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Genetic structure and population differentiation of endangered *Scrophularia takesimensis* (Scrophulariaceae) in Ulleung Island, Korea

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ABSTRACT: As a part of the on-going effort to conserve endangered *Scrophularia takesimensis* Nakai in Korea, its genetic structure and diversity from 3 population, consisted of 14 subpopulations in Ulleung Island were analyzed using RAPD band patterns. Out of 60 primers tested, 33 generated amplified bands with its genome, including 149 polymorphic and 67 monomorphic bands. The highest number (146) was found in northern population, especially, 64 in HY subpopulation; the smallest (40) in eastern population. An examination of its genetic structure with AMOVA revealed that about 60% of all variations could be assigned to among subpopulations within populations. Population differentiation among populations and subpopulations is seriously going now because of habitat fragmentation due to human activities, such as road and small port construction. Although the habitats of *S. takesimensis* in Ulleung Island, Korea are disappeared at an alarming rate, significant levels of genetic variation still exist at species level, and population level, especially northern population. Therefore, three conservation strategies should be needed urgently; 1) preservation of populations as it stands, 2) establishment of recovery plan to connect population and subpopulations genetically, and 3) long-term monitoring.

Keywords: endangered, genetic structure, *Scrophularia takesimensis*, Ulleung Island

Islands frequently have distinctive and often unique assemblages of species. In general, they have lower species diversity than equivalent continental areas, but tend to have elevated numbers of endemic species; about one in six plant species grows on oceanic islands (Groombridge, 1992). These island endemic species, however, are vulnerable because of; 1) the difficulties or impossibilities of finding refuge areas during large-scale ecological changes; 2) the often reduced size of their populations; 3) the major habitat changes caused by human impacts; 4) the large number of exotic species (Quilichini and Dubusche, 2000); and 5) their long isolation from some of the selective forces that have influenced the evolution of continental organisms (Wiles et al., 1996).

On the other hand, habitat fragmentation have restricted an increasing number of plant species to small and isolated populations (Markus and Matties, 1998), and whatever the process, habitat fragmentation generally leads to a significant increase in the extinction risk of populations (Gigord et al., 1999). So, habitat fragmentation has become a major topic in conservation biology and conservation genetics (Juan et al., 2004; Tomimatus and Ohara, 2003). Especially, the severe and fast fragmentation in islands has disrupted the genetic cohesion of island endemics, and the survival of the extant populations of these endemics might be seriously threatened (Bouza et al., 2002). These factors make island floras notoriously fragile and many are seriously threatened (Fransicso-Ortega et al., 2000; Hughes et al., 2003; Waldren et al., 1995), and currently one in three of all known threatened plant species are island endemics (Groombridge, 1992).

Ulleung Island is a volcanic island located 150 km east of Korea in the East Sea between Korean peninsula and Japan Islands, and is 73 km^2 in area and most of the seashore is composed of steep cliffs. The maximum age of the island is estimated as approximately 1.8 million years old. Ulleung Island has never been connected to any other land mass, and contains about 700 species of vascular plants, and of which 37 angiosperms are endemic (Sun and Stuessy, 1998). Many endemic species in Ulleung Island, however, are under severe endangerment, such as *Cotoneaster wilsonii* Nakai (Rosaceae), *Trillium tschonoskii* Max. (Liliaceae), *Bupleurum latissimum* Nakai (Apiaceae), and *Scrphularia takesimensis* Nakai (Schrophulariaceae), and these are protected by Wild Life Protection Act in Korea. Especially, *B. latissimum* was presumed to be extinct for a long time (Ku et al., 2004).

Scrophularia takesimensis (Scrophulariaceae), a perennial

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herb, is a critically endangered endemic plant in Ulleung Island. This species is dominant at coastal vegetation consisted of the shingles or sands, and its vegetation patches run parallel with the coastal line. Therefore, the constructions of coastal route and small harbor become the most serious threats to the viability of *S. takesimensis*, and many populations were already disappeared in this island. The conservation strategy, however, is not programmed yet, and little study has been devoted to declining populations.

In this study, the genetic variability of *S. takesimensis* encompassing the natural distribution of the species in Ulleung Island, Korea was examined using RAPD with the following objects; 1) to investigate the genetic diversity and structure of populations, 2) to clarify the genetic relationships among the populations, and 3) to provide a base for the development of conservation programs for this endangered species.

Materials and Methods

Plant material

Plants of *S. takesimensis* were sampled in 2001 from 14 different subpopulations in Ulleung Island, Korea (Table 1). Of them, 3 were from East population of Ulleung Island, 1 from South, and 10 from North. The western region is an inaccessible rocky places. The total number of individuals of each site was counted in field to estimate the population census size, and 3 to 8 fresh leaves per subpopulations were collected at the rate of its size, which were selected randomly with care being taken not to sample from obvious clones and young individuals. Samples were transported to the laboratory in a plastic bag contained silica gel, and kept frozen at −70°C. To estimate the distance between populations, the coordinates were recorded using GARMIN GPS VI in field, and conversed into the geographical distance.

To estimate the relationship between genetic diversity and plant's life stage, the individuals were counted as 3 groups on the basis of life stages, juveniles, vegetative adults, and generative adults, since the actual age of individual plants of *S. takesimensis* could not be determined. The juvenile stage means the immature plants whose height are below 5 cm and have not branched, vegetative adults are non-flowering individuals whose height are more than 5 cm, and generative adults are the plants baring one or more flowers.

DNA isolation and RAPD analysis

Genomic DNA was extracted according to the CTAB method (Dolye and Dolye, 1987), then measured with a Biotech photometer and agarose gel electrophoresis, and stored at 4°C prior to RAPD analyses. DNA amplification were carried out using a Gene-Amp PCR system 9700, following the method of Williams et al. (1990). Sixty primers (OPA 1 through 20, OPB 1 through 20, and OPAF 1 through 20) were purchased from Operon Technologies, and the amplification products were separated on a 1.4% agarose gel. The resulting RAPD bands were visualized with ethidium bromide and photographed over ultraviolet light.

Data analysis

Bands were scored manually on the photographs and confirmed with stained gels with eye. Polymorphic amplification products were scored with a 1 when present, and a 0 when absent. Genetic diversity was measured as the percentage of polymorphic bands at the subpopulation, population and species level (Chen et al., 2006; Sales et al., 2001), and genetic structures were analyzed by Arlequin ver. 2.000 (Stefan et al., 2000), adapted an analysis of molecular variance (AMOVA)

Table 1. Geographic location and regional distribution of 14 subpopulations for the analyses of genetic diversity of *S. takesimensis*.

Population	Subpopulation site	County	Code	Latitude	Longitude
South	Namyang	Semyeon, Namyang	NA	37°27′41′′	130°50′48′′
East	Salgunam 1	Ulleungeup, Dodong	SA	37°28′50′′	130°54′48′′
	Salgunam 2	"	SG	37°28′53′′	130°55′11′′
	Naesujun	Ulleungeup, Jedong	NS	37°30′15′′	130°54′57′′
North	Hyunpo	Bukmyeon, Hyunpo	HY	37°31′43′′	130°50′22′′
	Mt. Songgot, Chusan	Bukmyeon, Nari	CH	37°31′53′′	130°51′17′′
	Pyungri	"	PY	37°31′54′′	130 50′45′′
	Chusan, Street No. 1	"	CN	37°31′54′′	130°51′38′′
	Chunnyunpo	Bukmyeon, Chunbu	CU	37°32′05′′	130°52′15′′
	Sunchang	"	SU	37°32′17′′	130°54′51′′
	Amsu rock region	"	AR	37°32′19′′	130°04′57′′
	Chunbu	"	CB	37°32′22′′	130°53′08′′
	Danbau	"	DA	37°32′35′′	130°53′59′′
	Jukam Bridge	"	JU	38°32′20′′	130°53′45′′

analysis (Stewart and Excoffier, 1996). The unweighted pair group method with arithmetic averages (UPGMA) dendrogram was constructed with PAUP ver. 4.0b10 (Swofford, 2000). Using the geographic distance conversed from GPS coordinates and coastal-line distance calculated using X-plan 360dII, Mantel tests were carried out test for correlation between geographic and genetic distance using implemented in Arlequin ver. 2.000.

Results

Population size variations

There were 1,807 individuals occupied at 3 populations, northern, eastern and southern, including 32 subpopulations. Of them, 14 subpopulations consisted of above 10 individuals were selected for the analyses of genetic diversity of *S. takesimensis* (Table 2).

The most large subpopulation was CN, consisted of 360 individuals, whereas subpopulation PY and CH were consisted of only 9 individuals. Relatively large subpopulations (> 100) was 5, and total individuals of these 5 subpopulations is 1,222 (75.8%). About 85% of plants were in northern population of Ulleung Island, this trends were also showed in generative and vegetative adults. However, young individuals were 37.8% to the total number, and 96% of them mainly distributed in northern population, especially sandy areas. Most of larger subpopulations have more vegetative individuals than generative ones, except two subpopulations, HY and CN (Table 2).

RAPD profile and genetic diversity

Of the 60 primers tested, 33 generated 216 amplified bands, for an average of 6.5 per primer, identifying 76 different RAPD phenotypes among 77 individuals. Two identical phenotypes were observed in plants belonging to the same subpopulation, CU. Among amplified bands, 149 (69%) bands were polymorphic (Table 2). The number of polymorphic bands varied from 1 (OPA14, OPB8) to 13 (OPAF5), with an average of 4.5 polymorphic bands per primer. The northern population produced 146 polymorphic bands, whereas the smallest number, 40, was identified in samples from the southern population.

Each subpopulation has, at average, 29.9 polymorphic bands, and subpopulation HY, one of northern population containing

Table 2. Number of individuals per population and examined individuals, percentage of polymorphic RAPD bands and Nei's genetic diversity within *S. takesimensis*. Acronym of subpopulations refer to Table 1.

Population	Subpopulation	Number of individuals				Number of examined individuals	Number of polymorphic bands		Genetic diversity[5]
		GA[1]	VA[2]	Juv.[3]	Total		Number of bands	%	
North	AR	23	35	11	69	7	33(29)[4]	15.3	0.056
	SU	122	169	9	300	8	37(33)	17.1	0.062
	DA	5	43	116	164	8	22(20)	10.2	0.039
	HY	53	10	0	63	6	64(38)	29.6	0.139
	PY	8	1	0	9	3	15(14)	6.9	0.025
	CH	7	2	0	9	3	21(21)	9.7	0.043
	CN	79	27	254	360	4	4(4)	1.9	0.005
	CU	19	23	0	42	5	17(16)	7.9	0.032
	CB	9	56	12	77	4	44(38)	20.4	0.081
	JU	49	67	176	292	8	53(40)	24.5	0.091
Subtotal	Subtotal	374	433	578	1,385	56	146(98)	67.6	0.173
East	NS	13	65	0	78	7	44(27)	20.4	0.087
	SA	6	6	22	34	3	13(11)	6.0	0.022
	SG	7	3	0	10	3	11(5)	5.1	0.021
Subtotal	Subtotal	26	74	22	122	13	89(63)	41.2	0.147
South	NA	44	52	10	106	8	40(33)	18.5	0.075
Total	Total	444	559	610	1,613	77	149(100)	69.0	0.176

1. GA; generative adult, 2. VA; vegetative adult, 3. Juv.; Juvenile plant, 4. Number in the parenthesis are numbers fo polymophic bands after pruning the null alleles, 5. Nei's genetic diversity

63 individuals and of them 53 (84%) was generative, has the highest genetic diversity at 29.6%. In contrast, subpopulation CN has the lowest at 1.9%, which has 360 individuals at most, but, of them only 21.9% was generative, and 70.6% was juvenile plants. Genetic diversity and population size was not significantly correlated (Table 3).

However, the relationships between genetic diversity and life stages had revealed that the generative and vegetative individuals contributed to the genetic diversity more than juveniles (Table 3). When 3 subpopulations, DA, CN and JY, which contained more than 100 juvenile individuals were excluded, the correlation coefficients were more or less increased, especially in case of generative individuals, from 0.238 to 0.434. The genetic diversity of subpopulations, SU and CN, which are two largest subpopulations contained more than 300 individuals, have relatively low genetic diversity compared to relatively small subpopulations (Table 2).

Table 3. Correlation coefficient between number of individuals and number of polymorphic bands.

Life stage	Correlation coefficient	
	Total	3 populations excluded*
Total	0.089	0.398
Generative individuals	0.238	0.436
Vegetative individuals	0.385	0.362
Generative plus vegetative individuals	0.347	0.411
Juvenile individuals	−0.184	−0.050

*Populations contained more than 100 juvenile individuals, DA, CN and JU

Genetic structure of *Scrophularia takesimensis*

AMOVA revealed that about two-thirds variation (64.9%) was found among subpopulations within populations (Table 4), and the 38.3% was found within subpopulations. Differentiation among northern, eastern and southern population was not significant (−3.2%). The Fst index, which describes that genetic differences among subpopulations, was 0.62, and Fsc was 0.63. The average number of individuals exchanged between subpopulations per generation (N_em) was estimated as 0.31. When all three populations were grouped together, this proportion of the variation and Fst index were very similar as 62.3% and 0.64. For the within-population analyses, the similar results were also obtained (Table 4); more than 60% of the variation was found among subpopulations.

There was not significant differences in AMOVA values and Fst indices when the RAPD fragments showing the low frequency (greater than (1 − 3/n), where n is the population sample size) were removed to avoid the effects these low null allleles (Lynch and Milligan, 1994; Isabel et al., 1999); the variation of among subpopulations within populations was 70.2% ($P < 0.001$), those of within subpopulations was 34.6% ($P < 0.001$), and those of among populations was −6.6% ($P > 0.05$); Fst, Fsc, and Fct were 0.64 ($P < 0.001$), 0.66 (P,0.001) and −0.07 (> 0.05), respectively.

Seventy-seven individuals were clustered into 4 groups by UPGMA analysis (Fig. 1 and 2), without forming a population- and subpopulation-specific cluster; eastern and northern individuals were dispersed into 4 groups and eight southern individuals were also dispersed into several groups of cluster III. This clustering pattern revealed that a similar lack of strong regional genetic pattern was found. However, UPGMA

Table 4. Summary of analyses of molecular variance (AMOVA) at different hierarchical levels for 77 *S. takesimensis* individuals, based on RAPD data.

Source of variation	Variance components			Fixation index	
	d.f.	Variance components	%	Phi value	P value
Three populatons					
Among populations	2	−0.53	−3.2	Fct = −0.03	> 0.01
Among subpopulations within populations	11	10.87	64.9	Fsc = 0.63	< 0.001
Within subpopulations	60	6.42	38.3	Fst = 0.62	< 0.001
One population					
Among subpopulations	13	10.62	62.3	-	-
Within subpopulations	60	6.42	37.7	Fst = 0.64	< 0.001
Northern population					
Among subpopulations	9	10.83	62.9		
Within subpopulations	44	6.40	37.1	Fst = 0.63	< 0.001
Eastern population					
Among subpopulations	2	11.24	64.6		
Within subpopulations	9	6.17	35.4	Fst = 0.65	< 0.001

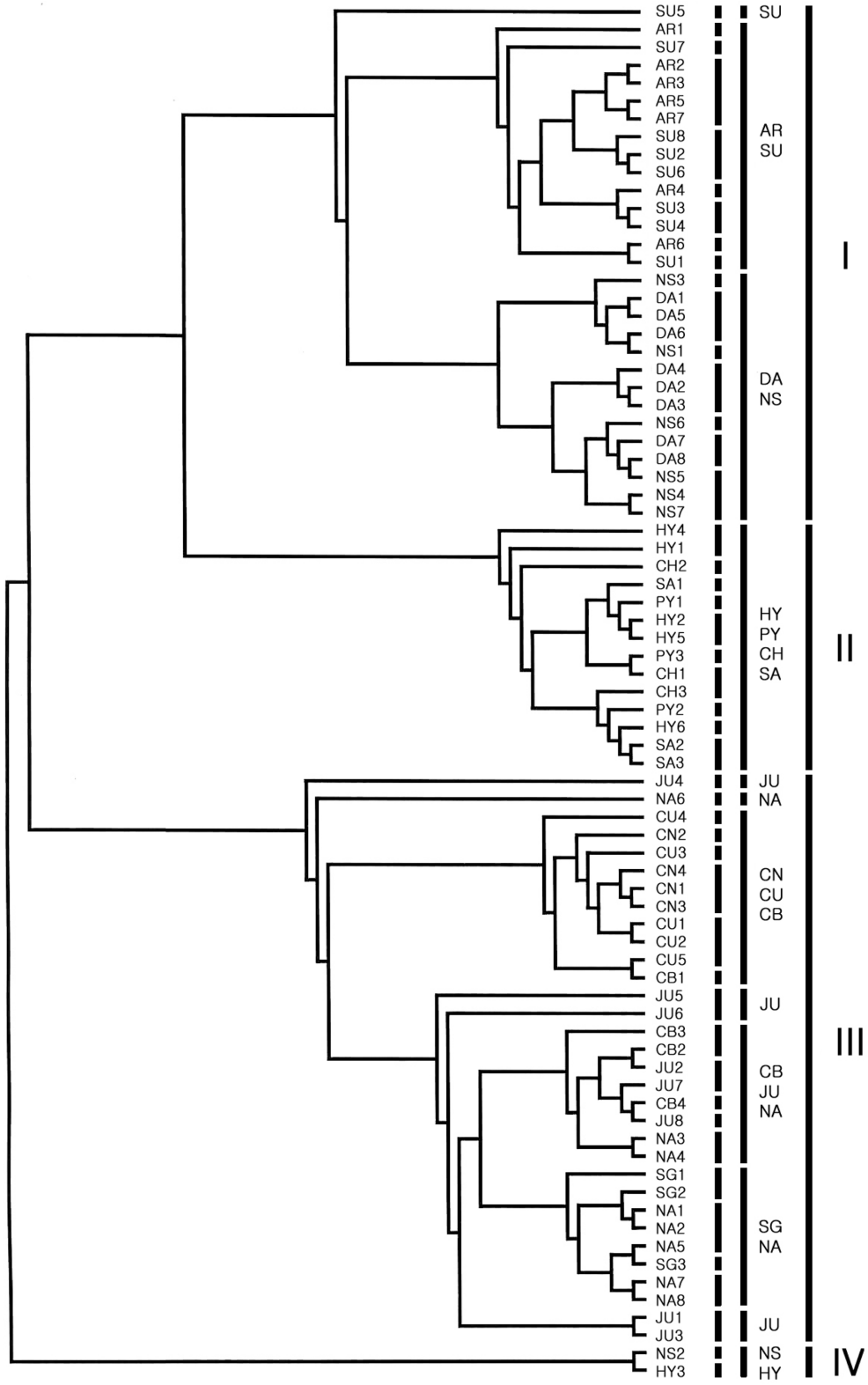

Fig. 1. Simplified UPGMA dendrogram showing the clustering pattern of 77 individuals of *S. takesimensis* from Ulleung Island.

dendrogram revealed some clustering patterns; subpopulations spatially near each other tended to be genetically similar, in part (Fig 1). The matrix of the pair-wise geographic distance (Km) was weakly correlated with the corresponding matrix of

Table 5. Percentage of polymorphic bands (PPBs) of *S. takesimensis* and those of other endangered species occupied in island and mainland at species, population and subpopulation levels. The average, minimum and maximum values of percentage of polymorphic bands were obtained from 45 previous studies. The studies analyzed were listed in Appendix.

	Species level		Population level		Subpopulation level	
	PPBs	No. of studies	PPBs	No. of studies	PPBs	No. of studies
Total	(30.2)70.0 ± 23.4(100)*	45	(9.1)53.5 ± 21.7(83.2)	25	26.0-50.5	2
Island	(41.0)73.4 ± 21.0(100)		(21.0)64.8 ± 19.7(83.2)		50.5	
Mainland	(30.2)71.4 ± 24.8(100)		(9.1)47.1 ± 20.7(82.8)		26.0	
This study	69.0		42.4		13.8	

* : (minimum)mean ± standard deviation(maximum)

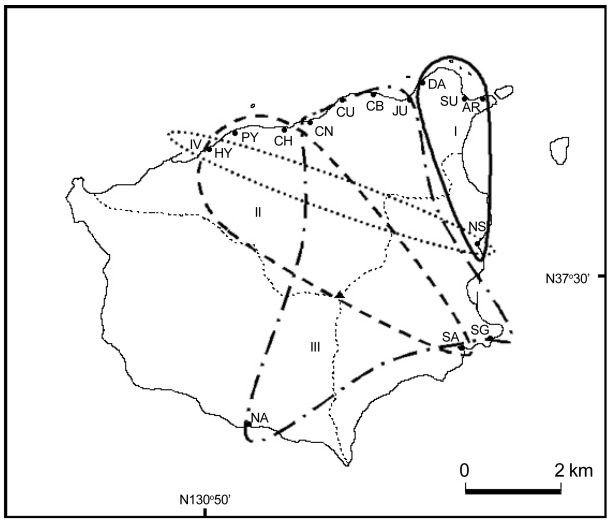

Fig. 2. Location of subpopulations of *S. takesimensis* grouped on the basis of UPGMA dendrogram.

pair-wise Fst (Mantel test; r = 0.150, P = 0.079), however, those of coastal line distance was not significantly correlated (Mantel test; r = 0.106, P = 0.114).

Discussion

Genetic diversity based on RAPDs

Since dominant genotypes cannot be unambiguously scored and thereafter the observed heterozygosites are not available, and normal segregation and linkage disequilibrium, which are prerequisites to the use of these markers in population genetics studies, cannot be assessed (Isabel et al. 1995), comparisons with levels of genetic variation in other rare plants, which are based on allozyme data, are difficult (Fischer and Matthies, 1998). In addition, the accuracy of gene diversity estimates derived from dominant RAPD fingerprints is questionable, especially when population sample sizes are uneven and small, which is a common problem in studies of rare or endangered species (Isabel et al., 1999).

The genetic diversity based on RAPD analysis, however, was generally estimated by 1) number or percentage of polymorphic bands (PPB) (Bartish et al., 1999; James and Ashburner, 1997; Sydes and Peakall, 1998; Torres et al., 2003), 2) Nei's gene diversity (Hensen et al., 2005; Maguiure and Sedgle, 1997; Zahreddine et al., 2004) and 3) Shannon's genetic index (Bouza et al., 2002; Cardoso et al., 1998; Fu et al., 2003; Jimenez et al., 2002; Martin et al., 1999) or 4) both of Nei and Shannon's indices (Allnut et al., 2003; Chen et al., 2006, Matin et al., 2003; Wu et al., 2004).

Among these indices, Shannon's index was considered to be the most biased estimator of the many studies (Isabel et al., 1995), although the reason for this remains unclear. This make Shannon's index not be used appropriately in the case of the varied number of individuals per population (Bussell, 1999). In addition, unfortunately, comparison with Shannon's indices derived for other species is complicated by the fact that other studies have employed different approaches for its calcualtion (Renau-Morata et al., 2005). Whereas, although there is a suggestion that Nei and Li's method is considered as more appropriate for RAPD data sets (Maguire and Sedgley, 1997), Nei's index need strict dominant and recessive allelic frequency, and the data from RAPDs do not depend on these criteria (Fu et al. 2003).

Therefore, genetic diversity of *S. takesimensis* was estimated by the percentage of polymorphic bands (PPB), at the subpopolation, populatin and species level in this study (Chen et al., 2006; Sales et al., 2001), instead of Nei's and Shannon's index. However, the comparison of the percentage of polymorphic bands (PPB) of RAPD are hindered by the fact that 1) primers are usually pre-selected for their ability to produce a high rate of polymorphic bands (Torres et al. 2003), and 2) estimations based on a small number of primers may be seriously biased (Bartish et al. 1999), 3) different criteria calculating the ratio of monomorphic and polymorphic loci (Nybom and Bartish, 2000), and 4) biased results may be occur due to the effects of very low frequencies of null alleles (Lynch and Milligan, 1994; Isabel et al., 1999). In this study, these problems were overcame by 1) using the 33 primers without pre-selection, 2)

defining the monomorphic bands as the bands occurred in all individuals, and 3) adapting Lynch and Milligan's method.

Genetic diversity of Endangered *Scrophularia takesimensis*

Genetic variations of endangered plants, especially distributed in islands, are generally considered to have low genetic diversity with high proportion of monomorphic markers (Morden and Loeffler, 1999; Nybom and Bartish, 2000), for a variety of reasons, such as a consequence or cause of the rarity (Ku et al., 2004), small size and potential isolation of population (Hogbin et al., 1998), severe fragmentation and degradation of habitats (Bouza et al., 2002), human activities, such as over-exploitation, habitat destruction and degradation and exotic species introduction (Wang et al., 2005), and shared common ancestry and similar selective regimes (Schaal et al., 1998).

To compare the percentage of polymorphic bands (PPB) of *S. takesimensis* to other plant species, 45 studies using RAPDs markers were analyzed (Table 5). Among them, 20 showed only PPB at species level, 25 at species and population level, and only 2 showed at species, population and subpopulation level. At species level, there is not any significant differences not only between genetic diversity of *S. takesimensis* and those of other species, but also between those of island species and those of mainland species. In addition, the percentage of polymorphic bands of *Bupleurum latissimum*, which is a endangered endemic species in Ulleung island and once reported as a extinct species, was estimated as 75.6, at species level (Ku et al., 2004), whereas those of *Campanula takesimana*, widely distributed endemic species in Ulleung Island, was 72.5 (Kim et al., 1998). Whereas, the PPB of *Aldrovanda vesiculosa*, a water plants evaluated as extinct in wild, was estimated as 37% (Martin et al., 2003).

This means that the genetic diversity of *S. takesimensis* is not seriously reduced or more or less balanced between persistence and extinction at species level, because of 2 factors; 1) positively, the high production of seeds, and 2) negatively, the reduction of individuals or subpopulations. The plants of *S. takesimensis* were reproduced with seed, which were made by cross-pollination, and germinated easily, especially in sand. Seed production by one individual was estimated as more than 30,000; one capsule contained more than 150 seeds, one inflorescence had about 20 flowers averagely, and one individual made more than 10 inflorescences (personal observation). However, juvenile plants were usually swept by typhoons or surfs. On the contrary, the reduction of individuals or subpopulations was occurred recently; several populations were discovered near Tunggumi, located at southern coast of Ulleung Island during 1990-94, but now was disappeared because of the construction of harbor and road, and 4 subpopulations, consist of more than 10 individuals, were destroyed due to typhoon and road construction between 2001 and 2005 (personal observation).

Meanwhile, genetic diversity at population level was relatively low, compared to other endangered species (Table 5), especially southern population; NA, had showed only 18.5% based on ther number of polymorphic bands (Table 2), and three eastern populations, NS, SA, SG, showed 41.2%. The eastern and southern populations were isolated to each other and, also, northern population (Fig. 1 and 2), largest population of *S. takesimensis*. Moreover, PPB at subpopulation level was 13.8 averagely, ranged from 1.9 at minimum in CN to 29.6 at maximun in HY (Table 2). The average value of PPB was lower than those of previously studies; 26.0% in mainland species, *Caldesia grandis* (Chen et al., 2006) and 50.5% in island species *Digitalis minor* (Sales et al., 2001). These results suggested that genetic diversity of *S. takesimensis* was more or less higher at species level, but lower at subpopulation level, because of severe fragmentation of population and subpopulation, which maybe bring about the interruption of gene flow between population and subpopulation as well as the population differentiation.

Population size and genetic diversity

In general, small populations tend to maintain low genetic diversity, i.e., population size is positively correlated with the level of diversity, primarily due to random genetic drift over generations (Hartl and Clark, 1997; Wang et al., 2005). Levels of genetic diversity however, were not always correlated to population size (James and Ashburner, 1997; Lee et al., 2004; Zawko et al., 2001), and genetic diversity of *S. takesimensis* also did not showed these general trends.

There are several reasons for the lack of positive correlation between population size and genetic diversity in endangered species; 1) fluctuation in size due to frequent extinctions and re-colonization (Maki, 2003), 2) individuals within single populations are genetically very closely related, i.e., population has recently been established by few propagules (Jimenez et al., 2002), 3) recent fragmentation (human disturbance) of a once continuous genetic system (Rossetto et al., 1995); 4) extensive gene flow due to the combination of bird pollination and high outcrossing rates (Maguire & Sedgely, 1997), and 5) insufficient length of time for genetic diversity to be reduced following a natural reduction in population size and isolation (Coates, 1988).

From 2001 to 2005, the total number of individuals fluctuated as 1807, 820, 2705, 2206 and 1500, and the percentage of juvenile individuals also fluctuated from 13% at 2002 to 72.4% at 2004 (personal observation). This means that the population size is unstable yearly, and many young plants, maybe genetically very closely related, are recruited, therefore, the positive correlation between population size and genetic diversity is not occurred in *S. takesimensis*. In addition, although there is not strong evidences that the populations and subpopulations were once totally connected, the recent fragmentations owing to construction of roads and small ports, especially in south and east coast of Ulleung Island, occurred, and this fragmentation seemed to make an serious barrier of gene flow between populations

Population differentiation of *S. takesimensis*

Generally, Wright's Fixation index, FST means the inbreeding effect of population structure, and values of FST above 0.25 indicates very great genetic differentiation (Hartle and Clark, 1997). The value of FST or GST value, modified F-statistics using AMOVA based on RAPD, were effected by several factors; 1) sexual system, GST value of inbreeding species was usually > 50%, and of outbreeding species was < 20% (Bussell, 1999); 2) populations located in geographically or ecologically marginal areas of the species with a center of diversity showed most genetic variations between populations (Wu et al., 2004); 3) the geographically isolated populations exhibited high genetic differentiation among populations (Oiki et al., 2001); 4) genetic drift due to isolation of populations (Shrestha et al., 2002), and 5) habitat fragmentation and deterioration under human disturbance (Wu et al., 2004).

In contrast, the cluster pattern (Fig. 1, and 2) and genetic structure estimated by AMOVA suggested that genetic differentiation between subpopulations have happened. The plants of *S. takesimensis* were presumed as outbreeder, based on floral structure including the nectar and visiting animals. Therefore, fragmentation or isolation of habitat of *S. takesimensis* may be a key factor leading to high genetic variation among subpopulations within populations. This was supported by relatively low N_em value, which means the average number of individuals exchanged between populations or subpopulations per generation. However, fragmentation of habitat was not enough long to interrupt gene flow between subpopulations, so this make the genetic differentiation not complete until now (see Fig.1 and 2). Though, many of plants of *S. takesimensis* form a cohesive cluster, and this suggesting that some genetic restructuring is taking place.

Conservation implications

The plants of *S. takesimensis* grow in seaside area in Ulleung Island, Korea, especially along the seacoast consisted of sand or shingles. These habitats are under severe stress, such as construction of road and small ports along the seacoast, and discharge of wastes, or seacoast regeneration. In addition, periodic typhoon and severe surfs are the another main threatening factors; these swept the young plants or seeds deposited in their habitats, especially plants rooted in sands. Finally, fragmentation as a result of these two factors may be evaluated as the key factors.

For the conservation of *S. takesimensis*, 3 actions will be needed; 1) preservation of populations as it stands, 2) establishment of recovery plan to connect populations and subpopulations genetically, and 3) long-term monitoring. In 2001, there were 32 subpopulations of *S. takesimensis* in Ulleung Island, and among them 9 were disappeared until now. The best way is maybe to conserve every populations, because 1) the long-term effects of the recent reduction in plant numbers are yet to be established (James and Ashburner, 1997), 2) the prevention of further substantial loss of genetic diversity is needed (Wang et al., 2005), 3) pollinator maintenance for *S. takesimensis* is essential for genetic diversity through preservation of coexisting vegetation to support pollinators (Maguire and Sedgley, 1997), and 4) even though road verge populations may be small and highly fragmented, they may still retain links with neighbouring populations via gene flow and are thus able to maintain considerable genetic variability (Ellstrand and Elam, 1993).

Next, the reintroduction of plants to the recently breakdown areas, which will connect the populations or subpopulations, will be needed especially in southern and eastern seacoast region, because only habitat preservation might be insufficient if these populations represent genetically isolated “habitat islands” resulting from the fragmentation of a once widespread population (Bouza et al., 2002). For the reintroduction, since *S. takesimensis* showed significant level of population differentiation, germplasm from different populations should be separately collected and propagated (Bekessy et al., 2002). Also, because the germplasm having high genetic diversity should be selected, accurate and critical estimates of genetic diversity are useful for optimizing sampling strategies driven at the conservation and management of genetic resources (Bouza et al., 2002; Olfelt et al., 2001).

Finally, for monitoring of populations is necessary for information on flowering, seed set and growth rate, long-term monitoring sites should be established and population mapped (James and Ashburner, 1997). This provides an ideal

opportunity for monitoring population dynamics so that any effects of the recent population depletions can be noted in the future (James and Ashburner, 1997). From 2001 to now, monitoring was continued, and varied number of subpopulations and individuals were counted year by year.

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Appendix. Taxa included in our data compilation (Table 5) and the references, which was not shown fully.

Taxa	References
Neolitsea sericea	Wang et al. 2005. Ann. Bot. 95: 305-313
Haloragodendron lucasii	Sydes et al. 1998. Mol. Ecol. 7: 87-93
Bupleurum latissimum	Ku et al., 2004. Kor. J. Bio. Sci.8: 289-294
Dorycnium specatabile, Isoplexis chalcantha	Bouza et al. 2002. Int. J. Pl. Sci. 163: 619-630
Astelia australiana	James et al., 1997. Biol. Conserv. 82: 253-161
Banksia cuneata	Maguire et al. 1997. Heredity 79: 394-401
Leucopogon obtectus	Zawko et al. 2001. Mol. Ecol. 10: 2389-2396
Digitalis minor	Sales et al. 2001. Am. J. Bot. 88: 1750-1759
Alphitonia ponderosa, Colubrina oppositifolia	Kwon et al. 2002. Mol. Ecol. 11: 991-1001
Haplostachys haplostchya	Morden et al. 1998. Mol. Ecol. 8: 617-625
Toucharida latifolia	Loeffler et al.. 2003. Biochem. Syst. Evol. 31: 1323-1335
Campanua microdonta	Oiki et al., 2001. Ann. Bot. 87: 661-667
Grevillea barklyana	Hogbiin et al. 1998. Heredity 80: 180-186
Antirrhinum subbaeticum	Jimenez et al. 2002. Heredity 89: 387-393
Oryza granulata	Wu et al. 2004. Pl. Sci. 167: 35-42
Potamogeton maackinaus	Li et al. 2004. Aquat. Bot. 80: 227-240
Elaeocarpus williamsianus	Rossetto et al. 2004. Biol. Conserv. 117: 33-39
Prunus mahaleb	Jordano et al. 2000. Mol. Ecol. 9: 1293-1305
Changium smyrnioides	Fu et al. 2003. Bot. Bull. Acad. Sin. 44: 13-18
Zostera japnoica	Lee et al. 2004. J. Pl. Biol. 47: 275-281
Caesalpinia echinata	Cardoso et al. 1998. Mol. Ecol. 7: 601-608
Primula farinosa	Reisch et al. 2005. Basic Appl. Ecol. 6: 35-45
Caldesia grandis	Chen et al. 2006. Aquat. Bot. 84: 301-307.
Goodyera procera	Wong et al. 1999. Am. J. Bot. 86: 1406-1413
Hemigenia exilis	Mattner et al. 2002. Biol. Conserv. 107: 37-45
Antirrhinum microphyllum	Torres et al. 2003. Am. J. Bot. 90: 85-92
Araucaria araucana	Bekessy et al. 2002. Heredity 88: 243-249
Wyethia reticulata	Ayres et al. 1997. Mol. Ecol. 6: 761-772
Pinus oocarpa	Diaz et al. 2001. Mol. Ecol. 10: 2593-2603
Halodule wrightii	Angel, 2002. Aquat. Bot. 74: 165-174
Aldrovanda vesiculosa	Maldonado et al. 2003. Aquat. Bot. 75: 159-172
Oryza glumaeptatula	Buso et al. 1998. Mol. Ecol. 7: 107-117
Pancratium maritimum	Zahreddine et al., 2004. Biol. Conserv. 120: 11-18
Gentianella germanica	Fischer et al. 1998. Am. J. Bot. 85: 811-819
Erodium paularense	Martin et al. 1999. Mol. Ecol. 8: S31-S40
Wyethia bolanderi, Wyethia reticulata	Ayres et al. 1999. Am. J. Bot. 86: 344-353
Pulsatilla vulgaris	Hensen et al. 2005. Flora 200: 3-14
Leucadendron elimense	Tansley et al. 2000. Biol. Conserv. 95: 39-48
Pilgerodendron uviferum	Allnutt et al. 2003. Biol. Conserv. 114: 245-253
Acacia raddiana	Shrestha et al. 2002. Biol. Conserv. 108: 119-127
Ranunculus reptans	Fischer et al., 2000. Am. J. Bot. 87: 1128-1137
Metasequoia glyptostroboides	Li et al. 2005. Conserv. Biol. 19: 224-231