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Genetic diversity of the threatened Chinese endemic plant, *Sinowilsonia henryi* Hemsi. (Hamamelidaceae), revealed by inter-simple sequence repeat (ISSR) markers

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ABSTRACT

Sinowilsonia henryi Hemsi., the only representative of the monotypic genus *Sinowilsonia* Hemsi. (Hamamelidaceae), is a threatened plant endemic to China with high phylogenetical, ecological and economical values. In the present study, inter-simple sequence repeat (ISSR) markers were employed to investigate the genetic diversity and differentiation of 214 individuals sampled from 14 populations. Fifteen selected primers yielded a total of 178 bright and discernible bands. The genetic diversity was low at the population level ($h = 0.1025$; $I = 0.1506$; PPL = 26.7%), but quite high at the species level ($h = 0.2449$; $I = 0.3690$; PPL = 72.5%). In line with the limited gene flow ($N_m = 0.3537$), the hierarchical analysis of molecular variance (AMOVA) revealed pronounced genetic differentiation among populations ($\Phi_{ST} = 0.6639$). Furthermore, the Mantel test revealed a significant correlation between genetic and geographic distances among populations ($r = 0.688$, $P = 0.001$), indicating the role of geographic isolation in shaping its present population genetic structure. The present patterns of genetic diversity of *S. henryi* were assumed to result largely from its evolutionary history and geographic factors. Based on these findings, conservation strategies were proposed to preserve this threatened plant.

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1. Introduction

Nowadays, conservation of threatened and endangered species has aroused global concerns because of their ecological, educational, historical, esthetic, recreational and scientific values. Since genetic diversity in natural populations can significantly affect the long-term survival and evolution of species or populations in changing environments (Futuyma, 1986), any conservation effort should aim to preserve maximum genetic variability within the target gene pool. Thus, an accurate estimate of its levels and distribution patterns is essential to the formulation of effective conservation strategies for threatened and endangered species, which can only be obtained by detailed population genetic studies (Hamrick and Godt, 1996).

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Sinowilsonia henryi Hemsi., the only representative of the monotypic genus *Sinowilsonia* Hemsi. (Hamamelidaceae), is a threatened plant endemic to China (Ying and Zhang, 1994; Zhang et al., 2003). This plant is mainly distributed in central China, occurring primarily in shady and moist valleys with an elevation of 600–1400 m. Morphological, anatomical and molecular data support the genus *Sinowilsonia* as being primitive or ancestral ones within the family Hamamelidaceae; plants of this family once dominated in temperate forests from Cretaceous to early Tertiary, and eventually survived the Quaternary glacial age (Qian et al., 2006; Wu et al., 2005; Zhang and Lu, 1995). The fossil of *S. henryi* was discovered in strata of Paleocene in Europe (Wolfe, 1973). Possessing high phylogenetic study value, *S. henryi* is an ideal material for studying the occurrence and evolution of the flora of China. With a developed root system, the species has adapted to moist habitats, even can tolerate intermittent flooding. Hence, it can be of important utility for the conservation of water and soil. Furthermore, it is also an ideal raw material for manufacturing furniture. However, *S. henryi* is highly vulnerable to environmental changes. The habitats of this plant have been severely deteriorated and fragmented, so its natural populations have shrunk to small sizes. For example, during our field surveys across its geographic range, most populations observed only have 5–50 individuals (Table 1). In addition, all extant natural populations are located in anthropogenically unaffected or slightly affected areas. Thus, *S. henryi* has been listed as a threatened species by both local and central governments in China (Di and Yu, 1989; Yu and Li, 1989), calling for urgent protection and restoration.

To date, previous studies of *S. henryi* have been mainly focused upon its morphology and anatomy (Fu et al., 1993; Fu and Gao, 1992), cytology (Oginuma and Tobe, 1991), systematics (Endress, 1989; Li and Fu, 1997; Zhang et al., 2001; Zhang, 1999) and reproductive biology (Linghu et al., 2009). No effort has been reported on its population genetics, although such information is essential to the formulation of effective conservation strategies for this threatened species. In this study, inter-simple sequence repeat (ISSR) markers were employed to investigate the genetic diversity and differentiation in *S. henryi*. Owing to its Mendelian inheritance, hypervariability and its time-, cost- and labor-efficiencies (Wolfe and Liston, 1998), this microsatellite-derived marker system has found wide and successful applications in population genetic studies (Feyissa et al., 2007).

The objectives of this paper were to: (i) characterize the level of genetic diversity in this endemic species; (ii) reveal the partitioning of genetic variability within and among populations; and (iii) discuss the possible implications for its conservation.

2. Materials and methods

2.1. Plant materials

A total of 214 individuals, which corresponded to 14 populations, was sampled across its entire geographic range in China, including Shaanxi, Henan, Gansu, Hubei and Chongqing Provinces (Fig. 1; Table 1). Fresh leaves were collected, dried in a ziplock bag with silica gel, transported back to laboratory and kept in −80 °C freezer. Corresponding to each population, parameters such as longitude, latitude and altitude were recorded for further analyses (Table 1). Due to the limited availability of individuals, sample sizes of some populations in this study were relatively small. For example, only five and eight individuals were available for populations MTZ and QS, respectively (Table 1).

2.2. DNA isolation and ISSR–PCR amplification

Total genomic DNA was extracted using a modified 2× CTAB method (Doyle and Doyle, 1987), and then dissolved in 0.1× TE buffer (10 mM pH 8.0 Tris–HCl; 1 mM EDTA) for the subsequent use.

Table 1
Geographic localities, sample sizes and genetic diversity of *S. henryi* populations in this study.

Population code	Geographic localities	Longitude (°E)	Latitude (°N)	Altitude (m)	Population size	Sample size	Genetic diversity[a]		
							h	*I*	PPL (%)
DC	Dongcha, Tianshui, Gansu Province	106.64	34.31	1300	100	19	0.0582	0.0865	16.29
MTZ	Miaotaizi, Liuba, Shaanxi Province	106.80	33.65	1550	5	5	**0.0579**	**0.0840**	**14.04**
HY	Huayang, Yangxian, Shaanxi Province	107.51	33.67	1370	>200	21	0.0792	0.1157	20.22
XYB	Xunyangba, Ningshan, Shaanxi Province	108.54	33.55	1500	50	21	0.0807	0.1185	21.35
CHS	Cuihuashan, Chang'an, Shaanxi Province	109.02	33.97	1300	10	10	0.0622	0.0896	14.61
JSX	Jiangsixia, Shangnan, Shaanxi Province	110.55	33.35	840	50	20	0.1313	0.1954	35.39
LJS	Laojunshan, Luanchuan, Henan Province	111.63	33.74	1342	12	12	**0.1863**	**0.2727**	**46.63**
HLG	Heilonggou, Jiyuan, Henan Province	112.09	35.27	880	>200	20	0.1330	0.1968	35.39
JLG	Jiuligou, Jiyuan, Henan Province	112.43	35.21	905	100	20	0.1234	0.1824	33.15
QS	Qingshui, Zhengba, Shaanxi Province	107.73	32.67	1350	8	8	0.1122	0.1603	27.53
HLS	Hualongshan, Pingli, Shaanxi Province	109.20	32.06	1200	10	10	0.1096	0.1603	28.09
WDS	Wudangshan, Shiyan, Hubei Province	111.01	32.41	1030	16	16	0.1288	0.1923	37.08
SNJ	Shennongjia, Hubei Province	110.40	31.50	1405	12	12	0.0843	0.1238	21.91
HH	Honghua, Xingshan, Hubei Province	110.56	30.09	1300	50	20	0.0882	0.1294	22.47
Mean value	–	–	–	–		–	***0.1025***	***0.1506***	***26.72***
Species level	–	–	–	–		–	***0.2449***	***0.3690***	***72.47***

The lowest, the highest, the mean of population diversity and the diversity in species level for *S. henryi* were pointed out with bold and italics number.
[a] Three genetic diversity indices were presented, i.e., Nei's gene diversity (*h*), Shannon's information index (*I*) and the percentage of polymorphic loci (PPL, %).

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