HIGH GENETIC DIVERSITY IN THE NATURALLY RARE PLANT TAIHANGIA RUPESTRIS YÜ ET LI (ROSACEAE) DWELLING ONLY CLIFF FACES

ABSTRACT: Cliffs are the primary habitat of many rare and endangered plant species, but few studies have investigated the genetic diversity of these species. *Taihangia rupestris* Yu et Li (Rosaceae) is the only species of the genus *Taihangia*, which occurs exclusively in small crevices on the faces of north-facing vertical cliffs at altitudes ranging from 600 to 1500 m above sea level. It is a perennial herb endemic to the southern part of the Taihang Mountains in northern China. We sampled fresh leaves from 11 to 38 individuals of *T. rupestris* in each of eight cliff-face populations located in the provinces of Hebei, Shanxi and Henan in China. The leaves were dried by silica gel and DNA was extracted. We then assessed the genetic variability within and among the eight populations of *T. rupestris* using random amplified polymorphic DNA (RAPD). Within the 150 plants sampled, 81.2% of the 117 RAPD markers detected were polymorphic, and Nei’s diversity \((H)\) was on average 0.21 in the eight populations and 0.28 in the species as a whole. The grouping of the eight populations by clustering analysis agreed with their pattern of geographical distribution and with the separation of the species into two varieties (\(T. rupestris\) var. ciliata and \(T. rupestris\) var. rupestris). Genetic distances (\(\Phi_{st}\)) were significantly correlated with geographic distances. Although significant genetic differentiation existed between groups (varieties) and among populations, a high proportion (65.4%) of the total genetic variation was maintained within populations. Therefore, high genetic diversity is preserved in the cliff-dwelling populations, and \textit{in situ} protection of *T. rupestris* should focus on the protection of the habitat of both varieties, which may be of particular importance for the long-term survival of this species.

KEY WORDS: cliff ecology; clonal plant; endemic species; restricted habitat; vertical cliffs

1. INTRODUCTION

Geographical distribution and habitat stability are two important factors shaping the genetic structure of plant species (Hamrick and Godt 1989). It is generally believed that narrowly distributed plant species from stable habitats are likely to possess low levels of genetic variability, whereas widely distributed ones from unstable habitats tend to have high levels of genetic variability (Fischer 2000). However, there is also evidence that some narrow endemic plant species from relatively stable habitats possess the same level of genetic variability as their widespread congeners (Gitzendanner and Soltis 2000). Some studies have further revealed that naturally rare species, especially those from glacial refuges, may harbour high levels of genetic variability (Huenneke 1991, Schmidt and Jensen 2000).
Recent studies have demonstrated that cliffs support special ecosystems composed of many rare and endemic plant species (Colas et al. 1997, 2001, Larson et al. 2000, de Lange and Norton 2004, Tang et al. 2004). The habitats of cliffs are much more stable than other habitats such as forests and grasslands, because cliffs are free of massive disturbances caused by competing plant species, grazing animals, fire and human activities (Bunce 1968, Larson et al. 2000, Colas et al. 2001, de Lange and Norton 2004). This, on the one hand, may enable the rare and endemic species to physiologically acclimate to and morphologically adapt to the harsh cliff habitats and, on the other hand, may have reduced the genetic variability of populations in the long run (Ellstrand and Elam 1993). However, up to date, only little attention has been paid to the genetic variability and conservation biology of these naturally rare plant species dwelling only on cliffs (Freville et al. 2001, Liu et al. 2007).

One of such naturally rare, cliff-dwelling species is *Taihangia rupestris* Yü et Li (Rosaceae), a perennial herb endemic to the southern part of the Taihang Mountains in northern China (Yü and Li 1980). *Taihangia rupestris* is the only species of the genus *Taihangia* and occurs exclusively in small crevices on the faces of north-facing vertical cliffs at altitudes ranging from 600 to 1500 m above sea level (Yü and Li 1983, Shen 1994). This species consists of two varieties, *T. rupestris* var. *rupestris* and *T. rupestris* var. *ciliata*. The subdivision into these two varieties is mainly based on the differences in leaf morphology, including the abundance of leaf pubescence, width of leaf blade and the occurrence of glands on the leaf blade. The populations of *T. rupestris* var. *ciliata* occur in the northern part of the species distribution range, while those of *T. rupestris* var. *rupestris* are found in the southern part (Table 1; Yü and Li 1983). Because of the limited number of populations, the narrow distribution and the special habitat, *T. rupestris* was not known to science until 1980 and has been included in the China plant red data book (Fu 1992).

*Taihangia rupestris* is self-compatible and can reproduce both sexually and asexually (Shen 1994). It forms both bisexual flowers and unisexual male flowers. The bisexual flowers produced seed which could develop into new plants under greenhouse conditions (Shen 1994). However, seed setting and seedlings were rarely observed in the wild (Shen 1994, Lu et al. 1995). Lu (1996) found that, when temperature was too low, the pistils of the bisexual flowers of *T. rupestris* tended to abort, resulting in a high proportion of male flowers. The species can also reproduce vegetatively by forming short rhizomes that produce roots and leaves at the tips. However, for lack of soil on the cliff faces, the expansion of the population by vegetative reproduction may be limited. Vegetatively reproduced plants can only establish themselves very close to the mother plants and extend along the crevices on the cliff faces (Tang 2004).

A previous experiment indicated that *T. rupestris* still possessed a large capacity to physiologically acclimate and morphologically adapt to the changing environments (Tang 2004, Tang et al. 2004). Further examination and understanding of the genetic structure and population differentiation of this naturally rare, cliff-inhabiting species are needed.

### Table 1

The sampling sites and numbers of plants collected (n) of the eight cliff-face populations (P1-P8) of *Taihangia rupestris* (see Fig. 1).

<table>
<thead>
<tr>
<th>Code</th>
<th>Location</th>
<th>Variety*</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m asl.)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Lianggou, Hebei Province</td>
<td>ciliata</td>
<td>36°55’ N</td>
<td>113°46’ E</td>
<td>1525</td>
<td>24</td>
</tr>
<tr>
<td>P2</td>
<td>Liejiang, Hebei Province</td>
<td>ciliate</td>
<td>36°53’ N</td>
<td>113°46’ E</td>
<td>1500</td>
<td>22</td>
</tr>
<tr>
<td>P3</td>
<td>Liejiang, Hebei Province</td>
<td>ciliat</td>
<td>36°54’ N</td>
<td>113°46’ E</td>
<td>1292</td>
<td>14</td>
</tr>
<tr>
<td>P4</td>
<td>Nanyao, Shanxi Province</td>
<td>ciliat</td>
<td>36°45’ N</td>
<td>113°31’ E</td>
<td>1427</td>
<td>15</td>
</tr>
<tr>
<td>P5</td>
<td>Sanshimu, Shanxi Province</td>
<td>ciliat</td>
<td>36°40’ N</td>
<td>113°24’ E</td>
<td>779</td>
<td>38</td>
</tr>
<tr>
<td>P6</td>
<td>Jiaogou, Hebei Province</td>
<td>ciliat</td>
<td>36°30’ N</td>
<td>113°38’ E</td>
<td>910</td>
<td>11</td>
</tr>
<tr>
<td>P7</td>
<td>Huayuan, Shanxi Province</td>
<td>rupestris</td>
<td>35°59’ N</td>
<td>113°40’ E</td>
<td>1310</td>
<td>14</td>
</tr>
<tr>
<td>P8</td>
<td>Baiyuns, Henan province</td>
<td>rupestris</td>
<td>35°26’ N</td>
<td>113°21’ E</td>
<td>640</td>
<td>12</td>
</tr>
</tbody>
</table>

* The subdivision into these two varieties is according to Yü and Li (1980).
Using random amplified polymorphic DNA (RAPD) markers, we investigated the genetic variability in eight populations of *T. rupestris* across its geographic range. We specifically addressed the following questions: (1) How high is genetic variability within and among natural populations of *T. rupestris*? (2) Is the level of genetic differentiation among populations correlated with geographic distances? (3) Do the genetic data support the taxonomic separation of two varieties within *T. rupestris*? Based on the results reported, we also discuss indications for the conservation strategy of this species.

2. MATERIALS AND METHODS

2.1. Plant material and DNA extraction

We sampled eight populations of *T. rupestris* across its geographical range in Taihang Mountains (Fig. 1). The location, altitude and sampling size of each population are outlined in Table 1. Individual plants were selected at intervals of 1 m in large and easily reached populations (P1, P3, P5), and at intervals of 0.5 m in populations that are small and difficult to reach (P2, P4, P6, P7, P8). Green leaves were sampled and dried using silica gel. Total genomic DNA was extracted using the CTAB method of Doyle and Doyle (1987).

2.2. RAPD protocol

We tested 120 10-base primers (Shengong Inc.) using three randomly selected samples, from which we selected 16 that could give strong, distinct and reproducible bands. DNA amplification was performed in Rapidcycler 1818 (Idaho Tech.), with the PCR reaction mixture composed of 50 mmol L⁻¹ Tris-HCl (pH 8.3), 725 μg mL⁻¹ BSA, 1% Ficoll 400, 1 mmol L⁻¹ Tartrazine, 2 mmol L⁻¹ MgCl₂, 200 μmol L⁻¹ dNTP, 1 μmol L⁻¹ primer, 0.5 U Taq polymerase and 10 ng of DNA template in a total volume of 10 μL. The reaction was run with two cycles of 1 min at 94°C, 10 s at 37°C, 20 s at 72°C, followed by 40 cycles of 2 s at 94°C, 10 s at 37°C, 70 s at 72°C, and a further 5 min at 72°C. The amplification products were separated on 1.5% agarose gels in 0.5 × TBE (Tris-Borate-EDTA) buffer (pH 8.3), and stained with ethidium bromide. Images were taken using Bio-Rad imaging devices (Gel Doc 2000 Gel Documentation System) supported by Quantity One (version 4.2). Molecular weights were estimated with a 100–3000 bp DNA ladder. To test the repeatability, the

Fig. 1. Geographical distribution of the eight cliff-face populations (P1-P8) of *Taihangia rupestris*. The lines show the borders between the administrative providences (e.g. the providences of Hebei, Shanxi and Henan) in China.
amplifications of some samples were repeated at different times using the same protocol.

2.3. Statistical analysis

Amplified fragments were scored as 1 (presence) and 0 (absence) to compile a binary matrix of RAPD phenotypes for further analysis. Using the program POPGENE (Yeh et al. 1997), the percentage of polymorphic bands (PPB) and Nei’s genetic diversity ($H$) (Nei 1978) were calculated to assess the genetic variability within populations. With the program MVSP 3.1b, principal coordinate analysis (PCO) was performed. The dendrogram was constructed by cluster analysis based on Nei’s genetic distance using UPGMA.

Variation in RAPD patterns were analyzed by analysis of molecular variance using the software of AMOVA (Excoffier et al. 1992).

Table 2. Genetic diversity in the eight cliff-face populations (P1-P8) of Taihangia rupestris (see Table 1, Fig. 1). $H$, Nei’s genetic diversity; PPB, percentage of polymorphic bands.

<table>
<thead>
<tr>
<th>Species</th>
<th>$H$</th>
<th>PPB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0.2297</td>
<td>57.26</td>
</tr>
<tr>
<td>P2</td>
<td>0.2589</td>
<td>61.54</td>
</tr>
<tr>
<td>P3</td>
<td>0.2109</td>
<td>52.14</td>
</tr>
<tr>
<td>P4</td>
<td>0.2318</td>
<td>52.99</td>
</tr>
<tr>
<td>P5</td>
<td>0.2265</td>
<td>59.83</td>
</tr>
<tr>
<td>P6</td>
<td>0.1813</td>
<td>41.03</td>
</tr>
<tr>
<td>P7</td>
<td>0.1816</td>
<td>44.44</td>
</tr>
<tr>
<td>P8</td>
<td>0.1809</td>
<td>43.59</td>
</tr>
<tr>
<td>Mean</td>
<td>0.2127</td>
<td>51.60</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td>81.20</td>
</tr>
</tbody>
</table>

Fig. 2. The dendrogram of the eight cliff-face populations (P1-P8, see Fig. 1, Table 1) of Taihangia rupestris produced by UPGMA clustering based on Nei’s (1978) genetic distance.

Fig. 3. Ordination diagram by principal coordinate analysis of RAPD data for the eight cliff-face populations (P1-P8, see Fig. 1, Table 1) of Taihangia rupestris.
Since two main groups were revealed by the cluster analysis, we included the group factor in the AMOVA model. Overall genetic variation was thus partitioned into three hierarchical levels: between groups, among populations and within populations. The correlation between the matrix of geographical distances and genetic distances ($\Phi_{ST}$) was investigated by Mantel's test (NTSYSpc 2.02a, Rohlf 1997).

3. RESULTS

The amplifications of the same samples at different times using the same protocol produced the same results, suggesting that the method was robust.

3.1. Variation within populations

With the 16 primers, a total of 117 fragments were amplified and each individual sampled had a distinct RAPD phenotype. For the species as a whole, the fraction of polymorphic bands (PPB) was 81.20% and Nei's genetic diversity ($H$) was 0.2890 (Table 2). For the eight populations, PPB ranged from 41.03% to 61.54% and $H$ was on average 0.2127 (range: 0.1809–0.2589, Table 2). The two populations (P7-P8) in the southern part of the species' distribution range, as well as the population (P6) closest to them, had relatively smaller PPB and $H$ than the other five populations (P1-P5; Table 2).

3.2. Variation among populations

The eight populations were separated into two main groups at a genetic distance of 0.88 (Fig. 2). P7 and P8 in the southern part of the geographical range formed one group and the other six populations another. This was supported further by the result of the PCO analysis, in which P7 and P8 were clearly separated from the other populations on the first principal axis (explaining 19.5% of the total variance; Fig. 3). The separation into the two main groups agreed with the separation into the two varieties of *T. rupestris* (Yü and Li 1980).

AMOVA revealed significant genetic differentiation between the two groups (17.81%) and among populations within groups (16.84%), although greater genetic diversity (65.35%) was partitioned within populations of *T. rupestris* (Table 3). Mantel's test showed that genetic distances was significantly positively correlated with geographical distances ($r = 0.79, P< 0.001$; Fig. 4).

4. DISCUSSION

The present study revealed high levels of genetic variability in the naturally rare, cliff-dwelling herb *T. rupestris*.

4.1. Variation within populations

With its limited range of distribution and special habitats, *T. rupestris* was expected to be vulnerable to environmental changes and habitat destruction (Shen 1994). Furthermore, the low frequencies of sexual reproduction and the dependence at least partially on vegetative reproduction for population expansion may have markedly reduced the genetic variability within its populations. However, the mean value of Nei's genetic diversity ($H$, 0.213) in the eight populations of *T. rupestris* was as high as those in populations of 41 higher plant species analysed in other case studies (0.214, Nybom and Bartish 2000). Also a high proportion (81.2%) of the markers was polymorphic and each sample repren-
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presented a distinct genotype. These results indicate high levels of genetic variability in *T. rupestris*.

The exact mechanisms through which the relatively high levels of genetic diversity were maintained in the *T. rupestris* populations were not clear. Based on the finding that rare and endemic species growing on rocky outcrops exhibited great morphological polymorphism, Baskin and Baskin (1988) argued that lack of genetic diversity might not characterize those species. The genetic diversity in the cliff populations of *Thuja occidentalis* L. and *Lloydia serotina* (L.) Reichenb was found to be as high as or even higher than that in populations in their main geographical ranges (*i.e.*, non-cliff habitats, Young 1996 cited by Larsson et al. 2000, Jones et al. 2001). Young (1996) and Jones et al. (2001) attributed the high genetic diversity of the populations on cliffs to the fixed heterozygosity of ancient clones. Therefore, one likely mechanism is that the relatively stable environments on cliff faces aided the natural populations of *T. rupestris* to maintain the genetic variability from ancestral clones.

*T. rupestris* produces male flowers in addition to bisexual flowers. This mating system can reduce selfing to some extent and increase outcrossing between different genotypes. A controlled experiment showed that the rate of fruit set was higher in outcrossed individuals than in selfed individuals of *T. rupestris* (Lu et al. 1995). Therefore, an alternative mechanism is that a relatively high outcrossing rate resulting from the formation of male flowers may have reduced the probability of inbreeding depression and thus helped maintain genetic diversity in *T. rupestris*.

4.2. Variation between populations

The clustering of the eight populations of *T. rupestris* into the two major groups agreed with the geographical distribution and its separation into the two varieties (Figures 1, 2 and 3), and also consistent with the significant genetic differentiation detected between these two varieties (Table 3). This is in contrast to the findings of some other studies (*e.g.*, Max et al. 1999). For instance, almost no genetic differentiation was found between the two subspecies of *Dryas octopetala* L. (Rosaceae) despite their significant morphological differences (Max et al. 1999). According to Yü and Li (1980), the present distribution range of *T. rupestris* in Taihang Mountains was also the original centre of this species. Thus, the time was long enough for the evolution of both morphological and genetic differentiation between the two varieties of *T. rupestris*. However, the factors that were responsible for the morphological and the genetic divergence between the two varieties are unclear. The relationship between the two varieties still needs more exhaustive examination and historical climatic data of this region may help to explain these divergences.

AMOVA also revealed a significant genetic differentiation between populations within each variety of *T. rupestris* (Table 3). The result of Mantel’s test indicates that the process of isolation by distance may have been partly responsible for the population differentiation of *T. rupestris* (Fig. 4), which in turn may have strengthened the division between the two varieties. A significant correlation between genetic and geographical distances was also found in another rare, cliff-dwelling species, *Centaurea corymbosa* Pourret (Freville et al. 2001). However, geographical distances could not explain the genetic differentiation between populations in the two narrow endemics *Antirrhinum charidemi* Lange and *A. valentinum* Front Quer and the endangered *Arctomecon humilis* Covi (Allphin et al. 1998, Mateu-Andrés and Segarra-Moragues 2000).

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Fig. 4. The relationship between pairwise genetic distances (Φst) and geographic distances among the eight populations of *Taihangia rupestris*.

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It was argued that violent disturbances by human activity may at least partly be responsible for lack of correlation between genetic and geographic distances in these species (Allphin et al. 1998, Mateu-Andrés and Segarra-Moragues 2000). In T. rupestris, however, the relatively stable environmental conditions on cliffs but the constant environmental differences between cliffs along the geographic range may have strengthened this correlation (Fischer et al. 2000).

4.3. Conservation strategies

The habitat of T. rupestris seems to be quite stable, since it has been only weakly affected by such common disturbance factors as competition, herbivory, fire and human activity. However, recent increases in human activity in Taihang Mountains, such as road construction and mountain climbing, have greatly increased the risk of damaging the limited natural populations of T. rupestris. For instance, the population in Xiuwu, Henan (P8) has been partly destroyed by cave drilling for road construction. Ex situ conservation of T. rupestris has been carried out in the Botanic Garden of Institute of Botany, Chinese Academy of Science. However, since genetic diversity exists mainly within populations and great genetic differentiation was detected between the two varieties, in situ protection of T. rupestris should focus on the protection of the habitat of both varieties, which may be of particular importance for the long term survival of this species.

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