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INFERRING GENETIC DIVERSITY AND DIFFERENTIATION OF THE ENDANGERED CHINESE ENDEMIC PLANT *SAUVAGESIA RHODOLEUCA* (OCHNACEAE) USING MICROSATELLITE MARKERS

XIAO-YI LI[1, 2, 3], ZONG-YOU CHEN[1*], ZHENG-FENG WANG[2],HONG-LIN CAO[2], XIAO WEI[1], YUN-SHENG JIANG[1] AND SHENG-FENG CHAI[1]

[1] *Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuangzu Autonomous Region and the Chinese Academy of Sciences, Guilin 541006, P.R. China*
[2] *Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems & Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, P. R. China*
[3] *Graduate University of the Chinese Academy of Sciences, Beijing 100049, P.R. China*
[*]*Corresponding author's e-mail: chenzong_7839@qq.com*

Abstract

Sauvagesia rhodoleuca is one of the most endangered species in China. It has a narrow distribution in the evergreen broadleaved forest of southern China. Up to now, only six populations remained in two provinces. In this study, eight microsatellite loci were used to examine genetic diversity in these populations. We found very low levels of genetic diversity within populations of *S. rhodoleuca* with average observed and expected heterozygosity (H_O and H_E) of 0.069 and 0.186, respectively. Estimated inbreeding coefficients (F_{IS}) within populations were high suggests the probable selfing in the species.Combination of the UPGMA dendrogram and the INSTRUCT analysis show that six extant populations could be classified into three distinct genetic groups and no pattern of isolation by distance was detected among populations. The low genetic variation within populations and high genetic differentiation among populations indicate that the management for the conservation of genetic diversity in *S. rhodoleuca* should aim to preserve every population.

Key words: *Sauvagesia rhodoleuca*, Genetic diversity, Genetic differentiation, Selfing, Conservation.

Introduction

Medicinal plants play a critical role in health care, especially in developing countries. Out of 26092 native plant species in China, 4941 are used as drugs in Chinese traditional medicine (Duke & Ayensu, 1985). Currently, many medicinal plant species are severely threatened by over exploitation and habitat loss due to deforestation. Anthropogenic destruction would lead to severe loss of genetic variation, particular for endangered species (Young *et al*., 1996; Wang *et al*., 2006). Thus, the identification of genetic diversity and genetic structure is an important step to establish management plans to preserve genetic resources and biodiversity.

Population genetic analysis provides information on the level of genetic diversity, knowledge of which is fundamental for conservation biology (Hamrick *et al*., 1991; Frankham *et al*., 2002; Leimu *et al*., 2006; Al-Qurainy *et al*., 2014; Chen *et al*., 2010). In theory, a high level of population genetic diversity in a species allows to better adapt to environment changes and determines its evolutionary capacity (Frankham *et al*., 2002). However, endemic plant species with narrow distribution range (Barrett & Kohn, 1991), small population size (Hamrick & Godt, 1996) and short dispersal (Willi *et al*., 2006) usually possess low levels of genetic variation and high levels of genetic differentiaton. Low genetic diversity would consequently reduce specises' reproductive fitness and evolutionary potential and, ultimately, lead to extinction (Frankham, 2002; Spielman *et al*., 2004).

Nanling Mountain Range, the most largest mountain range in south of China, is situated in the region of boundaries among four provinces (Guangdong, Guangxi, Hunan, Jiangxi Province). It harbors a subtropical flora and is characteristic of floristic transition from tropical to temperate (Chen & Chang, 1994). In addition, it is partly located inside the Mountains of South-Central China biodiversity hotspot (Myers *et al*., 2000). A large number of plant species endemic to the Nanling Mountain Range or rare to China, are threatened or endangered, mainly due to on-going human activities such as excessive exploitation and deforestation (Su & Liao, 1999), like *Sauvagesia rhodoleuca*. So far, few studies have been conducted with regard to conservation genetics of plants in this hotspot, particularly for endemic species (Li *et al*., 2002).

Family Ochnaceae is widely distributed in tropical areas and comprises 35 genera among which *Sauvagesia* is one of the most important genera in studies of biogeography and phylogeny (Amaral, 1991). *Sauvagesia rhodoleuca* (Diels) M.C.E. Amaral is a small erect shrub, endemic to China. It is exclusively confined to the special habitat of shady and moist valleys between 400 - 800 m above sea level within Nanling Mountain Range in Guangxi Zhuangzu Autonomous Region and Guangdong Provinces, China (Fen & Amaral, 1984). It is included in National Protected Species (Class I) in China (Liang, 2006) due to its rarity and vulnerability, since local residents are used to exploit them for pharmaceuticals, such as suppressing itching. However, *S. rhodoleuca* did not draw attention until a vegetation investigation occurred in 1998 (He & Li, 2005). Recently, the biological characteristics (Liang, 2006), seed germination (Chai *et al*., 2010) and cutting propagation (Zeng *et al*.,

2010) of this endangered species have been studied, and a genetic study based on ISSR markers (Chai *et al*., 2014) indicates a low genetic diversity within populations and high genetic differentiation among populations. Fine population genetic structure, however, remained unresolved by the study of Chai *et al*. (2014). Moreover, the previous genetic studies may have underestimated the genetic consequences of past demographic events as it used traditional standard approaches of assessing population structure (Selkoe & Toonen, 2006; Kang *et al*., 2008). The critical situation of *S. rhodoleuca* populations urges further analyses of its genetic structure as a support for the definition of a conservation plan. Nuclear microsatellite markers are powerful genetic markers because of their high level of variability (Zane *et al*., 2002). In the present study, eight polymorphic microsatellite markers developed by Li *et al*. (2010) were used to: (1) investigate the degree of genetic diversity within the remnant *S. rhodoleuca* populations, and compare it with that identified previously on the basis of ISSR markers; (2) infer potential evolutionary factors that could have led to present genetic differentiation and population structure. Based on these data the implications of conservation measures for *S. rhodoleuca* are discussed.

Materials and Methods

Species biology and sampling: *Sauvagesia rhodoleuca*, (Syn: *Sinia rhodoleuca* Diels), normally grows to a height of 80-150 cm (Liang, 2006). It flowers from April to May, and the flowers are 4-6.5 mm in diameter with white to slightly pink tepals (Fen & Amaral, 1984). Many seeds per fruit ripen from June to July and are probably dispersed by wind or some insects (Personal observation of Cao H-L). Stemmed trees can be observed frequently in all populations but not each individual, suggesting the likelihood of vegetative propagation. Additionally, cutting propagation is feasible but not the main reproductive strategy, while the narrow suitable range of temperature limited the seed germination, the low germination velocity, uneven germination and slow growth made this species disadvantage in interspecies competition, leading to the poor regeneration of *S. rhodoleuca* populations (Chai *et al*., 2010; Zeng *et al*., 2010) (Fig. 1).

According to historical records, eight *S. rhodoleuca*populations were discovered in the midlands and the north of Guangxi Zhuang Autonomous Region, and in Huaiji, Fengkai, Guangning and Lianshan counties of Guangdong province (Fen & Amaral, 1984). However, we could only find six populations of them (Fig. 2, Table 1) and totally sampled leaves of 227 individuals in 2007 and the number of samples in each population was variable according to plant density, while failed to find any individuals in Huaiji and Gaoyao counties, Guangdong Province. Three out of six extant populations (Table 1) are located in the protected areas where *S. rhodoleuca* is currently relatively well preserved, nevertheless, a new road crossed beside the population JXX was constructed for local residents by the local government in 2010, and it has led to an increase in tourist visits on the area of this population, this could negatively affect the survival of *S. rhodoleuca*.

Microsatellite genotyping: Eight high polymorphic microsatellite loci (SR3, SR4, SR5, SR6, SR7, SR8, SR10 and SR11) were used for genetic characterizations of all collected samples and the microsatellite procedure was performed as described by Li *et al.* (2010).

Fig. 1. *Sauvagesia rhodoleuca* (Ochnaceae).

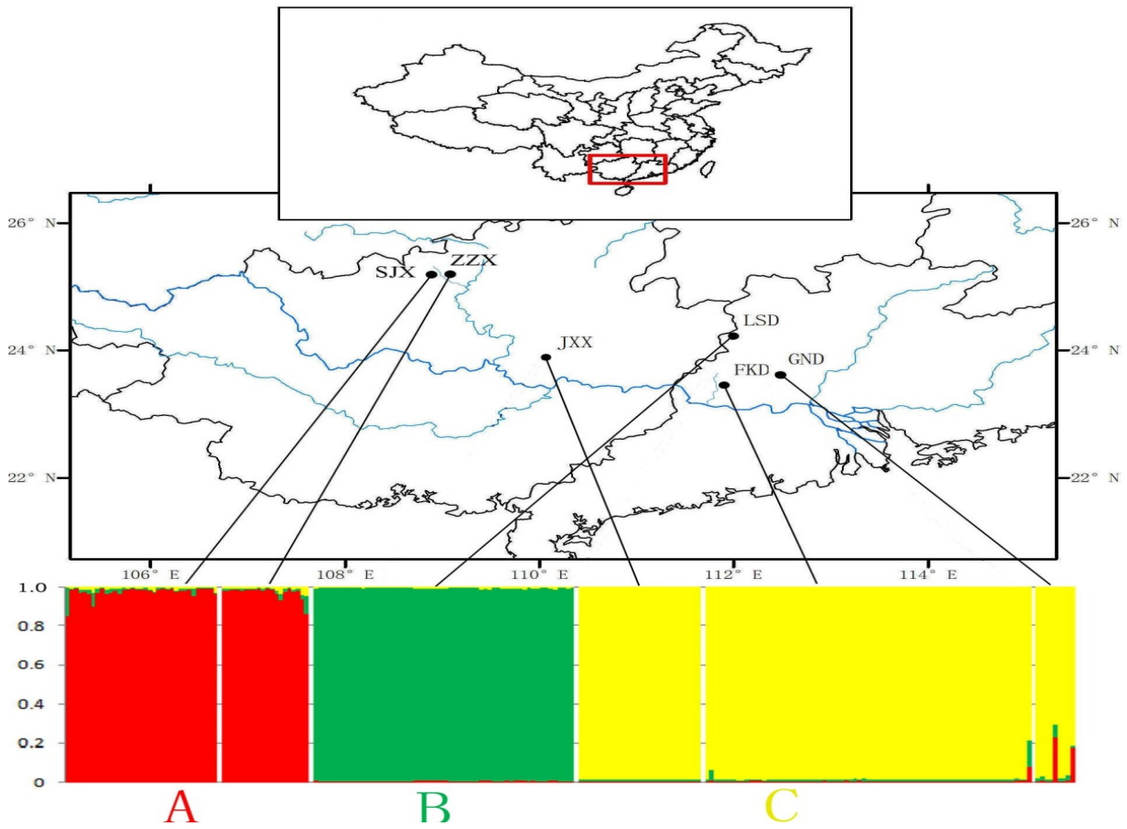

Fig. 2. Sample locations of *Sauvagesia rhodoleuca* in China and clusters of the six populations from INSTRUCT analysis.

Table 1. Genetic variation in the six populations of *Sauvagesia rhodoleuca*, and comparing with the species having different life-history traits.

Population	Locations	Sample size	*P*(%)	N_A	A_P	A_R**	H_O	H_E	F_{IS}	$F_{IS;\,IIM}$	F_{ST}	F_{ST}(C)
LSD*	Lianshan, Guangdong	60	50.00	1.63	3	1.70	0.052	0.097	0.464	0.538		
FKD*	Fengkai, Guangdong	75	62.50	2.25	1	1.96	0.080	0.197	0.593	0.602		
GND	Guangning, Guangdong	9	37.50	1.63	1	-	0.014	0.153	0.914	0.868		
JXX*	Jinxiu, Guangxi	28	12.50	1.38	1	1.36	0.004	0.087	0.950	0.925		
ZZX	Zhongzhai, Guangxi	20	62.50	1.88	1	1.87	0.082	0.189	0.572	0.507		
SJX	Sijian, Guangxi	35	100.00	3.75	6	3.25	0.182	0.393	0.522	0.514		
	Mean	-	54.17	-	-	-	0.069	0.186	0.669	0.659		
	Overall	-	-	4.88	-	3.72	-	-	-	-	0.684	0.648

Within-population genetic diversity in plant species with different life-history traits using microsatellite markers***

Breeding system	H_O	H_E	F_{IS}
Selfing	0.05	0.41	0.88
Mixed	0.51	0.60	0.15
Outcrossing	0.63	0.65	0.03

P, percentage of polymorphic loci; N_A, the average number of allele across all loci in each population; A_P, private allele per population; A_R, allele richness corrected for variation in sample size (Leberg 2002); H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; F_{IS}, inbreeding coefficient uncorrected with null alleles;F_{IS}; IIM, inbreeding coefficient corrected with null alleles using the individual inbreeding mode (IIM); F_{ST}, genetic differentiation uncorrected with null alleles; F_{ST}(C), genetic differentiation corrected with null alleles;*, located in the protectedarea; **, not calculated for GND population due to its low sample size, ***,$H_O$and $H_E$from Nybom (2004) and F_{IS} was calculated with the formula $(H_E - Ho)/ H_E$.

Data analysis: Genetic diversity parameters, the percentage of polymorphic loci (*P*), the average numbers of allele across all loci in each population (N_A), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) (Nei, 1978) and the numbers of private alleles per population (A_P) were calculated using GenAlEx 6.3 (Peakall & Smouse, 2006). Inbreeding coefficients (F_{IS}) without considering null alleles and allelic richness (A_R) for each population were estimated using F_{STAT} 2.9.3 (Goudet, 1995).

Genotypic linkage disequilibrium (LD) between all pairs of loci was tested using software F_{STAT} 2.9.3. Deviations from Hardy-Weinberg equilibrium (HWE) were assessed using the exact test in GENEPOP 4.0 (Raymond & Rousset, 1995; Rousset, 2008). The possible presence of null alleles for each locus and population and inbreeding coefficients which are robust to a presence of null alleles were examined using the individual inbreeding model (IIM) implemented in INEST 2.0 (Chybicki & Burczyk, 2009).Null allele frequencies for each locus and population were also calculated based on the Expectation Maximization (EM) algorithm (Dempster *et al*., 1977), implemented in FreeNA (Chapuis & Estoup, 2007).

F_{ST} statistics for each pair of populations and overall were calculated both with and without null allele exclusion with FreeNA (Chapuis & Estoup, 2007). According to values of genetic differentiation with excluding null alleles, gene flow (N_m) between populations was then estimated indirectly from the formula $N_m = [(1/F_{ST})-1]/4$ (Wright 1969). F_{ST}, as estimated from a typical size sample, is capable of estimating N_m roughly (Whitlock & McCauley, 1999). The correlation between geographical and genetic distances ($F_{ST}/(1-F_{ST})$) was analyzed (Rousset, 1997) using a Mantel test implemented in IBDWS 2.0 obtained at http://ibdws.sdsu.edu (Jensen *et al*., 2005).

Population genetic structure was analyzed using Bayesian individual based clustering methods implemented in the software INSTRUCT (Gao *et al*., 2007), which takes inbreeding into account and does not assume Hardy-Weinberg equilibrium. We did 5 independent runs on the mode 4 (inferring population structure and population inbreeding coefficients) in INSTRUCT for each *K* (putative cluster numbers, from 2 to 6) with 10^6 iterations after a burn-in period of 5×10^5. The software gave the optimal *K* automatically based on Deviance Information Criterion (DIC) values. Based on the inferred *K*, CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) was used to calculate average membership coefficient for each individual by aligning the results of the above 5 runs because clustering algorithms in INSTRUCT results in slightly different outcomes in each independent stochastic simulation.

The genetic distances for all individual pairs and population pairs were calculated using the chord distance (Cavalli-Sforza & Edwards, 1967) with the software POPULATION1.2.28 (Langella, 2000). This genetic distance is unbaised to the existence of null alleles, making no assumption regarding constant population size or mutation rates among loci in order to obtain a correct tree topology. These distances were subsequently used to generate dendrogram based on the unweighted pair-group method with arithmetic mean (UPGMA) with FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree) to visualize genetic relationship among individuals and populations.

Results

Genetic diversity: The values of gene diversity parameters for each population are summarized in Table 1. The percentages of polymorphic loci (*P*) ranged from 12.50% (JXX) to 100.00% (SJX) within the six natural populations, with an average of 54.17%. The number of alleles (N_A) in each population, averaged across loci, ranged from 1.38 (JXX) to 3.75 (SJX). The number of private alleles (A_P) varied between 1 and 6 among six populations. The lowest allelic richness (A_R) was found in JXX with 1.36 and the highest in SJX with 3.25. The global mean observed (H_O) and expected (H_E) heterozygosity were 0.069 and 0.186, respectively. Estimated inbreeding coefficients (F_{IS}) within populations were high, the global mean F_{IS} values with and without null allele correction were 0.659 and 0.699, respectively.

Significant deviations from HWE were evident for all loci at least in one population due to heterozygote deficits. The frequencies of null alleles ranged from 0.023 to 0.362 with IIM method, from 0.001 to 0.386 with EM method (Table 2). No linkage disequilibrium was found for any locus pair in any population ($p<0.05$).

Genetic differentiation and population structure: The overall F_{ST} among populations changed slightly from 0.684 including null alleles to 0.648 after excluding null alleles (Table 1). All pair wise F_{ST} values were significantly high no matter with or without null allele correction (Table 3). Mantel test did not detect a significant pattern of isolation by distance ($r = 0.1532$, $P = 0.2410$).

In INSTRUCT analysis, there were three optimal group numbers in our data set: one consists of two populations SJX and ZZX in the A group, another contains only LSD in the B group, and the other one consists of the rest three populations JXX, FKD and GND in the C group (Fig. 2). UPGMA dendrogram for population pairs also indicates three groups for *S. rhodoleuca*, which was generally congruent with the INSTRUCT results (Fig. 3). From the dendrogram it is also clear that individuals in LSD population were most divergent from the others without mixture with the others (Fig. 3). UPGMA dendrogram for all sampled individuals shows five individuals from SJX population were assigned to the genetic pool as the C group, where individuals from FKD, GND and JXX had mixed, and the individuals in LSD population were most divergent from the others without mixture with the others (Fig. 3).

Discussion

Null alleles and breeding system: Null alleles in microsatellite have been found in a wide range of taxa (Dakin & Avise, 2004). Thus, we estimated the

frequencies of null alleles for eight loci in six populations using the IIM and EM method (Table 2). They are expected to cause significant problems in genetic diversity and structure analyses when their frequencies > 0.2 (Dakin & Avise, 2004; Chapuis & Estoup, 2007) in locus where significant deviation from HWE expectations are detected. We found that results from two methods were slightly different null allele frequencies, but most of which were lower than 0.2 and no consistent high null frequencies with > 0.2 per locus appear across all populations, indicating that null allele does not affect other estimates, including the inbreeding coefficient. Therefore, high values of F_{IS} for the study populations most likely result from the breeding system of the species. The F_{IS} values of six populations are much higher than that of species with breeding system mixed suggest the probable selfing in the species (Table 1). In fact, selfing (partial or complete self-fertilization) is main breeding system in endemic plant species (Cole, 2003) and generally associates with small/isolated/marginal populations which are less visited by pollinators. Such a breeding system provides reproductive assurance for these populations (Martén-Rodríguez & Fenster, 2010). In field, *Sauvagesia rhodoleuca* individuals are highly scattered distributed and its small and light color flowers have little attraction to pollinators. This is not conducive to reproduction, and the selfing is a probable form of "evolutionary rescue" (Lynch & Lande, 1993; Gomulkiewicz & Holt, 1995; Bodbyl Roels & Kelly, 2011) for the species to prevents extinction. Further studies are needed to understand the breeding system and its interactions with evolution of *S. rhodoleuca*.

Genetic variation and structure: Endemic and restricted geographic distributions species harbor relatively lower genetic diversity within populations than that of widespread species (Hamrick & Godt, 1989; Cole, 2003; Nybom, 2004). Our results are in agreement with summary results from Nybom (2004), but with much lower genetic diversity (mean H_O = 0.069, mean H_E = 0.186), comparing with H_O(0.32) and H_E (0.42) for the endemic species. This result consistent with Chai *et al*.'s (2014) observations. Low genetic diversity in *S. rhodoleuca* may attribute to the fluctuation of population size, and this has been observed in previous studies for other endangered species (Barrett & Kohn, 1991; Godt *et al*., 1996; Butcher *et al*., 2009). During the past several decades, *S. rhodoleuca* has been degraded due to its medical utilizations and the loss of habitats for deforestation, and all these cause its population size shrink rapidly. Rapid declines in population size can lead to a loss of genetic diversity and elevated levels of inbreeding (Gilpin & Soulé, 1986). In addition, selfing plant species with relatively more homozygous individuals and reduced effective population size, usually display lower genetic variation within populations than outcrossers (Hamrick & Godt, 1989; Nybom & Bartish, 2000; Spielman *et al*., 2004). Therefore, it did not surprise us to find that *S. rhodoleuca* displaya very low levels of genetic diversity within populations.

Table 2. Estimation of null allele frequencies and testing deviations from Hardy-Weinberg equilibrium (HWE) for eight microsatellite loci of *Sauvagesia rhodoleuca*. IIM: null allele frequency estimated using the Individual Inbreeding Model (Chybicki and Burczyk 2009, Yasuda 1968). EM: null allele frequency using Expectation Maximization method of (Dempster et al. 1977); *P*: P-value of probability for departure from Hardy-Weinberg equilibrium using exact test with GENEPOP 4.0 (Raymond and Rousset 1995, Rousset 2008); NA: not available

Locus	SJX			ZZX			JXX			LSD			FKD			GND		
	IIM	EM	*P*	IIM	EM	*P*	IIM	EM	*P*	IIM	EM	*P*	IIM	EM	*P*	IIM	EM	*P*
SR3	0.131	0.223	<0.001	0.126	0.001	NA	0.109	0.001	NA	0.066	0.000	1.000	0.023	0.079	0.004	0.303	0.279	0.012
SR4	0.090	0.148	0.006	0.126	0.001	NA	0.111	0.001	NA	0.072	0.001	NA	0.024	0.001	NA	0.198	0.001	NA
SR5	0.070	0.000	1.000	0.124	0.001	NA	0.107	0.001	NA	0.221	0.226	<0.001	0.034	0.238	<0.001	0.275	0.251	0.012
SR6	0.131	0.221	<0.001	0.164	0.157	0.031	0.109	0.001	NA	0.074	0.001	NA	0.025	0.001	NA	0.198	0.001	NA
SR7	0.067	0.112	<0.001	0.209	0.185	0.002	0.111	0.001	NA	0.053	0.009	0.717	0.027	0.163	<0.001	0.197	0.001	NA
SR8	0.126	0.220	<0.001	0.147	0.159	0.022	0.108	0.001	NA	0.073	0.001	NA	0.027	0.141	<0.001	0.197	0.001	NA
SR10	0.063	0.073	0.157	0.149	0.128	0.077	0.106	0.001	NA	0.073	0.001	NA	0.025	0.001	NA	0.198	0.001	NA
SR11	0.116	0.208	<0.001	0.166	0.149	0.026	0.362	0.386	<0.001	0.137	0.124	<0.001	0.025	0.180	<0.001	0.314	0.298	0.004

Evidence from Bayesian clustering method and UPGMA dendrogram indicate the presence of significant population structuring within the restricted range of *S. rhodoleuca*. For many endangered plants (e.g., Tang *et al*., 2006; Kaneko *et al*., 2008; Moreira *et al*., 2009; Dunbar-Co & Wieczorek, 2011), the factor of geographic distance played an important role to influence to the genetic structure. While our data does not show a significant relationship between genetic distance and spatial distance of *S. rhodoleuca*, the low correlation coefficient (r = 0.1532; R^2 = 0.0235) indicates that about 97.65% of the variation in genetic distance was related to factors other than geographic distance. This was also reflected in some anomalous clustering of populations in the INSTRUCT analysis and UPGMA dendrogram. Although the LSD population was geographically close to the FKD and GND populations, it did not cluster with them, but the JXX population have showed the opposite. Moreover, UPGMA dendrogram for all sampled individuals (Fig. 3) shows that five individuals from SJX population were the most distinct from the other individuals of genetic pool A group, and clustered to the individuals of genetic pool C group with also ignoring the geographical isolation. A similarly result of anomalous clustering of populations appear in another endangered plant *Sarracenia leucophylla* (Wang *et al*., 2004). According to generalizations proposed by Wang *et al*. (2004), the other factors like selection, founder effects, loss of intervening populations, and possibly human-mediated gene flow have probably also influenced the species genetic structure. In addition, the mating system by which a given population reproduces plays an important role in determining its genetic structure (Brown & Allard, 1970). Compared with outbreeders, inbreeding species showed markedly greater variation among populations in average values of Nei's gene diversity statistic (Schoen & Brown, 1991).We observed high genetic differentiation among populations with pair wise F_{ST} values ranging from 0.162 to 0.804 (Table 3), which is most likely to be attributable to selfing. We suspect that the probable selfing has played an important role in the genetic structure for *S. rhodoleuca*.

Conservation suggestions and management: Although anthropogenic disturbances on *S. rhodoleuca* have attracted conservation attention since 1998 (He & Li, 2005), the species still faces an uncertain future and its endangered status has not been ameliorated. To our sampling in 2007, two of the eight populations found by Fen and Amaral (1984) have been lost and there were only several individuals in GND population during sampling. We infer that the situation of other populations is not optimistic. If effective conservation programs were not designed and implemented, decreases in population sizes and loss of suitable habitats for *S. rhodoleuca* would continue and ultimately the species may become extinct in the near future.

Genetic diversity is critically important for a species to maintain its evolutionary potential. Considering low genetic variation within populations and high genetic differentiation between populations, we recommend that proper management by *in situ* conservation strategy should involve in protection and maintenance of genetic diversity throughout the range of *S. rhodoleuca*. Populations JXX, FKD, and LSD, respectively, have been protected in the foundations of the Dayaoshan National Nature Reserve, Heishiding and Bijiashan Provincial Nature Reserve, but the other three populations (GND, ZZX and SJX) located outside the nature reserve have been almost completely destroyed through deforestation. Each of these unprotected populations harbor its own private alleles, the decline of these populations may cause the irretrievable loss of unique alleles. Additionally, the populations SJX have high conservation priorities, because it exhibited the highest level of genetic diversity in the six remnant populations (Table 1). Therefore, habitat protection of these three unprotected populations is particularly urgent. Collection for pharmaceuticals is a critical threat to the persistence of *S. rhodoleuca,* three unprotected populations (GND, ZZX and SJX) are in vulnerable, and the other three populations (JXX, FKD, and LSD) located inside the nature reserve are still under threat from cut for suppressing itching by local residents. Obviously, an increased legal protection will undoubtedly be required if this species is to survive in the wild. For the population JXX, the road recently constructed by the local government has led to an increase in tourist visits in recent years. It is believed that tourism has severe impacts on threatened plants (Kelly *et al*., 2003; Kang *et al*., 2005), the effects of which need to be investigated and monitored for this population.

In addition to *in situ* conservation efforts, *ex situ* conservation should be planned for *S. rhodoleuca*, it could help to re-colonize new areas for the species. The individuals of small populations of endangered plants are vulnerable to stochastic environmental factors and anthropogenic activity, and protected reserves of individuals in other locations would provide insurance for species persistence and provide a source for population augmentation (Straub & Doyle, 2009). *Ex situ* conservation based on seed harvest of key genotypes should be carried out. Furthermore, a multidisciplinary project on protection of *S. rhodoleuca* that includes biological studies (e.g., demography, breeding system, and in vitro propagation) as well as work with people in local communities (e.g., environmental education programs) is urgently needed to reach a sustainable management of this species.

Table 3. Matrix of pair wise comparisons of *Sauvagesia rhodoleuca* population genetic differentiation (pair wise F_{ST} values with and without excluding null allele (in parenthesis) which in the below diagonal) and gene flow (N_m value which in the above diagonal).

	ZZX	FKD	GND	SJX	JXX	LSD
ZZX		0.135	0.123	0.662	0.064	0.076
FKD	0.650*(0.699*)		1.293	0.174	0.335	0.106
GND	0.670*(0.713*)	0.162*(0.210*)		0.226	0.097	0.073
SJX	0.274*(0.310*)	0.589*(0.640*)	0.525*(0.560*)		0.123	0.127
JXX	0.796*(0.800*)	0.427*(0.448*)	0.720*(0.686*)	0.671*(0.680*)		0.061
LSD	0.768*(0.810*)	0.702*(0.746*)	0.773*(0.813*)	0.663*(0.693*)	0.804*(0.819*)	

*: p<0.05

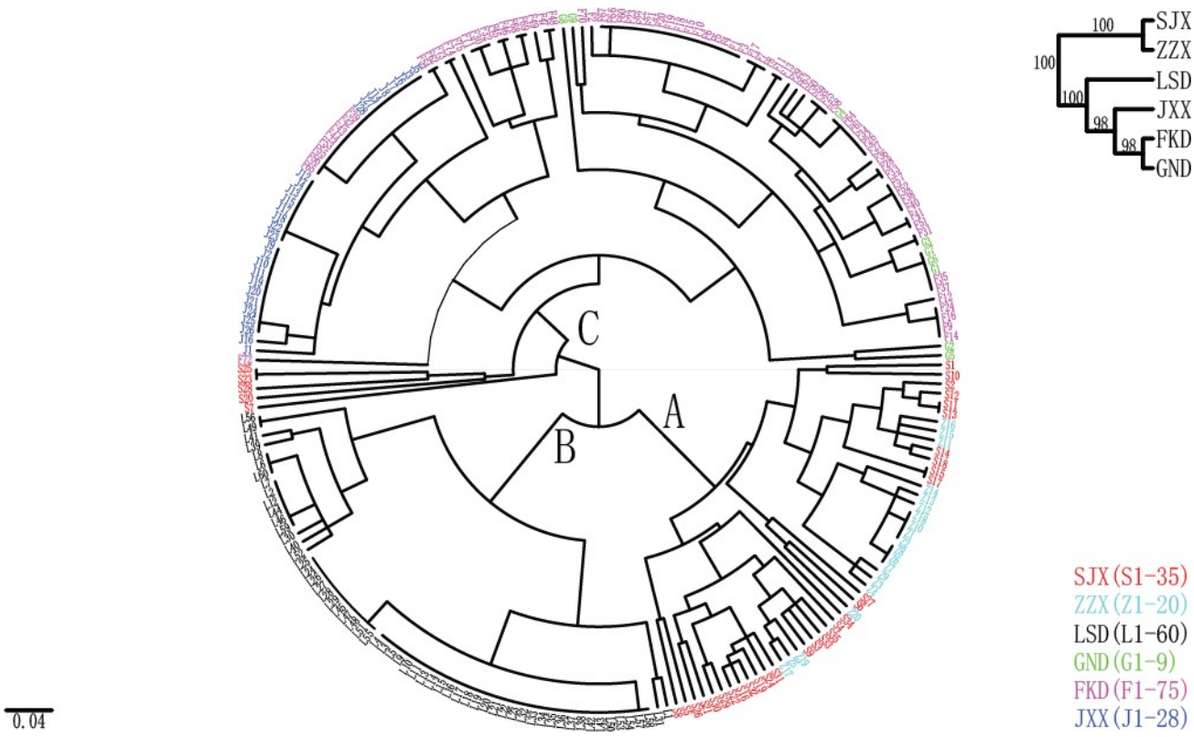

Fig. 3. UPGMA dendrogram based on genetic distances showing the genetic relationships among populations and all sampled individuals.

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