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Genetic diversity in endangered *Notopterygium forbesii* Boissieu based on intraspecies sequence variation of chloroplast DNA and implications for conservation

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ABSTRACT

A thorough understanding of the levels and partitioning of genetic variation across populations and geographical regions of endangered species is a prerequisite to ensure effective conservation and/or restoration activities. Here, we examined chloroplast DNA (cpDNA) *trn*H-*psb*A intergenic spacer sequences variation within *Notopterygium forbesii*, an endangered and endemic perennial herb in China. Sequence data obtained from 141 individuals in 14 populations revealed twenty-two haplotypes. A high level of haplotype diversity ($Hd = 0.81$) and low level of nucleotide diversity ($Pi = 0.0047$) were detected. Low genetic differentiation among populations and also among regions was consistently indicated by both hierarchical analyses of molecular variance (AMOVA) and the structure of a neighbor-joining tree. Low level of population differentiation between populations or between regions in cpDNA sequences may be due to effects of the abundance of ancestral haplotype sharing and the high number of private haplotypes fixed for each population. Based on our results, we proposed some conservation strategies.

1. Introduction

Notopterygium forbesii Boissieu, belonging to the *Notopterygium*, Umbelliferae, is an endangered perennial herb endemic to China. According to the historical record, it had a relatively wide distribution throughout alpine forest and sub-alpine shrubs between 1700 m and 4500 m in China (Zhou et al., 2003). As a traditional Tibetian medicine, “Zhuma” was used by local people in small quantities. However, in recent decades, wild populations of *N. forbesii* in China have rapidly decreased due to habit fragmentation, excessive exploitation, and a low rate of natural regeneration. At present, wild populations of *N. forbesii* can only be found above 4000 m. Consequently, the species is regarded as endangered in the Chinese Plant Red Book (Wang and Xie, 2004).

The long-term survival and evolution of species depends on the maintenance of sufficient genetic variability within and among populations to accommodate new selection pressures brought about by environmental changes (Barrett and Kohn, 1991). Therefore, knowledge about the genetic diversity and variation within and between populations in rare and endangered plants not only enhances our understanding of population dynamics, adaptation and evolution but also provides information useful for developing conservation strategies (Schaal et al., 1991).

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To date, previous studies of *N. forbesii* have mainly focused on its morphology, anatomy (Wang et al., 1996; She and Pu, 1997), systematics (Pu et al., 2000), ecology (Jiang et al., 2005) and pharmacognosy (Yang et al., 2006). The genetic diversity of different populations of *N. forbesii* have not yet been reported. In the present study, we used chloroplast DNA (cpDNA) trnH-psbA to examine the genetic diversity of *N. forbesii*. Our aims were to reveal the genetic diversity and the partitioning of genetic diversity within and among populations and to provide baseline genetic information pertinent to the conservation and restoration of this endangered species.

2. Material and methods

2.1. Plant materials

A total of 140 individuals, which corresponded to 14 populations of *N. forbesii*, were sampled across 3 provinces in China; Qinghai, Gansu, and Sichuan Provinces (Table 1; Fig. 1). Due to the limited availability of individuals in wild populations, samples sizes of populations in this study were relatively small, from 8–12 individuals. Neighboring sampled individuals were at least 10 m apart to avoid resampling from the same individual. Leaf material was dried in silica gel and stored at room temperature. Parameters such as longitude, latitude and altitude were recorded for each population (Table 1).

2.2. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted with the CTAB method (Doyle, 199l). DNA quality was checked on a 1% agarose gel. The intergenic spacer between *trn*H and *psb*A genes of the cpDNA was amplified with a pair of universal primers (5′-ACTGCCTTGATCCACTTGGC-3′; 5′-CGAAGCTCCATCTACAAATGG-3′) (Hamilton, 1999). Polymerase chain reaction (PCR) was performed in a 25 μL volume, which contained 1 μL (10–40 ng) plant DNA, 2.5 μL 10×reaction buffer, 1.5 μL $MgCl_2$ (25 mM), 0.5 μL dNTP mix (10 mM), 1 μL of each primer (10 pmol), and 1 U of *Taq* polymerase (Promega, MBI, USA). All reactions were performed with the following program: 5 min at 95 °C, 35 cycles of 1 min at 94 °C, annealing for 1 min at 52 °C, 1.5 min at 72 °C, a final extension for 7 min at 72 °C, and then the reactions were held at 4 °C until further steps. PCR products were resolved electrophoretically on 1.5% agarose gels run at 200 V in 1×TAE, visualized by staining with ethidium bromide, and photographed under ultraviolet light.

All successfully amplified DNA fragments were purified using a TIANquick Midi Purification Kit according to the recommended protocol (TIANGEN), prior to sequencing. Then, PCR products were sequenced directly using an ABI3730XL automated DNA sequencer, applying the PCR-primers as sequencing primers. Sequence electropherograms were edited using Chromas version 2.33 (http://www.technelysium.com.au/chromas.html).

2.3. Data analysis

DNA sequences were aligned with the CLUSTAL X program (Thompson et al., 1997) and then manually refined. Arlequin, version 3.01 (Excoffier et al., 2006), DnaSP, version 4.0 (Rozas et al., 2003), and MEGA, version 4.0 (Tamura et al., 2007), were used to calculate statistical values, such as the nucleotide composition, number of polymorphic sites (S), haplotype diversity (*Hd*), nucleotide diversity (*Pi*), and average number of pairwise nucleotide differences (*k*). These values were calculated for each geographic population and geographic region. The phylogenetic trees were constructed by the neighbor-joining (NJ)

Table 1
Location data, number of individuals analyzed for *trn*H-*psb*A (n) and haplotype diversity (*Hd*), nucleotide diversity (*Pi*) of the investigated populations of *Notopterygium forbesii*.

Code	Population	Voucher	Location	Altitudem (m)	Sample number	Hd	Pi
HY	Huangyuan, QH[a]	G.Y. Zhou001	101.36°E36.73°N	3032	9	0.69 ± 0.147	0.0070 ± 0.00243
HZH	Huangzhong, QH[a]	G.Y. Zhou002	101.69°E36.30°N	2912	11	0.60 ± 0.154	0.0023 ± 0.00020
PA	Pingan, QH[a]	G.Y. Zhou003	101.90°E36.31°N	2945	12	0.53 ± 0.136	0.0036 ± 0.00274
LD	Ledu, QH[a]	G.Y. Zhou004	102.40°E36.69°N	2704	9	0.75 ± 0.079	0.0029 ± 0.00410
MH	Minhe, QH[a]	G.Y. Zhou005	102.64°E36.17°N	2405	8	0.46 ± 0.200	0.0020 ± 0.00189
HZ	Huzhu, QH[a]	G.Y. Zhou006	102.43°E36.87°N	2800	8	0.46 ± 0.200	0.0014 ± 0.00154
MY1	Mengyuan1, QH[a]	G.Y. Zhou009	101.40°E37.45°N	2945	12	0.86 ± 0.079	0.0064 ± 0.00429
MY2	Mengyuan2, QH[a]	G.Y. Zhou010	102.00°E37.28°N	2770	10	0.53 ± 0.180	0.0022 ± 0.00196
ZK	Zeku, QH[a]	G.Y. Zhou014	101.94°E35.31°N	2907	11	0.69 ± 0.086	0.0024 ± 0.00208
TZ1	Tianzhu1, GS[b]	G.Y. Zhou007	102.66°E36.97°N	2923	10	0.51 ± 0.164	0.0031 ± 0.00249
TZ2	Tianzhu2, GS[b]	G.Y. Zhou008	102.61°E36.93°N	2693	9	0.83 ± 0.098	0.0050 ± 0.00360
XH	Xiahe, GS[b]	G.Y. Zhou011	102.90°E35.37°N	2234	10	0.36 ± 0.159	0.0010 ± 0.00122
HZU	Hezuo, GS[b]	G.Y. Zhou012	102.91°E35.06°N	2919	10	0.00 ± 0.000	0.0000 ± 0.00000
REG	Reergai, SC[c]	G.Y. Zhou013	103.18°E33.60°N	3254	11	0.60 ± 0.154	0.0030 ± 0.00244
Total					140	0.81 ± 0.021	0.0047 ± 0.00308

[a] QH: Qinghai Province China.
[b] GS: Gansu Province, China.
[c] SC: Sichuan Province, China.