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Genetic Variation in Geographically Peripheral Populations of *Bupleurum euphorbioides* (Apiaceae) with Comparison to a Widespread Congener, *B. longiradiatum*

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Abstract: *Bupleurum euphorbioides* is isolated and restricted to high mountains in Korea northeastern China. Its conservation depends on whether it is threatened by inbreeding or a loss of genetic diversity. We compared the genetic variability in *B. euphorbioides* with *B. longiradiatum*, a widespread congener, to understand how they differ in their population genetic structure. Although *B. euphorbioides* showed a little lower genetic variability than *B. longiradiatum*, F_{IS} statistics for most loci were strongly positive in both *B. euphorbioides* (0.445) and *B. longiradiatum* (0.553). In addition, *B. euphorbioides* showed higher mean F_{ST} value than *B. longiradiatum* (0.297 vs 0.194). It might be due to the polycarpic nature of *B. longiradiatum*, which holds higher genetic potentials effectively in homogeneous environment than the monocarpic *B. euphorbioides*. The results suggested that *B. euphorbioides* is a genetically viable species, and that they are threatened primarily by environmental factor.

Key words: *Bupleurum euphorbioides*, *Bupleurum longiradiatum*, conservation priority, inbreeding, monocarpic, polycarpic

To establish a conservation program for rare and endangered plants, it is important to understand their biological characteristics including ecology and genetics. Habitat preservation practices and other ecological considerations have been emphasized in the past, and the management action plans for rare plants have been gradually changed to be more integrative including population genetic study (Falk, 1990). Studies on the population genetic structure in plants can offer a wide range of information on the level and distribution of genetic variation (Hamrick et al., 1991), sufficiency of genetic variation being the major issue in evolutionary conservation genetics (Frankel and Soulé, 1981). Many theoretical works have revealed that genetic mechanisms such as inbreeding or genetic drift in small population caused by genetic bottlenecks and founder effects are important factors in reducing genetic variability (Charlesworth and Charlesworth, 1987; Barrett and Kohn, 1991).

The long-term survival of populations will be enhanced if management efforts aim not only at propagating plants, but also at conserving genetic variations within populations. Thus, one aspect of the criteria for self-sustaining designation is that populations must contain sufficient genetic variations to adapt to natural habitat changes (Kress et al., 1994).

Bupleurum euphorbioides Nakai (Apiaceae) is a long-lived monocarpic herb, which has a restricted occurrence in the Korean peninsula and Mt. Chang-pai region in northeastern China (Jirin province) (Shan and Sheh, 1979; Fig. 1). Throughout its distribution, it is restricted to alpine environments (ca. 1,500 m above sea level). In South Korea, among the six known populations, five populations of *B. euphorbioides* inside National Parks area have been managed by Ministry of Environments. Populations of this species ranging in size from 100 to 1,200 individuals are distributed patchily. The species was classified as "Regionally Vulnerable" on the recent proposition for the Korea Red List of vascular plants (Chang et al., 2001). This herbaceous perennial consists of rosette with its radical leaves arising from single deep root on open bare rocks and has 10-40 small green umbels. Little is known of its breeding system, but small fly pollinators and the floral morphology facilitate

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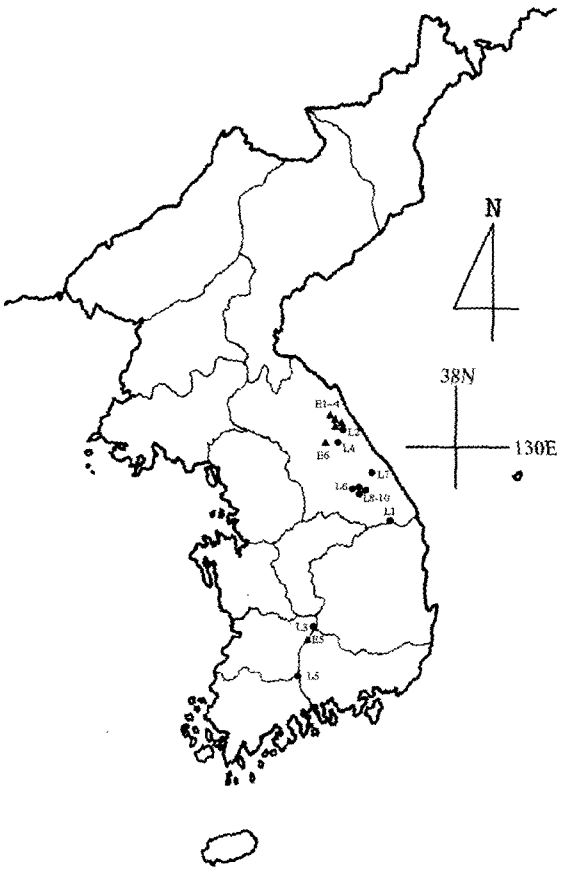

Fig. 1. Locations of 16 populations of *Bupleurum euphorbioides* and *B. longiradiatum* in Korea from which samples were collected for electrophoretic analysis: six populations of *B. euphorbioides* (▲), ten populations of *B. longiradiatum* (●).

selfing (personal observation in 1995-1997). Moreover, a preliminary study indicated that the species was at least partially self-compatible and geitonogamous (Kim, 1996). The field and demographic studies have also demonstrated that the adult stage individuals, less than 5% of populations, bloomed between late June and early August. The existing populations apparently fluctuate year by year, but sizes of some populations were presumed to increase continuously during our monitoring (Kim, 1996). Another congener, *B. longiradiatum*, is a polycarpic perennial with a long stem (50-120 cm) and both radical and cauline leaves. *B. longiradiatum* has a fibrous root without rhizome and runner, grows under deciduous forests above 1,000 m sea level, and is widely distributed in Korea.

The choice of species to be compared provides a limited perspective because it represents a serious bias against species that are rare, endemic, and/or difficult to grow in cultivation. For these reasons we needed to make a special effort to study locally endemic species and compare *B. euphorbioides* to its more widespread relative, *B. longiradiatum*. The two species share similar floral morphology, but are distinguished from each other on monocarpic vs polycarpic nature, short vs long stem, and alpine vs deciduous forest distribution. Our major goal was to compare populations of *B. euphorbioides* with those of *B. longiradiatum*, and to understand how the two species might differ in their population genetic structure. The second objective was to show the amount of temporal difference of genetic diversity between adults and juveniles of *B. euphorbioides* by investigating the potential dependence of genetic variation on ecological factors. The last aim was to provide guideline information on conservation of *B. euphorbioides*.

MATERIALS AND METHODS

Population samples

Six populations of *B. euphorbioides* and 10 populations of *B. longiradiatum* in Korea were collected during the spring and summer of 1995 and 1996 (Fig. 1, Table 1). For each population, we collected 21 to 53 individuals. Only adult individuals were sampled by removing one or two leaves to prevent the possibility that leaf removal might significantly reduce the probability of the plant's survival. To compare the differences in genetic structures between flowering adults and non-flowering juveniles of *B. euphorbioides*, the plants were grouped based on the presence/absence of flowers.

Extraction, electrophoresis, and genetic inference

Leaf tissues were kept cool and moist until enzyme extraction. Leaves were homogenized in the extracting buffer (Wendel and Weeden, 1989). The homogenized extracts were absorbed into filter paper (Whatman 3MM, 4 mm×10 mm) and were kept frozen at -70°C until electrophoresis was performed on 12% starch gels. Samples were electrophoresed for 4-6 hour on four gel and buffer systems (Conkle et al., 1982; Wendel and Weeden, 1989). Aconitase (ACO), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), and shikimate dehydrogenase (SKDH) were resolved on a continuous Tris-citrate buffer (pH 7.0) system. A system of lithium borate gel/ Tris-citrate electrode resolved catalase (CAT) and phosphoglucoisomerase (PGI). Glutamate dehydrogenase (GDH) and glutamate-oxaloacetate transaminase (GOT) were resolved on a sodium-borate electrode/ Tris-citrate buffer system. A continuous morpholine buffer system was used to resolve glucose-6-phosphate dehydrogenase (G6PDH), menadione reductase (MNR), and 6-phophogluconate dehydrogenase (6-PGDH). Enzymes were visualized using staining methods detailed by Weeden and Wendel (1989). Most staining recipes were modifications of the widely used assays presented in earlier manuals (Conkle et al., 1982; Soltis et al., 1983).

For enzymes with more than one locus, allozymes were

Table 1. Location data for populations of *Bupleurum euphorbioides* and *B. longiradiatum* sampled from Korea

Species	Code	Size	Population
Bupleurum euphorbioides	E1	<1,200	Jungcheongbong (alt. 1,600 m), So-rak-san, Gangwon-do Province
	E2	200<	Socheongbong (alt. 1,600 m), So-rak-san, Gangwon-do Province
	E3	<400	Madeungryong (alt. 1,327 m), So-rak-san, Gangwon-do Province
	E4	<1,000	Hwachaebong (alt. 1,300 m), So-rak-san, Gangwon-do Province
	E5	300<	Duk-yu-san (alt. 1,508 m), Jeollabuk-do Province
	E6	<200	Garibong (alt. 1,519 m), Gangwon-do Province
B. longiradiatum	L1	Continuous populations more than 5,000 for each population	Taebaeksan (1,560 m), Gangwon-do Province
	L2		So-rak-san (alt. 1,708 m), Gangwon-do Province
	L3		Duk-yu-san (alt. 1,614 m), Jeollabuk-do Province
	L4		Jeombongsan (alt. 1,200 m), Gangwon-do Province
	L5		Jirisan (alt. 1,734 m) Jeollanam-do Province
	L6		Joong-wang-san (alt. 1,300 m), Gangwon-do Pronvince
	L7		Gye-bang-san (alt. 1,577 m), Gangwon-do Province
	L8		Mt. Ka-ri-wang-C (alt. 1,561 m), Gangwon-do Province
	L9		Mt. Ka-ri-wang-B (alt. 1300 m), Gangwon-do Province
	L10		Mt. Ka-ri-wang-A (alt. 1000 m), Gangwon-do Province

numbered sequentially, with the most anodal designated as 1. Loci were considered putative because no previous genetic analyses were performed on this species.

Genetic parameter analysis

Allele frequencies for each locus in each population and genetic structure of the entire set of populations were analyzed using BIOSYS-1 (Swofford and Selander, 1981). The genetic parameters including the number of alleles per locus (A), the percent of polymorphic loci (P) using the 95% criterion, the observed (H_o) and expected (H_e) heterozygosity, and Wright's (1951) fixation index (F) were calculated. A goodness-of-fit (χ2-test) test of genotypic frequencies to Hardy-Weinberg equilibrium was performed on each variable locus.

Population structure was analyzed using Wright's (1965) F-statistics where F_{IT} represents the overall inbreeding coefficient, F_{IS} the levels of inbreeding due to nonrandom mating within populations, and F_{ST} population subdivision. Nei's (1973; 1978) indices were also calculated: the total allelic diversity (H_T), the allelic diversity within populations (H_S), and the proportion of total diversity distributed among populations (G_{ST}) were measured.

To analyze whether there was a relationship between the geographical distance of populations and their degree of genetic similarity, Nei's (1978) unbiased identity measure was used in cluster analysis by the unweighted pair group method (UPGMA), and the results were compared to the spatial distance between populations. Genetic variations among life stages were evaluated by calculating mean values separately for the adults and juveniles. A nonparametric Wilcoxon signed-rank test (Siegel, 1956) was used to test significance of the difference of H_T, F_{IS}, F_{IT} and F_{ST} between the adults and juveniles of *B. euphorbioides*.

RESULTS

The 11 enzyme systems show variation and interpreted as being encoded by 17 putative loci. The patterns observed, however, were typical of the same enzyme systems studied in other species and were consistent with the expected banding patterns of those species for which formal analyses have been carried out (Gottlieb, 1981; Weeden and Wendel, 1989; Kepart, 1990). Frequencies of all alleles at all loci polymorphic in at least one population for both species were summarized in Table 2. Seven loci (MDH-3, PGI-1, GOT-1, 6PG-1, 6PG-2, G6P-1, and G6P-2) were monomorphic in all populations of *B. euphorbioides* and *B. longiradiatum*. Alleles unique to one or the other species were few. MDH-1^d and GDH-1^e were alleles restricted to populations of *B. euphorbioides*, whereas nine alleles (MDH-1^a, IDH-1^a, CAT-1^a, PGI-2^a, PGI-2^e, GDH-1^a, GDH-2^b, GOT-2^d, MNR-1^a) were restricted to populations of *B. longiradiatum*. At MNR-1, *B. euphorbioides* was monomorphic, while in *B. longiradiatum* was polymorphic at population level. Overall *B. longiradiatum* differed from *B. euphorbioides* by the most common allele four loci (MDH-1^b, MDH-2^b, SKD-1^a, and PGI-2^c). Although the percentage of polymorphic loci had positive correlation with the population size (r_s=0.63), no other measure of genetic diversity (allelic richness, observed and expected heterozygosity) had a noteworthy correlation with population size (=the number of estimated individuals; r_s=-0.26, 0.23 and 0.20, Spearman rank correlation).

The levels of genetic variation and deviation from Hardy-Weinberg expectations are summarized in Table 3. There were 28 alleles at ten putative polymorphic loci of *B. euphorbioides*, whereas 35 alleles were detected in *B. longiradiatum* for these same ten loci. The mean number of alleles per locus, A (1.63 vs 1.80), the percent polymorphic loci,

Table 2. Summary of allele frequencies for ten polymorphic loci for the 16 populations sampled. Populations E1-E6 are *Bupleurum euphorbioides* and L1-L10 are *B. longiradiatum*

Locus	*Bupleurum euphorbioides*						*B. longiradiatum*									
	E1	E2	E3	E4	E5	E6	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
ACO-1																
A	0.041	0	0.057	0.058	0	0	0	0	0	0	0	0	0.152	0	0.119	0
B	0.905	0.948	0.887	0.837	1	1	1	0.161	0.269	1	0.817	1	0.761	1	0.738	1
C	0.054	0.052	0.057	0.105	0	0	0	0.839	0.731	0	0.183	0	0.087	0	0.143	0
MDH-1																
A	0	0	0	0	0	0	0.675	0.464	0.218	0.470	0.283	0.370	0.435	0.500	0.524	0.152
B	0.027	0.362	0.208	0.116	0.613	0.083	0.162	0.393	0.513	0.530	0.533	0.174	0.435	0.355	0.262	0.485
C	0.865	0.500	0.792	0.523	0.339	0.250	0.162	0.143	0.269	0	0.183	0.457	0.130	0.145	0.214	0.364
D	0.108	0.138	0	0.360	0.048	0.667	0	0	0	0	0	0	0	0	0	0
MDH-2																
A	0.892	0.483	0.915	0.860	0.742	0.867	0.325	0.214	0.064	0.227	0.117	0.326	0.391	0.387	0.024	0.091
B	0.095	0.431	0.085	0.140	0.258	0.133	0.675	0.786	0.936	0.773	0.883	0.652	0.565	0.597	0.500	0.909
C	0.014	0.086	0	0	0	0	0	0	0	0	0	0.022	0.043	0.016	0.476	0
IDH-1																
A	0	0	0	0	0	0	0	0.089	0.013	0	0	0	0.087	0	0.500	0.069
B	1	1	0.972	0.884	1	1	0.837	0.911	0.949	0.909	1	1	0.870	1	0.262	0.931
C	0	0	0.028	0.116	0	0	0.162	0	0.038	0.091	0	0	0.043	0	0.238	0
SKD-1																
A	0.284	0.897	0.868	0.791	0.083	0.017	0.013	0.107	0.141	0.167	0.033	0.109	0.217	0.194	0	0.258
B	0.716	0.103	0.132	0.209	0.917	0.983	0.625	0.857	0.859	0.833	0.967	0.870	0.783	0.710	1	0.727
C	0	0	0	0	0.016	0	0.363	0.036	0	0	0	0.022	0	0.097	0	0.015
CAT-1																
A	0	0	0	0	0	0	0.013	0.125	0.103	0.030	0.133	0.022	0	0.097	0	0.015
B	0	0.155	0.104	0.047	0.177	0	0	0	0	0	0	0	0	0	0	0.015
C	1	0.845	0.896	0.983	0.823	1	0.987	0.875	0.897	0.970	0.867	0.978	1	0.903	1	0.970
PGI-2																
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.227
B	0	0	0.057	0	0.113	0	0.275	0.321	0.218	0.318	0.350	0.500	0.261	0.210	0.381	0.470
C	0.514	0.414	0.208	0.465	0.371	0.567	0.650	0.393	0.782	0.439	0.617	0.457	0.413	0.339	0.524	0.273
D	0.486	0.586	0.736	0.535	0.516	0.433	0.075	0.250	0	0.242	0.033	0.043	0.326	0.452	0.071	0.030
E	0	0	0	0	0	0	0	0.036	0	0	0	0	0	0	0.024	0
GDH-1																
A	0	0	0	0	0	0	0	0.018	0.051	0	0.183	0	0	0	0	0.030
B	0	0	0	0	0	0	0.050	0.089	0.167	0	0.200	0.174	0	0.242	0.095	0.106
C	0.905	0.845	0.887	0.977	0.977	0.964	0.950	0.893	0.769	1	0.617	0.826	1	0.758	0.905	0.848
D	0.081	0.155	0.113	0.023	0.023	0.036	0	0	0.013	0	0	0	0	0	0	0.015
E	0.014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOT-2																
A	0	0.069	0.047	0	0.018	0	0	0.304	0.051	0.045	0.117	0	0	0.065	0.024	0.015
B	0.432	0.431	0.557	0.523	0.393	0.300	0	0.268	0.051	0.061	0.133	0.022	0.196	0.048	0.071	0.015
C	0.568	0.500	0.396	0.477	0.589	0.700	1	0.429	0.897	0.894	0.750	0.978	0.804	0.855	0.881	0.970
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0.032	0.024	0
MNR-1																
A	0	0	0	0	0	0	0	0.393	0	0	0.033	0	0	0	0	0
B	0	0	1	1	1	1	1	0.607	1	0.939	0.033	0.761	0.500	1	1	0.939
C	1	1	0	0	0	0	0	0	0	0.061	0.900	0.239	0.500	0	0	0.061
D	0	0	0	0	0	0	0	0	0	0	0.033	0	0	0	0	0

P (41.20 vs 44.72), and the average expected heterozygosity, H_e (0.151 vs 0.174) were all lower in *B. euphorbioides* than *B. longiradiatum*. Fixation indices suggested comparable levels of inbreeding in the two species; in *B. euphorbioides* 30 (63.8%) of 47 were significant, while 65 of 83 (78.3%) were significant in *B. longiradiatum*.

Genetic identities among the *B. euphorbioides* populations ranged from 0.851-0.988 and averaged at 0.914. Populations MAD (Madeungryong) and HWA (Hwachaebong) had the highest mean genetic identity. Genetic identities among the *B. longiradiatum* populations ranged 0.884-0.991 and averaged at 0.948 and JEO (Jeombongsan) and KAR (Mt. Ka-ri-wang-C) populations had the highest genetic identity. Fig. 2 shows the cluster diagrams (generated by the UPGMA

Table 3. Summary of genetic variabilities in the 16 populations sampled. The values shown are means with standard errors in the parentheses

Species populations	Sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[a]	Mean heterozygosity	
				Observed	H-W Expected[b]
Bupleurum euphorbioides					
E1	37	1.6 (0.2)	41.2	0.072 (0.025)	0.130 (0.045)
E2	29	1.6 (0.2)	47.1	0.116 (0.038)	0.181 (0.058)
E3	53	1.7 (0.2)	47.1	0.067 (0.023)	0.137 (0.041)
E4	43	1.6 (0.2)	41.2	0.088 (0.030)	0.165 (0.051)
E5	31	1.8 (0.2)	41.2	0.057 (0.024)	0.166 (0.056)
E6	30	1.5 (0.2)	29.4	0.096 (0.038)	0.125 (0.048)
Mean	37.2	1.63	41.2	0.083	0.151
B. longiradiatum					
L1	40	1.6 (0.2)	41.2	0.076 (0.028)	0.151 (0.050)
L2	28	1.9 (0.2)	58.8	0.084 (0.029)	0.231 (0.061)
L3	39	1.8 (0.2)	52.9	0.069 (0.027)	0.153 (0.046)
L4	33	1.6 (0.2)	41.2	0.041 (0.023)	0.138 (0.049)
L5	30	1.9 (0.2)	47.1	0.059 (0.024)	0.182 (0.054)
L6	23	1.7 (0.2)	35.3	0.082 (0.030)	0.156 (0.055)
L7	23	1.8 (0.2)	47.1	0.069 (0.032)	0.214 (0.062)
L8	31	1.8 (0.3)	41.2	0.104 (0.034)	0.179 (0.059)
L9	21	1.9 (0.3)	41.2	0.115 (0.044)	0.189 (0.063)
L10	33	2.0 (0.3)	41.2	0.064 (0.022)	0.146 (0.053)
Mean	30.1	1.8	44.7	0.076	0.174

[a]A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95, [b]Unbiased estimate (Nei, 1978)

method; Sneath and Sokal, 1973) that graphically summarize the overall genetic similarity among the population, using the pairwise genetic identity values.

A summary of *F*-statistics for the two species (Table 4) showed similar F_{IT} in both species, while F_{ST} was somewhat higher in *B. euphorbioides,* and F_{IS} higher in *B. longiradiatum.* Evidence of pronounced inbreeding in both species was also provided by the high mean of F_{IS} and F_{IT} values. Total genetic diversity at the polymorphic loci (H_T) for *B. euphorbioides* was 0.224, but the proportion of allozyme diversity among populations (G_{ST}) was 0.153

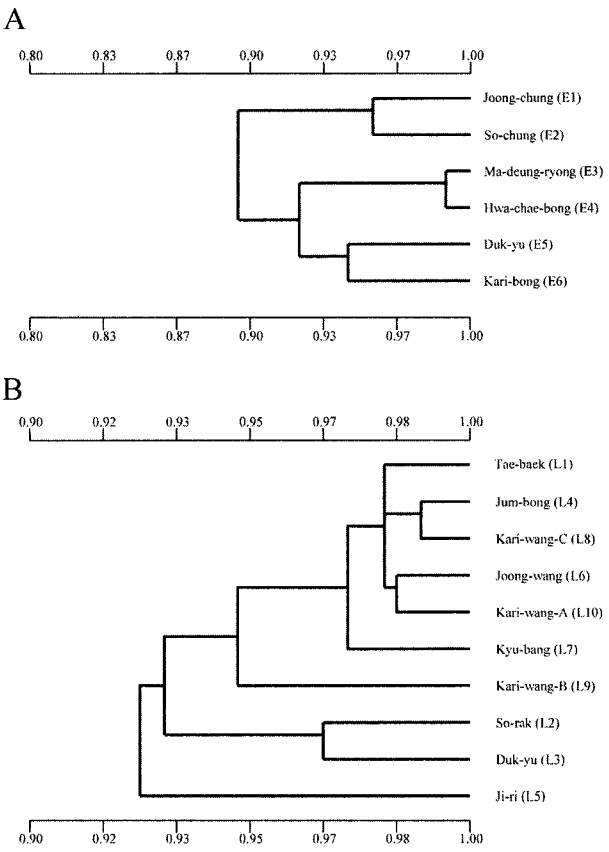

Fig. 2. The UPGMA phenograms of Nei's genetic identity coefficient for *Bupleurum euphorbioides* (A) and *B. longiradiatum* (B) populations sampled.

(Table 5). This result indicated the presence of considerable genetic differentiation among the populations. Likewise, *B. longiradiatum* had a high mean total genetic diversity ($H_T = 0.212$). The ratio of among population variation to total variation was 0.116 (G_{ST}), lower than the value of *B. euphorbioides* (Table 5).

Table 4. *F*-statistics at polymorphic loci for *Bupleurum euphorbioides* and *B. longiradiatum*

Locus	*B. euphorbioides*			*B. longiradiatum*		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
ACO-1	0.600	0.616	0.042	0.708	0.856	0.507
MDH-1	0.470	0.595	0.237	0.482	0.526	0.086
MDH-2	0.224	0.407	0.235	0.728	0.766	0.137
IDH-1	0.507	0.540	0.068	0.471	0.629	0.298
SKD-1	0.688	0.860	0.551	0.557	0.602	0.100
CAT-1	0.639	0.663	0.067	0.769	0.781	0.049
PGI-2	0.589	0.630	0.099	0.597	0.634	0.090
GDH-1	-0.071	-0.015	0.052	0.210	0.279	0.087
GOT-2	0.422	0.441	0.033	0.457	0.535	0.144
MNR-1	---	1.000	1.000	0.640	0.810	0.473
Mean	0.445	0.610	0.297	0.553	0.639	0.194

Table 5. Genetic diversity statistics (Nei, 1973, 1978) and estimates of gene flow for *Bupleurum euphorbioides* and *B. longiradiatum*

Species	Number of loci	H_T	H_S	D_{ST}	G_{ST}
Bupleurum euphorbioides	17	0.227	0.158	0.069	0.153
B. longiradiatum	17	0.212	0.171	0.047	0.116

Genetic diversity parameters were calculated for polymorphic and monomorphic loci. H_T = total genetic diversity, H_S = gene diversity within populations, D_{ST} = gene diversity among populations, G_{ST} = the proportion of gene diversity apportioned among populations

To compare the genetic diversity between adults and juveniles within the same population, levels of diversity were presented for each life cycle stage in Table 6. Genetic variability for adult populations were similar to that of juveniles, but all expected heterozygosities of adult individuals were less than those of juveniles. The mean values of *A*, *P*, H_o, and H_e in adults versus juveniles were 1.50 vs 1.60, 36.78% vs 45.63%, 0.08 vs 0.09, and 0.13 vs 0.16, respectively. In matched-pair comparisons between juveniles and adult populations, the single-locus estimates of F_{IS}, F_{IT}, and F_{ST} values for polymorphic loci were significantly different (Wilcoxon signed-rank: F_{IS}, T_S = 15, $p<0.05$, F_{IT}, T_S =23, $p<0.05$, F_{ST}, T_S = 7, $p<0.05$).

DISCUSSION

Comparisons of genetic variability between the two congeners

Species with restricted geographic ranges have generally lower diversity parameters compared to widespread congeners: The parameters include lower percentages of polymorphic loci, fewer alleles per locus, and lower heterozygosity (Karron et al., 1988; Loveless and Hamrick, 1989; Pleasant and Wendel, 1989; Sherman-Broyles et al., 1992; Purdy and Bayer, 1995). The given population sizes and fragmentations, *B. euphorbioides* showed a surprisingly large amount of genetic variation. The values for all genetic diversity measures were higher than those of narrowly endemic taxa, which were compiled by the previous reviews (Hamrick and Godt, 1989; Loveless and Hamrick, 1989; Hamrick et al., 1991). Genetic diversity within species (H_{es}) and the effective number alleles (A_{es}) indicated that *B. longiradiatum* (H_{es} =0.360, A_{es}=1.563) had similar genetic variation with *B. euphorbioides* (H_{es}= 0.359, A_{es}=0.1559). Total genetic variation at species level (H_T) had no significant difference between the two species. Furthermore, when compared with the same sample sizes from larger populations, genetic diversities in smallest populations (E6) were not particularly low.

Table 6. *F*-statistics for adult and juvenile individuals of *Bupleurum euphorbioides* in Korea

Locus	Adult			Juvenile		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
ACO-1	1.000	1.000	0.055	0.465	0.470	0.008
MDH-1	0.569	0.620	0.118	0.510	0.565	0.113
MDH-2	-0.248	-0.092	0.125	0.061	0.292	0.246
IDH-1	-0.077	-0.018	0.055	0.628	0.652	0.066
SKD-1	0.287	0.855	0.796	0.711	0.739	0.098
CAT-1	0.854	0.866	0.080	0.266	0.297	0.043
PGI-2	0.253	0.324	0.095	0.651	0.676	0.072
GDH-1	-0.138	-0.109	0.026	-0.204	-0.109	0.079
GOT-2	0.491	0.518	0.053	0.365	0.402	0.058
MNR-1	----	1.000	1.000	----	1.000	1.000
Mean	0.374	0.576	0.322	0.425	0.556	0.228

At individual locus, there was a dominant deficiency of heterozygosity both in *B. euphorbioides* and *B. longiradiatum*. This result from limited observations suggested that these two species have similar breeding systems. Due to protandrous flowers, the frequency of selfing was low, but the possibility of selfing and geitonogamy was not excluded since different flowering time of inflorescences existed within the individual. Biparental inbreeding between local neighbors would be frequent because the two species are pollinated by small fly species. These pollinators might be less effective than bees and butterflies as their visits were extremely scarce even under the normal weather condition (personal observations in 1995-1997).

The mean F_{ST} of *B. longiradiatum* (0.194) was lower than that of *B. euphorbioides* (0.297). In all species, the largest contribution to the overall inbreeding coefficient (F_{IT} = 0.639 vs 0.610) was F_{IS} (0.553 vs 0.445), which measured the reduction in heterozygosity of an individual due to nonrandom mating within a population. These results suggested that there was greater genetic differentiation, and lower gene flow, among populations of *B. euphorbioides* than *B. longiradiatum*. This was an expected result, primarily because the much smaller stature of *B. euphorbioides* and its smaller umbels on short plants suggested that it would get fewer pollinator visits than the more visible *B. longiradiatum* (Linhart and Premoli, 1993).

The movement of genes across populations has a significant influence on the distribution of genetic variation. The amount of gene flow and, hence, the extent of genetic differentiation among population subdivisions are broadly correlated with several ecological and life-history characteristics, particularly the breeding system, life form, and mode of seed dispersal (Williams and Guries, 1994). Monocarpy of *B. euphorbioides* reduces flowering individuals in each habitat patch and mating possibility by asynchronous phenology. Because of its effects on the efficiency of pollination, monocarpy reduces effective population size and promotes population differentiation (Loveless and Hamrick, 1989). Because the *B. euphorbioides* grows only on the

shallow crack of naked rocks or pebble ground divided by conifer forest and rough topography, it is too difficult for the Diptera pollinator to fly through, while *B. longiradiatum* grows on an open habitat of deciduous forest floor. Greater opportunities for pollen exchange, seed production and seedling establishment suggested that gene flow might be more extensive in *B. longiradiatum*. Therefore, spatial arrangement of populations and demographic factors (effective population size) were important in determining gene flow rates among populations of each of the two species.

Populations did not form clusters based on pairwise genetic distances as predicted by geographic proximity alone (Fig. 2). Mean genetic identity value (0.914) of *B. euphorbioides* was outside the 0.95-1.00 ranges reported for most other conspecific populations (Crawford, 1983). Within the *B. euphorbioides* species, although the Duk-yu (E5) population was located in the most disjunct area, Duk-yu (E5) and Ka-ri-bong populations (E6) were joined on the cluster against other populations (E1, E2, E3, and E4) of So-rak area (Fig. 2). Also, *B. longiradiatum* of Mt. Duk-yu (L3) population was separated from Mt. Ji-ri population (L5) and was joined with Mt. So-rak (L2), which was also not correlated with geographic distance.

Some populations that are geographically distant are genetically more closely related to one another than to more adjacent populations. This may be due to effects of drift, to be expected in small populations occupying unstable, repeatedly disturbed montane habitats (Chang et al, 2003). Geographic limits on interpopulation gene exchange and inner factors (genetic drift or loss of intervening populations, and/or natural selection) resulted in genetic divergence of local population (Hamrick and Godt, 1989). Alpine plants like *B. euphorbioides* in the Korean peninsula were isolated in small and restricted area at a mountain peak above 1,500-m sea level. During the previous glacial period, it may be assumed that vegetation zones tended to shift to the lower elevation, but the shifts were complicated and strongly influenced by geographic feature such as mountains, ocean and prevailing winds. In the subsequent retreat to high elevation refugia during inter glacial, *B. euphorbioides has* appeared to manage to survive fragmentation (Kong, 1989). As the isolation distance was too great to exchange genetic elements, distantly located populations underwent a genetic drift and natural selection within their habitat without recolonization occurring by midway population

Comparison of congeneric species is useful for studying the genetic structures between a rare species and a more widely distributed congener, which could be affected by life historical characters including mating system, life form, and seed dispersal mechanism as well as evolutionary events (Loveless and Hamrick, 1989). Their similar breeding systems and other resembling biological makeups make possible a valid comparison and strengthen their empirical data (Karron et al., 1988; Baskauf et al., 1994).

It is argued that the perceived characteristics of rare species is the maintenance of low levels of genetic variation. The simple facts regarding the lack of genetic variation of a rare species offer limited information on management when we do not know how this level compares to that of a widespread relative. While managers will attempt to conserve as much diversity as possible, their management strategies should be changed if it is known that, for example both a rare and a widespread congener have similar levels of diversity (Gitzendanner and Soltis, 2000). As levels of genetic diversity may not be directly related to survival, a widespread species also exhibits low diversity. Therefore, our emphasis should switch from attempting to increase genetic diversity to focusing on other factors leading to population decline.

Temporal genetic structure in *B. euphorbioides*

According to pairwise comparisons between juveniles and adult populations, the results showed that the juveniles had smaller F_{ST} value than the adults, indicating that genetic differentiation among the populations in the adults cohorted when compared to juvenile individuals. The increased genetic divergence of populations detected in adult individuals appeared to be the result of postemergence events (Tonsor et al., 1993). The estimated heterozygosity values indicated that adult individuals had less heterozygosity than juvenile individuals. An increase in heterozygosity with age is common in other species (Schaal and Levin, 1976; El Kassaby et al., 1987; O'Mally et al., 1988; Hamrick et al., 1993; Tonsor et al., 1993). The decreased heterozygosity throughout the life cycle of *B. euphorbioides* could be due to selection in favor of particular homozygous genotypes among the outbred individuals or to selection against heterozygous individuals.

Conservation implications

Through this study, Jeom-bong population of *B. euphorbioides* was found to be extinct, but there was no solid evidence to verify the cause. The fate of the remaining six populations was also precarious. These populations are restricted within small patches in bare rocks of mountain peak and are extremely vulnerable to local catastrophes. The fine and small-scaled disturbances induced by human activities such as mountain tourism can easily lead to demise of the population.

This study showed that *B. euphorbioides* revealed a substantial amount of allozyme variation at both species and population levels. The percentage of polymorphic loci and numbers of alleles per locus indicated that *B. euphorbioides* had no history of sufficiently severe or long-lasting population bottlenecks to cause a loss of genetic diversity. The large amount of differentiation among

populations suggested either a lack of gene flow among populations or that fragmented populations have been isolated long enough for genetic drift (or selection) to have caused population differentiation (Ellstrand, 1992). Because *Bupleurum euphorbioides* is a genetically viable species based on our data, reserve designs based on ecological factors would likely suffice to maintain genetic diversity. Conservation of this species could be accomplished through a combination of protecting sites and restoring degraded habitat. Only the sufficient self-sustaining population can justify *in situ* conservation efforts.

In natural habitats, a long-term monitoring is required to check whether a population would lose an extensive amount of initial heterozygosity by the diminishing effective population size.

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