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High Genetic Diversity *vs*. Low Genetic Differentiation in *Nouelia insignis* (Asteraceae), a Narrowly Distributed and Endemic Species in China, Revealed by ISSR Fingerprinting

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• *Background and Aims Nouelia insignis* Franch., a monotypic genus of the Asteraceae, is an endangered species endemic in Yunnan and Sichuan Provinces of China. Most of the populations are seriously threatened. Some of them are even at the brink of extinction. In this study, the genetic diversity and differentiation between populations of this species were examined in two drainage areas.
• *Methods* DNA fingerprinting based on inter-simple sequence repeat polymorphisms was employed to detect the genetic variation and population structure in the species.
• *Key Results* Genetic diversity at species level was high with $P = 65{\cdot}05\,\%$ (percentage of polymorphic loci) and $H_t = 0{\cdot}2248$ (total genetic diversity). The coefficient of genetic differentiation among populations, G_{st}, which was estimated by partitioning the total gene diversity, was 0·2529; whereas, the genetic differentiation between populations in the Jinsha and Nanpan drainage areas was unexpectedly low ($G_{st} = 0{\cdot}0702$).
• *Conclusions* Based on the genetic analyses of the DNA fingerprinting, recent habitat fragmentation may not have led to genetic differentiation or the loss of genetic diversity in the rare species. Spatial apportionment of fingerprinting polymorphisms provides a footprint of historical migration across geographical barriers. The high diversity detected in this study holds promise for conservation and restoration efforts to save the endangered species from extinction.

Key words: Conservation, gene flow, genetic diversity, genetic differentiation, ISSR fingerprinting, *Nouelia insignis*.

INTRODUCTION

Nouelia insignis Franch., a monotypic genus of the Mutisieae (Asteraceae), is restricted in distribution to the Jinsha and Nanpan drainage areas in south-western China. This region has been recognized as a biodiversity hotspot that maintains high species diversity and is characterized by high endemism in the floristic composition. About 8000 species of flowering plants have been recorded (Li and Li, 1993; Wang *et al*., 1993; Wang and Zhang, 1994), of which numerous genera and species, e.g. *Nouelia insignis*, *Trailliaedoxa gracilis* W. W. Sm. et Forrest (Rubiaceae) and *Acanthochlamys bracteata* P. C. Kao (Velloziaceae), are indigenous to the region. Floristic characteristics of the hoptspot include adaptive radiation with a large number of plant groups, and the occurrence of many relict plant lineages (Wu, 1988; Wilson, 1992; Li and Li, 1993; Ying *et al*., 1993; Wang and Zhang, 1994; Boufford and van Dijk, 1999; Sun, 2002). The area has been referred to as the South-Central China Hotspot (Myers *et al*., 2000) or the Hengduan Mountains Hotspot (Boufford and van Dijk, 1999). Undoubtedly, it provides a natural laboratory for studying the origin and conservation of biodiversity.

Nouelia insignis grows in dry valleys, ranging from 1000 to 2800 m a.s.l., in northern Yunnan and western Sichuan Provinces (Wang, 1989). The species is a diploid shrub ($2n = 54$) (Peng *et al*., 2003) with abundant branches and a height of 3–5 m. In recent decades, *Nouelia insignis* has become endangered due to rapid habitat destruction and fragmentation. Most of the extant populations consist of fewer than 50 individuals. According to observations made for this study, no more than 5000 individuals survive in the wild. Moreover, this species suffers from reproductive failure because of low seed productivity and seed germination rates. Accordingly, very few seedlings could be located in the natural habitats.

Declining populations are predicted to possess low levels of genetic diversity because of the high probability of inbreeding within populations driven by random genetic drift (Ellstrand and Elam, 1993). In this species, given both small population number and size, genetic diversity is likely to be reduced. As long-term population viability is associated with the levels of genetic variability (Barrett and Kohn, 1991), the erosion of genetic diversity would eventually harm the health of populations and species. Considering the unusual woody life-form and limited population number and size, this endemic and rare Asteraceae species should be assigned a high priority for protection.

Genetic variation at the intraspecies level is a prerequisite for future adaptive change or evolution, and has profound implications for species conservation

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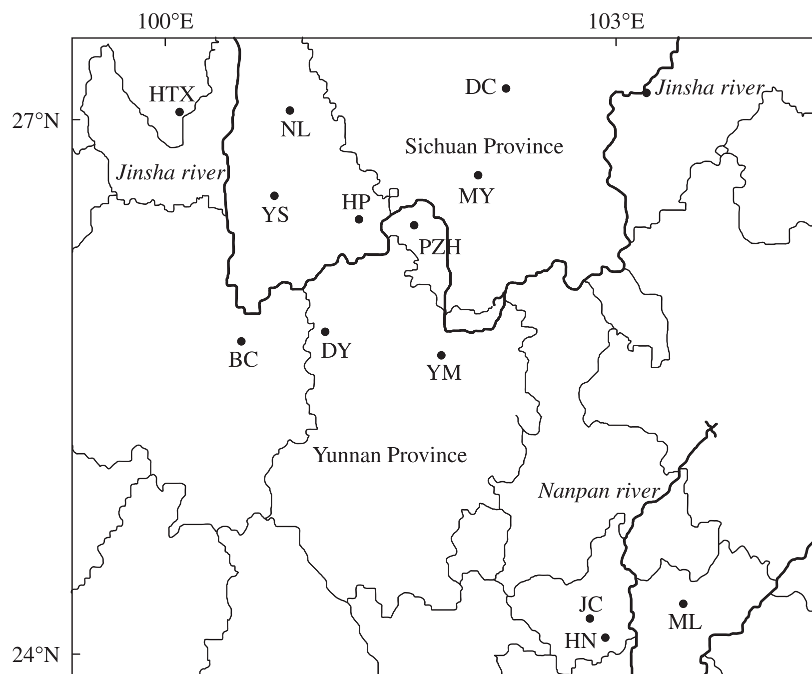

FIG. 1. Map showing locations of the populations of *Nouelia insignis* sampled. Population symbols follow Table 1.

(Schaal *et al.*, 1991). Understanding genetic variation within and between populations is essential for the establishment of effective and efficient conservation practices for rare species. Several aspects of conservation biology, such as loss of genetic diversity in conservation programmes and restoration of threatened population, can only be addressed by detailed population genetic studies (Hamrick and Godt, 1996). So far, the genetic variation of *Nouelia insignis* has been estimated based on allozyme analysis (Peng *et al.*, 2003). Because the number of allozyme loci that can be probed is limited, and they only correspond to coding sequences, allozyme variation may not be able to provide an accurate or complete measure of nucleotide variation in the genome (Clegg, 1990; Xiao *et al.*, 2004). In contrast, molecular fingerprinting allows noncoding DNA sequences to be examined, thereby providing more penetrating insights into population genetic structures. Among DNA fingerprinting techniques, inter-simple sequence repeats (ISSR) represent noncoding sequences between simple sequence repeats, which supply an effective means to assess genetic diversity. ISSR studies of natural populations demonstrated a hyper-variable nature of these DNA fingerprints and their potential use for population-level studies (Ge and Sun, 1999; Culley and Wolfe, 2001).

The objectives of this study are to examine the levels of ISSR variation and genetic differentiation among *Nouelia insignis* populations between different drainage areas. Based on genetic analyses, the evolutionary factors that influenced the spatial apportionment of genetic diversity are estimated. This molecular information will provide effective and efficient measures for protecting *Nouelia insignis*.

TABLE 1. *Sites and sample size for 13 populations of* Nouelia insignis *examined in the ISSR analysis*

Code	Drainage	Population	Sample size	Latitude (N)	Longitude (E)
BC	JS	Bingchuan	17	25°50'	100°36'
DC	JS	Dechang	17	27°38'	102°17'
DY	JS	Dayao	17	25°51'	101°06'
HP	JS	Huaping	17	26°35'	101°21'
HTX	JS	Hutiaoxia	17	27°19'	100°08'
MY	JS	Miyi	17	26°54'	102°13'
NL	JS	Ninglang	17	27°21'	100°51'
PZH	JS	Panzhihua	17	26°24'	101°46'
YM	JS	Yuanmou	17	25°46'	101°50'
YS	JS	Yongsheng	17	26°34'	100°48'
HN	NP	Huaning	17	24°17'	102°51'
JC	NP	Jiangchuan	16	24°21'	102°43'
ML	NP	Mile	17	24°41'	103°40'

MATERIALS AND METHODS

Sample collection

Ten populations from the Jinsha drainage area and three extant populations (JC, HN and ML) from the Nanpan drainage area were surveyed (Fig. 1). Of these populations, PZH, MY and DC are distributed in Sichuan Province, while the others occur in Yunnan Province. From each population, leaf tissue of 17 individuals (only 16 from the Jiangchuan population) was randomly sampled (Table 1). In total, young, healthy leaflets of 220 individuals were collected, dried with silica gel and stored at 4 °C until they were processed.

DNA extraction and polymerase chain reaction (PCR)

Leaf tissue of the above materials was ground to a powder in liquid nitrogen. Genomic DNA was extracted

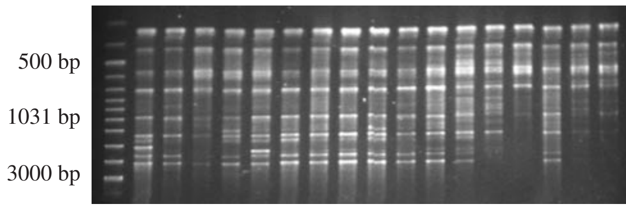

FIG. 2. Electrophoresis of PCR products amplified with primer UBC # 857 for the Ninglang population.

TABLE 2. *List of ISSR primers and the sequences used in this study, number of reliable bands and polymorphic bands of each primer generated and the* Nm *value for each primer*

Primer code	Nucleotide sequence 5′ to 3′	No. of bands analysed	No. of polymorphic bands	*Nm*
807	$(AG)_7T$	11	7	1·0835
811	$(GA)_8C$	9	7	0·8086
822	$(TC)_8A$	6	3	0·7178
827	$(AC)_8G$	13	10	0·5656
840	$(GA)_8YT$	11	8	0·6921
855	$(AC)_8YT$	9	7	1·2411
856	$(AC)_8YA$	10	6	0·5735
857	$(AC)_8YG$	9	6	0·6254
881	$(GGGGT)_3$	10	8	0·6103
889	$DBD(AC)_7$	7	3	1·9153
890	$VHV(GT)_7$	8	2	1·1687
Total		103	67	

Y = (A, C); D = (A, G, T); *B* = (C, G, T); *H* = (A, C, T); *V* = (A, C, G).

from the powdered tissue following CTAB procedures (Doyle, 1991). The DNA was amplified with PCR using ISSR primers from the University of British Columbia (UBC). Of 100 ISSR primers, 11 produced clear and reproducible bands, and were selected for the subsequent experiment (Fig. 2 and Table 2). PCR amplification was carried out in a total volume of 20 µL, consisting of 20 ng template DNA, 2·0 µL 10× PCR buffer, 2·2 mM $MgCl_2$, 0·12 mM dNTPs, 3 % formamide, 400 nM primer, 0·5 U of *Taq* polymerase (Takara) and double-distilled water. PCR was programmed on an ABI Prism Reaction Dye Terminator Cycle with a hot bonnet (Perkin-Elmer) as one cycle of denaturation at 94 °C for 5 min, 38 cycles of 30 s denaturation at 94 °C, 1 min 30 s annealing at 53 °C, and 1 min extension at 72 °C, followed by a 7-min extension at 72 °C. A negative control, in which template DNA was omitted, was included in every PCR protocol for testing contamination. Amplification products were electrophoretically separated on 1·5 % agarose gels buffered with 0·5× TBE. A DNA ladder was applied as a size marker (100–2500). After staining with ethidium bromide for 30 min, DNA fragments were identified by image analysis software for gel documentation. Only those gels that showed consistent and clear bands were considered; while those smeared and weak were excluded.

Data analysis

ISSR bands were scored as 1 (present) or 0 (absent) binary characters. The software program POPGENE v. 1·31 (Yeh *et al*., 1999) was used to obtain the genetic diversity parameters, percentage of polymorphic loci (P), allele number per locus (A), effective allele number per locus (A_e), and expected heterozygosity (H_e). Genetic diversity measures (H_t, total gene diversity; G_{st}, coefficient of gene differentiation) were tested using Nei's (1973) gene diversity statistics. Nei's genetic identity, I ranging from 0·0 to 1·0, and distance D, ranging from 0.0 to infinity, were computed for each population pair. The Shannon diversity index was calculated as $H_o = -\Sigma P_i \log_2 P_i$ (Lewontin, 1972), in which P_i represents the frequency of the given ISSR fragments. This index was then used to measure the total diversity (H_{sp}) and the mean intra-population diversity (H_{pop}).

The proportion of diversity among populations was calculated as $(H_{sp} - H_{pop})/H_{sp}$. AMOVA (analysis of molecular variance) was also employed to estimate the hierarchical apportionment of variation. Additionally, to test for putative correlations between genetic distances (D) and geographical distances among populations, a Mantel test was performed with Tools for Population Genetic Analysis (Miller, 1997), by computing 10 000 permutations, and the Arlequin program (Schneider *et al*., 2000) was used to test the significant values of pairwise genetic distances.

The gene flow estimates (Nm) were calculated as $Nm = (1 - G_{st})/4G_{st}$ (Slatkin and Barton, 1989), where Nm is the number of migrants per generation. The significance of the association between G_{st} and geographical distance was determined by a regression F-test using the SPSS program (Norusis, 1994). A UPGMA dendrogram based on unbiased genetic distance (Nei, 1972) was generated using POPULATIONS (Langella, 2000). Bootstrapping based on the fingerprinting data was conducted with 1000 replicates.

RESULTS

Genetic diversity

In this study, genetic diversity was examined in *Nouelia insignis*, based on ISSR fingerprinting. In total, 11 primers produced 103 reliable ISSR bands, of which 67 (65·05 %) were polymorphic. The percentage of polymorphic loci (P) averaged 36·89 with a range from 31·07 % (NL) to 41·75 % (YM and HP) at the population level. The mean number of alleles (A) per locus was 1.65, while the effective number (A_e) was 1.33. Assuming Hardy–Weinberg equilibrium, the expected heterozygosity within populations (H_{ep}) was 0·1491 (Table 3) on average, and the genetic diversity within the species (H_{es}) was 0·2248. Shannon indices (I) were 0·2245 and 0·3024, respectively. Among the 13 populations examined in this study, population YM had the highest level of variability, with H_e = 0·1820 and I = 0·2614, while the Dayao population exhibited the lowest diversity, with H_e = 0·1265 and I = 0·1842. No private bands were detected in either

TABLE 3. *Genetic variability within populations of* Nouelia insignis *revealed by ISSR analysis*

Population	P	H_e	H_o
BC	34·95	0·1488 (0·2083)	0·2148 (0·2980)
DC	32·04	0·1311 (0·2015)	0·1899 (0·2857)
DY	32·04	0·1265 (0·1975)	0·1842 (0·2824)
HP	41·75	0·1771 (0·2174)	0·2554 (0·3096)
HTX	39·81	0·1658 (0·2200)	0·2377 (0·3102)
MY	33·01	0·1405 (0·2050)	0·2029 (0·2937)
NL	31·07	0·1352 (0·2065)	0·1940 (0·2938)
PZH	39·81	0·1659 (0·2124)	0·2402 (0·3036)
YM	41·75	0·1820 (0·2195)	0·2614 (0·3130)
YS	34·95	0·1419 (0·2045)	0·2062 (0·2920)
JC	37·86	0·1650 (0·2174)	0·2367 (0·3089)
ML	34·95	0·1328 (0·1948)	0·1957 (0·2808)
HN	33·01	0·1260 (0·1912)	0·1857 (0·2767)
Mean	35·92 (8·74)	0·1491 (0·0560)	0·2158 (0·1857)

P is the percentage of polymorphic loci; H_e is the expected heterozygosity; H_o is the observed heterozygosity. Values in brackets are s.d.

population. That is, the ISSR divergence among populations was mainly attributed to differences of the DNA-fragment frequency rather than allele fixation. When the populations within the Jinsha or Nanpan drainage area were pooled together, the percentage of polymorphic loci of group Jinsha was 62·14, and of group Nanpan was 48·54. Other indexes supplied by POPGENE also indicated that group Jinsha had higher genetic diversity than Nanpan. Taking Shannon's diversity index as an example, the value was 0·3398 and 0·2783, respectively, for the two groups with a mean value of 0·3090.

Genetic divergence

Genetic analysis showed that the highest identity (0·9746) existed between populations BC and MY, while the lowest (0·8918) occurred between populations HTX and HN (Table 4). The percentage of the total genetic variation found among populations (G_{st} = 0·2529) was moderate, which means 74·71 % of the variation existed within populations. This result was further confirmed by AMOVA analysis among populations, which showed that 22·25 % of the variance existed among populations.

No significant genetic differentiation was found between the populations of Jinsha and Nanpan drainage areas. The level of genetic differentiation between these two areas was low, as revealed by G_{st} = 0·0702. Deduced from the G_{st} value, the level of gene flow (Nm) was estimated at 3·3130, indicating a very high migration rate between populations of the two drainage areas. These results agreed with the genetic structure estimated by Shannon's diversity index and AMOVA analysis, with 10·38 % and 13·10 % (Table 5) of the genetic variation existing between areas, respectively. A Mantel test showed a positive correlation between genetic differentiation and geographical distance (P = 0·002) (Fig. 3).

A UPGMA dendrogram (Fig. 4) was reconstructed based on the pairwise genetic distances between populations, none of which was identified as significant based on the Arlequin analysis. In total, two major clusters were identified, i.e. (ML, HN) *vs*. all others, of which (YM, DY), [YS, PZH, (HP, HTX)] and [(NL, JC), (DC, (BC, MY))] were further clustered. Nevertheless, only the clustering of ML and HN was significantly supported with a bootstrap value of 99 %; while all others were not unambiguously resolved.

DISCUSSION

High levels of genetic diversity within the rare species

In this present study, the genetic variance within *Nouelia insignis* was investigated. Despite its rarity, unexpectedly high levels of genetic diversity (P = 65·05 %, H_{ep} = 0·1491, H_e = 0·2248) were detected, as compared with a previous allozyme investigation, with A = 1·6, P = 37·5 %, H_o = 0·143, H_e = 0·141 in Yunnan populations (Peng *et al.*, 2003), and also to the means of allozyme variation for 100 endemics (P = 40·0 %, H_{ep} = 0·096, H_{es} = 0·063) (Hamrick and Godt, 1989). Generally, species with small geographic ranges tend to maintain less genetic diversity than geographically widespread species (Hamrick and Godt, 1989). However, exceptions are not uncommon (Gitzendanner and Soltis, 2000; Lopez-Pujol *et al.*, 2002; Wang *et al.*, 2004). For example, *Castilleja levisecta* is a rare species, with a distribution restricted to two island populations of British Columbia and nine populations in Washington State. Despite its small range and the few extant populations, this species has high genetic diversity with P = 100 %, H_{ep} 0·285 and H_{es} = 0·213 (Godt *et al.*, 2005).

High genetic diversity maintained in rare plants is attributable to a number of factors (Zawko *et al.*, 2001), such as recent reduction of population size plus insufficient time for isolation, or extensive, recurrent gene flow (Maguire and Sedgley, 1997; Chiang *et al.*, 2006). Field observations showed that plants of *Nouelia insignis* are perennials pollinated by a small bee-fly, which largely promotes outcrossing. In addition, sepals of this species develop specialized pappi, a structure facilitating fruit dispersal via wind. Both mechanisms help to maintain genetic polymorphisms across populations, even in this rare species.

Population genetic theory predicts that larger populations tend to maintain higher allelic diversity (Hedrick, 1985; Ellstrand and Elam, 1993). High levels of genetic diversity based on allozyme and ISSR evidence suggest a large effective population size of the species. However, populations of *Nouelia insignis* in the Jinsha drainage area remain fragmented in the wild and are separated from each other by less than 50 km. The rarity of this long-life perennial species is likely to be attributable to large-scale habitat destruction caused by human activities. During the past few decades, this plant has been over-exploited for firewood use, inevitably causing a dramatic decline of this species. Many plants now are scattered along roadsides where habitats are accessible. Such habitat fragmentation and population isolation of *Nouelia insignis* in the Jinsha drainage area may have occurred only recently as indicated by high levels of genetic diversity and the lack

TABLE 4. *Nei's pairwise genetic identity (above diagonal) and genetic distance (below diagonal) between* Nouelia insignis *populations*

Population	BC	DC	DY	HP	HTX	MY	NL	PZH	YM	YS	HN	JC	ML
BC	–	0·9598	0·9524	0·9430	0·9457	0·9746	0·9479	0·9467	0·9367	0·9358	0·9301	0·9449	0·9225
DC	0·0410	–	0·9418	0·9352	0·9420	0·9740	0·9627	0·9482	0·9303	0·9361	0·9227	0·9398	0·9110
DY	0·0654	0·0600	–	0·9458	0·9402	0·9554	0·9519	0·9540	0·9524	0·9363	0·9233	0·9415	0·9443
HP	0·0586	0·0546	0·0558	–	0·9684	0·9469	0·9459	0·9703	0·9437	0·9624	0·8962	0·9474	0·9197
HTX	0·0558	0·0597	0·0616	0·0321	–	0·9363	0·9444	0·9592	0·9246	0·9611	0·8918	0·9287	0·8995
MY	0·0257	0·0264	0·0456	0·0546	0·0659	–	0·9563	0·9554	0·9514	0·9447	0·9336	0·9612	0·9291
NL	0·0535	0·0380	0·0493	0·0557	0·0572	0·0447	–	0·9487	0·9357	0·9576	0·9428	0·9603	0·9397
PZH	0·0548	0·0532	0·0471	0·0302	0·0417	0·0423	0·0527	–	0·9458	0·9608	0·9118	0·9467	0·9200
YM	0·0654	0·0723	0·0488	0·0579	0·0784	0·0498	0·0665	0·0557	–	0·9356	0·9125	0·9327	0·9245
YS	0·0664	0·0661	0·0658	0·0383	0·0397	0·0569	0·0433	0·0400	0·0666	–	0·9007	0·9440	0·9013
HN	0·0724	0·0804	0·0798	0·1096	0·1145	0·0687	0·0589	0·0924	0·0916	0·1045	–	0·9531	0·9483
JC	0·0566	0·0621	0·0603	0·0541	0·0739	0·0395	0·0406	0·0548	0·0697	0·0573	0·0480	–	0·9587
ML	0·0807	0·0932	0·0573	0·0837	0·1059	0·0736	0·0622	0·0834	0·0785	0·1039	0·0531	0·0422	–

TABLE 5. *Nested AMOVA for* Nouelia insignis

Source of variance	d.f.	Sum of squares	Mean squares	Variance component	% total variance	*P* value
Among areas	1	114·1001	114·100	1·113	13·10	<0·0010
Among populations within areas	11	311·5356	28·321	1·314	15·46	<0·0010
Within populations	207	1256·7279	6·071	6·071	71·44	<0·001

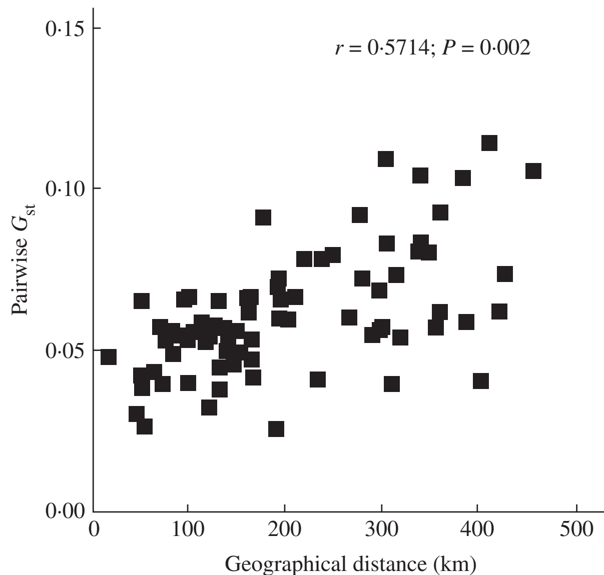

FIG. 3. Correlation between pairwise G_{st} of *Nouelia insignis* and geographical distance.

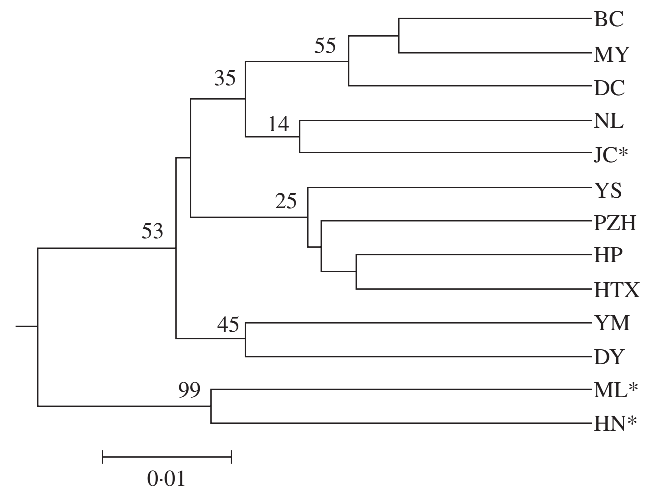

FIG. 4. UPGMA dendrogram based on Nei's (1972) genetic distance. * Populations found in the Nanpan drainage area.

of genetic differentiation. In other words, the present isolated and fragmented populations were likely to be derived from a previously large population.

Lack of genetic differentiation between populations and between geographical regions

The percentage of total genetic variation among populations (G_{st}) was 25·29 %, which is close to the mean G_{st} values found for 52 endemic plants (25 %) (Godt *et al*., 2005). Within Jinsha and Nanpan drainage areas, a positive correlation (P = 0·002) was found between genetic distance and geographic distance, agreeing with an 'isolation by distance' model, which suggests that the genetic exchangeability is constrained between geographically distant populations. In contrast, as the possibilities of pollen and seed flow across the long distance between the two geographical regions, which is over 150 km, are expected to be low, high *Nm* values were unexpectedly detected between the Jinsha and Nanpan groups (3·3130), revealing an unusual migratory mode deviated from the regular stepping-stone, Wright's island, or isolation by-distance models (Hamrick and Nason, 1996).

It is known that, as current gene flow is limited between populations, high *Nm* values estimated from genetic structure tend to represent historical genetic exchange, and are not indicative of current migration rates. For rare species, it is of greater concern when gene flow estimates are high since they may reflect previous intermingling of

populations; and they should not be interpreted as indicating the present state of population isolation (Godt *et al*., 2005). In this study, the high estimated *Nm* values between populations, plus high levels of genetic diversity within geographical regions, suggest a likely migrant-pool migratory model (Wade and McCauley, 1998), which describes a migratory pattern with colonists recruited from a random sample of previously existing populations. This model is usually associated with glaciation or vicariance events (Huang *et al*., 2001; Chiang and Schaal, 2006).

According to the geological evidence, following the uplifting of the Yunnan-Guizhou Plateau, the climate in this region has remained relatively steady since the Tertiary (Peng *et al*., 2003) in the Jinsha drainage area. The refugia thereby provided shelters for *Nouelia insignis* during the Quaternary glaciations. Subsequently, as Pleistocene glaciers retreated, plants from refugia expanded and colonized newly available habitats (Hewitt, 1996, 2001). Random samples of *Nouelia insignis* thereby migrated into Nanpan from the Jinsha drainage area. The high level of deduced gene flow between these two areas may reflect such ancient expansion events. Furthermore, the UPGMA dendrogram (Fig. 4), revealing a close phenetic relationship between population JC of the Nanpan drainage area and all populations of the Jinsha drainage area, also supports such historical migration.

Implications for conservation

The maintenance of genetic variation is one of the major objectives for conserving endangered and threatened species (Avise and Hamrick, 1996). Knowledge of genetic variation between and within populations provides essential information in the formulation of appropriate management strategies directed towards their conservation (Milligan *et al*., 1994). From the results obtained from this study, it is possible to draw inferences on the conservation of *Nouelia insignis*. Severe habitat loss and fragmentation predominantly ascribed to anthropogenic pressures caused local extinction of this species. The reduced size of most extant populations makes the species susceptible to the loss of genetic polymorphisms shaped by effects of random genetic drift and inbreeding.

Action for conserving this rare species for its long-term survival should be taken immediately. High levels of genetic polymorphisms maintained in this species ensure the conservation practices, despite the limited size of most populations. Among the extant populations, population YS has the largest size, with about 1000 individuals. Therefore, this population should be considered as the high priority for *in-situ* conservation to expand the population range and enlarge the population size. Additionally, adult individuals in the JC population were much taller than those in other populations, i.e. 8–20 m *vs*. <5 m. Besides, the age structure of this population is much healthier than others, altogether indicating less anthropogenic disturbance in JC. This population is distributed along slopes of a small mountain, where a Buddhist Biyun Temple is located. Proximity to the temple may have protected plants from human exploitation and ensured survival of a great number of old individuals of *Nouelia insignis*. Therefore, introduction of foreign germplasm to enhance the genetic diversity of this population may be a good way to protect this species as well.

In contrast, as *ex-situ* conservation is conducted, sampling from Jinsha drainage area may be sufficient considering the genetic diversity of the Jinsha group is higher than the Nanpan group. The absence of private DNA bands suggests that the Jinsha population can provide almost all the genetic polymorphisms of this species. In addition, because of its long life-cycle, seed collection to preserve the germplasm resource in botanical gardens or other institutions is also practical. The safest way may be to use a combination of the three methods mentioned above to protect the major components of genetic diversity of this species.

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LITERATURE CITED

Avise JC, Hamrick JL. 1996. *Conservation genetics: case histories from nature*. New York, NY: Chapman and Hall.

Barrett SCH, Kohn JR. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE, eds. *Genetics and conservation of rare plants*. New York, NY: Oxford University Press, 3–30.

Boufford DE, van Dijk PP. 1999. South-central China: In: Mittermeier RA, Myers N, Robles Gil P, Mittmeier CG. *Hotspots: earth's biologically richest and most endangered terrestrial ecoregions.* Mexico City, Mexico: CEMEX, 338–35.

Chiang TY, Schaal BA. 2006. Phylogeography of plants in Taiwan and the Ryukyu Archipelago. *Taxon* **51**: 31–41.

Chiang YC, Hung KH, Schaal BA, Ge XJ, Hsu TW, Chaing TY. 2006. Contrasting phylogeographical patterns between mainland and island taxa of the *Pinus luchuensis* complex. *Molecular Ecology* **15**: 765–779.

Clegg MT. 1990. Molecular diversity in plant populations. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sunderland, MA: Sinauer Associates, 98–115.

Culley TM, Wolfe AD. 2001. Population genetic structure of the cleitogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity* **86**: 545–556.

Doyle J. 1991. DNA protocols for plants—CTAB total DNA isolation. In: Hewitt GM, Johnston A, eds. *Molecular techniques in taxonomy*. Berlin: Springer, 283–293.

Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review Ecology and Systematics* **24**: 217–242.

Ge XJ, Sun M. 1999. Reproductive biology and genetic diversity of a cryptoviviparous mangrove *Aegiceras corniculatum* (Myrsinaceae) using allozyme and intersimple sequence repeat (ISSR) analysis. *Molecular Ecology* **8**: 2061–2069.

Gitzendanner MA, Soltis PS. 2000. Patterns of genetic diversity and population structure in *Tradescantia hirsuteicaulis* (Commeliniaceae). *American Journal of Botany* **80**: 959–966.

Godt MJW, Caplow F, Hamrick JL. 2005. Allozyme diversity in the federally threatened golden paintbrush, *Castilleya levisecta* (Scrophulariaceae). *Conservation Genetics* **6**: 87–99.

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 resourses*. Sunderland, MA: Sinauer Associates, 43–63.

Hamrick JL, Godt MJW. 1996. Conservation genetics of endemic plant species. In: Avise JC, Hamrick JL, eds. *Conservation genetics: case histories from nature.* New York, NY: Chapman and Hall, 281–304.

Hamrick JL, Nason JD. 1996. Consequences of dispersal in plants. In: Rhodes Jr OE, Chesser RK, Smith MH, eds. *Population dynamics in ecological space and time*. Chicago, IL: Chicago University Press, 203–236.

Hedrick PW. 1985. *Genetics of populations*. Boston, MA: Jones and Bartlett.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247–276.

Hewitt GM. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. *Molecular Ecology* **10**: 537–549.

Huang S, Chiang YC, Schaal BA, Chou CH, Chiang TY. 2001. Organelle DNA phylogeography of *Cycas taitungensis*, a relict species in Taiwan. *Molecular Ecology* **10**: 2669–2681.

Langella O. 2000. *POPULATIONS (Logiciel de Genetique Des Populaitons)*. Paris: Centre National de la Recherche Scientifique.

Lewontin RC. 1972. The apportionment of human diversity. *Evolutionary Biology* **6**: 381–398.

Li XW, Li J. 1993. A preliminary floristics study on the seed plants from the region of Hengduan Mountain. *Acta Botanica Yunnanica* **15**: 217–231.

Lopez-Pujol J, Bosch M, Simon J, Blanche C. 2002. Allozyme variation and population structure of the very narrow endemic *Seseli farrenyi* (Apiaceae). *Botanical Journal of the Linnean Society* **138**: 305–314.

Maguire TL, Sedgley M. 1997. Genetic diversity in *Banksia* and *Dryandra* (Proteaceae) with emphasis on *Banksia cuneata*, a rare and endangered species. *Heredity* **79**: 394–401.

Miller MP. 1997. *Tools for population genetic analysis* (TFPGA), Version 1.3. Flagstaff, AZ: Department of Biological Science, Northern Arizona University.

Milligan BG, Leebens-Mack J, Strand AE. 1994. Conservation genetics: beyond the maintenance of marker diversity. *Molecular Ecology* **12**: 844–855.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GA da, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

Nei M. 1972. Genetic distance between populations. *Nature* **106**: 283–292.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the USA* **70**: 3321–3323.

Norusis MJ. 1994. *SPSS for Windows 6·0*. Englewood Cliffs, NJ: Prentice Hall.

Peng YL, Hu YQ, Sun H. 2003. Allozyme anaysis of *Nouelia insignis* and its meanings of biogeography and conservation biology. *Acta Botanica Yunnanica* **25**: 563–571.

Schaal BA, Leverich WJ, Rogstad SH. 1991. Comparison of methods for assessing genetic variation in plant conservation biology. In: Falk DA, Holsinger KE, eds. *Genetics and conservation of rare plants*. New York, NY: Oxford University Press, 123–134.

Schneider S, Roessli D, Excoffier L. 2000. *ARLEQUIN, Version 2.000: a software for population genetics data analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva.

Slatkin M, Barton NH. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**: 1349–1368.

Sun H. 2002. Tethys retreat and Himalayas-Hengduanshan Mountains uplift and their significance on the origin and development of the Sino-Himalayas elements and alpine flora. *Acta Botanica Yunnanica* **24**: 273–288.

Wade MJ, McCauley DE. 1998. Extinction and recolonization: Their effects on the genetic differentiation of local populations. *Evolution* **42**: 995–1005.

Wang HS. 1989. Origins of endemic genera of vascular flora of China. *Acta Botanica Yunnanica* **11**: 1–16.

Wang HS, Zhang YL. 1994. The biodiversity and characters of spermatophytic genera endemic to China. *Acta Botanica Yunnanica* **16**: 209–220.

Wang WT, Wu SG, Lang KY, Li PQ, Pu FT, Chen SK. 1993. *Vascular plants of the Hengduan Mountains*. Vol. 1. *Pteridophyta, Gymnospermae, Dicotyledoneae (Saururaceae to Cornaceae)*. Beijing: Science Press.

Wang ZF, Hamrick JL, Godt MJW. 2004. High genetic diversity in *Sarracenia leucophylla* (Sarraceniaceae), a carnivorous wetland herb. *Heredity* **95**: 234–243.

Wilson EO. 1992. *The diversity of life*. Cambridge, MA: Belknap Press of Harvard University Press.

Wu CY. 1988. Hengduan Mountains flora and its significance. *Journal of Japanese Botany* **63**: 297–311.

Xiao LQ, Ge XJ, Gong X. 2004. ISSR variation in the endemic and endangered plant *Cycas guixhouensis* (Cycadaceae). *Annals of Botany* **94**: 133–138.

Yeh FC, Yang RC, Boyle T. 1999. *POPGENE. Microsoft Windows-based freeware for population genetic analysis.* Release 1.31. Edmonton: University of Alberta.

Ying TS, Boufford DE, Zhang YL. 1993. *The endemic genera of seed plants of China*. Beijing: Science Press.

Zawko G, Krauss SL, Dixon KW, Sivasithamparam K. 2001. Conservation genetics of the rare and endangered *Leucopogon obtetus* (Ericaceae). *Molecular Evolution* **10**: 2389–2396.