J. Plant Biol. 37(2) : 141~149 (1994)

Allozyme Diversity and Population Genetic Structure in Korean Endemic Plant Species: II. *Hosta yingeri* (Liliaceae)

Chung, Myong Gi* and Hye Gi Chung[1]

Department of Biology, Gyeongsang National University, Chinju 660-701, Korea; and

[1]School of Landscape Architecture, University of Georgia, Athens, Georgia 30602, U.S.A.

Levels of genetic diversity, population genetic structure, and gene flow in *Hosta yingeri*, a herbaceous perennial endemic to Taehuksan, Sohuksan, and Hong Islands, were investigated. Starch gel electrophoresis was conducted on leaves for 101 plants collected from three populations. Although the distribution of the species is restricted in the islands, it maintains high levels of genetic variation; 64% of polymorphic loci in at least one population (P_S), the mean number of alleles per locus (A_P) of 1.92, and the mean effective number of alleles per locus (Ae_P) of 1.52. Overall, mean genetic diversity (He_P=0.250) was substantially higher than mean estimate for species with very similar life history traits (0.102). Large population size, the persistence of multiple generations within populations, high fecundity, predominantly outcrossing breeding system, large size of pollinator visitation areas may be explanatory factors contributing the higher level of genetic diversity maintained within populations. Analysis of fixation indices showed an overall slight excess of heterozygotes (mean F_{IS}=−0.066) relative to Hardy-Weinberg expectations, which may in part be due to the near self-incompatible breeding system in the species. Significant differences in allele frequencies among populations were found for 14 out of 16 polymorphic loci ($P<0.05$). Slightly more than 80% of the total variation in the species was common to all populations (G_{ST}=0.198). As expected, indirect estimate of the number of migrants per generation (Nm=0.45, calculated from mean G_{ST}) and nine private alleles found in the three populations indicate that gene movement among three isolated island populations was low.

Key words : *Hosta yingeri*, Korean endemic species, genetic diversity, population genetic structure, gene flow

The genus *Hosta* Tratt. is composed of ca. 22 to 25 species of horticulturally important rhizomatous herbaceous perennials from China, Japan, and Korea (Chung *et al.*, 1991a). *Hosta yingeri* S. B. Jones, first described in 1989 (Jones, 1989), is only restricted to remote islands off the southwestern coast of Korea such as Taehuksan, Sohuksan, and Hong Islands (Chung and Kim, 1991). This species is distinct from other five native Korean *Hosta* species by its morphology, enzyme banding patterns, and chloroplast DNA restriction site variation (Chung, 1991; Chung *et al.*, 1991a, b). *Hosta yingeri* is characterized by unique morphological features such as a short transparent line (ca. 5 mm) on perianth and 3+3 stamens, funnel-shaped flowers, a short length of infated lobe (ca. 10 mm), flat bracts, delicate raceme of 20-30 flowers spreading evenly around the central axis of the inflorescence, and relatively thick, adaxially dark green leaves. This feature may be an adaptation to its island habitat, growing at 2-300 m above sea level. *Hosta yingeri* grows mainly on rocky, open grasslands or talus slopes along coast with *Hemerocallis hongdoensis* (Chung and Kang, 1994a) and *Pinus densiflora*, etc. *Hosta yingeri* is one of the abundant pla-

*Corresponding author : Fax +82-591-54-0086

nts on the coastal vegetation in the three islands in Korea (Chung, pers. obs.). This species is insect-pollinated; flowers are visited by bees (Chung, pers. obs.), and seeds are wind dispersed. The species is diploid ($2n=60$) (Chung *et al.*, 1991a). As *H. yingeri* is an attractive and interesting species with horticultural potential (Jones, 1989), it has been used in breeding programs as a gene pool since 1990 (S. B. Jones, pers. comm.).

The accumulation of data obtained from electrophoretic studies for describing levels and distribution of genetic variation has made it possible to provide insights into the relationships between allozyme diversity and life history traits (Hamrick and Godt, 1989; Hamrick *et al.*, 1991; Loveless and Hamrick, 1984). For example, species with narrow or endemic distributions maintain lower level of genetic diversity than species with widespread distributions. In general, geographically restricted or endemic species are known to contain fewer polymorphic loci, fewer alleles per polymorphic locus than widespread congeneric species (Hamrick and Godt, 1989). Knowledge of geographic range, however, may not always be predictive of levels of genetic diversity (Karron *et al.*, 1988; Olmstead, 1990; Soltis and Soltis, 1991). This is in part due to relatively few studies on the allozyme diversity of rare or endemic and endangerd species, particularly on the comparison of genetic diversity between endemic species and its more widespread congeners (Sherman-Broyles *et al.*, 1992).

Generalizations derived from the plant allozyme literature provide a basis on which to build a sound program for conservation of genetic diversity of rare or endemic and endangered species (Hamrick *et al.*, 1991; Soulé, 1986). Allozyme diversity can also be used as a "yardstick" to measure the effectiveness of *in situ* and *ex situ* conservation program (Hamrick *et al.*, 1991). Despite the importance of knowledge concerning genetic variation providing information for conservation purposes, detailed studies of the levels and distribution of genetic variation are not available for most native taxa in Korea (Chung and Kang, 1994b). Previous studies (Chung *et al.*, 1991a, b) on electrophoretic variation at six enzyme systems for seven species of *Hosta* in Korea and on Tsushima Island, Japan for inferring their systematic implications among species of *Hosta* were just based on the use of the band presence/absence and phenotype data, not genotypes inferred directly from enzyme phenotypes. Chung *et al.* (1991a, b) did not apply Wright's F-statistics (Wright, 1951, 1965) or Nei's (1973, 1977) methods of gene diversity analysis. Thus, they failed to compare the results of their study to previously published data that were calculated using these methods.

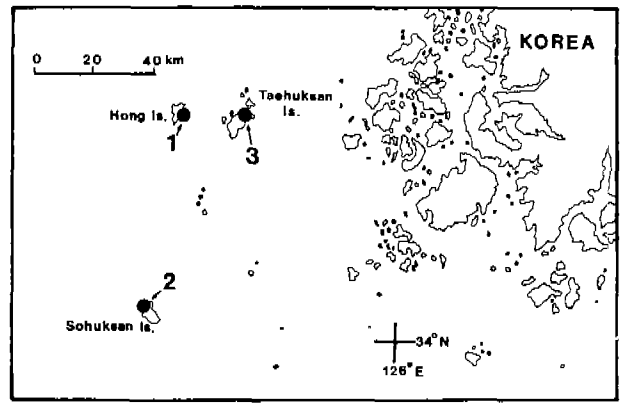

Fig. 1. The locations of the three sampled populations of *H. yingeri* in Korea.

The purpose of this study was; 1) to estimate how much total genetic diversity is maintained in the species, 2) to describe how genetic variation is distributed within and among populations, and 3) to compare our estimates with those for species having very similar life history traits.

MATERIALS AND METHODS

One hundred-one samples of *Hosta yingeri* rootstocks were collected from three populations, each from the three islands in Korea (Fig. 1). Table 1 lists the three populations, their numeric codes, sample sizes, and vouchers used in this study. Collections of rootstocks from each site were made at least 2 m apart to reduce the likelihood of collecting clones. The rootstocks were transported to the Botany Plant Growth Facility at the University of Georgia where plants were grown under uniform conditions. Voucher specimens of all collections were deposited at GA, GNUC, KYO, MO, and SNU.

Crude leaf tissue extacts for horizontal starch gel electrophoresis were prepared using liquid nitrogen. The extaction buffer was that of Mitton *et al.* (1979). Wicks were stored at −60℃ until needed for analysis. Electrophoresis was performed using 11% starch

Table 1. Location of *H. yingeri* populations sampled for electrophoretic analysis

Code	Locality (elevation)	N	Collection numbers[a]
1	Hong Island, Prov. Chollanam-do (ca. 290 m)	32	1340
2	Sohuksan Island, Prov. Chollanam-do (ca. 150 m)	30	1363
3	Taehuksan Island, Prov. Chollanam-do (ca. 15 m)	39	1383

[a]All collections are by MGC.

gels. Except as noted, gel and electrode buffers and enzyme staining procedures from Soltis *et al.* (1983) were used to assay 10 enzyme systems: phosphoglucomutase (PGM) was resolved on system 6; diaphorase (DIA) and glutamate oxaloacetate transaminase (GOT) on system 7; isocitrate dehydrogenase (IDH) on system 2; 6-phosphogluconate dehydrogenase (PGD) and malate dehydrogenase (MDH) on system 11; leucine aminopeptidase (LAP), triosphosphate isomerase (TPI), β-galactosidase (β-GAL), and fluorescent esterase (FE) on a modification (Haufler, 1985) of system 8. The staining procedures for DIA followed the method described by Cheliak and Pitel (1984). Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, and so on. Likewise, alleles were designated sequentially with the most anodally migrating alleles designated a. All *H. yingeri* isozymes expressed phenotypes that were consistent in subunit structures and genetic interpretation with most isozyme studies in plants as documented by Weeden and Wendel (1989).

Measures of allozyme diversity were estimated for individual populations and for the species as a whole using a computer program developed by M. D. Loveless and A. Schnabel. Subscripts refer to species (S) or population (P) level parameters. A locus was considered polymorphic if two or more alleles were observed. The percentage of polymorphic loci (P) and for each population separately (P_P), then averaged. Mean number of alleles per locus (A) was calculated by summing all the alleles scored and dividing by the total number of loci for the species (A_S) and for each population (A_P). Gene diversity was calculated for each locus by $He=1-\Sigma P_i^2$ and averaged overall loci, where P_i is the mean frequency of the *i*th allele at that locus (Nei, 1973), to obtain the species value (He_S) and over all loci in each population to obtain the within populaton value (He_P). The effective number of alleles per locus (Ae) at the species level (Ae_S) was calculated as $1/(1-He_S)$, then averaged across loci. At the population level, Ae_P was calculated as $1/\Sigma P_i^2$, averaged over all loci for each population (Hamrick *et al.*, 1979; Hamrick and Godt, 1989; Hamrick *et al.*, 1992).

Observed heterozygosity was compared to Hardy-Weinberg expected values using Wright's (1922) fixation indices (F) or the inbreeding coefficients. These indices were used to test significant deviations from an expected value ($F=0$) using the x^2-statistic following Li and Horvitz (1953): $x^2=F^2N$ $(a-1)$, $df=a(a-1)/2$, where N is the total sample size and a is the number of alleles at the locus.

Genetic variation among populations was examined in three ways. First, total genetic diversity (H_T), genetic diversity within populations (H_S), genetic diversity among populations (D_{ST}), and the proportion of genetic diversity found among populations (G_{ST}) were calculated following Nei's (1973, 1977) genetic diversity formula. Second, a x^2-statistic was used to detect significant differences in allele frequencies among populations for each locus: $x^2=2NG_{ST}(a-1)$, $df=(a-1)(n-1)$, where n is the number of populations (Workman and Niswander, 1970). Third, Nei's (1972) genetic identity (I) was calculated for each pairwise combination of populations.

Wright's (1965) F-statistics (F_{IT}, F_{IS}, and F_{ST}) were used to analyze genetic structure in populations. These measures represent relative excess of homozygotes or heterozygotes compared with panmictic expectations relative to all populations (F_{IT}), within populations (F_{IS}), and among populations (F_{ST}). F_{IT} was calculated using the direct method: $F_{IT}=1-Ho/He$, where Ho is the observed number of heterozygotes in the whole population and He is the expected number, based on mean allele frequencies. F_{ST} is equivalent to G_{ST} as calculated in this study. The relationships between these measures are: $(1-F_{IT})=(1-F_{IS})(1-F_{ST})$, which assumes random population differentiation. Deviations of F_{IT} and F_{IS} from zero were also tested using the x^2-statistic (Li and Horvitz, 1953). A correlation between genetic distance and geographical distance was calculated using PC-SAS (SAS Institute, Inc., 1989). Estimate of Nm (the num-

Table 2. Allele frequencies for 16 polymorphic loci estimated in three populations of *H. yingeri* from Korea. Abbreviations for populations and enzyme loci are given in Table 1 and text, respectively

Locus	Allele	Populations 1	2	3
Pgm-1	a	0.000	0.000	0.192
	b	0.938	0.400	0.808
	c	0.062	0.600	0.000
Pgm-3	a	0.609	0.667	0.000
	b	0.391	0.333	0.800
	c	0.000	0.000	0.067
	d	0.000	0.000	0.133
Dia-1	a	0.000	0.000	0.205
	b	0.156	0.800	0.718
	c	0.844	0.067	0.026
	d	0.000	0.133	0.051
Dia-2	a	0.172	0.233	0.180
	b	0.828	0.767	0.820
Dia-3	a	0.219	0.117	0.218
	b	0.781	0.883	0.782
Got-a	a	0.000	0.000	0.077
	b	0.109	0.533	0.577
	c	0.891	0.467	0.346
Got-3	a	0.033	0.083	0.039
	b	0.867	0.550	0.846
	c	0.100	0.367	0.115
Pgd-1	a	0.391	0.183	0.797
	b	0.125	0.400	0.000
	c	0.375	0.367	0.162
	d	0.109	0.050	0.040
Pgd-2	a	0.000	0.094	0.346
	b	1.000	0.062	0.000
	c	0.000	0.844	0.654
Idh-2	a	0.188	0.633	0.500
	b	0.750	0.367	0.500
	c	0.062	0.000	0.000
Mdh-2	a	0.250	0.483	0.051
	b	0.219	0.000	0.385
	c	0.031	0.000	0.000
	d	0.172	0.000	0.385
	e	0.328	0.517	0.179
Lap-3	a	0.000	0.283	0.000
	b	1.000	0.717	1.000
Tpi-2	a	0.109	0.217	0.128
	b	0.000	0.000	0.077
	c	0.672	0.650	0.436
	d	0.219	0.133	0.359
Tpi-3	a	0.422	0.667	0.539
	b	0.578	0.333	0.461
Fe-2	a	1.000	0.517	0.936
	b	0.000	0.483	0.064
Fe-3	a	0.641	0.000	0.346
	b	0.000	0.800	0.449
	c	0.359	0.200	0.205

Table 3. Percentage of polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (Ae), observed heterozygosity (Ho), and Hardy-Weinberg expected heterozygosity or genetic diversity (He) for three populations of *H. yingeri*

Pop[a]	P_P	A_P	Ae_P	Ho_P (SD)	He_P (SD)
1	52	1.84	1.46	0.222 (0.054)	0.207 (0.048)
2	64	1.88	1.54	0.271 (0.064)	0.279 (0.046)
3	60	2.04	1.57	0.319 (0.054)	0.265 (0.051)
Mean	58.7	1.92	1.52	0.271 (0.033)	0.250 (0.028)

[a]Numerical codes as in Table 1.

ber of migrants per generation) was obtained using Wright's (1951) equation as modified by Crow and Aoki (1984): $F_{ST}=1/(4Nm\alpha+1)$, where $\alpha=[n/(n-1)]^2$ and n is the number of populations.

RESULTS

Sixteen of the 25 loci examined (P_S=64%) were polymorphic in at least one of the three populations. *Pgm-2, Got-1, Idh-1, Mdh-1, Lap-1, Lap-2, Tpi-1,* β-*gal,* and *Fe-1* were monomorphic in all three populations sampled. Allele frequencies for 16 polymorphic loci were presented in Table 2. The mean percent of polymorphic loci within populations (P_P) was 58.7% (Table 3). The mean number of alleles per locus was 2.32 for the species (A_S) with a mean of 3.37 alleles per polymorphic locus. At the population level, the mean number of alleles per locus was 1.92 with a mean 2.44 alleles per polymorphic locus (Table 3). The highest number of alleles detected per locus was five for *Mdh-2*. The mean effective number of alleles per locus at the species level (Ae_S) and at the population level (Ae_P) were 1.73 and 1.52, respectively.

Expected heterozygosity or genetic diversity for the species (He_S) was 0.313, whereas mean population expected heterozygosity was 0.250. Mean observed heterozygosity was lower than expected only for population 2 (Table 3). Fifteen of 44 fixation indices were significantly different ($P<0.05$) from zero. Nine of these indices were negative, indicating an excess of heterozygotes at these loci and in these populations (Table 4). The mean F_{IS} over all loci was −0.066, ranged from −0.059 (*Mdh-2*) to 0.593 (*Fe-2*) (Table 5). At the level of the sample as a whole,

Table 4. Wright's fixation indices for three populations of *H. yingeri*. Chi-square tests were used to determine if fixation indices were different from an expected value (F=0). Populations that were monomorphic for a particular locus are indicated with a dash

Locus	Populations		
	1	2	3
Pgm-1	−0.050	−0.366*	−0.222
Pgm-3	−0.486***	0.263	0.047
Dia-1	−0.167	0.224	−0.153
Dia-2	−0.189	−0.283	−0.203
Dia-3	−0.260	−0.113	−0.262
Got-2	−0.105	0.210	−0.356*
Got-3	0.173	−0.297	0.154
Pgd-1	−0.042	−0.224	−0.189
Pgd-2	−	0.330	−0.510***
Idh-2	0.074	−0.553***	−0.974***
Mdh-2	−0.146	−0.116	0.092
Lap-3	−	0.435***	−
Tpi-2	0.559***	0.169	0.616***
Tpi-3	−0.703***	−0.180	−0.528***
Fe-2	−	0.803***	−0.055
Fe-3	0.666***	0.795***	−0.391**

*=P<0.05; **=P<0.01; ***=P<0.001.

however, Wright's F ; coefficients showed that significant deficiencies of heterozygotes exist for six (*Dia-1, Pgd-2, Lap-3, Tpi-2, Fe-2*, and *Fe-3*) of the 16 polymorphic loci (Table 5). In contrast, four loci (*Dia-2, Dia-3, Idh-2*, and *Tpi-3*) at the sample as a whole showed significant excess of heterozygosity.

Heterozygosity chi-square analyses indicated significant allele frequency differences among populations at 14 polymorphic loci (P<0.05) (Table 5). The proportion of variation found among populations (G_{ST}) ranged from 0.005 (*Dia-2*) to 0.576 (*Pgd-2*). On average, 80% of the variation resided within populations. The number of migrants per generation (Nm) was estimated to be 0.45 based on the mean G_{ST}. Nine private alleles were found in the three populations: 1 (*Idh-2*^{c} and *Mdh-2*^{c}), 2 (*Lap-3*^{a}), and 3 (*Pgm-1*^{a}, *Pgm-3*^{c,d}, *Dia-1*^{a}, *Got-2*^{a}, and *Tpi-2*^{b}). This and relatively low gene flow estimate are consistent with the significant population differentiation in allele frequencies.

Average genetic identity for all pairs of populations was 0.850 (SD=0.021), below the range of values expected for conspecific population (Crawford, 1989). Interestingly, highest genetic identity was found between the most isolated populations (ca. 76 km) (populations 2 and 3; I=0.873), while lowest genetic identity was found between the next most isolated populations (ca. 69 km) (populations 1 and 2; I=0.821). The mean genetic identity between populations 1 and 3 (ca. 23 km) showed an intermediate value (I=0.854). Thus, genetic distance and linear geographic distance was not significantly correlated (r=0.06, P<0.05).

Table 5. Total genetic diversity (H_T), genetic diversity within populations (H_S), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}) and within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST}) of *H. yingeri*

Locus	H_T	H_S	F_{IS}[a]	F_{IT}[a]	G_{ST}[b]
Pgm-1	0.426	0.299	−0.289***	0.093ns	0.296***
Pgm-3	0.609	0.498	−0.073ns	0.122ns	0.182***
Dia-1	0.584	0.354	−0.065ns	0.355***	0.394***
Dia-2	0.312	0.310	−0.245*	−0.239*	0.005ns
Dia-3	0.306	0.301	−0.249*	−0.232*	0.014ns
Got-2	0.519	0.419	−0.135ns	0.084ns	0.193***
Got-3	0.381	0.347	−0.078ns	0.019ns	0.090***
Pgd-1	0.654	0.548	−0.161ns	0.026ns	0.161***
Pgd-2	0.606	0.257	−0.247**	0.471***	0.576***
Idh-2	0.514	0.457	−0.581***	−0.405***	0.111***
Mdh-2	0.746	0.645	−0.059ns	0.084ns	0.135***
Lap-3	0.154	0.121	0.425***	0.550***	0.218***
Tpi-2	0.586	0.562	0.471***	0.493***	0.418***
Tpi-3	0.497	0.479	−0.511***	−0.455***	0.037*
Fe-2	0.280	0.195	0.593***	0.717***	0.305***
Fe-3	0.654	0.487	0.146ns	0.364***	0.256***
Mean[c]	0.489	0.392	−0.066	0.128	0.198
	(0.311)	(0.250)	(−0.092)	(0.124)	(0.188)

[a]Asterisks indicate F-coefficients significantly different from zero (*=P<0.05; **=P<0.01; ***=P<0.001). [b]Asterisks indicate significant allele frequency heterogeneity among populations based on a x^2 test (*=P<0.05; **=P<0.01; ***=P<0.001). [c]Means including four monomorphic loci are given in the parentheses.

DISCUSSION

Based on recent reviews of the plant allozyme diversity literature (Hamrick and Godt, 1989), features such as breeding system, patterns of geographical distribution, taxonomic status, life form, seed dispersal, and successional status influence amount and distribution of genetic diversity within and among populations. Levels of allozyme variation maintai-

ned by *H. yingeri* are substantially high compared with values for species with similar life history characteristics (Hamrick and Godt, 1989). For example, short-lived herbaceous perennials with a predominantly animal-outcrossing mode of reproduction and wind-dispersed seed, and endemic geographic range have a mean percent polymorphic loci (P_S) of 47%, mean number of alleles per locus (A_S) of 1.90, 1.18 mean effective alleles per locus (Ae_S), and genetic diversity (He_S) of 0.131. In contrast, for *H. yingeri*, P_S is 64%; A_S, 2.32; Ae_S, 1.73; and He_S, 0.313. The same trend is observed at the population level. Species with these traits have P_P of 33%, A_P of 1.51, Ae_P of 1.14, and He_P, 0.102. Within *H. yingeri* populations, P_P is 59%; A_P, 1.92; Ae_P, 1.52; and He_P, 0.250. It is of interest to note that 10 islands populations of *Campanula microdonta* Koidzumi, a herbaceous perennial endemic to the Izu Islands, Japan, were found to have considerably low amount of genetic diversity (P_S=76%, P_P=61%, A_S=2.53, A_P=1.89, He_S=0.128, He_P=0.088; Inoue and Kawahara, 1990) than that of *H. yingeri*. The possible factors contributing these low estimates may be: 1) dominant inbreeding system, 2) gravity dispersed seeds, 3) stochastic events in small island populations, and 4) asynchronous seed germination (Inoue and Kawahara, 1990).

Usually, plant species with widespread distributions tend to maintain higher level of allozyme diversity than plants more restricted ranges, whereas endemic plant species tend to exhibit the lowest amounts of allozyme variation (Hamrick and Godt, 1989). Contrary to the expectation, *H. yingeri*, a species with a restricted geographic range (ca. 76 km range), has higer level of genetic variation than mean estimates of genetic diversity for more widespread species (0.202 and 0.159, at the species and population levels, respectively; Hamrick and Godt, 1989). Thus, as Holsinger and Gottlieb (1991) suggested, endemic species that have restricted distributions but locally common, may resemble more widespread congeners in their ecological and genetic traits. In other words, the restricted distributions of *H. yingeri* may reflect adaptation to island habitats, but may not have a major influence on their population genetic structure within populations. Factors contributing to the maintenance of the higher level genetic variation may be: 1) large population sizes, 2) the persistence of multiple generations within populations, 3) high fecundity (Chung *et al.*, 1991b), 4) predominantly outcrossing breeding system, 5) large size of pollinator visitation areas (Chung, pers. obs.), and 6) seed dispersal by wind.

A number of significant deviations from Hardy-Weinberg expectation were found for some populations and at some loci. Only two deviations out of 44 tests would be expected on the basis of chance alone (given P=0.05), yet 15 were found significant, among these nine were negative, indicating overall excess of heterozygotes (F_{IS}=−0.066). This is not surprising considering the near self-incompatibility (Chung, unpubl. data) and the pronounced spatial separation (herkogamy) of mature anther and stigmas impeding self-pollination in the species. Other possible reasons for the overall excess of heterozygotes within populations may be: 1)large neighborhood (Ne) size, 2) large size of pollinator visitation areas in the open island habitats, and 3) long distance seed dispersal within each island by wind causing presumably genetically dissimilar matings. In this regard, gene flow estimates among subpopulations in each island would be expected to be substantially higher than estimates among populations.

Genetic differentiation among populations is principally a function of gene flow among populations via pollen and seeds dispersal (Ellstrand and Marshall, 1985). Species with naturally isolated, island populations as *H. yingeri* might be expected to exhibit higher level of genetic divergence among populations due to limited gene flow. Significant differences were found in allele frequencies among the three populations of *H. yingeri* for 14 out of 16 polymorphic loci. Of the total variation in *H. yingeri*, slightly less than 20% is due to differences among populations. This levels of divergence is comparable with, and lower and higher than the means for outcrossing, animal-pollinated species (G_{ST}=0.179), endemic species (G_{ST}=0.248) and short-lived herbaceous perennials (G_{ST}=0.233), and species with seed dispersal by wind (G_{ST}=0.143), respectively (Hamrick and Godt, 1989). Mean genetic identity among populations (I=0.850) was considersbly below the mean identity (I=0.946) reported by Crawford (1983) for 32 species. This is partly due to the presence of the nine private alleles with their mean frequency of 0.125. Previous study has shown, however, that the

three populations of *H. yingeri* are somewhat homogenous in their morphology based on the individuals grown under uniform conditions (Chung *et al.*, 1991 b) and morphologically the species is well characterized. In contrast to this, mean Nei's genetic identity values for the 10 populations of *Campanula microdonta* sampled from the Izu Isalnds which occur 20-250 km distance from Honshu, Japan, was 0.95, ranged from 0.89 to 1.00 (Inoue and Kawahara, 1990). Despite this relatively high mean identity, of the total variation in *C. microdonta*, 31% of the diversity exists among island populations. Indirect gene flow estimate of Nm (0.46, calculated using Wright's [1951] equation as modified by Crow and Aoki [1984]) was low and comparable with estimate of *H. yingeri*.

It has been generally known that morphology is a product of differential selection in response to abiotic pressures, while polymorphisms found at the molecular level are probably selectively neutral. If the neutral theory (Kimura, 1979) is true for the populations of *H. yingeri*, genetic variation among the three island populations of the species may be determined largely by the process of random genetic drift. For neutral genes, an Nm value<1 can be considered low, and, as a result, the value is considered insufficient to prevent divergence due to genetic drift (Wright, 1951; Slatkin, 1987). Indirect estimate of gene flow for *H. yingeri* (Nm=0.45), based on the G_{ST} value, was far below this value. The nine private alleles, in which population 3 was found to have six private alleles, also support that gene movement is very low among populations.

The correlation between genetic distance and geographic distance was not significant and indicated that nearly 100% (r^2=0.004) of the variation in genetic distances was due to other factors rather than distance such as history of species, presumable stochastic events, and isolation by sea, etc. Thus, mutation and genetic drift may play major roles in shaping the genetic structure among populations. Presence of six private alleles in the Taehuksan Island (population 3), the closest island to the mainland, indicate that ancestral stocks of *H. yingeri* went from this island to the two other islands by seed dispersal over sea in relatively ancient periods. Sea depth between the three islands and the mainland is only ranged 50-100 m. It is highly probable that sufficient time has passed since the origin of the species to harbor high levels of genetic divergence at isozyme loci among populations through the processes of mutation and genetic drift coupled with restricted gene movement among populations under the relatively uniform island habitats. Ecotypic differentiation among populations on quantitative characters might be expected be low in species, like *H. yingeri*, growing under the relatively homogenous environmental conditions. The results from this study would support the hypothesis that morphological and allozyme evolution occur independently at different tempos or rates (Crawford *et al.*, 1987). For *H. yingeri*, the different rates on quantitative characters and allozyme evolution may has been sustained by very low gene movement among the three island populations.

ACKNOWLEDGMENTS

We thank J.L. Hamrick, S.B. Jones, W. Anderson, R. Wyatt, S.S. Kang and S. Sherman-Broyles for assistance. We are also grateful to the staff members of the Office of National Monument of Korea for permission to collect samples from Hong Island. This research was supported in part by a NSF Dissertation Improvement Grant BSR-8914430 to MGC, a Non Directed Research Fund, Korea Research Foundation, 1992 to MGC, and a grant from KOSEF (grant no. 931-0500-031-2) to MGC.

LITERATURE CITED

Cheliak, W.M. and J.A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest species. Information Report PI-X-42, Petawawa National Forestry Institute, Agriculture Canada, Canadian Forestry Service, Chalk River, Ontario. pp. 1-49.

Chung, M.G. 1991. Notes on the phylogenetic relationships among species of Korean *Hosta* (Liliaceae) based on chloroplast DNA restriction site data. *Plant Species Biol.* **6**: 71-74.

Chung, M.G., J.L. Hamrick, S.B. Jones and G.S. Derda. 1991a. Isozyme variation within and among populations of *Hosta* (Liliaceae) in Korea. *Syst. Bot.* **16**: 667-684.

Chung, M.G., S.B. Jones, J.L. Hamrick and H.G. Chung. 1991b. Morphometric and isozyme analyses of the genus *Hosta* (Liliaceae) in Korea. *Plant Species Biol.* **6**: 55-69.

Chung, M.G. and S.S. Kang. 1994a. *Hemerocallis hongdoensis* (Liliaceae): A new species from Korea. *Novon* (in press).

Chung, M.G. and S.S. Kang. 1994b. Genetic variation and population structure in populations of *Eurya japonica*

(Theaceae) in Korea. *Am. J. Bot.* (in press).

Chung, M.G. and J.W. Kim. 1991. The genus *Hosta* Tratt. (Liliaceae) in Korea. *Sida* **14**: 411-420.

Crawford, D.J. 1983. Phylogenetic and systematic inferences from electrophoretic studies. *In*, Isozymes in Plant Breeding, part A.S.D. Tanksely and T.J. Orton (eds.). Elsevier, Amsterdam. pp. 257-287.

Crawford, D.J. 1989. Enzyme electrophoresis and plant systematics. *In*, Isozymes in Plant Biology. D.E. Soltis and P.S. Soltis (eds.). Dioscorides Press, Portland. pp. 146-164.

Crawford, D.J., R. Witkus and T.F. Stuessy. 1987. Plant evolution and speciation on oceanic islands. *In*, Differentiation Patterens in Higher Plants. K. Urbanska (ed.). Academic Press, New York. pp. 183-199.

Crow, J.F. and K. Aoki. 1984. Group selection for a polygenic behavioral trait: Estimating the degree of population subdivision. *Proc. Natl. Acad. Sci. USA* **81**: 6073-6077.

Ellstrand, N.C. and D.L. Marshall. 1985. Interpopulational gene flow by pollen in wild radish, *Raphanus sativus*. *Am. Nat.* **126**: 606-616.

Hamrick, J.L. and M.J.W. Godt. 1989. Allozyme diversity in plant species. *In*, Plant Population Genetics, Breeding and Genetic Resources. A.D.H. Brown, M.T. Clegg, A.I. Kahler and B.S. Weir (eds.). Sinauer, Sunderland. pp. 43-63.

Hamrick, J.L., M.J.W. Godt, D.A. Murawski and M.D. Loveless. 1991. Correlations between species traits and allozyme diversity: Implications for conservation biology. *In*, Genetics and Conservation of Rare Plants. D.A. Falk and K.E. Holsinger (eds.). Oxford Univ. Press, New York. pp. 76-86.

Hamrick, J.L., M.J.W. Godt and S.L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* **6**: 95-124.

Hamrick, J.L., Y.B. Linhart and J.B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Rev. Ecol. Syst.* **10**: 173-200.

Haufler, C.H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). *Syst. Bot.* **10**: 92-104.

Holsinger, K.E. and L.D. Gottlieb. 1991. Conservation of rare and endangered plants: Principles and prospects. *In*, Genetics and Conservation of Rare Plants. D.A. Falk and K.E. Holsinger (eds.). Oxford Univ. Press, New York. pp. 195-208.

Inoue, K. and T. Kawahara. 1990. Allozyme differentiation and genetic structure in island and mainland Japanese populations of *Campanula punctata* (Campanulaceae). *Am. J. Bot.* **77**: 1440-1448.

Jones, S.B. 1989. *Hosta yingeri* (Liliaceae/Funkiaccae): A new species from Korea. *Ann. Missouri Bot. Gard.* **76**: 602-604.

Karron, J.D., Y.B. Linhart, C.A. Chaulk and C.A. Robertson. 1988. The genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). *Am. J. Bot.* **75**: 1114-1119.

Kimura, M. 1979. The neutral theory of molecular evolution. *Sci. Amer.* **241**: 98-126.

Li, C.C. and D.G. Horvitz. 1953. Some methods of estimating the inbreeding coefficient. *Am. J. Human Genet.* **5**: 107-117.

Loveless, M.D. and J.L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. *Annual Rev. Ecol. Syst.* **15**: 65-95.

Mitton, J.B., Y.B. Linhart, K.B. Sturgeon and J.L. Hamrick. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *J. Hered.* **70**: 86-89.

Nei, M. 1972. Genetic distance between populations. *Am. Nat.* **106**: 283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA* **70**: 3321-3323.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. *Ann. Human Genet.* **41**: 225-233.

Olmstead, R.G. 1990. Biological and historical factors influencing genetic diversity in the *Scutellaria angustifolia* (Liliaceae). *Evolution* **44**: 54-70.

SAS Institute. 1989. SAS/STAT® User's Guide, Ver. 6, 4th ed., Vol. 1. SAS Institute, Cary. 943 pp.

Sherman-Broyles, S.L., J.P. Gibson, J.L. Hamrick, M.A. Bucher and M.J. Gibson. 1992. Comparisons of allozyme diversity among rare and widespread *Rhus* species. *Syst. Bot.* **17**: 551-559.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* **236**: 787-792.

Soltis, D.E., C.H. Haufler, D.C. Darrow and G.J. Gastony. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* **73**: 9-27.

Soltis, P.S. and D.E. Soltis. 1991. Genetic variation in endemic and widespread plant species: Examples from Saxifragaceae and *Polystichum* (Dryopteridaceae). *Aliso* **13**: 215-223.

Soulé, M.E. 1986. Conservation biology: The science of scarcity and diversity. Sinauer, Sunderland. 584 pp.

Weeden, N.F. and J.F. Wendel. 1989. Genetics of plant isozymes. *In*, Isozymes in Plant Biology. D.E. Soltis and P.S. Soltis (eds.). Dioscorides Press, Portland. pp. 46-72.

Workman, P.L. and J.D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Human Genet.* **22**: 24-49.

Wright, S. 1922. Coefficients of inbreeding and relationship. *Am. Nat.* **56**: 330-338.

Wright, S. 1951. The genetic structure of populations. *Ann. Eugen.* **15**: 313-354.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**: 395-420.

(Received February 5, 1994)

韓國 特產植物의 同位酵素 多樣性 및 集團遺傳的 構造: II. 흑산도비비추 (백합科)

鄭 明 基* · 鄭 孝 基[1]
慶尙大學校 自然科學大學 生物學科, [1]美國 Georgia大學校 造景學科

적 요

한반도 남서해안으로부터 떨어져 있는 대흑산도, 소흑산도, 홍도 및 인근 섬에만 자생하는 한국 특산종인 흑산도비비추는 다년생 초본이며 이 식물을 대상으로 유전적 다양성, 집단 유전적 구조 및 유전자 이동이 조사되었다. 세 도서의 자생지에서 채집된 101개체를 대상으로 각 개체로부터 한 개의 잎을 채취하여 전분전기영동법을 수행하였다. 비록 흑산도비비추는 이들 섬에만 국한되어 분포하지만, 집단내의 유전적 다양도의 수준은 높았다. 조사된 유전좌위 중 64%가 적어도 한 집단 이상에서 다형성을 보였고, 유전자 좌위 당 대립인자의 수는 1.92였으며, 유전자 좌위 당 효과적인 대립인자 수는 1.52였다. 집단내의 평균 유전적 다양도 (He_P=0.250)는 이 종과 매우 비슷한 생활환을 지니는 식물들의 평균 측정치 (He_P=0.102)보다 높았다. 이 이유들로 흑산도비비추는 집단의 크기가 크며, 각 집단내에 세대가 중복되어 있으며, 타가수분이 주된 교배계이며, 수분매개체의 활동범위가 넓으며, 종자가 바람에 의해서 산포된다는 점 등을 들 수 있다. 자가수정 계수 분석에 의하면 전반적으로 집단내에 이형접합자들이 조금 많이 존재하는 것으로 나타났다 (평균 F_{IS}=−0.066). 이는 아마도 본 종이 지니는 자가불화합성의 교배계 때문일 것이라고 부분적으로 해석된다. 조사된 16군데 유전자 좌위 중 14군데에서 집단간의 대립인자 빈도의 차이가 통계학적으로 유의성이 있는 것으로 나타났다 ($P<0.05$). 이 종이 지니는 전체 유전적 다양도 중 약 80% 이상이 조사된 모든 집단에 공통으로 나타났다 (G_{ST}=0.198). 기대되는 바와 같이, 평균 G_{ST}값으로부터 간접적으로 구하는 세대당 집단간 이동개체수 (Nm=0.45) 및 3집단내에 9개의 단독 대립인자의 존재는 바다로 격리된 이들 세 섬간에 유전자 이동이 낮다는 것을 의미한다.

주요어: 흑산도비비추, 한국 특산식물, 유전적 다양성, 집단유전적 구조, 유전자 이동

*교신저자: Fax (0591) 54-0086