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ORIGINAL RESEARCH

High Genetic Differentiation in Endangered *Sedum ussuriense* and Implications for Its Conservation in Korea

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Abstract *Sedum ussuriense* Kom. (Crassulaceae) is a succulent perennial herb localized to rocky valleys in southeastern Korea. Although it is an important natural resource with high economic value as an ornamental plant, it is currently endangered because of land-use changes and illegal exploitation. To initiate a proper conservation plan, we selected four populations (Juwang, Okgye, Jeolgol, and Haok) around Mt. Juwang, characterized their phenotypic traits, and evaluated patterns of random amplified polymorphic DNA variation. Despite its small population size, Okgye had the greatest proportion of flowering plants and higher seed production than from the other populations. This population also harbored the greatest genetic diversity. However, recent fragmentation between Okgye and Haok appeared to cause genetic divergence, leading to close genetic relationships of Okgye to Juwang vs. Haok to Jeolgol. In the long term, this raises concerns about the loss of genetic variation and the possibility of a demographic crash in those fragmented populations. Because our results indicated a high degree of divergence among populations, we suggest that conservation activities should focus on maintaining and propagating all populations throughout this species' range.

Keywords Genetic diversity · Habitat fragmentation · RAPD · Rare species

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Introduction

The protection and conservation of endangered species are a primary issue for humans facing the global loss of biodiversity. According to the Korean Red Lists (Ministry of Environment 2005), eight plant species are at the edge of extinction (first-class endangered species) and 56 are considered vulnerable (second class). The number of endangered species is rapidly increasing due to habitat destruction and illegal exploitation. In particular, habitat fragmentation leads to diminished connectivity among populations, ultimately leaving many small, isolated populations (Ellstrand and Elam 1993). Because such populations tend to have lower levels of genetic variation than do larger, widespread populations (Young et al. 1996), they become vulnerable to genetic drift, founder effects, and inbreeding depression (Hedrick and Kalinowski 2000; Reed 2005). Those processes are the major factors determining the demographic fate and long-term persistence of populations. Therefore, population genetics is useful for assessing the genetic variation among endangered populations when devising an effective conservation plan (Holsinger and Gottlieb 1991; Ellstrand and Elam 1993).

Sedum ussuriense Kom. is an insect-pollinated, polycarpic perennial herb in the Crassulaceae family. To our knowledge, its natural distribution is limited to northeastern Asia. Russian herbarium records have shown that this species is found along the Tumen River (North Korea–China border) and the basin of the Ussuri River in Russia

(Kim et al. 2000). In Korea, it grows in rather inaccessible crevices on the steep cliffs of Mt. Juwang and its vicinity (approx. 100 to 500 m elevation). This is regarded as the southernmost limit of its geographical range (Hyun 2001). Because of its restricted distribution, *S. ussuriense* has been included in the red list as a second-class endangered species in Korea (Ministry of Environment 2005). Plants occupy somewhat arid soil with large amounts of humus (Jeong 1999a). Such habitats are usually populated by desiccation-tolerant or succulent species, e.g., mosses, *Selaginella* spp., *Sedum kamtschaticum*, and *Parthenocissus tricuspidata*. Among them, cliff-covering vines (*P. tricuspidata*) appear to reduce the recruitment and growth of *S. ussuriense* in some areas (Y-B Ku, personal observation). Plants of *S. ussuriense* produce bright magenta pink flowers that are desirable for ornamental and horticultural uses. Previous studies of *S. ussuriense* in Korea have mainly dealt with its taxonomy, morphology, and physiology in horticultural applications (Kwon and Jeong 1999; Jeong 1999a, b, 2000; Jeong and Kwon 2003a, b). However, the extent of genetic diversity and patterns of genetic variation among populations are still unknown.

Populations of endemic species are often characterized by a low level of genetic diversity (Hamrick et al. 1991; Gitzendanner and Soltis 2000). The loss of genetic variation in rare and endangered species may reduce their ability to tolerate environmental changes and demographic fluctuations (Ellstrand and Elam 1993; Matthies et al. 2004). Thus, it is important to preserve their genetic variation to maintain their evolutionary potential to survive and adapt (Barrett and Kohn 1991; Hamrick et al. 1991; Schaal et al. 1991). The levels of genetic variation within and among natural populations provide useful information for their conservation (Milligan et al. 1994; Haig 1998). In this study, we first surveyed the ecological characteristics of four populations of endangered *S. ussuriense* in Korea. We then used random amplified polymorphic DNA (RAPD) analysis to assess their genetic diversity and population differentiation.

Materials and Methods

Field Survey

We chose study sites in four valleys (Juwang, Okgye, Jeolgol, and Haok) of Mt. Juwang and Mt. Naeyeon (Fig. 1 and Table 1). Although plants of *S. ussuriense* plants occurred continuously along the valleys, we located disjunct and densely growing population in each valley. Habitat size was determined from the longitudinal length of populations and population size by counting all individuals. Randomly drawn quadrats were used to estimate the percentage of flowering plants. From each population, we randomly marked 13 to 17 plants to measure their heights and to count the number of shoots per plant. To estimate seed production, we counted seeds from one flowering shoot per plant. From those individuals, we also collected leaf samples for RAPD analysis. Trait differences between populations were tested with analysis of variance and Duncan's multiple range tests using SAS (SAS institute, Cary, NC).

DNA Isolation and RAPD Analysis

Leaf samples were stored in silica gel to extract DNA using a PureGene DNA Isolation Kit (Gentra) according to the manufacturer's protocol. We conducted polymerase chain reaction in a total volume of 25 μl containing 25 mM $MgCl_2$, 10× reaction buffer, 1 U of Taq polymerase (M1861; Promega), 2 mM dNTPs (S240; Promega), 5 pM of primer, and template DNA. Reactions were performed in a thermocycler programmed for an initial incubation at 94°C for 2 min; followed by 45 cycles of 94°C for 5 s, 36°C for

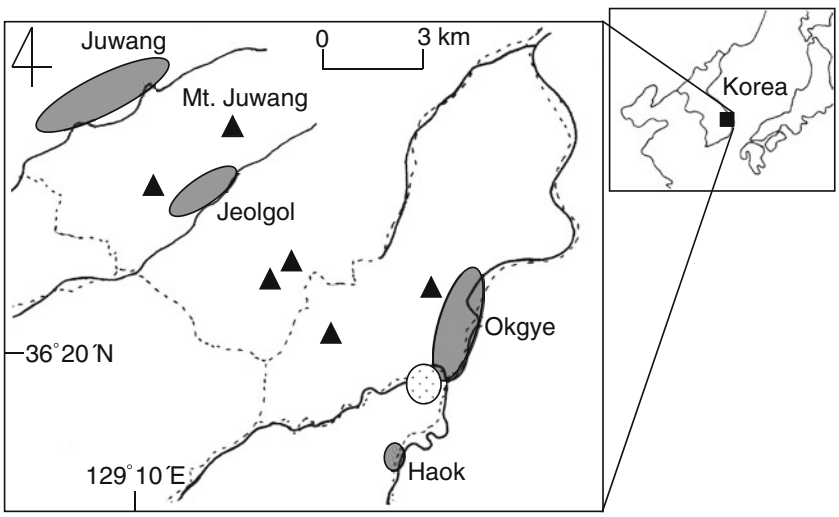

Fig. 1 Map of Mt. Juwang and its vicinity, with *black triangles* indicating summits. *Shaded areas* indicate *Sedum ussuriense* populations (Juwang, Jeolgol, Okgye, and Haok). *Dotted area* represents a local highway construction area. *Solid and dotted lines* indicate streams and local roads, respectively

Table 1 Habitat size, plant height, number of shoots and seeds per plant, and the percentage of flowering plants (± standard deviation) of four populations of *Sedum ussuriense*

Population	Habitat length (m)	n	n_1	n_2	n_3	Height (cm)	Number of shoots	Number of seeds	% of flowering plants
Juwang	3,100	1,000	17	9	291 (8)	13.2±9.2a	1.2±0.6a	47±32a	34±18a
Jeolgol	1,400	1,800	15	9	118 (6)	16.6±8.3a	1.8±0.9ab	44±15a	36±13a
Okgye	2,600	600	13	5	55 (3)	16.5±8.0a	2.7±1.9b	153±42b	68±16b
Haok	200	100	14	–	28 (2)	18.8±11.3a	2.0±1.3ab	–	34±27a

Different lowercase letters indicate significant differences among populations at 5% level using Duncan's multiple range test

n total number of plants in the population, n_1 number of plants to survey height and number of shoots, n_2 number of plants to survey number of seeds, n_3 number of plants with study quadrats in parenthesis to survey the percentage of flowering plants

1 min, and 72°C for 70 s; then ending with a 5-min extension step at 72°C. Amplified products were resolved by electrophoresis on 1.5% (*w*/*v*) agarose gels at 120 V in 1× TBE, visualized by ethidium bromide staining, and photographed under UV light. Ten primers for RAPD analysis (Table 2) were purchased from Operon Tech (Alameda, CA). A set of standard conditions was maintained to improve the reproducibility of RAPD and to exclude all possible spurious variations.

RAPD Data Analysis

To assemble a matrix of RAPD phenotypes, we scored amplified fragments according to the presence (1) or absence (0) of a band. RAPD is a dominant marker, and previous knowledge is scarce about the breeding system or population genetics of *S. ussuriense*. Therefore, we used statistical approaches that relied upon RAPD phenotypes and avoided analyses based on allele frequency, the latter of which requires previously known estimates of an inbreeding coefficient or an assumption of Hardy–Weinberg equilibrium (Bonin et al. 2007). Moreover, although every effort was made to optimize conditions for normal amplification and to maximize band detection capability, our data included 3.5% of missing data due to the absence of some loci in some samples. To account for that missing data and to minimize uncertainty in our analysis, we adopted resampling approaches implemented in FAMD 1.23 (Schlüter and Harris 2006). Within-population genetic diversity was estimated by using the number of polymorphic fragments (*F-POLY*), number of private fragments (*F-PRIV*), and Shannon's diversity index (*I*) using FAMD. Shannon's index ($I=-\Sigma[p_i \cdot \log_2(p_i)]$, where p_i is the relative frequency of the ith fragment in a population), was estimated by 1,000 bootstrapping over loci and compared among populations by t tests using FAMD. For comparison purposes, we also calculated Nei's unbiased gene diversity adopting Lynch and Milligan (1994) correction and assuming that *S. ussuriense* is completely outcrossing and its populations are under Hardy–Weinberg equilibrium.

To determine the similarities among samples, we calculated Dice's index (s_{ij}, Dice 1945) by averaging 1,000 datasets randomly drawn from the interval of possible similarity values as implemented in FAMD. This index is represented as $s_{ij}=2n_{ij}/(2n_{ij}+n_i+n_j)$, where n_{ij} is the number of bands shared by samples i and j, n_i is the number of bands found only in sample i, and n_j is the number found only in sample j. This similarity index (s_{ij}) was converted to genetic distance (calculated as $d_{ij}=1-s_{ij}$) to run analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA). Those analyses were performed using GenAlEx 6.2 (Peakall and Smouse 2006) to quantify and visualize the partitioning of genetic variation within and among populations. The degree of among-population differentiation was correlated with geographical distances among populations by applying a Mantel test. A neighbor-joining tree was then drawn from the distance matrix with branch support produced by 1,000 bootstrapping over loci, utilizing PHYLIP 3.69 (Felsenstein 2005) and TREEVIEW 1.6 (Page 1996).

Table 2 Ten random primers used for RAPD analysis

Primer	Sequence (5′ to 3′)	Primer	Sequence (5′ to 3′)
OPA-08	GTGACGTAGG	OPN-03	GGTACTCCCC
OPAF-12	TCGGCGATAG	OPO-01	GGCACGTAAG
OPAF-16	AGCCAGCGAA	OPO-02	ACGTAGCGTC
OPN-01	CTCACGTTGG	OPO-03	CTGTTGCTAC
OPN-02	ACCAGGGGCA	OPO-04	AAGTCCGCTC

Results

Population Size and Plant Performance

The size of habitat and population varied considerably among our four populations. Although Juwang population occupied the largest habitat, Jeolgol had the greatest number of individuals (Table 1). Plant height did not differ significantly among populations. Plants at Okgye produced the greatest

Table 3 Genetic diversity of *S. ussuriense* populations

Population	*n*	*F-POLY*	*F-PRIV*	*I*	$H_{unbiased}$
Juwang	17	86	1	4.28±0.07bc	0.182±0.016b
Jeolgol	15	66	2	4.45±0.06ab	0.151±0.016b
Okgye	13	104	7	4.54±0.06a	0.255±0.017a
Haok	14	62	2	4.06±0.09c	0.139±0.016b
Total	59	134	–	4.80±0.03	0.182±0.008

Different lowercase letters indicate significant differences among populations at 5% level

n number of samples, *F-POLY* number of polymorphic fragments, *F-PRIV* number of private fragments, *I* Shannon's diversity index ± standard deviation, $H_{unbiased}$ Nei's unbiased gene diversity ± standard error

number of shoots, followed by Haok and Jeolgol. Also, Okgye population had more flowering plants, which produced more seeds than plants from other populations.

Population Genetic Structure

The ten primer sets generated a total of 154 bands that ranged from 350 to 1,750 bp, of which 134 (87%) were polymorphic. Despite its small population size, Okgye generated the greatest number of polymorphic fragments and private fragments, and it harbored the highest Shannon's diversity and Nei's gene diversity (Table 3). AMOVA indicated that most of this variability was distributed among individuals within populations (63%), with the remainder (37%) being attributed to differences among populations (Φ_{ST}=0.37; Table 4). Among-population differentiation from AMOVA ranged from 0.277 to 0.432 (P<0.001 for all pairs), which was not significantly correlated with geographic distances among populations (R= 0.02, P=0.627; Table 5). Our principal coordinate plot showed that most individuals from a given population tended to cluster together, making them more genetically similar than individuals from different populations (Fig. 2). Axes PC1 and PC2 together explained 55.2% of the total genetic variation, while PC2 and PC3 together explained 43.1%. The PC1 vs. PC2 plot (Fig. 2a) provided a visual representation of genetic similarity between Juwang and Okgye that was well differentiated from Jeolgol and Haok on PC2. The PC2 vs. PC3 plot (Fig. 2b) revealed a close relationship between Jeolgol and Haok. The unrooted neighbor-joining tree resolved two clusters—one formed by Juwang and Okgye, the other by Haok and Jeolgol—and it supported the results given by our principal coordinate plot (Fig. 3).

Discussion

Population Size, Fitness Traits, and Genetic Diversity

The expected levels of Nei's gene diversity ranged from 0.139 to 0.255, which are comparable to or lower than values reported for species that are perennial (0.242), endemic (0.191), or outcrossing (0.260) (Nybom and Bartish 2000). Although population size and genetic diversity are often positively correlated (Leimu et al. 2006), our study demonstrated that the greatest genetic diversity was found in the Okgye population, despite its small size compared with some others. In contrast, the lowest genetic diversity was found at Haok, the smallest population.

The high level of fecundity and genetic diversity at Okgye may be attributed to its quality of habitat that facilitated sexual reproduction. Whereas the other populations were shaded because they were located near forest edges, plants in Okgye grew in a relatively open area with more sunlight and higher temperatures. Such conditions may have triggered flowering and attracted pollinators more often than for other populations. Okgye plants also had more flowering stems than did those of other populations (Y-B Ku, personal observation). Therefore, we cannot discount the possibility of local

Table 4 Analysis of molecular variance (AMOVA) of *S. ussuriense* populations

Source of variation	*df*	SSD	Variance component	% variation
Among populations	3	2.978	0.061	37%
Within populations	55	5.666	0.103	63%
Total	58	8.644	0.164	100%

The data show the degrees of freedom (*df*), sum of squared deviation (SSD), variance component estimates, and the percentage of total variance contributed by each component (% variation)

Table 5 Geographical distances in kilometers (above diagonal) and Φ_{ST} values (below diagonal) among *S. ussuriense* populations

	Juwang	Jeolgol	Okgye	Haok
Juwang	–	4.5	13.4	15.3
Jeolgol	0.333	–	9.0	10.9
Okgye	0.277	0.380	–	4.3
Haok	0.409	0.432	0.391	–

adaptation by Okgye genotypes under a favorable environment.

Historically, plants at Okgye and Haok once comprised a continuous population that was later divided by activities associated with road construction; further human disturbance significantly reduced their size (Fig. 1). Habitat fragmentation and a decline in population size due to recent construction may not always have an immediate impact on genetic diversity. However, a loss of genetic diversity eventually leads to long-term harmful effects on plant fitness and population viability (Young et al. 1996; Ayres and Ryan 1999). Our data revealed that Okgye continues to maintain a high level of genetic diversity not only because the characteristics of its habitat favor population growth but also because this fragmentation is only recent. Previous studies have also reported that high genetic diversity may still be detected in fragmented populations if insufficient

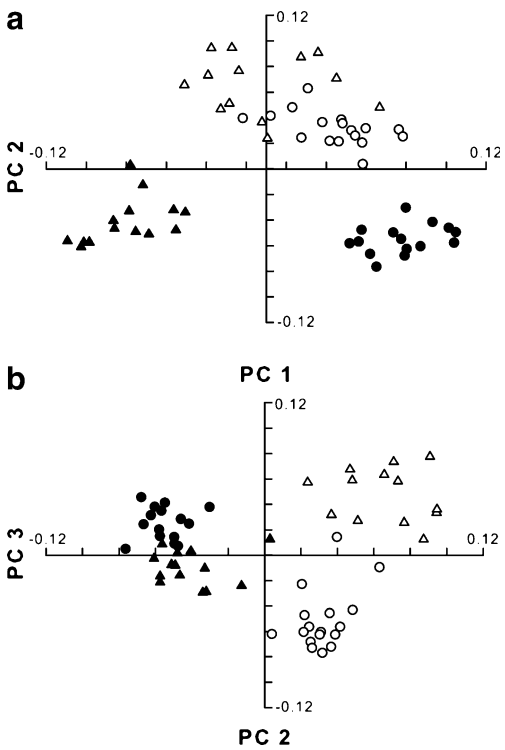

Fig. 2 Principal coordinate plots of four populations **a** between PC1 and 2 and **b** between PC2 and 3. *White circles* Juwang, *black circles* Jeolgol, *white triangles* Okgye, *black triangles* Haok

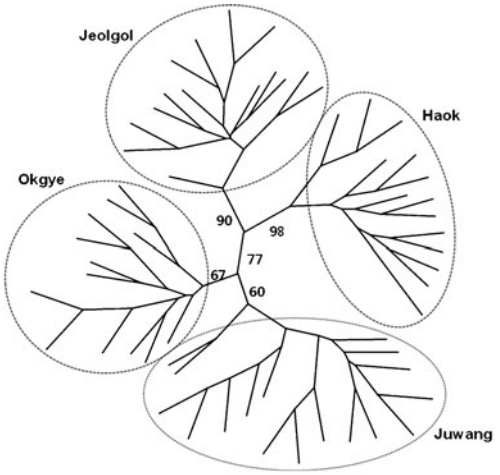

Fig. 3 Neighbor-joining concensus tree of RAPD phenotypes based on average Dice's similarity index. Values are percentage of bootstrap support between two clusters (Jeolgol and Haok vs. Okgye and Juwang; 77%) and for each population

time has elapsed for such reductions to occur (Coates 1988) or if human disturbances have only recently fragmented this type of continuous population (Rossetto et al. 1995). Thus, we suggest that a follow-up study be made of the genetic structure within *S. ussuriense* populations to assess the ultimate impact of habitat destruction on the evolutionary and demographic fate of this endangered species. Especially in small isolated populations such as Haok, our concern is that mating with relatives may promote inbreeding depression, a decrease in plant fitness, and inevitable local extinction (Charlesworth and Charlesworth 1995).

Genetic Differentiation of Populations

The four populations of *S. ussuriense* were highly divergent, although a low level of between-population variation is a characteristic of outcrossing perennial species (Hamrick and Godt 1996). Φ_{ST} values among populations were similar to or greater than the reported mean Φ_{ST} values of 0.25 for perennial species and 0.28 for outcrossing species (Nybom and Bartish 2000). This high degree of genetic divergence in *S. ussuriense* populations may be attributed to genetic drift and limited gene flow (Barrett and Kohn 1991; Ellstrand and Elam 1993). Although our study sites were located within a small geographic scale (4.3 to 15.3 km), they were isolated from each other by high mountains (Fig. 1), which restricted pollinator movement and seed dispersal across populations. For example, Mt. Juwang is a genetic barrier between Juwang and Jeolgol, a situation that may promote breeding between spatially close individuals within population, ultimately enhancing genetic divergence among populations.

In addition to the low levels of gene flow, habitat fragmentation and genetic drift may have contributed to the

divergence of *S. ussuriense* populations. In particular, the divergence between Okgye and Haok resulted in genetically close relationships between Juwang and Okgye vs. between Joelgol and Haok, as shown in the PCoA and cluster analysis (Figs. 2 and 3). Therefore, the fragmentation between Okgye and Haok may have promoted their genetic assimilation to Juwang and Jeolgol, respectively.

Conservation Implications

One important objective of conservation is to maintain the evolutionary potential of species by recovering their natural level of genetic variation (Holsinger and Gottlieb 1991). For *S. ussuriense*, the low genetic diversity associated with declining populations and the high degree of genetic divergence has been a consequence of geographical and topological isolation, limited gene flow, and habitat fragmentation. Furthermore, construction activities and human disturbance may have accelerated the fragmentation of remaining populations and the extinction of small populations. Thus, we propose that efforts toward conservation management should be aimed at preserving and increasing the size of current populations. Considering the high degree of genetic differentiation among populations, extinction of any population may lead to a considerable loss of genetic variation. Thus, management schemes should involve all populations simultaneously.

The outcome of our study suggests the urgency for both in situ and ex situ conservation. For *S. ussuriense*, the former approach should focus on increasing the size of extant populations by promoting the survival and recruitment of seeds and seedlings. By contrast, the latter approach must entail the collection and storage of seeds from representative samples of individuals from all populations to ensure the availability of genetic resources for future use in programs of reintroduction or reinforcement. Because this species produces only a few seeds per plant, the likelihood for successful establishment is enhanced once those seeds are located to suitable habitats. Therefore, we suggest collecting seeds from each population, then cultivating and transplanting them back to their original sites. However, we caution that plants must not be mixed between populations because that may lead to the loss of coadapted genetic structure via outbreeding depression (Templeton 1986; Lynch 1991). Both in situ and ex situ conservation efforts will necessitate an effective sampling plan to cover the full range of genetic variability in each population. This could be achieved by analyzing the spatial genetic structure of this species through more extensive sampling.

Because this species has a strong habitat preference, another focus should involve the conservation of unique habitats and ecosystems (UNEP 1995). Construction activity requires monitoring to minimize the impact on endangered species. In addition, any illegal collection of endangered species should be strictly prohibited by law. Finally, we highlight that only a few studies have been conducted on the ecology of endangered species in Korea. Because plant breeding system directly affects the degree and distribution of genetic variation (Charlesworth and Charlesworth 1995; Hamrick and Godt 1996), studies on the reproductive biology of endangered species should be accompanied by examinations of their population genetics or conservation. Finally, further investigations should concentrate on life history characteristics, mechanisms for pollen/seed dispersal, and impacts from herbivores and pathogens. Those studies will be a primary step toward deepening our understanding of the causes for rarity when composing proper conservation guidelines.

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