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High genetic diversity and weak population structure of *Rhododendron jinggangshanicum*, a threatened endemic species in Mount Jinggangshan of China

Mingwan Li [a, 1], Sufang Chen [a, 1], Shi Shi [a], Zhong Zhang [b], Wenbo Liao [a], Wei Wu [c], Renchao Zhou [a], Qiang Fan [a, *]

[a] Guangdong Key Laboratory of Plant Resources and Key Laboratory of Biodiversity Dynamics and Conservation of Guangdong Higher Education Institutes, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China
[b] Jinggangshan Administration of Jiangxi Province, Jinggangshan 343600, China
[c] South China Botanical Garden, Chinese Academy of Science, Guangzhou 510650, China

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ABSTRACT

Rhododendron is one of the largest and most valuable genera of woody plants. Species in this genus are often dominant shrubs in their habitats. However, many of these species are often narrowly distributed. Their current status calls for extensive scientific and social attention. In this study, nine microsatellite markers were used to investigate the genetic diversity and population structure of *Rhododendron jinggangshanicum*, a threatened *Rhododendron* species endemic to Mount Jinggangshan, Jiangxi, China. A total of 119 individuals were collected from five populations. The results showed that *R. jinggangshanicum* harbors a high level of genetic diversity ($A_R = 4.760 \pm 1.638$, $H_E = 0.642 \pm 0.200$) and that its genetic diversity is partitioned primarily within populations. AMOVA results demonstrated weak yet significant differentiation among the populations (4.60%, $P < 0.001$), which was consistent with the results of the pairwise F_{ST}. A marginally significant correlation was found between genetic differentiation and geographic distance, indicating that the genetic differentiation among the populations roughly follows a pattern of isolation by distance. STRUCTURE analysis showed that all of the individuals could be divided into two gene pools, which may be related to the local complex topography of Mount Jinggangshan. These results suggest that proper measures should be taken for the conservation of this threatened species.

1. Introduction

With beautiful vegetative forms and bright-colored flowers, *Rhododendron* (Ericaceae) species are important horticultural plants around the world. *Rhododendron* L. consisting of approximately 1025 species (Chamberlain et al., 1996), is one of the largest and most valuable genera of woody plants. As key components of alpine and subalpine communities, *Rhododendron*

* Corresponding author. Tel.: +86 20 84112802.
E-mail addresses: limw3@mail2.sysu.edu.cn (M. Li), chsuf@mail.sysu.edu.cn (S. Chen), shishi@mail2.sysu.edu.cn (S. Shi), syzz2010@126.com (Z. Zhang), lsslwb@mail.sysu.edu.cn (W. Liao), wuei@scbg.ac.cn (W. Wu), zhrench@mail.sysu.edu.cn (R. Zhou), fanqiang@mail.sysu.edu.cn (Q. Fan).
[1] These authors contributed equally to this work.

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species play a major role in preventing and controlling soil erosion and maintaining the stability of ecosystems (Monk et al., 1985). These ample wild germplasm resources are also widely applied to the introduction and development of new cultivars (Fang et al., 2005).

Many *Rhododendron* species are narrowly distributed in China. For example, among the 571 *Rhododendron* species recorded in China, approximately 314 of them are found in only one province, such as *Rhododendron albersenianum* in western Yunnan and *Rhododendron asterochnoum* in western Sichuan (Fang et al., 2005). Currently, up to 13 *Rhododendron* species are listed as threatened species according to IUCN Red List (IUCN, 2011). Unfortunately, many others have been missed from this list owing to our ignorance regarding their current status. Generally, these narrowly endemic species are more vulnerable to environmental disturbances than more widespread species, meaning that they require extensive scientific and social attention.

As one of the biodiversity hotspots in southeastern China, Mount (Mt.) Jinggangshan harbors up to 31 *Rhododendron* species (Wang et al., 2013a; Liao et al., 2014); three of them, *Rhododendron strigosum*, *Rhododendron xiaoxidongense* and *R. jinggangshanicum*, are considered to be endemic (Tam, 1982; Hu, 1990; Liu, 2001). During our field investigations from 2009 to 2013 (Liao et al., 2014), no individuals of *R. strigosum* or *R. xiaoxidongense* and only five populations of *R. jinggangshanicum* were found, which indicates the extremely serious status of these *Rhododendron* species.

Rhododendron jingangshanicum P. C. Tam, which is a diploid species ($2n = 26$) (Fang and Min, 1995), inhabits sheltered and moist areas above an altitude of approximately 1500 m, including the edges of forests and streams or jungle valleys (Fang et al., 2005). It has been listed as an endangered species on the China Species Red List (Wang and Xie, 2004) and there have been only a few studies on its cultivation and heat tolerance (Chen, 1998; Zhang et al., 2011).

Simple sequence repeats (SSRs; microsatellites), which are characterized by their reproducibility, multi-allelic nature, co-dominant inheritance, relative abundance and good genome coverage (Wang et al., 2013b), are useful tools for measuring changes in the genetic diversity of wild plants. Fortunately, ample microsatellite primers have been developed for *Rhododendron* species; for instance, 24 SSR primer pairs have been developed for *Rhododendron decorum* (Wang et al., 2013b), 12 pairs have been developed for *Rhododendron aureum* (Li et al., 2011), and nine pairs have been developed for *Rhododendron ferrugineum* (Delmas et al., 2011). In this study, we selected nine polymorphic SSR markers to investigate the genetic diversity and population structure of *R. jinggangshanicum*. Through these efforts, we hope to provide valuable advice for the conservation of this species.

2. Materials and methods

2.1. Sample collection

A total of 119 individuals of *R. jinggangshanicum* were sampled from five distinct wild populations on Mt. Jinggangshan (Table 1; Fig. 1). The geographic sampling locations were recorded using a Garmin GPS unit (GPSMAP 62sc, Taiwan) with an accuracy rate of 10 m. For each population, fresh leaf tissue was collected from 14 to 33 randomly selected adult individuals, which were at least 50 m apart from each other. The samples were then preserved and stored with silica gel in zip-lock plastic bags until DNA extraction. The herbarium specimens collected from each population were deposited in the Herbarium of Sun Yat-sen University (SYS).

2.2. DNA extraction and microsatellite analysis

Genomic DNA was extracted using the modified CTAB method (Doyle and Doyle, 1987). Nine single-locus nuclear SSR primer pairs that were previously published by Wang et al. (2009) were selected: RDW1, RDW6, RDW8, RDW11, RDW16, RDW33, RDW34, RDW44 and RDW51. The forward primer of each pair was labeled with one fluorescent dye (6-FAM, HEX or TAM; Table 2). Polymerase chain reaction (PCR) amplifications were performed in 20-μL reaction volumes containing 25 ng of genomic DNA, 2 μL of 10 × buffer (with Mg^{2+}), 0.25 mM of dNTPs, 0.2 μM of each primer, and 1 U of Easy-taq DNA polymerase (TransGen Biotech Co. Ltd, Beijing, China). The reaction cycles consisted of an initial denaturation for 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at a primer annealing temperature of 50 °C and 1 min at 72 °C, and a final step of 5 min at 72 °C. The reactions were carried out in a PTC-200 thermocycler (MJResearch Inc. Watertown, MA). The PCR products were run on an Applied Biosystems 3730XL DNA Analyzer (Applied Biosystems Inc., Foster, CA), and the raw data were collected using Peak Scanner Software v1.0 (Applied Biosystems).

Table 1
Sampled populations and their locations.

Population	Pop. ID	Region	Sample size	Latitude	Longitude	Altitude (m)
Nanfengmian, Jiangxi	NFM	North	14	26°17′51.5″N	114°03′51.7″E	1752
Jiangxi'ao, Jiangxi	JXA	South	31	26°25′58.9″N	114°04′57.1″E	1750
Dayuan, Hunan	DY	South	14	26°26′37.1″N	114°03′03.1″E	1567
Jingzhushan, Jiangxi	JZS	South	33	26°29′11.3″N	114°04′47.7″E	1371
Pingshuishan, Jiangxi	PSS	South	27	26°30′13.3″N	114°06′45.6″E	1601