

Genetic Diversity and Differentiation in Remnant Populations of *Bupleurum latissimum* Nakai, an Endangered Endemic Plant Species to Ulleung Island, Korea

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Bupleurum latissimum is a narrowly endemic and endangered plant, restricted to only two small populations on steep cliffs of a small island, Ulleung Island, in Korea. The genetic diversity and population differentiation in the two remnant populations of the species were investigated using RAPD (random amplified polymorphic DNA) analysis. The Nei's gene diversities were 0.146 in the smaller population of 45 individuals, and 0.151 in the larger population of 61 individuals. The genetic variation was not significantly different between these two populations. Genetic diversity within populations was not low considering the very small size of populations. Analysis of molecular variance (AMOVA) revealed higher variation within populations (65.9%) than genetic differentiation between them (34.1%). *B. latissimum* revealed higher population differentiation than other outbreeding species. The differentiation of the populations corresponded to low gene flow ($N_e m = 0.482$). The cluster and principal coordination analyses provide strong support for high population differentiation, showing that all individuals of the two populations have built up population-specific clusters. Although gene flow between the two populations of *B. latissimum* was limited, they have preserved relatively high levels of genetic variation.

Habitat fragmentation has a great effect on demographic and genetic aspects of plant populations (Schemske et al., 1994). Plants of islands are characterized by a large number of endemic species, and gene flow from the mainland to island populations is limited due to the spatial isolation (Baldwin et al., 1998). Isolation and fragmentation can cause genetic erosion owing to founder effects, genetic drift and inbreeding depression (Ellstrand and Elam, 1993). These responses could vary as a result of differences in habitat, distance among populations, population size, pollination patterns and microclimate conditions (Juan et al., 2004). Recently, many studies on the genetic consequences of fragmentation have been carried out in the floras of islands (e.g. Maki et al., 2003; Richardson et al., 2003; Tomimatsu and Ohara, 2003; Juan et al., 2004).

Species of genus *Bupleurum* (Apiaceae) occur in Asia, Europe, North Africa and North America. Despite the broad distribution, most species of the genus are rather

rare and restricted to small areas (Neves and Watson, 2004). *B. latissimum* is also a rare member of the genus which is endemic to Ulleung Island, a volcanic island located in East Sea between Korea and Japan. The area of the island is 72.6 km² and the shortest distance from the mainland is 137 km. This island has more than 30 endemic plant species and more than 25 rare plants (Kim et al., 2000; Kim and Lee, 2001).

Although *B. latissimum* appears to be naturally rare, habitat disturbance and fragmentation may have contributed to its extreme restriction. There are no records that *B. latissimum* has been collected or observed (Kim and Yoon, 1990; Choi et al., 1996; Kim et al., 2000), since Lee (1967) studied chromosome numbers of this species. This plant was presumed to be extinct for a long time. But recently, Yang et al. (2002) found extant populations. Currently, this plant is classified an endangered species by the Ministry of Environment (Hyun, 2001). Information on the degree of genetic variation within a population and its distribution within and between populations would aid in conservation planning. The objective of this study was to investigate the genetic variation and population differentiation in two

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remnant populations of *B. latissimum* using random amplified polymorphic DNA (RAPD) markers.

Materials and Methods

Studied species and populations

Bupleurum latissimum (Apiaceae) is a small herb with a chromosome number of $2n=16$ (Lee, 1967). This plant is a monocarpic perennial, lacking vegetative reproduction, and appears to be hermaphrodite and entomophilous. It is found in the shallow cracks of naked rocks or in stony soil in sparse thickets on steep rocky cliffs. The species is a narrow endemic, confined to precipitous cliffs on Ulleung Island. Only two populations were found in the whole island. The exact localities of the two populations are not disclosed here to prevent their habitats from being destroyed. The inaccessible locations of *B. latissimum* populations due to its growth on steep cliffs afford the species some protection from human disturbances. Yet, because of the fragile nature in their habitats and their small sized populations, accidental disturbances or changes could lead to their extinction.

We sampled two populations, small (S) and large size (L), separated geographically by 5 km. Each population had a limited number of individuals with no more than 30 flowering individuals and a few seedlings (Table 1). The population S consisted of 45 plants excluding seedlings. Its total distribution area was $20 \times 10 \text{ m}^2$, but most individuals were within $15 \times 5 \text{ m}^2$. The relative light intensity was below 5% on herbaceous layer of *B. latissimum* because the canopy of trees was almost closed. The growth of *B. latissimum* was interrupted by some vines such as *Parthenocissus tricuspidata*, which covered the cliff. The population L consisted of 61 plants, 25 of which were flowering. This population was larger in area ($50 \times 20 \text{ m}^2$) and had more flowering plants than the population S. Within the two natural populations, the recruitment of seedlings is rare, although an individual can produce more than 1,000 seeds.

DNA isolation and RAPD analysis

Leaf samples collected in the two populations of *B. latissimum* were stored with silica gel in zip-lock plastic bags until DNA isolation. DNA was extracted using NucleoSpin Plant kit (Macherey-Nagel). DNA amplification

Table 1. Distribution area, number of individuals, and sample size of RAPD analysis in the two populations of *Bupleurum latissimum*

Population	Area (m ²)	No. of plants*	No. of flowering plants	Sample size for RAPD
S	20×10	45	12	40
L	50×20	61	25	22

*Excluded seedlings

Table 2. Ten random primers used RAPD analysis

Primer	Sequence (5' to 3')	Primer	Sequence (5' to 3')
OPA-08	GTGACGTAGG	OPN-08	ACCTCAGCTC
OPA-13	CAGCACCCAC	OPN-11	TCGCCGCAAA
OPAF-08	CTCTGCCTGA	OPO-02	ACGTAGCGTC
OPAF-15	CACGAACCTC	OPO-16	TCGGCGGTTT
OPN-07	CAGCCCAGAG	OPW-03	GTCCGGACTG

for random amplified polymorphic DNA (RAPD) analysis was performed in a Programmable Thermal Cycler (GeneAmp 2700, AB), and commenced with a 2-min incubation at 94°C, followed by 45 cycles of 5 s at 94°C, 1 min at 36°C, 70 s at 72°C, and ended with 5-min incubation at 72°C. Reactions were carried out in a volume of 25 µl containing 25 mM MgCl₂, 10X reaction buffer, 1 U *Taq* polymerase (M1861, Promega), 2 mM dNTPs (S240, Promega), 5 pM primer, and template DNA. Products amplified by PCR were resolved electrophoretically on 1.5% (w/v) agarose gels run at 120 V in 1×TBE, visualized by staining with ethidium bromide, and photographed under ultraviolet light. Ten primers for RAPD analysis were purchased from Operon (Table 2).

Statistical analysis

The presence/absence of bands from PCR products were coded and the produced matrix was used for statistical analysis. Genetic similarity was calculated as Jaccard coefficient for all possible pairwise comparisons of individuals within and among populations (Sneath and Sokal, 1973). Associations among individuals were revealed by a cluster analysis with the unweighted pair group method using arithmetic averages (UPGMA) based on a Jaccard coefficient using NTSYS-pc software (Rohlf, 2000). Principal coordinate analysis (PCO) with Jaccard coefficient was also performed using NTSYS-pc. Genetic variation was measured by the percentage of polymorphic loci (P) and Nei's gene diversity (H; Nei, 1973) using the POPGENE program (Yeh et al., 1997). The genetic structure of the populations was analyzed by analysis of molecular variance (AMOVA) using program ARLEQUIN (Schneider et al., 2000). Genetic differentiation (Φ_{st}) between populations was also obtained using the ARLEQUIN package. Furthermore, gene flow between populations ($N_e m = 0.25 \times (1/\Phi_{st} - 1)$) was calculated from Φ_{st} values (Wright, 1951).

Results

With ten primers the RAPD analysis yielded a total of 168 bands ranging in size from 350 to 2,050 bp in the

Table 3. Genetic variation in the two populations of *Bupleurum latissimum*

Populations	Polymorphic RAPD bands		Nei's gene diversity
	No.	Polymorphism(%)	
S	105	62.5	0.146
L	94	56.0	0.151
Total	127	75.6	0.177
Mean	100	59.2	0.149

two populations of *B. latissimum*. Of these bands, 127 (75.6%) were polymorphic and 41 monomorphic (24.4%) among the 62 individuals (Table 3). Population S produced 105 polymorphic bands (polymorphism 62.5%) and the population L 94 (56.0%). The mean Nei's gene diversity was 0.149 in the two populations of *B. latissimum*. Genetic variation was not significantly different between the two populations.

The 62 sampled individuals comprised 62 RAPD phenotypes. The mean genetic similarity (Jaccard

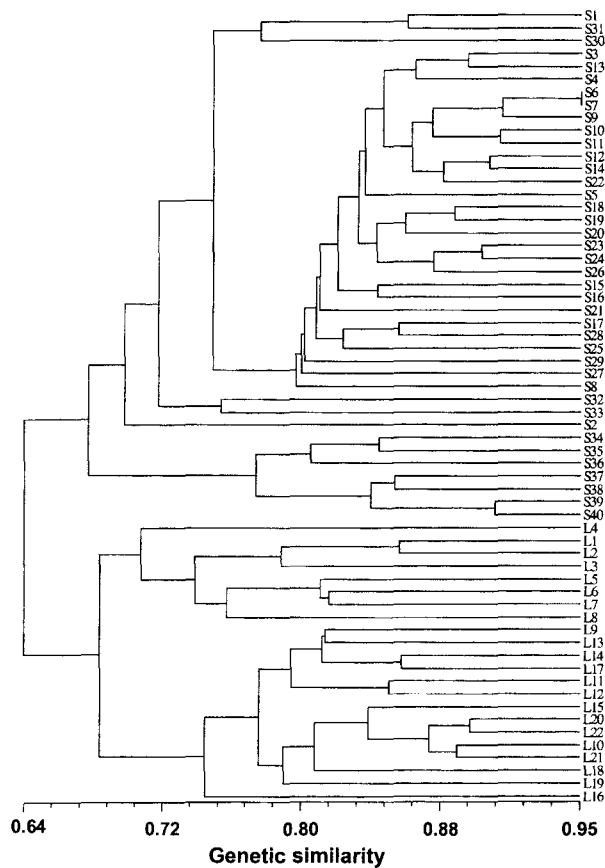


Fig. 1. UPGMA (unweighted pair group methods with arithmetic averages) dendrogram of RAPD (random amplified polymorphic DNA) markers based on the Jaccard genetic similarity in two populations of *Bupleurum latissimum*. S indicates the small population, L the large population, and numeral sample number.

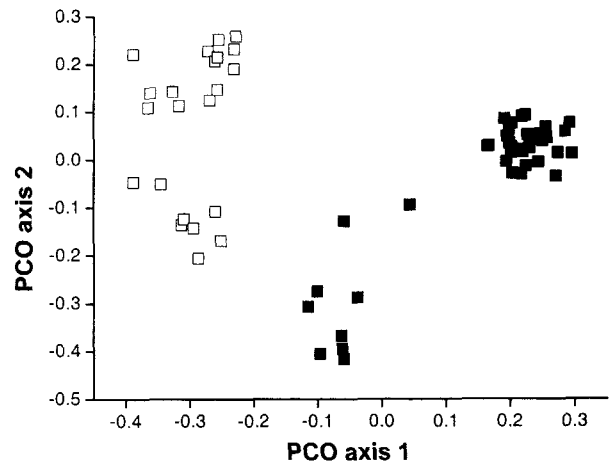


Fig. 2. PCO (principal coordinates) analysis of RAPD (random amplified polymorphic DNA) data in two populations of *Bupleurum latissimum*. Closed square indicates the small population and open square the large population.

coefficient) between all possible comparisons of individuals was, on average, 0.70, with the highest 0.95 and the lowest 0.48. Association among genetic similarities of all 62 individuals by cluster analysis revealed that all individuals of the two populations were arranged in population-specific clusters (Fig. 1). The two populations also segregated along a continuum of variation in principal coordinate (PCO) axis 1 (Fig. 2).

Variation in the RAPD banding pattern was highly significant between and within populations ($p < 0.001$). A large proportion of genetic variation (65.9%) resided within populations, whereas 34.1% resided between populations (Table 4). Genetic differentiation (Φ_{st}) between populations was 0.341, which was same as the proportion of genetic variation between populations. Estimate of $N_e m$ indicated that the number of individuals exchanged between populations per generation was low (0.482).

Discussion

Genetic variation

Genetic diversity within populations in small isolated population is very important for species conservation. Geographic range has been shown to have a significant effect on the levels of genetic diversity within species. In small isolated populations with restricted geographic

Table 4. Analysis of molecular variance for two populations of *Bupleurum latissimum* based RAPD variations

Source of variation	df	Variance	% Total	P^*
Between populations	1	5.23	34.1	<0.001
Within populations	60	10.09	65.9	<0.001

*Levels of significance are based on 3024 iteration steps

distribution, genetic variation within populations is reduced as a result of limited gene flow (Soejima et al., 1998; Gitzendanner and Soltis, 2000; Cole, 2003). Nei's gene diversity (H) was 0.149 and the percentage of polymorphic loci (P) was 75.6% in the two populations of *B. latissimum* (Table 3). According to Nybom and Bartish (2000), populations of outcrossing species have on average $H=0.260$ of gene diversity. *B. latissimum*, a narrowly endemic species, has lower levels of gene diversity. The decrease in genetic diversity could have occurred in for long term after isolation, or in short term with recent habitat fragmentation. Many studies of rare and geographically restricted plant species have reported no or little genetic variation as a consequence or cause of their rarity (Barrett and Kohn, 1991; Hamrick et al., 1991; Paschke et al., 2002). But the gene diversity in *B. latissimum* was somewhat higher than in other endangered plants, e.g. $H=0.11$ and $P=32.58\%$ in *Changium smyrnioides* (Qiu et al., 2004), $H=0.146$ and $P=45.2\%$ in *Paphiopedilum micranthum* (Li et al., 2002a), $P=32.46\%$ in *Vatica guangxiensis* (Li et al., 2002b). Therefore, the endangered species *B. latissimum* keeps relatively high genetic variation although this plant is found in only two small populations on a small island. There are also other examples of rare species that show high levels of variability (Young and Brown, 1996; Torres et al., 2003).

Based on RAPD variation, we could not find a significant difference in genetic variations between the large (L) and small (S) populations of *B. latissimum* (Table 3). Generally, genetic diversity in plants is significantly correlated with the actual number of individuals in a population (Fischer and Matthies, 1998; Fischer et al., 2000; Paschke et al., 2002). However, it should be noted that the actual sizes of populations were not different greatly between the two populations of *B. latissimum* (45 individuals in S and 61 in L; Table 1).

Population differentiation

A high degree of RAPD variation was found within rather than between populations of *B. latissimum*, an entomophilous perennial (Table 4). That most genetic diversity existed within populations is consistent with the general trend in other outcrossing species (Hamrick et al., 1991; Nybom and Bartish, 2000). The amount of variance between populations (34.1%) was lower than in some rare outcrossing species (Fischer and Matthies, 1998; Schmidt and Jensen, 2000; Paschke et al., 2002; Qiu et al., 2004), but higher than in others (Fischer et al., 2000; Li et al., 2002a; Ronikier, 2002). Bussell (1999) reviewed RAPD studies on population genetics of 38 plant species and indicated that the average genetic variance component among populations of the outbreeding species given by AMOVA was 14.4% (0-41.3%). Nybom and Bartish (2000) compiled 78 estimates of AMOVA-derived Φ_{st} using RAPD markers and showed that the

average Φ_{st} of the outcrossing species was 0.28. The fact that our results are higher than these two averages indicates that *B. latissimum* underwent higher population differentiation than other outbreeding species.

The differentiation between populations is related to expected value of $N_e m$. In *B. latissimum* population, $N_e m$ index (0.482) was below 1. Its value is similar to those for outcrossed and endangered species reported by Hamrick (1987). $N_e m$ represents cumulated historical average levels of gene flow (Lee et al., 2002). The UPGMA tree and PCO analysis provide strong support for high population differentiation, showing that all individuals of two populations built up population-specific clusters (Fig. 1 and Fig. 2). This indicates the possibility that the gene flow between the two populations has been very low and genetic differentiation is occurring.

Conservation implications

Resources available for conservation are limited, and it can be asked how many, or which populations of plants are worth conserving. Genetic studies may provide answers to this question. Given that only two populations of *B. latissimum* are known, however, both populations require preservation irrespective of genetic considerations (Hogbin and Peakall, 1999).

Small populations have an increased risk of extinction because of environmental, demographic, and genetic stochasticity (Lacy, 1987; Fischer and Matthies, 1998; Fischer et al., 2000; Schmidt and Jensen, 2000; Keller and Waller, 2002). A population size of more than 1,000 individuals has predicted to be required to sustain quantitative genetic variation (Lande, 1995). Because both populations of *B. latissimum* consist of less than 100 individuals, the levels of genetic variability are likely to be reduced in future. Though we cannot identify the actual genetic mechanism, it appears important to minimize further genetic erosion by genetic drift, and management should aim to increase the number of plants in the populations. However, such management must be applied with caution, because population differentiation between the two populations suggests that the local genotype should be maintained and not intermixed during the conservation program. That is, interpopulation crosses could result in reduced offspring fitness because of outbreeding depression (Barrett and Kohn, 1991). Because pollinator behavior is related with gene flow and genetic differentiation among populations (Souto and Premoli, 2003), it would be necessary to study pollination of *B. latissimum* in the future.

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