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Genetic Diversity of the Relict Plant *Taiwania cryptomerioides* Hayata (Cupressaceae) in Mainland China

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Abstract

The genetic diversity and differentiation of five populations of *Taiwania cryptomerioides* Hayata in mainland China were investigated using inter-simple sequence repeats (ISSR). In comparison with other coniferous species, *T. cryptomerioides* from mainland China possesses little genetic variation, particularly at the level of individual populations (the percentage of polymorphic loci, Nei's gene diversity and Shannon's indices of diversity at the species and population levels are 38.02%, 0.1326, 0.1986 and 9.27%, 0.035, 0.0518 respectively). In contrast, the level of population differentiation is much higher (G_{ST}: 0.7269; Shannon's genetic differentiation: 0.7392; Hickory θ^B: 0.668; AMOVA genetic differentiation: 72.37%). The genetic divergence of pairs of populations was not significantly correlated with the geographical distance separating them. Current patterns of

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genetic variation were related to biogeographic history and the small population size. On the basis of these findings, we discuss the development of conservation strategies for this endangered species.

Key words: Taiwania cryptomerioides; genetic diversity, ISSR, China, relict species, conservation

Introduction

Like most of the genera within the Cupressaceae that evolved since the Jurassic period, *Taiwania*, one of the so-called "living fossils", is considered to be an ancient genus in evolutionary terms with its origin dating back to the Tertiary. According to the fossil evidence, e.g. *Taiwania schaeferi* Schloem.-Jäg. discovered in Kleinsaubernitz (Germany) (WALTHER, 1999), Spitsbergen (Norway) and Alaska (LePage BA, unpublished data), this genus was widespread in what are now the temperate regions of the northern hemisphere (FLORIN, 1963). Today, *Taiwania*, a monotypic genus containing only *Taiwania cryptomerioides* Hayata, has a scattered distribution. Its distributional range covers mountain areas on the border between Burma and China (southwestern part of Yunnan province), the Hoang Lien Son mountain range in northern Vietnam, and Taiwan (FARJON, 2002; FARJON and GARCIA, 2003). Taiwan is an important refuge for *T. cryptomerioides*: it grows on the Central Mountain at the altitudes of 1600–2600 m, where the largest population and greatest timber yield in the world are found (HUANG, 1983). In addition, there are still a few individuals of *T. cryptomerioides* in mainland China: in the remote Lichuan mountains in Hubei Province, Leishan mountains in Guizhou Province, and Gutian-Pingnan mountains in Fujian Province. These areas are regarded as having acted as key refugia for relict species during Pleistocene glaciations (WU, 1980; WANG and LIU, 1994). The populations in mainland China have only been widely known to botanists since the early decades of the 20th century, and have sometimes been regarded as comprising a different species, namely *T. flousiana* Gaussen. Due to human overexploitation in the past century, the conservation status of this species is "vulnerable" according to the IUCN Red List criteria (FARJON, 2001) and it is a protected species in China (FU, 1995).

T. cryptomerioides is a component of evergreen broadleaf forests in mountainous areas. It occurs at an altitude of 500–2800 m in "coniferous, broad-leaved, or mixed evergreen valley forests on acid, red, or brown soils in warm or warm temperate regions with high summer and autumn rainfall but drier winters, usually scattered and associated with *Chamaecyparis formosensis*, *C. obtusa* var. *formosana*, *Cunninghamia lanceolata*, *Pinus wallichiana*, or *Tsuga dumosa*, but sometimes forming pure stands" (FU et al., 1999). It can grow as tall as 70 meters and is therefore known as the 'King of Asian Conifers'. This diploid species (2n = 22, LI et al., 2000) is wind-pollinated and monoecious, with male and female cones occurring on different branches (FU et al., 1999). With two subapical, unequal wings, seeds of *T. cryptomerioides* are light and small, and can be carried by wind over long distances.

Information about the genetic structure of a rare species such as this, as well as data on its overall level of genetic diversity, are particularly important for species conservation. Genetic analyses can provide valuable insights into the processes influencing extinction (CLARKE and YOUNG, 2000) and, increasingly, genetic data have been used to define conservation management units and to predict changes in population structure and dynamics (NEWTON et al., 1999). However, in comparison with the rarities from Europe and North America, few detailed studies of genetic variation have been undertaken on rare plants in China. Subtropical mainland Asia was one of the most important refugia for plants during the Pleistocene glaciations because it was not covered by ice sheets. Many species that became extinct elsewhere survived in this region, but in a state of isolation and in small populations. Strong genetic differentiation has been discovered in some relict conifer species in China, for example *Cathaya argyrophylla* (GE et al., 1998), *Glyptostrobus pensilis* (LI and XIA, 2005), *Metasequoia glyptostroboides* (LI et al., 2005) and *Ametotaxus argotaenia* (GE et al., 2005). The genetic diversity of *T. cryptomerioides* from Taiwan has been assessed previously by allozyme (LIN et al., 1993), ISSR and ITS sequence analysis (CHANG, 2005). A low genetic differentiation (G_{ST}: 5.2% based on allozyme data, LIN et al., 1993; Φ_{ST}: 15.26% using ISSR, CHANG, 2005) was found in the Taiwanese populations. Nevertheless, the intraspecific genetic variation across the distributional range still remains unknown.

Among various molecular tools, the inter-simple sequence repeats (ISSR) method has been widely used for studies of population genetics, because the repeats are highly variable, and the method is economical in terms of time, money, and labor (GUPTA et al., 1994; ZIETKIEWICZ et al., 1994; TSUMURA et al., 1996; WOLFE and LISTON, 1998). ISSRs have also been used to determine the genetic diversity of species of conservation concern (ESSELMAN et al., 1999). Technically, the ISSR reaction is more specific than RAPD amplification due to the longer SSR-based primers (WOLFE and LISTON, 1998). One limitation of the ISSR technique is that the bands are scored as dominant markers and that genetic diversity estimates are based on diallelic characters, thus the level of genetic diversity may be underestimated. The primary objective of this study was to use ISSR markers to investigate genetic variation within and between the extant *T. cryptomerioides* populations in mainland China. We wished to determine whether these populations are strongly differentiated, as is the case for the other relict conifer species mentioned above. The result of this investigation could assist in the development of conservation strategies, by identifying those populations of particular importance in terms of their genetic characteristics.

Material and Methods

Sample collection

A total of 104 individuals of *Taiwania cryptomerioides* was studied, representing four extant wild populations in mainland China and one plantation population (TC)

Table 1. – Genetic variability within populations of *Taiwania cryptomerioides* revealed by ISSR (N: sample size per population; P: percentage of polymorphic loci; H_E: Nei's gene diversity; H*pop*: Shannon indices).

Pop. code	Location	Coordinates (N/E)	Elevation (m)	Site type	Habitat Reference	N	P (%)	H_E	H*pop*
TC	Tengchong, Yunnan	25°01'/98°30'	1700	Hill, artificial forest	Feng et al., 1998	11	1.56	0.0052	0.0079
GS	Gongshan, Yunnan	27°43'/98°34'	2000	Valley, scattered amongst subtropical evergreen broadleaved forest	Liu et al., 1987	24	8.33	0.0316	0.0469
LS	Leishan, Guizhou	26°34'/108°15'	1040	Valley, scattered amongst subtropical evergreen broadleaved forest	Qiu et al., 1984	21	3.65	0.0121	0.0186
LC	Lichuan, Hubei	30°04'/109°05'	850	Valley, scattered amongst evergreen broadleaved forest	Ge, 1995	22	12.50	0.0455	0.0676
GP	Gutian and Pingnan, Fujian	26°35-56'/118°48'-119°11'	400-880	Scattered between village and valley	Yang and Jiang, 1999	26	20.31	0.0806	0.1179
Mean						21	9.27	0.035	0.0518
At species level						104	38.02	0.1326	0.1986

(*Table 1, Fig. 1*). Among the four wild populations, many individuals are several hundred years old with a DBH greater than 80 cm; their ecological and phytocoenological features have been described previously (*Table 1*; QIU et al., 1984; LIU et al., 1987; GE, 1995; FENG et al., 1998; YANG and JIANG, 1999). Individuals were sampled at random, regardless of their size and age. Young, healthy leaves were collected in the field, and dried in silica gel prior to DNA extraction. Total DNA was extracted using the CTAB method described by DOYLE (1991) then dissolved in 100 μL of TE buffer. DNA concentration was determined by comparison to uncut lambda DNA on 1% agarose gels; the average DNA concentration was about 20–30 ng/μl.

PCR amplification

PCR amplification was carried out in a volume of 20 μl; the reaction comprised 20 ng of template DNA, 10mM Tris-HCl (pH 8.0), 50mM KCl, 0.1% Triton x 100, 2.5mM MgCl$_2$, 0.1mM dNTPs, 2% formamide, 0.2 μM primer and 1.5 units of *Taq* polymerase. Amplifications were carried out in a PTC-200 thermal cycler (MJ research) following the method of GE and SUN (1999). One hundred primers, each 15–23 nucleotides long (UBC primer set # 9, Biotechnology Laboratory, University of British Columbia), were screened for the production of a high proportion of polymorphic and reproducible banding. Fifteen primers (UBC # 807, 808, 811, 826, 827, 828, 835, 836, 840, 846, 849, 855, 866, 880, and 886) fulfilled these requirements. PCR products were electrophoresed on 2.0% agarose gels buffered with 0.5 x TBE. A 100 bp DNA Ladder (New England Biolabs) was used as a size marker. After staining with ethidium bromide, the fragments were identified using image analysis software for gel documentation (LabWorks Software Version 3.0; UVP, Upland, CA 91786, USA).

Data analysis

Only bands that could be unambiguously interpreted across all the population samples were used in this study. ISSR profiles were scored as discrete characters (presence or absence of amplified products) for each
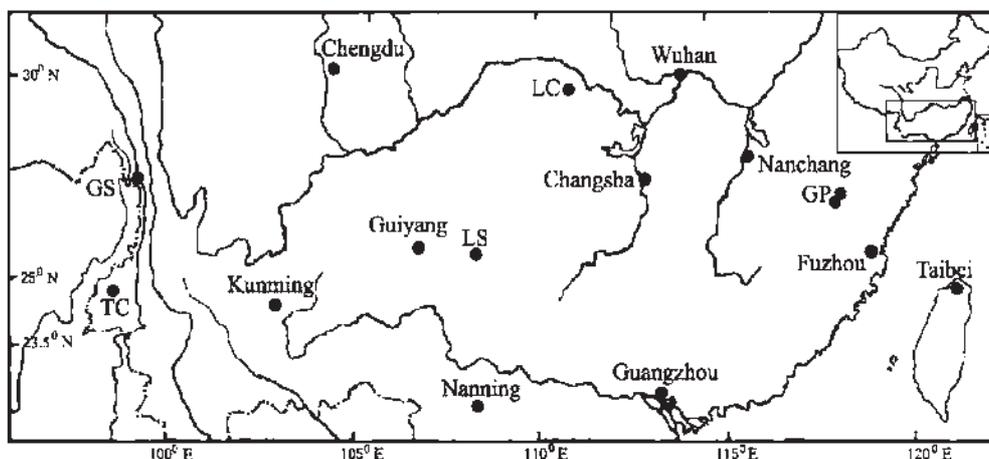
Figure 1. – The sampling locations of *Taiwania cryptomerioides* Hayata.

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individual. The resulting data matrix was analyzed using POPGENE v. 1.31 (YEH et al., 1999) to estimate genetic diversity parameters: the percentage of polymorphic loci (P) and expected heterozygosity (H_E). At the species level, genetic diversity measures (H_T: total gene diversity; G_{ST}: coefficient of gene differentiation) and the level of gene flow (Nm) were measured using Nei's (1973) gene diversity statistics. Genetic diversity was also estimated using Shannon's information statistic (LEWONTIN, 1972): H_{Sh} = -Σpi $\log_2 p_i$ where p_i is the frequency of a given ISSR fragment. H_{Sh} was calculated at two levels: the average diversity within the populations (Hpop), and the total diversity (Hsp). Then the proportion of diversity between the populations was estimated as: (Hsp-Hpop)/Hsp.

In addition, an analysis of molecular variance (AMOVA; EXCOFFIER et al., 1992) was used to estimate variance components for ISSR phenotypes, partitioning the variation between populations and between individuals. AMOVA analysis was performed using the Arlequin 2.000 program (SCHNEIDER et al., 2000). To test the presence of isolation by distance, a Mantel test between the pairwise genetic distance and geographic distance from each population pair was applied using Mantel's test in the software TFPGA (MILLER, 1997) (1000 permutations). Pairwise genetic distances (Φ_{ST}) were got from AMOVA analysis, which are analogous to traditional F statistics.

An alternative Bayesian method, allowing direct estimates of F_{ST} from dominant markers without any previous knowledge of F_{IS}, was employed to estimate θ^B (analogous to F_{ST}) using Hickory 1.0 (HOLSINGER and LEWIS, 2003). Using the default sampling parameters (burn-in = 5 000, sample = 25 000, thin = 5), θ^B was calculated under four different models. The first was a full model, in which both θ^B and f (i.e., F_{IS}, the inbreeding coefficient) were estimated. Two alternative models assumed either θ^B or f equal to zero. Finally, because estimates of f based on dominant markers are usually strongly biased (especially at low sample sizes, i.e., n < 10), HICKORY was used to construct a model in which f was allowed to vary. The deviance information criterion (DIC) was employed to determine which model was the best fit for the data: lower values indicate a better fit.

Results

In this study, 73 of the 192 electrophoretic bands (38.02%) were polymorphic within the populations. The percentages of polymorphic loci (P) for a single population ranged from 1.56% (TC) to 20.31% (GP), with an average of 9.27%. The average genetic diversity was estimated to be 0.0350 at the population level (H_S) and 0.1282 at the species level (H_T). The Shannon indices

were 0.0518 at the population level (Hpop) and 0.1986 at the species level (Hsp), respectively (Table 1). The cultivated TC population exhibited the lowest genetic diversity. Among the four wild populations, population GP had the greatest level of variability (P: 20.31%, H_E: 0.0806, Hpop: 0.1179); population LS had the lowest level of variability (P: 3.65%, H_E: 0.0121, Hpop: 0.0186) (Table 1). The average polymorphism of the four wild populations was 11.19%.

The coefficient of genetic differentiation between populations (G_{ST}) was 0.7269, estimated by partitioning the total gene diversity. The Shannon's diversity index analysis partitioned 73.92% of the total variation between populations, in broad agreement with the result of genetic differentiation analysis. The level of gene flow (Nm) was estimated to be 0.0939. The AMOVA analysis provided corroborating evidence for the genetic structure obtained from Nei's genetic diversity statistics and Shannon's diversity estimate. There were highly significant (P < 0.001) genetic differences between the five populations of *T. cryptomerioides*. Of the total molecular variance, 72.37% was attributable to between-population diversity and the rest (27.63%) to differences between individuals within populations (Table 2). A similar result was obtained from the Hickory calculation: θ^B was 0.668 for the f free model, which had the smallest DIC value.

Genetic distances (Φ_{ST}) between populations of *T. cryptomerioides* were calculated by Arlequin 2.000 (SCHNEIDER et al., 2000) (Table 3). The highest distance value was 0.9011 between TC and LS, and the lowest was 0.5037 between LS and GP. Geographically TC and GS are the closest pair of sites (269 km), however, the genetic distance between them, 0.7608, was not the lowest of all the pairs. The result of a Mantel test with 1000 permutations revealed that the genetic divergence of populations was not significantly correlated with geographical distance (Mantel test, r = -0.3124, P = 0.2170).

Discussion

Low level of genetic diversity and strong genetic differentiation

Correlations between genetic diversity and various attributes of different species have been examined based

Table 3. – The pairwise genetic distance (Φ_{ST}) (below diagonal) and geographic distance (km) (above diagonal).

	GP	LS	LC	GS	TC
GP	0.0000	1081	1052	2050	2080
LS	0.5037	0.0000	421	981	999
LC	0.6159	0.7871	0.0000	1085	1199
GS	0.6968	0.8604	0.7031	0.0000	269
TC	0.6471	0.9011	0.7832	0.7608	0.0000

Table 2. – Analysis of molecular variance (AMOVA) for five populations of *Taiwania cryptomerioides* d.f.: degrees of freedom; SS: sum of squares; MS: expected mean squares.

Source of variation	d.f.	SS	MS	Variance components	% Total	P-value[a]
Between populations	4	835.7448	208.936	10.0172	72.37	<0.001
Within population	99	378.5533	3.842	3.8234	27.63	<0.001

[a] Significance tests after 1000 permutations.

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on a vast amount of data relating to natural plant populations (HAMRICK and GODT, 1989; NYBOM, 2004). Most conifers have high levels of genetic diversity and low levels of differentiation between populations (HAMRICK et al., 1992). Examples include *Fitzroya cupressoides* (Hpop: 0.547, Φ_{ST}: 0.1438; ALLNUTT et al., 1999), *Juniperus rigida*, *J. coreana* (H_T: 0.224 and 0.199; G_{ST}: 0.173 and 0.118, respectively; HUH and HUH, 2000), *Cedrus atlantica* (Nei's gene diversity within population, H_S: 0.194; F_{ST}: 0.148; θ^B: 0.178; RENAU-MORATA et al., 2005) and *Juniperus phoenicea* (H_T: 0.148, H_S: 0.130, G_{ST}: 0.12; MELONI et al., 2006). Similar genetic diversity and genetic differentiation have also been reported for the Taiwanese populations of *T. cryptomerioides* (LIN et al., 1993; CHANG, 2005). In this study, however, ISSR polymorphisms revealed a low level of genetic diversity in the mainland populations of *T. cryptomerioides*, with an average of only 11.19% of ISSR bands being polymorphic in the four wild populations studied. In addition, strong population differentiation was demonstrated based on Nei's gene diversity, Shannon's information statistic, AMOVA and Bayesian estimates. About 70% of the total genetic variation was partitioned between the populations of *T. cryptomerioides* studied in mainland China.

The low genetic diversity within populations and high genetic differentiation among populations we observed in *T. cryptomerioides* are similar to that of many relictual endangered conifer species in mainland China. These include *Cathaya argyrophylla* (GE et al., 1998), *Ametotaxus argotaenia* (GE et al., 2005), *Glyptostrobus pensilis* (LI and XIA, 2005), *Metasequoia glyptostroboides* (LI et al., 2005), *Cupressus chengiana* (HAO et al., 2006), *Torreya jackki* (LI and JIN, 2007) and *Abies ziyuanensis* (TANG et al., 2007) (*Table 4*). Since these species are characterized by their relict status, biogeographic history may play an important role in determining their genetic diversity. *T. cryptomerioides* is one of the representatives of relict gymnosperms from the Tertiary period that are found in China. Fossil evidence indicates that the ancestor of *T. cryptomerioides* was much more widespread during the Tertiary than its current distribution suggests (WALTHER, 1999). Accordingly, a reasonable hypothesis is that the modern range of *T. cryptomerioides* was the result of population fragmentation and contraction after the Quaternary glacial cycles. The remnant populations contained only part of the genetic variation present before the range reduction. Genetic drift and inbreeding may have further decreased the genetic diversity and increased genetic differentiation in the small, isolated populations of *T. cryptomerioides* in mainland China.

In addition to historical influences, the present small population size of *T. cryptomerioides* may be important. According to the literature there are currently about 6000–7000 *T. cryptomerioides* individuals in mainland China (HU et al., 1995); populations LC and GP are composed of only a dozen individuals (GE, 1995; YANG et al., 2006). This suggests that this species is experiencing erosion of its genetic diversity. In this study, only fourteen electrophoretic bands (8%) with a frequency lower

Table 4. – Our results compared with those of relict gymnosperm species endemic to China.

Species	Sampling areas	Populations and individuals	P (%) population/species	H_E	H_{pop}	G_{ST}	Molecular marker	Reference
Ginkgo biloba	Zhejiang, Guizhou, Hubei, Jiangsu, New York	3 natural and 2 cultivated populations (66 individuals)	70.45/42.95	0.1622	0.2385	0.3248	ISSR	Ge et al., 2003
Abies ziyuanensis	Guangxi, Hunan	7 nantural populations (139 individuals)	35.73/83.60	0.136	0.200	0.482	AFLP	Tang et al., 2007
Ametotaxus argotaenia	Fujian, Zhejiang, Guangdong, Jiangxi, Hubei, Hunan, Sichuan	10 natural populations (201 individuals)	9.22/64.77	0.0379	0.0549	0.726	ISSR	Ge et al., 2005
Cathaya argyrophylla	Hunan, Sichuan, Guangxi, Guizhou	8 natural populations (101 individuals)	30.4/ 48.0	0.159		0.441	Allozyme	Ge et al., 1998
Cupressus chengiana	Sichuan, Gansu	8 natural populations (92 individuals)	47.83/98.55	0.3120	0.4740	0.4791	ISSR	Hao et al., 2006
Glyptostrobus pensilis	Guangdong, Jiangxi, Hunan, Fujian, Yunnan	14 natural populations (170 individuals)	10.2/24.7		0.058	0.4416	ISSR	Li and Xia, 2005
Metasequoia glyptostroboides	Hubei,	8 natural populations (81 individuals)	46.12/87.93	0.1832	0.2611	0.298	RAPD	Li et al., 2005
Torreya jackii	Zhejiang, Fujian	6 natural populations (120 individuals)	22.56/60.48	0.08	0.11	0.63	ISSR	Li et Jin, 2007
Taiwania cryptomerioides	Taiwan	4 Taiwan populations (327 individuals)	50.2/53.33			0.052	Allozyme	Lin et al., 1993
	Taiwan, Yunnan	8 Taiwan populations and one Yunnan population (120 individuals)	--/70.83			0.3854	ISSR	Chang, 2005
	Yunnan, Guizhou, Hubei, Fujian	5 populations from mainland China (104 individuals)	9.27/38.02	0.1326	0.1986	0.7237	ISSR	This study

P: percentage of polymorphic loci; H_E: Nei's gene diversity; H_{pop}: Shannon indices; G_{ST}: the coefficient of gene differentiation.

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than 30% were observed, suggesting a possible stochastic process of genetic drift, which could result in the loss of low-frequency alleles in the populations (BROYLES, 1998).

The genetic structure of plant populations reflects the interactions of various evolutionary processes. Low levels of genetic differentiation in gymnosperms are usually attributed to wind-pollination and breeding systems that promote outcrossing. *T. cryptomerioides* is dioecious, wind pollinated and has light seeds that are usually dispersed by wind (about 1000 seeds in 1.01 g; CHUNG and CHANG, 1990). It seems likely, therefore, that considerable gene flow should take place between populations. For Taiwan populations of *T. cryptomerioides*, little genetic differentiation is apparent, thus supporting this suggestion (LIN et al., 1993). In contrast to the geographic proximity of the populations in Taiwan, the populations in mainland China are separated from each other by several hundred kilometers (*Table 3*). This extreme isolation makes gene flow negligible, resulting in the lack of a correlation between genetic distance and geographical distance found in this study. For example, the genetic distance between populations that are relatively close geographically (i.e., populations TC and GS) was no lower than between geographically more distant ones. The present locations of *T. cryptomerioides* in mainland China coincide with the key refugia for other relict species during the Pleistocene glaciations (WU, 1980; WANG and LIU, 1994). For example, at the northern limit of this species, Lichuan (LC) in Hubei Province, there are many relict species, including *Davidia involucrata*, *Ginkgo biloba*, *Keteleeria fortunei*, *Taxus chinensis*, *Tetracentron sinensis*, *Emmenopterys henryi* and *Bretschneidera sinensis*. The famous relic plant *Metasequoia glyptostroboides* was also discovered in this region in the 1940s (see http://www.metasequoia.org/). This distribution pattern indicates that populations of *T. cryptomerioides* in mainland China may have been isolated from each other since the end of the Pleistocene glaciations.

The high genetic distance between the artificially cultivated TC population and the four wild populations demonstrated that the seed source for this population is unlikely to have been any of the wild populations studied. According to the Flora of Yunnan (Kunming Institute of Botany, 1986), there are still some wild populations of *T. cryptomerioides* deep in the mountains, on the border between China and Burma. The source of the TC population may be one of these unsampled populations or an extinct local wild population, since the local cultivation history of this species is very long and there are many aged trees in the TC population. According to Farjon (personal communication), the only endemic populations in mainland China are those near the border with Burma, such as population GS in this study. However, his opinion is not supported by the results of this study. In addition, the populations located in the remote mountains were inaccessible by road until the last two decades. More work is needed to identify the source of the propagules from which LC, LS and GP were established. For most conifer species, mtDNA is inherited maternally and is suitable for phylogeographical studies, which can be used to determine a species' natural range (BEEBEE and ROWE, 2004). Further study is needed to screen out the mtDNA fragments with intraspecific polymorphic variation in this species.

Conservation Implications

The maintenance of genetic diversity is crucial to the survival of organisms, because it allows them to evolve and adapt to changing environmental conditions. The high level of genetic differentiation between the studied populations of *Taiwania cryptomerioides* indicates that a considerable amount of the overall genetic variation of the species in mainland China would be lost if management concentrated only on the remaining large populations. Therefore, several populations throughout the entire range should be considered for conservation. At present three populations in mainland China (GS, LS and LC) are protected *in situ*. Despite being situated within a protected area, population LC has been severely affected by land reclamation, and should therefore be accorded a high priority for future conservation action. As for *ex situ* conservation in botanical gardens, further reduction of genetic variability by genetic drift should be avoided, therefore one additional measure could be artificial gene flow between populations, i.e., artificial introductions should include as many populations as possible.

Acknowledgments

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Genetic Variation Within Two Sympatric Spotted Gum Eucalypts Exceeds Between Taxa Variation

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Abstract

Population substructure and hybridization, among other factors, have the potential to cause erroneous associations in linkage disequilibrium (*LD*) mapping. Two closely related spotted gum eucalypts, *Corymbia variegata* and *C. henryi* (Myrtaceae) occur in sympatry in the east coast of Australia and potentially interbreed. They are morphologically similar but are distinguished as separate species based on capsule and foliage size. To determine whether they hybridize in nature and its implications for *LD* mapping, we investigated the level of molecular divergence between the two species at two sympatric locations separated by 300 kilometres. Very few individuals of intermediate morphology were identified, despite the two species occurring only metres apart. Analysis of genetic structure using 12 microsatellite loci showed that genetic differentiation between populations of the same species at different locations ($F_{ST} = 0.07$ for both species; $p = 0.0001$) was significantly higher than that observed between species at each location (mean $F_{ST} = 0.02$ and 0.04 for Cherry tree and Bunyaville respectively; $p = 0.0001$; all Mann-Whitney *U-test* $p \leq 0.01$). No species-specific alleles or significant allele frequency differences were detected within a site, suggesting recurr#ent local gene flow between the two species. The lack of significant allele frequency differences implies no population stratification along taxonomic lines. This suggested that there is little concern for cryptic hybridization when sampling from sites of sympatry for *LD* mapping.

Key words: panmixia, hybridization, gene flow, association mapping, population structure, admixture, reproductive isolation, linkage disequilibrium.

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