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Genetic Variation Within and Among Populations of *Rhodiola alsia* (Crassulaceae) Native to the Tibetan Plateau as Detected by ISSR Markers

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Genetic variation of 10 Rhodiola alsia *(Crassulaceae) populations from the Qinghai–Tibet Plateau of China was investigated using intersimple sequence repeat (ISSR) markers.* R. alsia *is an endemic species of the Qinghai–Tibet Plateau. Of the 100 primers screened, 13 were highly polymorphic. Using these primers, 140 discernible DNA fragments were generated with 112 (80%) being polymorphic, indicating pronounced genetic variation at the species level. Also there were high levels of polymorphism at the population level with the percentage of polymorphic bands (PPB) ranging from 63.4 to 88.6%. Analysis of molecular variance (AMOVA) showed that the genetic variation was mainly found among populations (70.3%) and variance within populations was 29.7%. The main factors responsible for the high level of differentiation among populations are probably the isolation from other populations and clonal propagation of this species. Occasional sexual reproduction might occur in order to maintain high levels of variation within populations. Environmental conditions could also influence population genetic structure as they occur in severe habitats. The strong genetic differentiation among populations in our study indicates that the conservation of genetic variability in* R. alsia *requires maintenance of as many populations as possible.*

KEY WORDS: *Rhodiola alsia*; ISSRs; genetic variation; population structure; Qinghai–Tibet Plateau.

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INTRODUCTION

Knowledge of how genetic variation is partitioned among populations may have important implications not only in evolutionary biology and ecology but also in conservation biology. Hence, reliable estimates of population differentiation are crucial to understanding the connectivity among populations and represent important tools in the development of conservation strategies (Balloux and Lugon-Moulin, 2002). Environmental barriers, historical processes, and life history (e.g., mating system) may all, to some extent, shape the genetic structure of populations (Donnelly and Townson, 2000; Gerlach and Musolf, 2000; Palsson, 2000; Tiedemann *et al*., 2000).

A low level or absence of gene flow among populations is a characteristic of many plant species (Slatkin, 1985). Consequently, genetic differentiation among populations has been reported for a number of endemic species and attributed to local adaptation (Erickson and Hamrick, 2003; Fu *et al*., 2003; Li and Ge, 2002; Travis *et al*., 1996). Most studies on genetic variation have reported that isolation increases the probability that populations will be affected by genetic drift (Ellstrand and Elam, 1993; Van Treuren *et al*., 1991; Young *et al*., 1996). Erosion of genetic diversity within populations has been considered as a cause for a decrease in adaptability to environmental variation (Young *et al*., 1996). Environmental conditions can also influence population structure. A severe environment can reduce recruitment of offspring and the movement of pollen, impact the breeding system and seed dispersal, and affect selection. Biogeographic origin of the populations can influence their level of divergence (Ambruster and Schwaegerle, 1996; Max *et al*., 1999).

Rhodiola alsia (Fröderström) S. H. Fu is a perennial herbaceous plant of the family Crassulaceae. It is distributed on the Qinghai–Tibet Plateau and can be found in the southeast of Qinghai, west of Sichuan, and east of Tibet in China at altitudes between 3400 and 4800 m above sea level (Fu and Ohba, 2001). In addition, as a traditional herbal remedy, the medicine of *Rhodiola* has been used by Tibetans in many ways, such as clearing heat in the lungs, eliminating toxins from the body, treating various epidemic diseases, edema of limbs, traumatic injuries, and burns. Earlier, the plants of *Rhodiola* were used only by local people in small quantities, but commercialization of some species in recent years has increased demand for them and consequently increased their exploitation. *R. alsia* is also used as an herbal remedy by Tibetan people as a substitute for popular species of *Rhodiola* in the Qinghai–Tibet Plateau. Heavy extraction of this plant from the wild, loss of habitat by deforestation, and excessive grazing pressure in high altitude pastures in the entire Qinghai–Tibet region now threaten its survival.

The populations of *R. alsia* are isolated from each other by several mountain ranges and rivers of the Qinghai–Tibet Plateau (e.g., the Tangula, Bayankala,

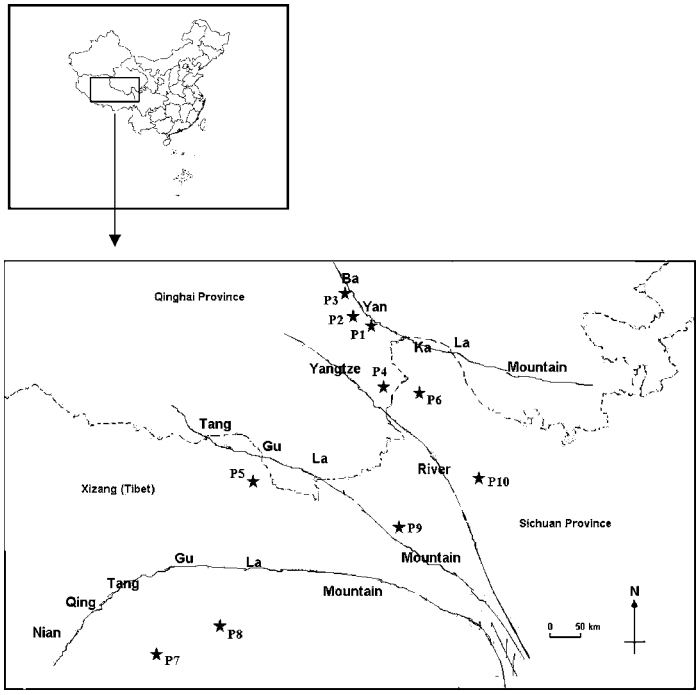

Fig. 1. Location of the 10 *R. alsia* populations sampled from the Qinghai–Tibet Plateau.

and Nianqingtangula mountains and Yangtze River), which are situated at about 29°–35°N and 92°–99°E (Fig. 1). The mean altitude of this area is about 4000 m. This area has three hypsozonal vegetation types: high alpine gelifluction-adapted communities, alpine steppe, and alpine Cyperaceae mats. In high alpine gelifluction-adapted communities frost-induced gelifluction of shattered bedrock is the predominant process, and even during the vegetation period night frosts occur regularly. The thawing depths of the permafrost layer are shallow, and the rhizosphere mostly stays water-saturated. Silty substratum is moved by needle-ice and is therefore often devoid of plants. The snow cover produced by advective disturbances of the weather and hail from convective local thunderstorms may persist for several days even in July or August, especially on shady slopes. The precipitation is between approximately 80 and 300 mm/year. Summer precipitation prevails, but periodically, heavy snowfall occurs in December. Typical annual mean temperatures range from −5°C to around zero. Mean monthly temperatures between November and February are below −10°C; mean minimum temperatures of January can be lower than −30°C. Plant distribution is irregular and diffuse. The

dominant species are cushion and rosette plants: *Thylacospermum caespitosum*, *Saussurea gnaphalodes*, *Stellaria decumbens,* and *Eriophyton wallichii* in the high alpine gelifluction-adapted communities (Miehe and Sabine, 2000). *R. alsia* can be found in the high alpine gelifluction-adapted communities and, rarely, in the alpine steppe. We found that *R. alsia* forms multiramet cushions and mats, which consist of hemispherical rosettes of 6–10 cm in diameter. Although the plant can sexually produce seeds, populations also rely on clonal propagation to maintain their populations.

In recent years, a number of PCR-based DNA markers, such as RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats), and ISSR (intersimple sequence repeats), have been widely used to investigate population genetic structure because they overcome the limitations of allozyme markers (Esselman *et al*., 1999; Rossetto *et al*., 1999; Tani *et al*., 1998). Of these, the most popular marker is RAPD, which has been successfully used in a wide variety of fields (Wolfe and Liston, 1998). As a less widely used PCR-based marker, ISSR has a few advantages over other markers as follows: (1) no prior information or lengthy mapping studies are required; (2) development costs are low; and (3) laboratory procedures can easily be transferred to any plant species. The sequences that ISSRs target are abundant throughout the eukaryotic genome and evolve rapidly. Consequently ISSRs may reveal a much higher number of polymorphic fragments per primer than RAPDs (Esselman *et al*., 1999; Fang and Roose, 1997). In addition, studies have indicated that ISSRs produce more reliable and reproducible bands compared with RAPDs because of the higher annealing temperature and longer sequence of ISSR primers (Nagaoka and Ogihara, 1997; Tsumura *et al*., 1996; Wang *et al*., 2001). Therefore, ISSRs have proved to be useful in population genetic studies (Barth *et al*., 2002; Esselman *et al*., 1999; Wolfe and Liston, 1998; Zietkiewicz *et al*., 1994).

In the present study, we used ISSR markers to examine the genetic structure of *R. alsia* throughout its known distribution. We were particularly interested in the level of differentiation among populations and the levels of diversity within populations, information that would help us decide which populations might have a higher conservation priority. We also used information on the levels and distribution of variation within populations to determine whether genetic drift has affected the species. This information will be used in conjunction with ecological and demographic data to propose an integral conservation strategy for the species.

MATERIALS AND METHODS

Population Sampling

Populations were collected along the mountains and rivers, representing the full range of *R. alsia* in the Qinghai–Tibet Plateau, including Qinghai, Xizang

(Tibet), and Sichuan Province of China, which included most of the species distribution (Fig. 1). Populations were sampled during the months of August and September 2002. Ten natural populations of *R. alsia* were collected from the Qinghai–Tibet Plateau (Fig. 1; Table I). The sites were divided into three regional groups according to their geographical locations. The three groups are N Tangula, N Nianqingtangula, and S Nianqingtangula. The distance between populations within the three regional groups varied between 50 and 200 km.

Young leaf tissue was collected from each sampled individual and dried in silica gel for subsequent DNA extraction. Fifteen to 25 plants were collected with an interplant distance of at least 10 m to increase the possibility of detecting potential among-individual variation. In total, 200 individuals of *R. alsia*, representing 10 populations, were included in the analyses (Table I). Voucher herbarium specimens from each population are housed at the Herbarium of Northwest Plateau Institute of Biology, Chinese Academy of Sciences, People's Republic of China (HNWP).

Total DNA Extraction

Total DNA was extracted using a modification of the protocol of Doyle and Doyle (1987). Dried leaf material was ground to a fine powder in a mortar with liquid nitrogen and then transferred to a 1.5 mL Eppendorf tube filled with 780 μL of preheated 2 $\times$ CTAB extraction buffer containing 0.2% mercaptoethanol. After being incubated at 65°C for 45 min, the homogenate was mixed with 680 μL chloroform: isoamylalcohol (24:1, v/v). Samples were shaken gently for 10 min and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was reserved and 600 μL chloroform: isoamylacohol (24:1, v/v) was added and the process repeated. The supernatant was reserved and mixed with 1/5 to1/2 volume of 5 M NaCl, inverting the tubes several times, then mixed with 2/3 volume ice-cold isopropanol. The tubes were held at -20°C for 1 h to allow precipitation of DNA. DNA was collected as a pellet by centrifugation at 10,000 rpm at 4°C, washed with 500 μL of 70% ETOH twice, dried, and dissolved in 150 μL of 1 $\times$ TE buffer. DNA quality and quantity were determined in 1.0% agarose gels.

ISSR PCR Amplification

One hundred primers from the University of British Columbia (UBC, Vancouver, Canada) were tested for PCR. DNA amplification was performed in a PTC-100 thermocycler (MJ Research Inc., Watertown, MA) using microtiter plates, beginning with 5 min at 94°C, followed by 38 cycles of 30 s at 94°C, 45 s at 50°C, 1 min 30 s at 72°C, and ended with 7 min at 72°C. Reactions were carried out in a volume of 20 μL, containing 50 mM Tris-HCl (pH 8.3), 500 μg mL^{-1} BSA, 10% Ficoll, 1 mM tartrazine, 2 mM $MgCl_2$, 200 μM dNTP, 0.9 μM primer, 20–100 ng

Table I. Geographical Characteristics of Sampled Populations of *R. alsia*

Geographic group	Population	No. of plants	Region	Altitude (m)	Latitude (°N)	Longitude (°E)
■ ▲	P_1	18	Qingshuihe (Qinghai)	4670	34°05′42″	97°37′24″
■ ▲	P_2	19	Maduo County (Qinghai)	4550	34°17′35″	97°58′54″
■ ▲	P_3	18	Chengduo County (Qinghai)	4200	34°52′60″	98°28′38″
■ ▲	P_4	22	Xiewu (Qinghai)	4350	33°12′39″	97°26′51″
□ △ ★	P_5	23	Dingqing County (Xizang or Tibet)	4900	31°41′28″	94°55′32″
■ ▲	P_6	19	Shiqu County (Sichuan)	4390	33°08′57″	97°33′20″
□ △ ☆	P_7	20	Jiacha County (Xizang or Tibet)	4820	29°01′41″	92°21′02″
□ △ ☆	P_8	21	Linzhi (Xizang or Tibet)	3470	29°41′58″	94°43′39″
■ △ ★	P_9	17	Changdu (Xizang or Tibet)	4590	31°17′18″	97°20′44″
■ ▲	P_{10}	23	Dege County (Sichuan)	4560	31°56′44″	98°54′36″

Note. ■ = Northern side of Tangula Mountains (N Tangula); □ = Southern side of Tangula Mountains (S Tangula); ★ = Northern side of Nianqingtangula Mountains (N Nianqingtangula); ☆ = Southern side of Nianqingtangula Mountains (S Nianqingtangula); ▲ = Northern side of Yangtze River; △ = Southern side of Yangtze River.

of DNA template, 1U *Taq* polymerase, and 4% DMSO. Amplification products were resolved electrophoretically on 1.5% agarose gels run at 200 V in 1 × TBE, visualized by staining with ethidium bromide, and photographed ultraviolet light. Molecular weights were estimated using a 100 bp DNA ladder.

Data Analysis

ISSR bands were scored as present (1) or absent (0) for each sample, and the Jaccard coefficient was employed to calculate pairwise band similarities for all 200 individuals using the NTSYS program (Rohlf, 1994). Genetic diversity was measured by the percentage of polymorphic bands (PPB), calculated by dividing the number of polymorphic bands at population and species levels by the total number of bands surveyed. The Shannon index of diversity (I) was also calculated using the POPGENE program (Yeh *et al*., 1997).

The nonparametric analysis of molecular variance (AMOVA program version 1.5) was used to describe the genetic structure and variability among populations as described by Excoffier *et al*. (1992). The matrices containing Jaccard distances between all pairs of phenotypes were used as input distance matrices constructed by the AMOVA-PREP program (Huff *et al*., 1993). A dendrogram was also constructed by an unweighted paired group method of cluster analysis using arithmetic averages (UPGMA).

RESULTS

ISSR Polymorphism

One hundred ISSR primers were screened on three randomly selected individuals by comparing the effects of magnesium concentrations and annealing temperature during amplification. Thirteen primers that produced clear and reproducible fragments were selected for further analysis (Table II). The 13 primers generated 140 bands ranging in size from 200 to 2100 bp, corresponding to an average of 10.7 bands per primer. Of these bands, 80% (112 in total) were polymorphic bands among 200 individuals, i.e., the percentage of polymorphic bands (PPB) for this species was 80%. Every primer produced polymorphic bands when all of the 10 populations were considered. Genetic diversity varied among populations, with PPB values ranging from 63.4% (P_8) to 88.6% (P_7). The Shannon index (I) shows the same trend (Table III).

Genetic Structure of Populations

To assess the overall distribution of genetic diversity, the AMOVA program was used to analyze the distance matrix given by the AMOVA-PREP program. AMOVA showed highly significant ($p < 0.001$) genetic differentiation among populations

Table II. Primers Used for ISSR Amplification

Primer	Sequence of primer (5′–3′)
807	AGA GAG AGA GAG AGA GT
809	AGA GAG AGA GAG AGA GG
812	GAG AGA GAG AGA GAG AA
826	ACA CAC ACA CAC ACA CC
836	AGA GAG AGA GAG AGA GYA
841	GAG AGA GAG AGA GAG AYC
857	ACA CAC ACA CAC ACA CYG
859	TGT GTG TGT GTG TGT GRC
861	ACC ACC ACC ACC ACC ACC
881	GGG TGG GGT GGG GTG
885	BHB GAG AGA GAG AGA GA
888	BDB CAC ACA CAC ACA CA
889	DBD ACA CAC ACA CAC AC

Note. Y = C/T, B = C/G/T, D = A/G/T, R = A/G, H = A/C/T.

(Table IV). A large proportion of genetic variation (70.3%) resided among populations, whereas 29.7% resided among individuals within populations. When populations were grouped based on their origins, genetic variation within groups (70.7%) was much higher than that among groups (29.3%), suggesting that the genetic differentiation occurred mainly among populations rather than between the two groups (the northern side of Tangula Mountains, populations P_1, P_2, P_3, P_4, P_6, P_9, and P_{10}, and the southern side of Tangula Mountains, populations P_5, P_7, and P_8). In a nested AMOVA analysis based on the two main groups, 18.4% of the total variation was found between groups (Table IV). In a nested analysis of the N Tangula group, the variance component among populations was 55.4% and among individuals was 35.4%; much less variation (9.27%) was found

Table III. Genetic Diversity for 10 *R. alsia* Populations

Population	No. bands	No. polymorphic bands	% polymorphic bands (PPB)	Shannon index (I)
P_1	107	74	69.16	0.0729 (0.1657)
P_2	97	73	75.26	0.1469 (0.2158)
P_3	94	77	81.91	0.1740 (0.2399)
P_4	104	75	72.12	0.1221 (0.2059)
P_5	104	73	70.19	0.1116 (0.2045)
P_6	116	73	73.63	0.1459 (0.2180)
P_7	105	93	88.57	0.2235 (0.2414)
P_8	101	64	63.37	0.1329 (0.2230)
P_9	94	74	78.72	0.1488 (0.2229)
P_{10}	96	67	69.79	0.0904 (0.1906)
Species	140	112	80.00	

Table IV. Analysis of Molecular Variance for 200 Plants From 10 Populations of *R. alsia* Using ISSRs

Source of variation	df	SSD	MSD	Variance components	% total variance
Among three main geographic regions	2	1007.6	503.8	3.3	13.6
Among populations within regions	7	3010.2	334.5	14.1	57.9
Among individuals within populations	190	1314.4	6.9	6.9	28.5
Among populations	9	2002.5	286.1	16.4	70.3
Among individuals	190	1314.4	6.9	6.9	29.7
Among N and S side of Tangula group	1	741.3	741.3	4.7	18.4
Among populations within groups	8	3010.2	334.5	13.9	54.4
Among individuals within populations	190	1314.4	6.9	6.9	27.4
Among groups	1	741.3	741.3	7.5	29.3
Within groups	198	3583.3	18.1	18.1	70.7
Among regions in N side of Tangula group	1	334.0	334.0	1.9	9.27
Among populations within regions	5	1242.4	248.5	11.5	55.4
Among individuals within populations	129	827.8	7.3	7.3	35.4
Among regions in S side of Tangula group	1	407.4	407.4	2.2	9.5
Among populations within regions	1	1026.4	342.1	15.1	63.9
Among individuals within populations	61	486.6	6.3	6.3	26.6
Among populations in N side of Tangula group	6	1242.4	248.5	12.2	62.5
Among individuals within populations	129	827.7	7.3	7.3	37.6
Among populations in S side of Tangula group	2	1026.4	342.1	16.6	72.5
Among individuals within populations	61	486.6	6.3	6.3	27.5

Note. All p values obtained were <0.001, except for one negative variance component. Levels of significance were based on 3000 iteration steps. Abbreviations: df—degrees of freedom; SSD—sum of squared deviations; MSD—mean-squared deviations.

between the northern and southern side of Yangtze River. Also in a nested analysis of the S Tangula group, most of the variation was found among (63.9%) and within (26.6%) populations; much less variation (9.5%) was found between the northern and southern side of Nianqingtangula Mountains. In an AMOVA analysis of all N Tangula populations, disregarding geographic region, most of the variation (62.5%) was found among populations and 37.6% within populations. Similarly, the variation among the S Tangula populations was 72.5 and 27.5% among individuals (Table IV).

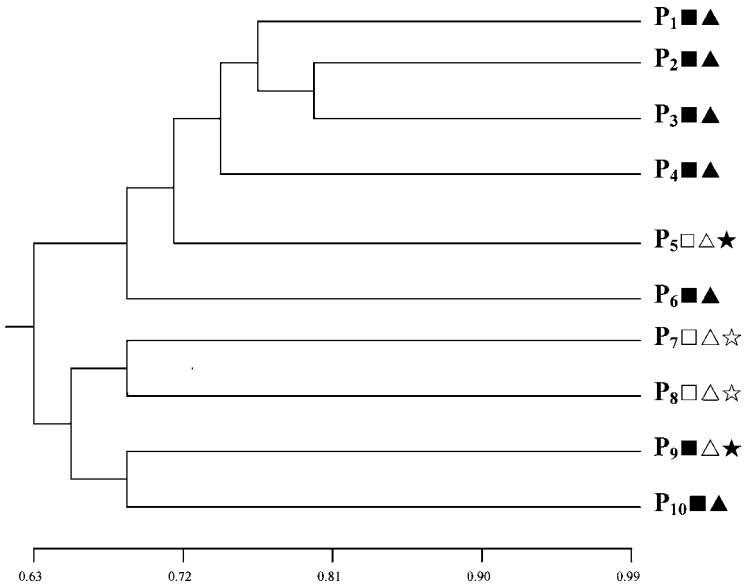

Fig. 2. UPGMA clustering of 10 *Rhodiola alsia* populations based on genetic similarity (Jaccard coefficient) calculated from 13 ISSR markers. ■ = Northern side of Tangula Mountains (N Tangula); □ = Southern side of Tangula Mountains (S Tangula); ★ = Northern side of Nianqingtangula Mountains (N Nianqingtangula); ☆ = Southern side of Nianqingtangula Mountains (S Nianqingtangula); ▲ = Northern side of Yangtze River; △ = Southern side of Yangtze River.

The UPGMA analysis indicated that there are two clusters (Fig. 2). One contains populations P_1, P_2, P_3, P_4, and P_6 from the northern side of Tangula Mountains and population P_5 from the southern side of Tangula Mountains. Populations P_7 and P_8 from the southern side of Nianqingtangula, along with populations P_9 and P_{10} from the northern side of Tangula, are grouped into a second cluster. Populations from two geographic regions (northern and southern side of Tangula) were to a large extent intermingled in the UPGMA. Both the AMOVA analysis and the cluster dendrogram demonstrated that there was no distinct genetic differentiation between geographical regions (northern and southern side of Tangula or Nianqingtangula). A dendrogram based on genetic similarity between individuals showed that all individuals from the same population formed a group (data not shown).

DISCUSSION

Analysis of Genetic Diversity

Intersimple sequence repeats are presumably noncoding loci and therefore have fewer mutational constraints than do isozyme loci (Wolfe and Liston, 1998). Thus,

it is not surprising that ISSRs have provided more polymorphisms than isozymes (Ge and Sun, 1999; Camacho and Liston, 2001). By using ISSR primers, we demonstrated a high genetic diversity for the species of *R. alsia* with 80% of bands being polymorphic in all 10 populations. The percentage of polymorphic bands (PPB) in each population ranged from 63.4 to 88.6%. The genetic variation detected was a little higher than other species in the same genus. By using 16 RAPD primers, Yan *et al.* (1999a) estimated the genetic diversity of *Rhodiola sachalinensis* for 12 populations from the Changbai Mountains of Jilin and Heilongjiang provinces of China and found that the PPB values were 69.6 and 78.8% for the Heilongjiang and Jilin populations. Using allozymes, Zu *et al.* (1998) also revealed low genetic diversity for four populations of *R. sachalinensis* from the Changbai Mountains of Jilin and Heilongjiang. The percentage of polymorphic loci (P) ranged from 22.2 to 44.4 within populations, with the average expected heterozygosity (H_e) being 0.122. Based on 17 allozyme loci encoding 10 enzymes, Yan *et al.* (1999b) found that genetic diversity within populations of *Rhodiola sachalinensis* at high altitude was greater than that of populations at low altitude. We also found that the PPB values were 63.37% in population P_8 at low altitude (3470 m) from Linzhi of Xizang and 88.57% in population P_7 at high altitude (4820 m) from Jiacha County of Xizang. In comparison, *R. alsia* presents high genetic diversity both within populations and among populations. The level and pattern of genetic diversity detected by ISSRs in the present study were in overall agreement with those studies.

Population Genetic Structure

The analysis of molecular variance indicated very pronounced genetic differentiation among populations of *R. alsia* (Table IV). As in our study, pronounced genetic differentiation among populations was found in several rare plant species and attributed to low or absent interpopulation gene flow (Brauner *et al.*, 1992; Dolan, 1994; Fischer and Matthies, 1998; Fischer *et al.*, 2000; Raijmann *et al.*, 1994; Schmid, 1984, 1986; Travis *et al.*, 1996). Observed genetic differentiation among populations of *R. alsia* both between and within regions suggests low gene flow among populations in accordance with the geographic isolation of the populations. Generally, geographical distances between populations of *R. alsia* are large; the median distance to the closest population in our sample was more than 50 km, and the harsh climate and mountainous environment are probable barriers to pollinator movement and seed dispersion. This situation is typical for alpine plants.

A small-scale differentiation in ISSR variation within populations of *R. alsia* was observed. This is consistent with the habitats and biological characteristics of *R. alsia*. This species grows in tundra, on rocky slopes or in rocky caves $\geq$4000 m above sea level, where the environments are very severe. The growing season is only approximately 120 d, the temperatures are extremely cold, the mean annual

temperature is −5°C to 0°C, and snowstorms are common. Although the seeds are winged at both ends for wind dispersal, the likelihood of seeding recruitment is low. Zu *et al.* (1998) found that one of the internal factors of endangered species of *R. sachalinensis* in Changbai Mountains is pollen infertility and external factors such as the severe climate and overcollection by local people. Tang *et al.* (2002) studied seed dispersal patterns and germination of *Rhodiola sachalinensis* from the Changbai Mountains. Geographical conditions, particularly the effects of the wind, are important factors for dispersal of seeds of *R. sachalinensis* in the Changbai Mountains. The seeds could not disperse because the plants usually grow in rocky caves or in leeward areas, even though the seeds are small (weight per thousand seeds is only 0.10–0.14 g). Germination rates are also very low under natural conditions (5–10%), although under experimental conditions rates of 80% have been achieved. *R. alsia* grows in similar habitats to *R. sachalinensis*, suggesting that germination and propagation will be very rare events even though the seeds can disperse. As a result of limited dispersal, the genetic diversity within population depends on the first colonizing plants. The low level of genetic variation within populations and high genetic differentiation among populations in *R. alsia* may be attributed to the clonal propagation of this species. Indeed, Hewitt (1996) suggested that strong genetic differentiation among populations may be related to fragmentation during glaciation periods. It is possible that populations in the Qinghai–Tibet Plateau are remnant populations of a more widely distributed species that have survived during the uplifting of the Qinghai–Tibet Plateau. The latest uplifting occurred at about 1.6 Myr ago. This event led to a colder, drier climate and the formation of the modern river systems. This uplift also was a turning point for climate change and vegetation replacements by alpine shrub, alpine meadow, and the expanded coniferous forest (Shi *et al.*, 1998).

We also found limited genetic differentiation among three groups, which suggests that the populations from different sides of the Tangula and Nianqingtangula mountains are poorly differentiated. Only 13.6% of the total variation was found among three main geographic regions (N Tangula, N Nianqingtangula, and S Nianqingtangula). The most reasonable interpretation of these data is that a very short time has been available for genetic divergence of the species *Rhodiola alsia* after the Qinghai–Tibet Plateau uplift. The lack of genetic divergence among different geographical regions of *R. alsia* is consistent with the general view on the origins of the Qinghai–Tibet Plateau flora. This view concludes that the flora receded from or was extinguished by the Quaternary ice sheet (Wulff, 1944) and that all endemics in the plateau originated in situ or from adjacently distributed genera at the time of uplift (Wu, 1987).

Conservation Implications

A major goal of conservation biology is to preserve genetic variation and evolutionary processes in viable populations of species in order to prevent potential

extinction. Loss of genetic diversity is thought, potentially, to lead to a decrease in a species' ability to survive environmental changes and demographic fluctuations both in the short and long term (Ellstrand and Elam, 1993; Milligan *et al*., 1994; Reisch *et al*., 2003). The maintenance of at least a constant level of genetic variation is therefore generally considered essential for long-term protection of a taxon (Simberloff, 1988). *R. alsia* is an endemic species that has isolated populations in the Qinghai–Tibet Plateau and therefore is considered to have a high priority for the conservation of biodiversity in China. Our investigation revealed that *R. alsia* had strong genetic differentiation among populations. The management for the conservation of genetic variability in *R. alsia* should aim to preserve every population. This is because extinction of any one population would reduce total genetic variability considerably. Detailed studies of the reproductive biology and population demography of this species are currently under way and should yield valuable information for the conservation management of *R. alsia*.

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