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Genetic Diversity and Spatial Structure of *Symplocarpus renifolius* on Mt. Cheonma, Korea

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Abstract - Genetic variation and structure of 9 subpopulations of *Symplocarpus renifolius* Schott ex Tzvelev on Mt. Cheonma, in Korea, were determined via starch-gel electrophoresis. The genetic diversity at 10 loci for 8 isozymes (P_{99}=66%, A=2.26, H_o=0.212, H_e=0.230) was found to be considerably higher than that seen in other long-lived perennial plants. On the whole, the genotype frequencies were in accordance with Hardy-Weinberg expectations. Approximately 5% (θ=0.049) of the total variability was among subpopulations. The high levels of observed genetic diversity in *S. renifolius* were attributed to a universal outcrossing system and other specific factors like differences in age classes and widely scattered individuals around the main distribution. Heterozygosity was highest at a mid-range of elevation (450m ~600m). The lowest heterozygosity at lower elevation was attributed to the possible origin of seeds transported by water from upstream regions during the monsoon season. Spatial structure in a subpopulation evidenced a strong autocorrelation between closer individuals within 3~4m of distance. This was assumed to be attributable to the restricted seed dispersal characteristics of *S. renifolius*. In accordance with the findings generated in this study, some implications regarding the conservation of *S. renifolius* at the Mt. Cheonma were also presented.

Key words - Isozyme, Conservation, Genetic diversity, Spatial differentiation, *Symplocarpus renifolius*

Introduction

The genus *Symplocarpus* represents a classical example of species disjunction between eastern Asia and eastern North America. It contains only three species: *S. renifolius* Schott ex Tzvelev, *S. nipponicus* Makino, and *S. foetidus* Nutt. *Symplocarpus renifolius* are distributed throughout northern Japan, Korea, northeastern China, and eastern Siberia. *Symplocarpus nipponicus* is also encountered in northern Japan, Korea, and northeastern China (Wen *et al.*, 1996). *Symplocarpus foetidus* is a common species that is distributed widely in eastern North America (Wilson, 1960).

The natural distribution of *S. renifolius* in South Korea is concentrated in the Gyeonggi and Gangwon provinces and their general vicinity. *Symplocarpus nipponicus*, however, is found primarily along the Taebaek Mountains in Gangwon province. *Symplocarpus renifolius* exhibits a characteristically patchy distribution. It is found in wetlands along mountain valleys and in shady sites under deciduous and mixed forest stands.

This species is one of the earliest plants to flower in spring, so much so that its spathes often emerge through a layer of snow remaining on the ground. The spathe functions as a bud, which holds and protects the flower while it emerges from the ground. The spathe surrounds a spherical head of flowers, which is referred to as a spadix. Even with the flowers are in full bloom, they remain enveloped by the spathe. Individual variations in both the color and the shape of the spathes, as well as of the spadices, are manifold and complicated. A rare variant of this species, characterized by the presence of a pure yellow spathe, has been observed only in a few sites, and the taxonomic position of this variant remains to be clearly elucidated.

Only a few studies concerning the phenology and ecology of *S. renifolius* have been, thus far, conducted (Uemura *et al.*, 1993; Wada and Uemura, 1994; Kang and Min, 1994; Hong and Son, 2003; Ito *et al.*, 2004). However, this plant species has yet to be genetically evaluated.

The mountain Cheonma region located approximately 30km northeast of Seoul is one of the few habitats of the yellow-colored variant, and also famous for its abundant plant species. It has been designated a public park by the regional county authority, and has become a popular place for not only the denizens of the county, but also for visitors from adjacent areas, including the Seoul metropolitan area. Habitat interference as the result of uncontrolled

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gathering of wild flowers, and soil stamping is steadily becoming a more serious problem. Concrete measures are urgently required in order to protect the natural habitat of *S. renifolius* in the Mt. Cheonma region from the dangers of decline and extinction.

In this study, we used isozyme markers to examine the genetic diversity of nine sub-populations of *S. renifolius* gathered from the Mt. Cheonma area. The genetic diversity data of the yellow spathe variants was compared with those of other subpopulations, in order to determine whether a significant difference exists between the two groups. A micro-spatial genetic autocorrelation study within one subpopulation was also conducted. Results of this study are expected to provide us with essential basic information to the establishment of concrete conservation measures, not only for the Mt. Cheonma population, but also for other Korean *S. renifolius* populations.

Materials and Methods

Study site and sampling of leaf materials

The mountain Cheonma study site is located in Gyeonggi province, in central Korea (N 37° 40' 44" E 127° 16' 19"). The vegetation of Mt. Cheonma is dominated by *Quercus mongolica* Fisch. ex Turcz., *Acer mono* Maxim., *Fraxinus rhynchophylla* Hance, and *Larix leptolepis* Hort. ex Endl. *Symplocarpus renifolius* exhibits a patchy distribution in the Mt. Cheonma zone. The plant occurs in small groupings on rocky wetlands along streams, and in shady sites under broad-leaved deciduous and mixed forest stands. Patches of the species are found primarily on gently sloping western or/and north-western aspects. The sizes of these patches differ between sites, ranging from 50㎠ to 3,000㎠, but in general with the range of 200㎠~300㎠. Density also varies considerably, with a range of 1~20 plants /㎡.

The basic idea for the sampling sites was to cover the entire area of distribution to as complete a degree as possible. Nine subpopulations were selected in 2003 (Fig. 1). In order to study the genetic diversity of the yellow spathe variants, individual variants found in some subpopulations (1, 2 and 6) were considered to be a separate subpopulation (Y). General information on the sampled sites is shown in Table 2. In April to June of 2003, small sections of leaves were collected from a minimum number of 36 individuals from each of the subpopulations. One large subpopulation (No. 2) was selected for microspatial genetic analysis. Leaf samples were collected from all plants (196) located in a 20m × 20m quadrant of the subpopulation.

Immediately after collection, labeled samples were maintained in an ice box, and transported to the laboratory.

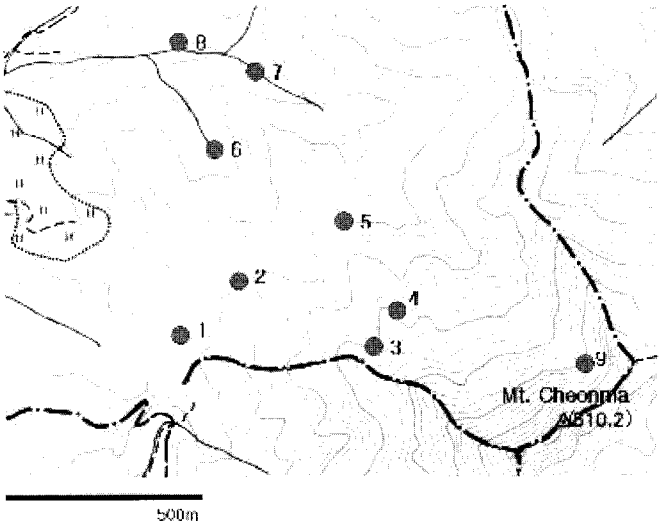

Fig. 1. Sampling sites of *Symplocarpus renifolius* at Mt. Cheonma.

Isozyme electrophoresis

Leaf samples were ground using a cold mortar and pestle. Extraction buffer [0.1M Tris-HCl pH 7.3; 10% PVP (wt. 40,000); 0.5% (v/v) 2-mercaptoethanol; 0.003M EDTA] was added to the tissue samples, and the extracts were absorbed into paper chromatography wicks (Whatman 3MM) and stored at -70℃ until electrophoresis.

The following eight isozymes were analyzed; catalase (*Cat*), fluorescent esterase (*Fst*), glutamate dehydrogenase (*Gdh*), glutamate-oxaloacetate transaminase (*Got*), malate dehydrogenase (*Mdh*), phosphoglucose isomerase (*Pgi*), shikimate dehydrogenase (*Skdh*) and superoxide dismutase (*Sod*). The isozymes were separated in 12% starch gels, using two buffer systems. Five isozymes (*Cat, Gdh, Got, Pgi* and *Sod*) were resolved with a Sodium-Borate buffer system (pH 8.2), and the other three (*Fst, Mdh* and. *Skdh*) were resolved using a Morpholine-Citrate buffer system (pH. 7.0). The recipes used for the preparation of the buffers and staining solutions were derived from those established by Conkle *et al.* (1982). Genetic interpretations of the banding patterns were predicated on the quaternary structures reported in other plants (Weeden and Wendel, 1989; Kephart, 1990; Jeong, 2003). Loci and alleles were designed on the basis of relative protein mobility, with lower numbers being assigned to those further away from the origin (Fig. 2).

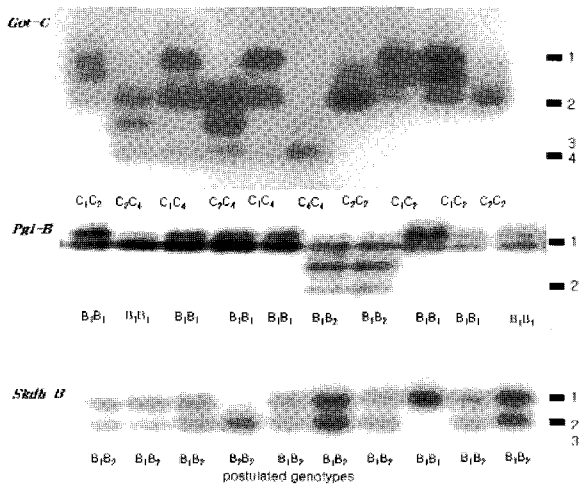

Fig. 2. Zymograms of three polymorphic isozyme loci, *Got-C, Pgi-B* and *Skdh-B*. Allele 3 at *Got-C* and *Skdh-B* was not shown on this picture. Abbreviations of locus names are same as in "Materials and Methods".

Data analysis

Measures of genetic diversity within and among subpopulations were calculated with the GDA v1.0 computer program (Lewis and Zaykin, 1999). For each of the subpopulations, the following measurements were taken; proportion of polymorphic loci (P, 99% level), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (A_P), and expected and observed heterozygosity (H_e and H_o).

Departures from Hardy-Weinberg expectations were examined for each of the polymorphic loci of each subpopulation, using the Chi-square test function of the BIOSYS-1 program (Swofford and Selander, 1989).

Nei's (1978) unbiased genetic distances and identities between all pairs of subpopulations were also estimated, in order to generate the average linkage clustering, via the UPGMA technique (unweighted pair group method with arithmetic mean). The UPGMA dendrogram was constructed using the TreeView program (v. 1.6.6).

Hierarchical F-statistics (Wright, 1951) were calculated in accordance with the method of Weir and Cockerham (1984) and Weir (1996). Weir and Cockerham' s f, which is analogous to Wright' s F_{IS}, measures the correlations of genes within individuals in populations. θ (similar to F_{ST}) measures the degree of differentiation among subpopulations, relative to total genetic diversity. Upper and lower 95% confidence intervals were generated for f and θ via bootstrapping across loci, using 1,500 replicates. The GDA software (Lewis and Zaykin, 1999) was also employed in the estimation of the F-statistics, as mentioned above.

Analyses of spatial autocorrelation between individual plants were conducted on single-locus allele frequency data, using the SGS, version 1.0c software (Degen, 2000; Degen *et al.*, 2001). The aggregation index (R) of Clark and Evans (1954) was calculated as an indicator of the spatial distribution between individuals. The autocorrelation coefficient, Moran's I, was calculated for all individuals within a 1m distance of one another, in order to generate a correlogram. For each of the spatial distance classes, the observed values were compared with the distributions obtained after 1,500 permutations, after which a 95% confidence interval for the parameters was constructed, as the interval from the lower and upper limit ordered permutation estimates.

Results

Allele frequencies and distribution

The 8 enzyme stains revealed the products of 10 putative loci, 2 of which (*Cat-A* and *Gdh-B*) were monomorphic. Zymograms of some polymorphic isozymes were shown in Fig. 2. A total of 28 alleles were described (Table 1). At 6 of the loci, all but *Got-C* and *Skdh-B*, the most frequently detected allele was common to all subpopulations. Three alleles, *Fst-*B_1, *Got-*C_3 and *Skdh-*B_3, all of which were unique to a single subpopulation, were detected in subpopulations 5, 8, and 1, respectively (Table 1). The average percentage of polymorphic loci fulfilling the 99% criterion (P) was 66%, with a range between 60% (subpopulation 1) and 80% (subpopulation 7) (Table 2). The average number of alleles per locus (A) was 2.26, which increased to 2.89 (A_P) when only the polymorphic loci were considered. The expected heterozygosity (H_e) ranged between 0.192 (subpopulation 6) and 0.264 (subpopulation 2). The mean value for 10 subpopulations was 0.230. The observed heterozygosity (H_o) ranged from 0.183 in subpopulation 6 to 0.256 in subpopulation 2, with a mean of 0.212. In all subpopulations but subpopulation 7, the average proportion of observed heterozygotes was lower than had been expected in accordance with Hardy-Weinberg equilibrium. The genetic diversity data of the yellow-colored spathe-exhibiting variants was not substantially different from those data in other subpopulations (Table 1, 2).

Genetic structure and differentiation

Of the 66 tests conducted in order to compare the observed genotypic frequencies at each locus with those expected by Hardy-

Table 1. Allele frequency for 8 polymorphic isozyme loci in 9 subpopulations of *Symplocarpus renifolious* on Mt. Cheonma

Locus[a] / allele	Subpopulation									
	1	2	3	4	5	6	7	8	9	Y[b]
Fst-B										
N	60	196	60	49	60	46	40	191	60	36
1	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
2	0.058	0.054	0.033	0.031	0.117	0.065	0.050	0.031	0.000	0.014
3	0.942	0.946	0.950	0.969	0.875	0.935	0.950	0.945	1.000	0.986
4	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.024	0.000	0.000
Got-A										
N	60	196	60	49	59	42	37	173^{+*}	60	36
1	0.092	0.107	0.083	0.255	0.085	0.036	0.000	0.078	0.100	0.028
2	0.542	0.577	0.700	0.500	0.678	0.714	0.784	0.705	0.667	0.667
3	0.367	0.316	0.217	0.245	0.237	0.250	0.216	0.217	0.233	0.306
Got-B										
N	60	196	60	49	60^{+**}	47	40	191	60	36
1	0.008	0.110	0.025	0.031	0.042	0.064	0.087	0.058	0.058	0.014
2	0.992	0.890	0.975	0.969	0.958	0.936	0.913	0.942	0.942	0.986
Got-C										
N	52	196	60	49	59^{+*}	47	40	191	59	36
1	0.058	0.207	0.025	0.010	0.076	0.011	0.013	0.055	0.034	0.153
2	0.558	0.526	0.367	0.663	0.576	0.777	0.637	0.330	0.669	0.417
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000
4	0.385	0.268	0.608	0.327	0.347	0.213	0.350	0.599	0.297	0.431
Pgi-B										
N	60	196	60	49	60	47	40	191	60	36
1	0.983	0.972	1.000	0.990	1.000	1.000	0.988	1.000	1.000	0.986
2	0.017	0.028	0.000	0.010	0.000	0.000	0.013	0.000	0.000	0.014
Sod-A										
N	60	196	60	49	60	47	40	191	59^{+**}	35
1	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.050	0.017	0.000
2	1.000	1.000	1.000	1.000	1.000	1.000	0.988	0.950	0.983	1.000
Mdh-B										
N	60	196	60	49	59	47	40^{+*}	191^{+**}	59	36^{-*}
1	0.025	0.008	0.050	0.071	0.059	0.043	0.075	0.086	0.102	0.000
2	0.417	0.523	0.375	0.602	0.695	0.755	0.637	0.654	0.475	0.389
3	0.042	0.000	0.033	0.010	0.008	0.000	0.013	0.000	0.034	0.000
4	0.150	0.184	0.258	0.143	0.102	0.149	0.025	0.003	0.136	0.222
5	0.033	0.003	0.042	0.041	0.034	0.000	0.013	0.065	0.017	0.028
6	0.333	0.283	0.242	0.133	0.102	0.053	0.237	0.191	0.237	0.361
Skdh-B										
N	60	196	60	48	59	47	40	189^{+*}	59^{+*}	36
1	0.492	0.487	0.363	0.594	0.364	0.319	0.594	0.701	0.517	0.458
2	0.500	0.513	0.637	0.406	0.636	0.681	0.406	0.299	0.483	0.542
3	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[a]Abbreviations of locus names were described in "Materials and Methods".
[b]Plants of yellow spathe variant were collected from 3 subpopulations (1, 2 and 6).
N: Number of individuals analyzed.
Genotype frequencies not at Hardy-Weinberg equilibrium are indicated by plus sign (significant heterozygote deficiency); χ^2, $p < 0.05$* and $p < 0.01$**.

Table 2. Description of sampling sites and genetic diversity at 9 loci in 9 subpopulations of *Symplocarpus renifolius* on Mt. Cheonma

Sub-population	Altitude (m)	Size (m^2)	Density	Slope	Direction	*N*	*P*	*A*	A_p	H_e	H_o
1	430	300	High	11°	NW	59.0	60.0	2.40	3.17	0.248	0.228
2	485	600	High	12°	W	196.0	70.0	2.20	2.71	0.264	0.256
3	600	250	High	24°	NW	60.0	60.0	2.30	3.17	0.234	0.242
4	590	600	Middle	9°	N	48.9	70.0	2.30	2.86	0.232	0.217
5	500	300	Middle	10°	N	59.5	60.0	2.30	3.17	0.229	0.211
6	375	800	Low	6°	NW	46.3	60.0	2.00	2.67	0.192	0.174
7	370	800	Low	8°	NW	39.6	80.0	2.30	2.63	0.213	0.183
8	340	300	High	30°	SW	188.5	70.0	2.50	3.00	0.224	0.199
9	710	400	Middle	22°	NW	59.5	60.0	2.20	3.00	0.230	0.211
Y	-	-	-	-	-	35.9	70.0	2.10	2.57	0.236	0.200
Mean	488.9	394	-	14.7°	-	79.3	66.0	2.26	2.89	0.230	0.212

N: Sample size, *P*: proportion of polymorphic loci (%), *A*: mean number of alleles per locus, A_p: mean number of alleles per polymorphic locus, H_e: expected heterozygosity, H_o: observed heterozygosity.

Weinberg equilibrium, 9 tests from 5 subpopulations showed significant departures from the expected distributions (Table 1). All of these significant departures reflected a deficiency in heterozygotes. The other 4 subpopulations evidenced no significance at all loci. Overall, the genetic structures of 10 *S. renifolius* subpopulations in the Mt. Cheonma region appeared to be in equilibrium.

The values of f at each of the loci varied, from -0.018 (*Pgi-B*) to 0.149 (*Sod-A*), with a mean value of 0.068, indicating a 6.8% deficiency in heterozygotes relative to the Hardy-Weinberg expectation within a subpopulation (Table 3). Heterozygote excess was observed only at the *Pgi-B* locus. The estimate of the total fixation index, F, was 0.113, exhibiting an 11.3% deficiency of heterozygotes, on average, in the Mt. Cheonma region populations of *S. renifolius* as a whole.

θ values for individual loci ranged between 0.010 (*Pgi-B*) and 0.085 (*Got-C*), with a mean of 0.049. θ measures the fixation of different alleles in different subpopulations, and therefore this value represents the level of differentiation among subpopulations. Thus, approximately 4.9% of total genetic variation was detected among the subpopulations (Table 3).

Genetic distance and cluster analysis

Table 3. Summary of *F-statistics* (Weir 1996) at 8 isozyme loci analyzed for 9 subpopulations of *Symplocarpus renifolius* on Mt. Cheonma. Upper and lower 95% confidence intervals (CI) were derived from bootstrapping across loci with 1,500 replicates

Locus	f	F	θ
Fst-B	0.005	0.016	0.011
Got-A	0.030	0.048	0.018
Got-B	0.009	0.025	0.016
Got-C	0.055	0.135	0.085
Pgi-B	-0.018	-0.008	0.010
Sod-A	0.149	0.175	0.030
Mdh-B	0.141	0.180	0.045
Skdh-B	0.058	0.114	0.059
Mean	0.068	0.113	0.049
Upper CI	0.111	0.154	0.069
Lower CI	0.031	0.054	0.018

Estimates of genetic identities and distances, calculated over 10 loci, are presented in Table 4. Genetic distance values ranged between 0.001 and 0.059. The dendrogram generated via UPGMA clustering exhibited no definitive geographic pattern (Fig. 3). However, subpopulations near the main ridge of the distribution tended to be separated from the subpopulations near the swampy, low-elevation areas (Fig. 1). The hypothetical subpopulation Y, consisting of 36 individuals with the yellow-colored spathe, built a subgroup with subpopulations 1 and 2, from which most of the variant individuals were collected.

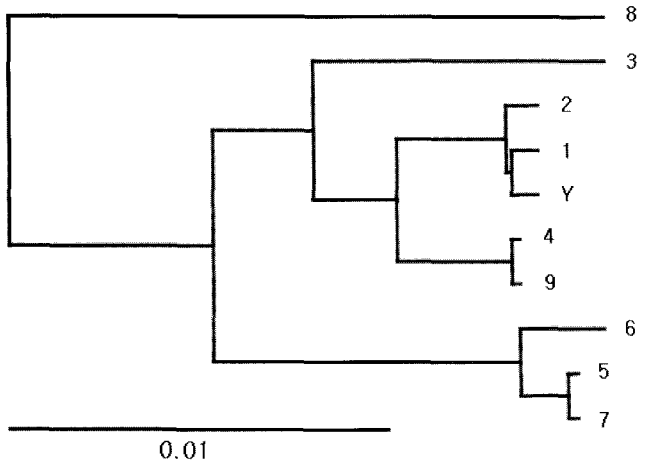

Fig. 3. Dendrogram constructed via the UPGMA method, based on Nei's (1978) unbiased genetic distance from isozyme data. Y denotes the assumed subpopulation of 36 variants with yellow-colored spathes.

Spatial structure

Spatial autocorrelation analyses conducted between individual plants revealed that the mean distance between adjacent individuals was 0.48m, in a range between 0m and 23.99m. The aggregation index (R), which refers to the distribution patterns of individuals within the study site, was 0.761.

The correlogram constructed using the autocorrelation coefficient, Moran' s *I*, revealed that closer individuals tended to appear more genetically similar within a distance of 4m. Over that distance, the autocorrelation coefficients were generally included within the confidence interval (95%) estimated for the case of random spatial distribution (Fig. 4).

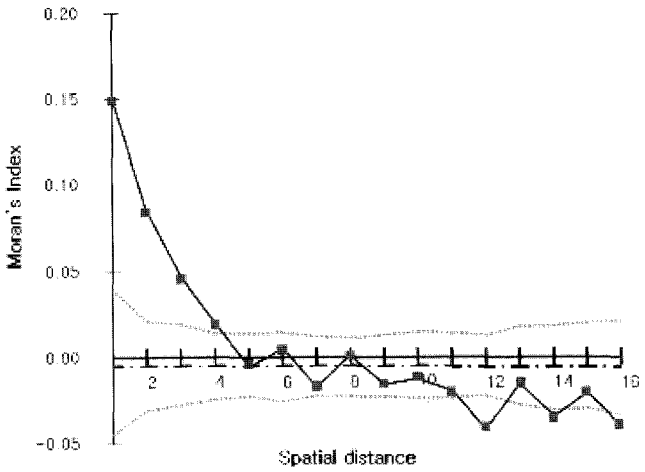

Fig. 4. Correlogram (solid line) simulated on the basis of 7 polymorphic loci (in subpopulation 2) at 1m distance interval. The horizontal broken line indicates the expected values for the case of no autocorrelation (-0.005), and dotted lines indicate the upper and the lower confidence interval of 95%, obtained by 1,500 permutations.

Table 4. Nei's (1978) unbiased genetic distance (above diagonal) and genetic identity (below diagonal) among 9 subpopulations of *Symplocarpus renifolius*

Subpopulation	1	2	3	4	5	6	7	8	9	Y
1	*****	0.005	0.011	0.010	0.016	0.029	0.015	0.029	0.004	0.001
2	0.995	*****	0.020	0.012	0.012	0.022	0.014	0.032	0.006	0.006
3	0.989	0.980	*****	0.025	0.026	0.049	0.026	0.017	0.017	0.004
4	0.990	0.988	0.975	*****	0.014	0.022	0.021	0.026	0.005	0.024
5	0.984	0.988	0.975	0.986	*****	0.003	0.002	0.032	0.011	0.019
6	0.971	0.978	0.952	0.978	0.997	*****	0.005	0.059	0.016	0.037
7	0.985	0.986	0.975	0.979	0.998	0.995	*****	0.033	0.007	0.017
8	0.972	0.968	0.98	0.975	0.968	0.943	0.967	*****	0.028	0.027
9	0.996	0.994	0.984	0.995	0.989	0.984	0.993	0.973	*****	0.010
Y	0.999	0.994	0.996	0.977	0.981	0.964	0.984	0.973	0.990	*****

Discussion

Symplocarpus renifolius growing in the Mt. Cheonma region exhibited high levels of genetic diversity. The percentage of polymorphic loci (*P*), the mean number of alleles per locus (*A*), and the expected heterozygosity (H_e) were estimated to be 66.0%, 2.26, and 0.230, respectively (Table 3). These values were considerably higher than those reported for other long-lived perennial plants (*P*=39.6, *A*=1.42, H_e=0.205) (Hamrick and Godt, 1989). This may be principally attributable to the obligatory outcrossing mode of this species. *Symplocarpus renifolius* is protogynous. Flowering progresses basipetally for each spadix, resulting in an expression of different sexual phases over time. The female and male phases take 6.8 ± SD 5.8 days and 16.7 ± SD 5.8 days, respectively, with a short transitional bisexual phase of 2.1 ± SD 0.9 days (Uemura *et al.*, 1993). This type of temporal sexual separation certainly contributes to the degree of outcrossing in this species. In fact, in a bagging test, 77.6% of cross-pollinated plants produced multiple fruits, whereas only 1 of 20 self-pollinated plants was shown to (Uemura *et al.*, 1993). As *S. renifolius* blooms in the very early spring, even prior to spring thaw in many cases, only a few fly and beetle species function as effective pollinators (Uemura *et al.*, 1993; Hong and Son, 2003). Wind pollination may be another factor contributing to gene flow (Camazine and Niklas, 1984), although the specific inflorescence and pollen structures of this plant do not appear very favorable in this regard.

The specific age structure of *S. renifolius* may also be an important factor in the maintenance of a high level of genetic variation at the Mt. Cheonma specimens. A perennial plant, the life span of *S. renifolius* is known to be several decades (Uemura *et al.*, 1993). Therefore, populations of *S .renifolius* tend to be composed of individuals of various ages. As older plants tend to bloom earlier (Wada and Uemura, 2000), pollens are transported by insects not only to other old plants, but also to younger plants. Although many plants produce pollen, the rate of fructification in *S. renifolius* ranges only from 8.1% to 13% (Kang and Min, 1994; Wada and Uemura, 1994). Additionally, only about 18% of produced seeds actually successfully germinate (Wada and Uemura, 1994). Therefore, the number of plants involved in fertilization and their relative contribution to seed production can differ each year, and the genetic structure of the progenies of each year can differ substantially from the parents. However, taking into consideration a lengthy period of time, the effects of the yearly unequal fructification of individual plants are diminished. Ultimately, the genetic structure of the entire progeny population would tend to approach equilibrium. In order to elucidate this phenomenon more clearly, researches into the mating system, as well as into genetic structures among different age classes in *S. renifolius* stands should be conducted.

As a whole, the genetic structures of the 9 subpopulations of *S. renifolius* collected from Mt. Cheonma appeared to be in equilibrium. However, approximately 6.8% homozygote excess was observed, as was suggested by the positive *f* mean values (Table 3). Of the 66 tests conducted to compare the observed genotypic frequencies at each of the loci with those predicted by the Hardy-Weinberg expectations, 36 evidenced heterozygote excess, and the other 30 evidenced homozygote excess (Table 1). However, for all 9 tests, where significant departures from Hardy-Weinberg expectation were detected, the homozygote excess was distinct. 5 out of 9 of these significant departures were observed at only two isozyme loci, *Mdh-B* and *Skdh-B*. This indicated that the homozygote excess in the *S. renifolius* growing on Mt. Cheonma was subject to the strong influences exerted by certain loci. These results suggest that some evolutionary processes are operating which are altering genetic structures in this population. Selection at specific loci and an insignificant degree of inbreeding could both be advanced as partial explanations for these results, although more reliable conclusions may be drawn after an investigation of the mating system, as well as genetic analyses of a variety of populations under differing environmental conditions

The mean θ value showed that approximately 5% of the total detected genetic variation was distributed among subpopulations. This degree of subpopulational differentiation is consistent with findings regarding inter-populational differentiation in other species with similar life history and mating systems (Williams, 1994). Generally, the degree of genetic divergence within and among populations in outcrossing species depends principally on the degree to which gene flow occurs (Gao *et al.*, 2001; Nakagawa, 2004). Although some *S. renifolius* subpopulations are rather small in size, they harbor lots of individuals in a broad distribution. Those individuals must have contributed to the maintenance of a population size sufficient for the prevention of significant genetic drift, and also sufficient for the promotion of gene exchanges among neighbored subpopulations. Water-dispersed seeds might also contribute to gene exchange between different sites, especially in those plants located along valleys and streams. Wright's (1951)

estimate of the number of migrants per generation (N_m = 4.852) also supported substantial gene flow among subpopulations of *S. renifolius* in Mt Cheonma.

Both expected and observed heterozygosity values were quite low in the subpopulations from lower elevations (up to 400m) and increased with altitude to around 500~600m (Fig. 5). It can be assumed that populations of *S. renifolius* at lower elevations, especially those near downstream regions, were established from seeds that had been transported by water from upstream regions, during monsoon season. Intense predation of inflorescences and fruits by some animals and birds during the spring and autumn months (Hong and Son, 2003) may have contributed to a reduction in the effective size of some populations, ultimately culminating in a decline of heterozygosity throughout the entire population. A somewhat lower level of genetic diversity at higher elevations, in comparison with those at middle elevation, could be related to shortages of large habitats. At higher elevation, *S. renifolius* is found primarily in small populations, and only on damp and concave sites with favorable moisture conditions. It is assumed that damage inflicted to flowers and fruits by animals tend to be less severe at higher elevations.

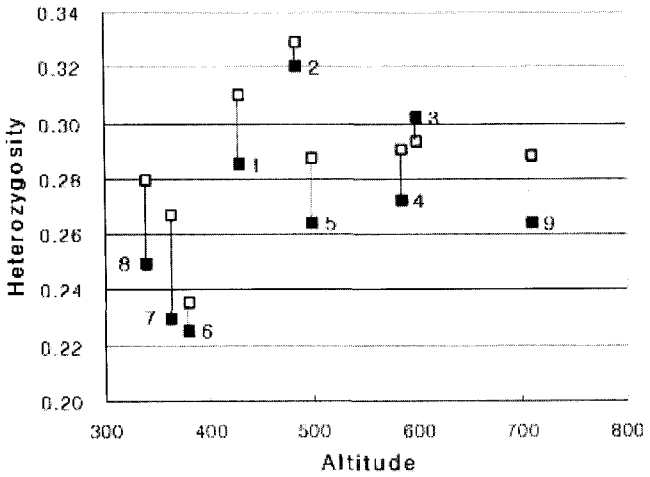

Fig. 5. Altitudinal variation in the heterozygosities of 9 subpopulations of *Symplocarpus renifolius* on Mt. Cheonma. (□ : Observed heterozygosity; ■ : Expected heterozygosity).

Spatial structure within a subpopulation evidenced a strong autocorrelation between closer individuals (within 3~4m from one another) (Fig. 4). This fine-scale genetic structure could be seen in cases in which pollen migration was unrestricted, but seed dispersal was highly localized (Hamrick and Nason, 1996; Susan *et al.*, 2001; Chung *et al.*, 2004; Chung *et al.*, 2005). *Symplocarpus renifolius* produces multiple fruits, harboring scores of large, malodorous seeds. These seeds can be harvested or moved by small rodents, and the mean dispersal distance of the hoarded seeds are as small as 9.6m ± SD 3.8m (Wada and Uemura, 1994). In reality, it can be very easily observed that a number of individuals aggregate in bundles. Consequently, the significant spatial autocorrelation observed at close distances in this study can be associated with restricted seed dispersal in *S. renifolius*.

The genetic diversity data of the yellow-colored individuals did not differ significantly from those measured in other subpopulations. The grouping of the yellow-colored variants with subpopulations 1 and 2 from cluster analysis (Fig. 3) was attributed to the fact that the two subpopulations were the main suppliers of the yellow-colored variants (26 of 36 individuals). Currently, the genetic clarification of the yellow-colored variant of *S. renifolius* remains a difficult proposition. In order to define it more clearly, other studies will be required, such as artificial hybridization and other genetic analyses that exploit different molecular markers, including mtDNA or cpDNA.

Symplocarpus renifolius in Mt. Cheonma is not currently under legal protection. Mt. Cheonma is the habitat of the most abundant numbers of the yellow-colored variant of the species. However, habitat damage and losses resultant from excessive human activity are worsening. The yellow-colored variants are being depleted as the result of illegal collection, and are facing possible extinction. Consequently, concrete measures to protect the natural habitat of *S. renifolius* in Mt. Cheonma from decline and extinction are urgently warranted. It is recommended that 2 to 4 subpopulations (at least 0.5 to 1 ha) at middle elevation, as well as the forest ecosystems surrounding them, should be registered as protected areas. The genetic characteristics of *S. renifolius* determined in this study may prove helpful in the establishment of further protective measures.

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