such allele with a certain probability of about 0.950 should be preserved to maintain the genetic diversity of the species.

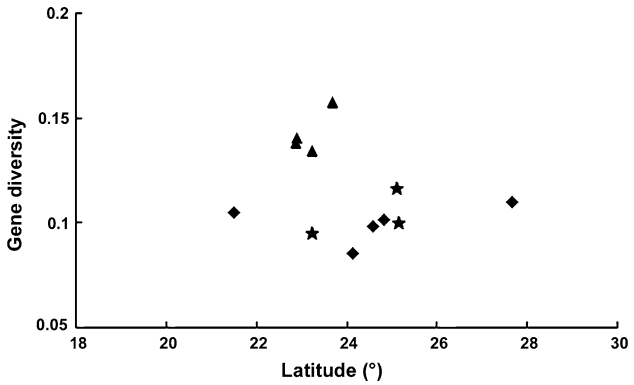

Fig. 5 Graph of the relationship between gene diversity (H_{pop}) and latitude for the populations of *Magnolia cathcartii* in Yunnan, China. *Triangles* southeast populations, *diamonds* western populations, *stars* cultivated populations

In the present study, among all of the *M. cathcartii* populations, both PB and GN harbor sufficient genetic diversity (100%) when calculated with the Marshall and Brown criterion, while JP harbors about 93.0% and WS 84.4%. When calculated as an integrated group, these southeast populations still possess representative genetic diversity (100%), whereas the other populations contain a low proportion of the total genetic diversity when analyzed separately (54.1–74.0%) or together (80.1%). Thus, although plants from most of the extant populations have been brought into nature reserves, not all of the in situ or ex situ populations contain the representative genetic diversity of the species, even in the southeast populations. Therefore, the extant in situ populations should be fully conserved to prevent further loss of genetic diversity. The southeast populations with the highest genetic diversity and the highest numbers of "private" fragments deserve special attention and priority in the conservation efforts.

As for the ex situ populations, only 69.9, 74.6 and 73.4% of the total genetic diversity of the species were contained in JZ, XC and BZ populations, respectively. These ex situ populations thus represent only limited levels of the genetic diversity of the species. The cultivated XC and BZ populations share the same source population, BS, based on our field survey and inquiries. The number of natural populations that are required to sample the representative proportion of genetic variation ($\geq$95.0%) can be calculated as $1 - (G_{st})^n$, where n is the number of populations proposed for sampling (Ceska et al. 1997). Based on our G_{st} value of 0.247, at least three natural populations would be required to sample more than 95.0% of the genetic variation $[1 - (0.247)^3 = 0.985]$ in *M. cathcartii*. It would be our suggestion to preserve the most genetically divergent population (PB, DU or JH) that possesses more specific, locally adapted genotypes as preferential source populations in the ex situ conservation program. The complex topography of this region may have helped to preserve a natural refugium of biodiversity for *M. cathcartii* in the past, but it will only be through careful, well-informed conservation efforts and in situ and ex situ preservation that the biodiversity of this rare tree will be maintained in the present.

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