Comparisons of Genetic Diversity in the Endangered Adenophora lobophylla and Its Widespread Congener, A. potaninii

SONG GE,*‡ KE-QING WANG,* DE-YUAN HONG,* WEN-HUI ZHANG,† AND YUAN-GANG ZU†

*Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
†Department of Forestry, Northeast Forestry University, Harbin 150040, China

Abstract: Starch-gel electrophoresis was used to examine the levels and distribution of genetic diversity in two Adenophora species: the narrow endangered Adenophora lobophylla and its widespread congener, A. potaninii. Based on allozyme variation at 18 putative loci, we measured high levels of genetic variability both in the endangered and the widespread species, with 83.3% of the loci being polymorphic. The mean expected heterozygosity within populations ($H_{ep}$) and within species ($H_{es}$) were 0.234 and 0.244 for A. potaninii and were as high as 0.210 and 0.211 for A. lobophylla. There was higher differentiation among populations in A. potaninii ($F_{ST} = 0.155$) than in A. lobophylla ($F_{ST} = 0.071$). The high levels of genetic diversity in the present allozyme survey are consistent with the morphological variation observed in these species and may be attributed to high outcrossing rates in the Adenophora species. In addition, A. lobophylla was identified as a distinct species on the basis of Nei's genetic distances and thus should be given a high priority for protection. It is noteworthy that the endangered A. lobophylla maintains much higher genetic diversity than most endemic or narrowly distributed plant species in spite of its restricted distribution. We hypothesize that A. lobophylla has become endangered for ecological and stochastic reasons, including habitat destruction or environmental changes, mud slides, and human disturbance such as grazing and mowing. Consequently, habitat protection is of particular importance for conserving this endangered species.
Introduction

Assessment of the level and distribution of genetic diversity within rare and endangered plant species may contribute to knowledge of their evolutionary history and potential and is critical to their conservation and management (Schaal et al. 1991; Hamrick & Godt 1996). Several recent surveys of allozyme variation in plants have shown that rare, endemic, or narrowly distributed plants tend to maintain less allozyme variation than widespread species (Hamrick & Godt 1989, 1996). Many rare and endangered species have been shown to be genetically depauperate, and in extreme cases no polymorphism was found at loci encoding soluble enzymes (Schwartz 1985; Waller et al. 1987; Lesica et al. 1988; P. S. Soltis et al. 1992; Crawford et al. 1994; Godt et al. 1997). But recent studies based on enzyme electrophoresis have demonstrated that rare and endangered species may actually contain high levels of genetic variation, even within extremely narrow distribution (P. S. Soltis & Soltis 1991; Cosner & Crawford 1994; Richter et al. 1994; Lewis & Crawford 1995; Ge et al. 1997). As indicated by Hamrick and Godt (1996), the accuracy of predicting genetic diversity in unstudied species based on such generalization is low because there is high variation in estimates of genetic diversity among species with similar life-history and ecological traits. As a result, growing interest in population genetics and conservation biology has directed attention to comparisons of widespread and restricted congenic species (Karron et al. 1988; P. S. Soltis & Soltis 1991; Edwards & Wyatt 1994; Lewis & Crawford 1995).

Adenophora lobophylla Hong (Campanulaceae) was described in 1982 and was included in the A. potaninii Korsh. complex, which consists of about six closely related species (Ge 1993). Biosystematic studies suggest, however, that A. lobophylla is a distinct species (Ge 1993). According to herbarium records, A. lobophylla has been found in a number of localities in two adjacent counties (Jinchuan and Barkam) in northwestern Sichuan Province of China. Recent expeditions in the northwestern part of the province from 1992 to 1995 indicated that several populations previously recorded in Barkam County are now extinct and that the extant populations in Jinchuan County have gradually been reduced in size, although quite a few populations at the type locality in Jinchuan were recently discovered (Zhang 1995). Therefore, the species is currently confined to moist or damp valleys in the mountains of Jinchuan County (Zhang 1995). In contrast, the widespread and sympatric A. potaninii Korsh. is distributed geographically from northwest to southwest China, including four provinces (Fig. 1). Because these two species are herbaceous perennials with similar life-history traits, they are well suited for a comparison of genetic variation using enzyme electrophoresis.

We analyzed the amount and distribution of genetic diversity in this endangered species by means of enzyme electrophoresis. We were particularly interested in whether the endangered A. lobophylla is distinct, as evidenced by the biosystematic work and whether it is genetically depauperate compared to its widespread congeners. Such information may contribute to a better understanding of the causes of the endangerment of A. lobophylla and may be used in developing recommendations for its recovery and management.

Methods

We sampled 15–30 individual plants randomly from each of five populations of A. lobophylla and seven populations of A. potaninii (Fig. 1); the plants were subsequently maintained in a garden in Beijing and a greenhouse in Harbin. The numbers of individuals sampled for each population are shown in Table 1. Young leaves were removed from each plant and ground immediately in Tris-maleate grinding buffer with 8% PVP and 0.2% v/v 2-mercaptoethanol (modified from D. E. Soltis et al. 1983). Three buffer systems were used for separating enzymes in 12% horizontal starch gels; the buffer numbers are provided in Table 1 of D. E. Soltis et al. (1983). Three buffer systems were used for separating enzymes in 12% horizontal starch gels; the buffer numbers are provided in Table 1 of D. E. Soltis et al. (1983). System 1 was used to resolve alcohol dehydrogenase (ADH), catalase...
(CAT), isocitrate dehydrogenase (IDH), and phosphoglucomutase (PGM). System 6 was used to resolve aspartate aminotransferase (AAT), diaphorase (DIA), phosphoglucoisomerase (PGI), and triosephosphate isomerase (TPI). Leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (PGD), and shikimate dehydrogenase (SKD) were resolved on buffer system 9. Staining procedures for all enzymes followed D. E. Soltis et al. (1983).

When more than one isozyme was observed for an enzyme, isozymes were numbered sequentially, with the most anodally migrating isozyme designated 1. Allelic variation at a putative locus was denoted alphabetically, with the most anodal designated a. Interpretation of the genetic basis of enzyme banding patterns relied on knowledge of previously determined compositions of active subunits of the enzymes and numbers of isozymes expected in diploid angiosperms (Gottlieb 1982; Weeden & Wendel 1989).

Genetic diversity statistics, including percentage of polymorphic loci (P), mean number of alleles per locus (A), observed heterozygosity (H_o) and expected heterozygosity (H_e), and Nei’s genetic distance (D) (Nei 1978) and hierarchical diversity statistics (Nei 1975) were calculated with BIOSYS-1 (Swofford & Selander 1989). We generated an unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis of genetic distance values to examine the species delimitation. Deviation from Hardy-Weinberg equilibrium (fixation index, F) was calculated manually for each population (Wright 1965).

Results

Eighteen putative alloyme loci were resolved with sufficient consistency and clarity for 13 enzyme systems surveyed. Of these, 15 loci were polymorphic, and the remaining three (Pgd1, Pgi1, and Tpi2) were monomorphic in both species. (The table of allelic frequencies for each population is available from the first author on request.)

For the species as a whole, both A. lobophylla and A. potaninii were highly polymorphic and shared the same percentage of polymorphic loci (83.3%) (Table 1). The mean A and H_e values were slightly higher in A. potaninii (2.89 and 0.244, respectively) than in A. lobophylla (2.72 and 0.211, respectively). At the population level, the species maintained comparable levels of variation. Although H_e was slightly higher in the widespread A. potaninii than in A. lobophylla (0.234 vs. 0.210), P and A were remarkably similar: for A. potaninii 71.8 and 2.04 and for A. lobophylla 73.1 and 2.06, respectively.

For the endangered A. lobophylla, F values were negative or slightly positive, with a mean of −0.029. For all A. potaninii populations, in contrast, H_e exceeded H_o with a mean F of 0.129, indicating a deficiency of heterozygotes. The results of F statistics (A. lobophylla F_is = −0.078; A. potaninii F_is = 0.050) parallel these findings.

In Nei’s (1978) unbiased genetic distance calculations (data not shown), the mean genetic distance between populations ranged from 0 to 0.041 in A. lobophylla, with a mean of 0.013, and ranged from 0.001 to 0.036 in A. potaninii, with the mean of 0.016. In contrast, the average distance between populations of two species was as high as 0.130 (range from 0.081 to 0.192), which was 8 to 10 times higher than the averages between populations within species (Fig. 2). It was evident that A. lobophylla populations were genetically differentiated from those of A. potaninii.

We studied the organization of genetic variation in both species using the F statistics of Wright (1965). Overall, mean F_ST was 0.155 for A. potaninii and 0.071 for A. lobophylla, which suggests that the widespread species possess a greater proportion of the overall genetic variation among populations than the endangered one.

### Table 1. Genetic variability statistics of A. lobophylla (L) and A. potaninii (P).

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>A (SE)</th>
<th>P (SE)</th>
<th>H_o (SE)</th>
<th>H_e (SE)</th>
<th>F (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L01</td>
<td>18</td>
<td>1.8 (0.20)</td>
<td>66.7</td>
<td>0.245 (0.062)</td>
<td>0.229 (0.056)</td>
<td>−0.070</td>
</tr>
<tr>
<td>L02</td>
<td>30</td>
<td>2.4 (0.20)</td>
<td>77.8</td>
<td>0.190 (0.052)</td>
<td>0.195 (0.051)</td>
<td>0.026</td>
</tr>
<tr>
<td>L03</td>
<td>15</td>
<td>2.1 (0.20)</td>
<td>72.2</td>
<td>0.234 (0.058)</td>
<td>0.246 (0.050)</td>
<td>0.049</td>
</tr>
<tr>
<td>L04</td>
<td>26</td>
<td>2.1 (0.20)</td>
<td>77.8</td>
<td>0.230 (0.046)</td>
<td>0.227 (0.047)</td>
<td>−0.013</td>
</tr>
<tr>
<td>L05</td>
<td>21</td>
<td>1.7 (0.10)</td>
<td>66.7</td>
<td>0.194 (0.050)</td>
<td>0.169 (0.041)</td>
<td>−0.148</td>
</tr>
<tr>
<td>Mean</td>
<td>2.06 (0.12)</td>
<td>73.1 (2.28)</td>
<td>0.215 (0.010)</td>
<td>0.210 (0.012)</td>
<td>−0.029 (0.031)</td>
<td></td>
</tr>
<tr>
<td>Species level</td>
<td>110</td>
<td>2.72 (0.13)</td>
<td>83.3</td>
<td>—</td>
<td>0.211 (0.032)</td>
<td>—</td>
</tr>
<tr>
<td>P01</td>
<td>23</td>
<td>2.0 (0.02)</td>
<td>72.2</td>
<td>0.219 (0.045)</td>
<td>0.256 (0.046)</td>
<td>0.145</td>
</tr>
<tr>
<td>P02</td>
<td>24</td>
<td>2.1 (0.02)</td>
<td>77.8</td>
<td>0.210 (0.049)</td>
<td>0.261 (0.049)</td>
<td>0.195</td>
</tr>
<tr>
<td>P03</td>
<td>15</td>
<td>2.0 (0.02)</td>
<td>72.2</td>
<td>0.275 (0.059)</td>
<td>0.293 (0.057)</td>
<td>0.061</td>
</tr>
<tr>
<td>P04</td>
<td>27</td>
<td>1.7 (0.02)</td>
<td>61.1</td>
<td>0.165 (0.039)</td>
<td>0.176 (0.043)</td>
<td>0.063</td>
</tr>
<tr>
<td>P05</td>
<td>26</td>
<td>2.4 (0.02)</td>
<td>83.3</td>
<td>0.201 (0.037)</td>
<td>0.243 (0.042)</td>
<td>0.173</td>
</tr>
<tr>
<td>P06</td>
<td>25</td>
<td>2.2 (0.02)</td>
<td>72.2</td>
<td>0.207 (0.050)</td>
<td>0.250 (0.051)</td>
<td>0.172</td>
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<tr>
<td>P07</td>
<td>16</td>
<td>1.8 (0.02)</td>
<td>60.5</td>
<td>0.162 (0.041)</td>
<td>0.169 (0.041)</td>
<td>0.041</td>
</tr>
<tr>
<td>Mean</td>
<td>2.04 (0.09)</td>
<td>71.8 (2.95)</td>
<td>0.203 (0.012)</td>
<td>0.234 (0.015)</td>
<td>0.129 (0.022)</td>
<td></td>
</tr>
<tr>
<td>Species level</td>
<td>156</td>
<td>2.89 (0.17)</td>
<td>83.3</td>
<td>—</td>
<td>0.244 (0.034)</td>
<td>—</td>
</tr>
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</table>

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Nevertheless, the genetic diversity values for the endangered endemic perennial, *Adenophora potaninii*, based on Nei’s (1978) unbiased genetic distance coefficient. Alphanumeric codes of populations correspond to those in Fig. 1 and Table 1.

Discussion

Our study demonstrates high levels of genetic variability both at the population level and at the species level in the two *Adenophora* species. As measured by mean expected heterozygosity within population (*H*$_{ep}$) and within species (*H*$_{es}$), the amounts of genetic variation in *A. lobophylla* (*H*$_{ep}$ = 0.210 and *H*$_{es}$ = 0.211) and *A. potaninii* (*H*$_{ep}$ = 0.234 and *H*$_{es}$ = 0.244) are comparable to or slightly higher than the averages reported for long-lived perennials (*H*$_{ep}$ = 0.084, *H*$_{es}$ = 0.213; Hamrick & Godt 1989). Because *Adenophora* species are protandrous and the mechanism of their out-pollination is fully developed (Ge 1993), it is not unexpected that they maintain high genetic variation. Our recent morphological studies have also demonstrated great variation in floral and vegetative traits in these two species (Ge 1993; Ge & Hong 1994; 1995). The higher differentiation among populations of *A. potaninii* (*F*$_ST$ = 0.155) than of *A. lobophylla* (*F*$_ST$ = 0.071) is expected because *A. potaninii* were sampled for a larger geographic area (Fig. 1). Nevertheless, the genetic diversity values for the endangered *A. lobophylla* (*P*$_s$ = 85.3, *P*$_p$ = 73.1, *H*$_{es}$ = 0.211, and *H*$_{ep}$ = 0.210) are much higher than those found in most geographically restricted plant species (the average diversity of about 100 endemic plant species was *P*$_s$ = 40.0, *P*$_p$ = 26.3, *H*$_{es}$ = 0.096, and *H*$_{ep}$ = 0.063, Hamrick & Godt 1989). Consequently, our results are somewhat surprising considering that all populations of *A. lobophylla* surveyed were sampled from a narrow zone in a single county.

Based on their allozyme survey of 11 *Polygonella* species, Lewis and Crawford (1995) found that two widespread species of *Polygonella* showed reduced within-population genetic diversity relative to their narrowly endemic congeners. They explained their results by high levels of selfing in the widespread species and the large-scale migration during Pleistocene glaciations, which removed much of the diversity of the more northerly distributed and widespread species. Recently, Ge et al. (1997) detected moderate genetic variation (*P*$_s$ = 46.2, *P*$_p$ = 37.5, *H*$_{es}$ = 0.116, *H*$_{ep}$ = 0.091) in an extreme endemic perennial, *Ophiopogon zylorrhizus*, which comprised only about 800 individuals and was distributed in an area of approximately 30 × 20 km. Moderate to high levels of genetic variation have also been documented in a number of other narrowly distributed species, such as in *Elmera racemosa* (P. S. Soltis & Soltis 1991), *Creopsis pulchra* (Cosner & Crawford 1994), and *Delphinium viridescens* (Richter et al. 1994). These data indicate that geographic distribution is not always a good predictor of genetic diversity, although endemic and narrowly distributed plants tend to maintain lower levels of genetic variation than more widespread species (Hamrick & Godt 1989).

As demonstrated by many workers (e.g., Davis & Goldman 1993; Chamberlain 1998), isozyme data have been used successfully for taxonomy and species delimitation, which in turn has important implications for the evaluation of species conservation (Vogler & Desalle 1994). *A. lobophylla* was traditionally considered a close relative of *A. potaninii* but was later suggested to be a distinct species based on detailed morphological analyses and crossing work (Ge 1993; Ge & Hong 1994; 1995). Our study, using cluster analysis of Nei’s genetic distances (Fig. 2), provides further evidence for the genetic distinctiveness between *A. lobophylla* and *A. potaninii*. *A. lobophylla*, therefore, is evolutionarily distinct and as such should be given a high priority for protection. 

Zhang (1995) conducted an ecological study on *A. lobophylla* and found that, unlike most other *Adenophora* species, this endangered species occurs in a variety of wetland habitats at elevations of 2200–3200 m. The most evident feature common to all these habitats is half-shade with a relatively high level of groundwater, such as among shrubs at forest edges by streams, which suggests that this species has specific environmental requirements (Zhang 1995). Given its habitat specificity and narrow distribution, *A. lobophylla* appears to be extremely vulnerable to habitat loss and fragmentation as well as to local catastrophes. Nevertheless, the habitats suitable for *A. lobophylla* have been destroyed or degraded from decades of agriculture, silviculture, and human disturbance. In addition, periodic mud slides and frequent grazing and mowing have significantly decreased both the extent and density of *A. lobophylla* (Zhang 1995). Consequently, the endangerment of *A. lobophylla* could be attributed mainly to ecological factors, and the high levels of genetic diversity in *A. lobo-
phylla may be ascribed to the recent reduction in population numbers and sizes.

Some researchers (Schemske et al. 1994; for a review see Hamrick & Godt 1996) have argued that populations and species more often go extinct for ecological and demographic reasons rather than for the lack of genetic variation. This is most likely the case with A. lobophylla. For the long-term survival of these species, therefore, habitat protection and in situ conservation are of particular importance. Also, ex situ efforts need to be undertaken for those small but highly variable populations, such as L03 of A. lobophylla, which are especially vulnerable to demographic stochasticity. With population genetics data now available, an appropriate strategy for the preservation and sampling of populations could be formulated when in situ and ex situ conservation are required.

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Literature Cited


