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Evaluations of the natural monument populations of *Camellia japonica* (Theaceae) in Korea based on allozyme studies

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Abstract. The allozyme diversity of 17 Korean *Camellia japonica* populations was investigated using starch gel electrophoresis in order to evaluate the levels of genetic diversity in natural monument (NAM) in comparison to other natural (NNM) populations. Although the approximate mean population size of NAM is smaller than that of NNM, the mean percentage of polymorphic loci, mean number of alleles per loci, and mean expected heterozygosity (He) were not significantly different between NAM and NNM populations. In addition, Wright's *F*-statistic values (F_{IS}, F_{IT}, and F_{ST}) and estimates of gene flow for polymorphic loci were not significantly different from each other in pairwise comparisons between the groups. Although Korean populations of *C. japonica* maintain high levels of genetic diversity within populations (mean He = 0.266) and the degree of population differentiation is moderate (mean F_{ST} = 0.126), the several isolated natural populations of the species in Korea, which are at the margin of the species range, coupled with habitat fragmentation by human disturbance may suffer an erosion of genetic diversity in the near future. The results of this study suggest that a large portion of genetic diversity for Korean populations of *C. japonica* could be conserved by maintaining a few populations. The populations could be used as resources of genetic diversity for the restoration of genetically depauperate populations in the future.

Keywords: Allozyme diversity; Conservation; Korean *Camellia japonica*; Natural monuments.

Introduction

Camellia japonica L., a broad-leaved evergreen woody species, is widely distributed from Taiwan northward through Ryu Kyu islands to the main Japanese islands (Honshu, Shikoku, and Kyushu) and the southern and western coastal parts of the Korean Peninsula. In Japan, the species is abundant over its range (Wendel and Parks, 1985), whereas most Korean populations of the species are relatively small and isolated, occurring on several islands in the southern coastal parts of the Korean Peninsula (Chung and Kang, 1994). *Camellia japonica* is an important type of coastal forest vegetation in Japan and Korea (Kira, 1991). Unfortunately, the natural habitats of Korean camellias have been severely affected by human disturbances like road construction and seed and seedling collection by horticulturalists. The Korean government has designated nine populations of *C. japonica* and several other broad-leaved evergreen forests as natural monuments in Korea to preserve their natural habitats.

One of the aims of conservation biologists is to preserve the evolutionary potential of species by maintaining natural levels of genetic diversity. Knowledge of the levels and distribution of genetic variation within and among populations thus becomes a prerequisite for the establishment of effective and efficient conservation practices (Frankel, 1983). Three major types of characters (morphometric, allozyme, and DNA sequences) have been used to estimate levels of genetic variation. Among these, studies of allozyme variation in plants using starch gel electrophoresis offer several advantages over other measurements of genetic variation because the technique is relatively inexpensive and can be applied to a variety of plants (Hamrick et al., 1991; Schaal et al., 1991).

The present study was undertaken to determine how the natural monument populations of Korean *C. japonica* compare with other natural populations in Korea in terms of levels, distribution of genetic variation, and population genetic structure.

Materials and Methods

Four hundred fifteen and 375 individuals from nine natural monument (NAM) and other natural (NNM) populations were sampled from the geographical range of the species in Korea (Table 1). The individuals collected were ca. 5–6 m high and 50–100 years old, depending on the population. On average, each population covered approximately 500 m^2. Except for five populations, mature leaves were collected from 50 individuals per population (Table 1). Leaf samples were put individually in plastic bags and placed on ice, transported to a laboratory, and stored in a refrigerator for 4–5 days.

Enzyme extraction was done by grinding two finely cut leaves under liquid nitrogen with a mortar and pestle and mixing the resulting powder with a phosphate-polyvinylpyrrolidone extraction buffer following the method described by Mitton et al. (1979). The crushed

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Table 1. Locations and sample sizes for 17 populations of *C. japonica*.

Population code[1]	Location	Sample size
Non-natural monuments		
1	Jo Island, Chindo-gun, Prov. Chollanam-do	50
2	Bogil Island, Wando-gun, Prov. Chollanam-do	50
3	Nam-myon, Yeoch'eon-gun, Prov. Chollanam-do	50
4	Kumho Island, Yeoch'eon-gun, Prov. Chollanam-do	50
5	Dolsan Island, Yeoch'eon-gun, Prov. Chollanam-do	50
6	Taehuksan Island, Shinan-gun, Prov. Chollanam-do	50
7	Dongbaek Island, Pusan city	50
8	Wan Island, Wando-gun, Prov. Chollanam-do	25
Natural monuments		
9	Cheju Island, Anduk-myon, Prov. Cheju-do	50
10	Cheju Island, Jungmun-dong, Prov. Cheju-do	34
11	Cheju Island, Seoki-dong, Prov. Cheju-do	66
12	Hong Island, Shinan-gun, Prov. Chollanam-do	50
13	Keojae Island, Dongbu-myon, Prov. Gyeongsangnam-do	50
14	Seo-myon, Seoch'eon-gun, Prov. Chollanam-do	50
15	Asan-myon, Koch'ang-gun, Prov. Chollabuk-do	50
16	Doam-myon, Kangchin-gun, Prov. Chollanam-do	40
17	Chin Island, Uishin-myon, Prov. Chollanam-do	25

extract was absorbed onto 4×6-mm wicks cut from Whatman 3 MM chromatography paper. Sample wicks were stored at -70°C until needed for analysis. Electrophoresis was performed using 10.5% starch gels. Sixteen putative loci were resolved from nine enzyme systems using four gel/electrode buffer combinations. A modification (Chung and Kang, 1994) of Soltis et al. (1983) system 11 was used to resolve phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKDH). A Poulik buffer system, a modification (Haufler, 1985) of Soltis et al. (1983) system 8, resolved fluorescent esterase (FE), leucine aminopeptidase (LAP), and triosephosphate isomerase (TPI). Two morpholine citrate buffer systems were used: a buffer system (pH 6.1) by Clayton and Tretiak (1972) was used to resolve peroxidase (PER), and a modification (Chung and Kang, 1994) of Clayton and Tretiak (1972) resolved isocitrate dehydrogenase (IDH) and 6-phosphogluconate dehydrogenase (PGD). The staining procedures followed the method described by Soltis et al. (1983). 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 allele designated a, and the next b, etc. All camellia isozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most isozyme studies in plants, as documented by Weeden and Wendel (1989). In addition, the genetic inference and description of most enzyme systems employed here are reported clearly in Wendel and Parks (1982).

A locus was considered polymorphic if two or more alleles were observed, regardless of their frequencies. Four genetic parameters were estimated using a computer program developed by M. D. Loveless and A. Schnabel: percent polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (Ae), and gene diversity (He) (Hamrick et al., 1992). T-tests were used to test for significant differences in these parameters between NAM and NNM populations.

Wright's (1965) *F*-statistics (F_{IT}, F_{IS}, and F_{ST}) were also used to analyze genetic structure in populations of *C. japonica*. 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}). An estimate of gene flow among populations was calculated from the multilocus value of F_{ST} as $Nm = (1-F_{ST})/4F_{ST}$ (Wright, 1951), where *Nm* represents the number of migrants exchanged between populations per generation. The differences in F_{IS}, F_{IT}, F_{ST}, and *Nm* between NAM and NNM populations were analyzed with the Wilcoxon signed-rank nonparametric test.

Results

Twelve and 13 of 16 loci analyzed were polymorphic in NNM and NAM populations, respectively, and the levels of genetic diversity are presented in Table 2. The overall levels of allozyme diversity in NNM populations were slightly higher than those of NAM populations. The mean values of P, A, Ae, and He for NNM versus NAM populations were 68.75 vs. 68.06%, 2.77 vs. 2.51, 1.63 vs. 1.55, and 0.278 vs. 0.254, respectively (Table 2). Populations 1, 3, 5 (non-natural monuments) and 9, 11 (natural monuments) had the highest He (0.302–0.310), while 12 and 15 (natural monuments) had the lowest (0.179–0.212) (Table 2). In pairwise comparisons between NNM and NAM populations, however, the means of P, A, Ae, Ho, and He were not significantly different from each other. In addition, the F_{IS}, F_{IT}, F_{ST} values for polymorphic loci and estimates of gene flow were not significantly different from each other in pairwise comparisons between

Table 2. Levels of genetic variability in Korean *Camellia japonica.*

Population code	P	A	Ae	Ho (SE)	He (SE)
Non-natural monuments					
1	68.75	2.75	1.74	0.231 (0.003)	0.310 (0.004)
2	68.75	2.94	1.69	0.230 (0.003)	0.295 (0.004)
3	68.75	2.88	1.84	0.256 (0.003)	0.304 (0.004)
4	68.75	2.75	1.56	0.186 (0.003)	0.256 (0.003)
5	68.75	2.94	1.69	0.243 (0.003)	0.304 (0.004)
6	68.75	2.63	1.52	0.189 (0.003)	0.257 (0.003)
7	68.75	2.81	1.49	0.201 (0.003)	0.239 (0.004)
8	68.75	2.38	1.54	0.207 (0.004)	0.258 (0.004)
Mean	68.75	2.77	1.63	0.217 (0.003)	0.278 (0.003)
Natural monuments					
9	68.75	2.63	1.64	0.241 (0.003)	0.300 (0.004)
10	75.00	2.75	1.48	0.206 (0.003)	0.244 (0.003)
11	68.75	2.56	1.73	0.222 (0.002)	0.302 (0.004)
12	62.50	2.38	1.43	0.139 (0.002)	0.212 (0.003)
13	75.00	3.06	1.64	0.215 (0.003)	0.289 (0.004)
14	56.25	2.31	1.65	0.205 (0.003)	0.260 (0.004)
15	62.50	2.31	1.37	0.108 (0.002)	0.179 (0.000)
16	68.75	2.44	1.54	0.221 (0.003)	0.258 (0.004)
17	75.00	2.19	1.46	0.212 (0.004)	0.237 (0.003)
Mean	68.06	2.51	1.55	0.197 (0.003)	0.254 (0.003)

NNM and NAM populations, though the former three values were higher and *Nm* was lower in NAM than in NNM populations (Table 3) (Wilcoxon signed rank: F_{IS}, T_S=29, n=11, $P > 0.05$; F_{IT}, T_S=18, n=12, $P > 0.05$; F_{ST}, T_S=15, n=12, $P > 0.05$; *Nm*, T_S=17, n=12, $P > 0.05$). Data on allele frequencies are available upon request from the corresponding author.

Discussion

Although Korean populations of *C. japonica* are at the margin of the species range, they maintain considerably higher levels of allozyme diversity than most other long-lived woody species. Since the percentage of polymorphic loci and the average number of alleles per locus are dependent on the number of loci and sample size, mean expected heterozygosity is thought to be the most effective index for comparing genetic variation (Nei, 1987). The levels of genetic diversity maintained in Korean populations are similar to those of the continuously distributed, mainland Japanese populations studied by Wendel and Parks (1985). The mean expected heterozygosity (He) based on 58 Japanese populations was 0.263. It may be of interest to note that other broad-leaved woody angiosperms endemic to East Asia such as *Eurya japonica* Thunb. (He = 0.462: Chung and Kang, 1994) and *E.*

Table 3. Deviations from Hardy-Weinberg equilibrium among individuals (F_{IS}), among populations (F_{ST}), and total deviation (F_{IT}), with migrants per generation (*Nm*) for 12 loci in non-natural monument populations (NNM) and 13 loci in natural monument populations (NAM) of *C. japonica* in Korea. Values in parentheses indicate that means include *Idh* for NAM.

Locus	F_{IS}		F_{IT}		F_{ST}		*Nm*	
	NNM	NAM	NNM	NAM	NNM	NAM	NNM	NAM
Pgm-1	0.069	0.171	0.204	0.318	0.143	0.178	0.149	1.154
Pgm-2	0.342	0.276	0.395	0.332	0.080	0.078	2.875	2.955
Pgi-2	0.165	0.160	0.216	0.342	0.061	0.217	3.848	0.902
Skdh	0.372	0.310	0.477	0.433	0.167	0.177	1.247	1.162
Tpi-1	0.274	0.222	0.319	0.269	0.062	0.060	3.782	3.917
Tpi-2	0.338	0.277	0.357	0.406	0.029	0.178	8.371	1.154
Tpi-3	-0.042	-0.042	-0.002	0.003	0.038	0.037	6.329	6.507
Lap	0.298	0.347	0.401	0.446	0.146	0.151	1.462	1.406
Fe	0.175	0.174	0.233	0.319	0.071	0.175	3.271	1.179
Per-1	0.439	0.487	0.638	0.616	0.354	0.251	0.456	0.746
Per-2	0.115	0.333	0.142	0.416	0.030	0.124	8.083	1.766
Pgd-2	0.068	0.224	0.157	0.330	0.095	0.137	2.382	1.575
Idh	–	-0.033	–	-0.013	–	0.020	–	12.250
Mean	0.218	0.245 (0.224)	0.295	0.352 (0.324)	0.106	0.147 (0.137)	3.634	2.035 (2.821)

emarginata (Thunb.) Makino (Theaceae) (He = 0.296: Chung and Kang, 1995) also maintain high levels of genetic variability within populations. Although mechanisms responsible for harboring high levels of genetic variation are unclear, Wendel and Parks (1985) suggested that the ability to regenerate, abundant populations, long generation times, wide geographic distributions, high rates of outcrossing with insect pollination, and occasional long distance pollen transfers mediated by birds might contribute to the high levels of allozyme variability within populations.

Although the mean He value in non-natural monument populations was slightly higher than that of the natural monument populations, the difference was not significant. The number of individuals in population 15 is approximately 250. Several trees were transported from unknown natural populations into population 15 at least one thousand years ago (Yim, 1992). It is likely that the low He value observed in population 15 (He = 0.179) was attributable to a genetic bottleneck followed by genetic drift because of the small effective population size (Nei et al., 1975). In addition, He in population 12 (He = 0.212) was also lower than most other populations. The size of this population is also small and located at Hong Island off the southwestern coast of the Korean Peninsula. The island habitat has been severely disrupted by tourists because of its scenic beauty. Populations 9, 10, 11, 16, and 17 are also located on islands, but islands with high genetic diversities. These populations are, in addition, much larger than populations 12 and 15.

Since genetic variation is said to be a necessary prerequisite for any future adaptive change or evolution (Beardmore, 1983), genetically depauperate populations are in general expected to be at greater risk of extinction than populations rich in genetic variation. Evolutionary processes that erode genetic variation, such as genetic drift, are more likely to impact small and isolated populations. Perhaps populations 12 and 15 need the most concern because populations under careful management usually recover (Premoli et al., 1994).

Although Korean *C. japonica* maintains high levels of genetic diversity, the several isolated island populations that currently characterize Korean populations of the species coupled with recent increased destruction of natural habitats (e.g., population 17, M. Chung, pers. obs.) may result in erosion of genetic diversity in the near future. Hamrick et al. (1991) suggested that knowledge of the proportion of the among population component of the total genetic variation (G_{ST}, Nei, 1977) is of primary importance for the conservation of genetic diversity or evolutionary potential of a species. For species with high F_{ST} or G_{ST} values, more populations will need to be preserved or sampled to ensure that allelic and genotypic diversity is retained. Although Korean populations of *C. japonica* are located on the edge of the species distribution, the levels of genetic diversity are high within populations and the degree of population differentiation is moderate (mean F_{ST} = 0.126). The results of this study suggest that a large portion of genetic diversity for Korean populations of *C. japonica* could be conserved by maintaining a few populations, such as populations with high levels of allelic richness and expected heterozygosity (e.g., populations 1, 5, and 9). These populations could be used as sources of genetic diversity for the restoration of genetically depauperate populations in the future. However, these suggestions, based solely on allozyme data, should be reevaluated and/or combined with other studies of geographical variation in morphometric or physiological traits to further refine management strategies (Moran et al., 1988; Hamrick et al., 1991). More studies on detailed, fine scale population genetic structure and breeding structure to infer the approximate patch size should be included as well. These studies are now in progress. The approximate gene flow boundary among Korean populations of *C. japonica* (using spatial autocorrelation analysis) was estimated to be 100 km (M. Chung and S. Kang, unpubl. data). Occasional long distance pollen transfer by birds is possible, especially by *Zosterops japonica* Temminck & Schlegel (Wendel and Parks, 1985). Thus, the interaction between *C. japonica* and its pollen carrying pollinators such as birds and *Bombus ignitus* Smith needs also to be conserved.

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韓國山茶花自然保育族群之異構酶評估研究

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本報告利用澱粉膠體電泳探討 17 個韓國山茶花 (*Camellia japonica*) 族群之異構酶差異性以評估自然保育區族群 (NAM) 其他天然族群 (NNM) 的遺傳歧異度。以平均族群大小而言，雖然一般 NAM 族群小於 NNM ，但在其平均多形性基因座百分比、平均每一基因座之因子數及平均異型結合度期望值 (He)，NAM 及 NNM 兩族群間並無顯著差異。此外，Wright's *F*-值 (F_{IS}, F_{IT} 和 F_{ST}) 和多形性基因座其因子流動之估算在兩族群間之駢對比較差異亦不顯著。雖然韓國之山茶花族群在族群內擁有高程度之歧異度 (平均 He=0.266) 且族群分化程度為中度 (平均 F_{ST}=0.126)，但若干天然族群亦已達種界邊緣，加上人為干擾之介隔，在未來將有遺傳歧異度受吞蝕之慮。本試驗結果建議，韓國山茶花之大部分遺傳歧異度可利用維持一小部份族群來保存。此些族群可供利用為將來恢復一些歧異度萎縮族群之資源。

關鍵詞：異構酶歧異度；資源保護；韓國山茶花；自然保育區。