Allozyme variation and population structure of *Chimaphila japonica* in Korea

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Enzyme electrophoresis was used to estimate genetic diversity and population structure of *Chimaphila japonica* Miquel in Korea. The percent of polymorphic loci within the enzymes was 66.7%. Genetic diversity at the species level and at the population level was high ($H_{es} = 0.279$; $H_{ep} = 0.256$, respectively), whereas the extent of the population divergence was relatively low ($G_{ST} = 0.086$). F_{IS} , a measure of the deviation from random mating within the 16 populations, was 0.374. An indirect estimate of the number of migrants per generation ($Nm = 2.65$) indicates that gene flow is high among Korean populations of the species. In addition, analysis of fixation indices revealed a substantial heterozygosity deficiency in some populations and at some loci. Mean genetic identity between populations was 0.973. It is highly probable that directional toward genetic uniformity in a relatively homogenous habitat is thought to be operated among Korean populations of *C. japonica*.

INTRODUCTION

Most plants, especially for rhizomatous and stoloniferous species, have physical connections among ramets although its level of persistency is highly variable among species and habitats (Sobey and Barkhouse, 1977).

Studies of the genetic structure of apomictic plant populations have received revitalized interest in the past decade as a result of electrophoretic techniques, which allow us to better assess the genotypic composition of populations. A well-established general belief has been that asexually reproducing species lack genetic diversity and can be considered as evolutionary “dead-ends”. Various studies have shown that asexually reproducing plants can be much more genetically diverse than originally thought (Ellstrand and Roose, 1987). Clearly, descriptive genetic work on both sexual and asexual plant populations is needed as well. Despite the importance of knowledge concerning genetic variation for providing information for conservation purpose and population genetic structure, detailed studies of the levels and distribution of genetic variation are not available for most species in Korea, particularly both sexual reproduction and asexually reproductive plants.

Chimaphila japonica Miquel (Pyrolaceae) is a widespread perennial herb occurring throughout boreal and temperate zones (Woodland, 1991). *C. japonica* reproduces extensively by vegetative rhizomes and potentially by sexually produced seed. The species in Korea is endemic to several

high mountains where it is found to elevations of 500 m above sea level. Leaves of this species are evergreen, alternate, simple with stipules lacking. Flowers are regular, perfect and hypogynous. In this species, we have compiled and quantitatively analyzed what is known of the genetic structure of populations to examine whether any trends occur both within and among populations.

Evolutionary theory predicts that self-compatible, short-lived perennial species with limited ranges and small population sizes are expected to exhibit low levels of genetic polymorphism (Loveless and Hamrick, 1984). In addition, the boreal forest and arctic tundra have low genetic variation (Odaze and Savolainen, 1996). Recently, however, contrasting results were encountered in the several plants (Aspinwall and Christian, 1992). The relationship between genetic diversity and modes of reproduction is not always coincide. Further study of the relationship between genetic diversity and the temperature of a period of reproduction in both sexual reproduction is necessary. The purposes of this study are; 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 assess genetic structure of *C. japonica*.

MATERIALS AND METHODS

Sampling procedure. *C. japonica* was collected from sixteen natural populations in Korea (Table 1). One leaf per plant was sampled during 1996 to 1997. More than 27 plants were collected from each population. Leaves

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Table 1. Population designation, collection localities, and winter temperature of *C. japonica* as source for isozyme analysis

Code	Collection locality	Winter temperature (°C)
CHH	Cheju Islands, Mt. Hanla, Cheju-do	5.2
GIR	Sancheng pref., Mt. Giri, Gyeongsangnam-do	-1.0
YOH	Haman pref., Mt. Yohang, Gyeongsangnam-do	1.5
WOC	Younam pref., Mt. Wolchul, Challanam-do	-0.5
KAG	Unmoon meon, Mt. Kagi, Gyeongsangnam-do	0.0
HWA	Haegeon pref., Mt. Juwang, Gyeongsangbuk-do	-0.5
YOU	Youngdong pref., Mt. Hackwha, Chungcheongbuk-do	-1.6
POH	Ankang meon, Pohang pref., Gyeongsangbuk-do	-2.0
CHL	Chengyang pref., Mt. Chilkap., Chungcheongbuk-do	-2.5
WOL	Wonju pref., Mt. Chiak, Chungcheongbuk-do	-4.8
SOL	Sokcho pref., Mt. Solak, Kwangwon-do	-4.5
SAW	Konmyeng meon, Sacheon pref., Gyeongsangnam-do	1.5
DAE	Daesang meon, Chemchon pref., Gyeongsangnam-do	1.5
TIL	Samjookmeon, Mt. Tilheon, Gyeonggi-do	-3.1
OAD	Sangwonsa, Mt. Odae, Kwangwon-do	-2.0
HAO	Sachangri, Mt. Haoack, Kwangwon-do	-5.2

gathered from natural populations were stored in plastic bags for several days in a refrigerator until electrophoresis was carried out.

Enzyme electrophoresis. Leaves were homogenized by mechanical grinding to release enzymes from cell and organellar membranes with Tris-HCl grinding buffer-PVP solution described in Soltis et al. (1983). Electrophoresis was performed using 10% starch gel. Buffer systems and enzyme staining procedures from Soltis et al. (1983) were used to assay twelve enzyme systems; peroxidase (PER), isocitrate dehydrogenase (IDH), glutamate oxaloacetate transaminase (GOT), fluorescent esterase (FE), 6-phosphogluconate dehydrogenase (PGD), phosphoglucosylmutase (PGM), malic enzyme (ME), malate dehydrogenase (MDH), acid phosphatase (ACP), shikimate dehydrogenase (SKD), phosphoglucose isomerase (PGI), and alcohol dehydrogenase (ADH). The procedures for starch gel electrophoresis were as reported by Soltis et al. (1983).

For enzymes which resolved more than one zone of activity, the most anodal isozyme is arbitrarily designated 1, with the others sequentially assigned higher numbers. Likewise, alleles were designated sequentially with the most anodally migrating allozyme designated 'a' and progressively slower forms 'b', 'c', and so on. All *C. japonica* 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).

Analysis of data. A locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. Four standard genetic parameters were estimated using a computer program developed by Loveless and Schnabel; % 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). Subscripts refer to species (s) or population (p) level parameters. Observed heterozygosity (Ho) was compared with Hardy-Weinberg expected value using Wright's fixation index (F) or inbreeding coefficients (Wright, 1922). These indices were tested for deviation from zero by χ^2 -statistics following Li and Horvitz (1953). Nei's gene diversity formulae (H_T , H_S , D_{ST} , and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations (Nei, 1973, 1977). In addition, χ^2 -statistics were used to detect significant differences in allele frequencies among populations for each locus (Workman and Niswander, 1970). Nei's genetic identity (I) was calculated for each pairwise combination of populations (Nei, 1972). We used the PC-SAS program (SAS Institute Inc., 1989) to conduct a cluster analysis on genetic distances via the unweighted pairwise groups method arithmetic average (UPGMA). The genetic structure within and among populations was also evaluated using Wright's (1965) F-statistics: F_{IT} , F_{IS} , and F_{ST} . The F_{IT} and F_{IS} coefficients measure excesses of homozygotes or heterozygotes relative to the panmictic expectations within the entire samples and within populations, respectively. The F_{ST} coefficient estimates relative population differentiation. Deviation of F_{IT} and F_{IS} from zero were tested using χ^2 -statistics (Li and Horvitz, 1953). Two indirect estimates of gene flow were calculated. One estimate of Nm (the number of migrants per generation) was based on G_{ST} (Wright, 1951) and the other estimate was based on the average frequency of "rare" alleles found in only one population (Slatkin, 1985; Barton and Slatkin, 1986). The absolute population differentiation (D_m) was calculated using Nei's (1973) statistics.

RESULTS

Genetic diversity. Fourteen of the 21 loci (66.7%) showed detectable polymorphism in at least two populations (Table 2). The remaining six loci (*Per-2*, *Per-3*, *Idh-1*, *Got-1*, *Fe-3*, and *Adh*) were monomorphic in all populations. An average of 55.1% of the loci were polymorphic within populations, with individual population values ranging from 42.9% to 66.7% (Table 3). The majority of the polymorphic loci (*Per-1*, *Skd*, *Pgd-1*, *Idh-2*, *Mdh-2*, *Got-2*, *Fe-2*, and *Pgm-1*) expressed three alleles, while the remaining ones expressed two (*Pgd-2* and *Me*), four (*Acp* and *Pgm-2*), and five (*Fe-1*). The average number of alleles per locus (A) was 1.96 across populations, varying from 1.71 for the

population with the lowest number of alleles and 2.19 for the population with the highest number of alleles. The effective number of alleles per locus (A_e) was similar at the species and the population levels ($A_{es} = 1.59$; $A_{ep} = 1.56$). The mean genetic diversity within populations was 0.256. Population SOL had the highest expected diversity (0.314), while population CHH had the lowest (0.194). Genetic diversity at the species level was high, whereas the value at the population level was lower than that of species level ($H_{es} = 0.279$; $H_{ep} = 0.256$).

Genetic structure. F_{IS} , a measure of the deviation from random mating within the 16 populations, was 0.374, and ranged from 0.164 for *Pgd-2* to 0.487 for *Skd*. The observed

Table 2. Allele frequencies at 14 polymorphic loci of *C. japonica*

		CHH	GIR	YOH	WOC	KAG	HWA	YOU	POH	CHL	WOL	SOL	SAW	DAE	TIL	OAD	HAO
<i>Per-1</i>	a	0.259	0.278	0.222	0.315	0.185	0.250	0.167	0.185	0.241	0.135	0.167	0.286	0.167	0.293	0.185	0.194
	b	0.704	0.556	0.556	0.685	0.556	0.518	0.630	0.556	0.638	0.615	0.533	0.518	0.611	0.569	0.556	0.548
	c	0.037	0.167	0.222	0.000	0.259	0.232	0.203	0.259	0.121	0.250	0.300	0.196	0.222	0.138	0.259	0.258
<i>Acp</i>	a	0.000	0.148	0.185	0.037	0.185	0.196	0.185	0.138	0.000	0.196	0.150	0.025	0.262	0.000	0.214	0.138
	b	0.593	0.611	0.593	0.574	0.574	0.482	0.426	0.431	0.411	0.429	0.467	0.675	0.405	0.500	0.464	0.500
	c	0.407	0.241	0.222	0.389	0.241	0.196	0.259	0.276	0.375	0.304	0.267	0.300	0.286	0.417	0.286	0.310
<i>Skd</i>	d	0.000	0.000	0.000	0.000	0.000	0.125	0.130	0.155	0.214	0.071	0.117	0.000	0.047	0.083	0.036	0.052
	a	1.000	1.000	1.000	1.000	1.000	0.589	0.500	0.407	0.733	0.482	0.450	1.000	0.821	0.685	0.448	0.538
	b	0.000	0.000	0.000	0.000	0.000	0.304	0.352	0.426	0.267	0.407	0.350	0.000	0.179	0.259	0.345	0.275
<i>Pgd-1</i>	c	0.000	0.000	0.000	0.000	0.000	0.107	0.148	0.167	0.000	0.111	0.200	0.000	0.000	0.056	0.207	0.187
	a	0.000	0.074	0.056	0.111	0.148	0.232	0.286	0.258	0.179	0.308	0.400	0.069	0.212	0.190	0.276	0.304
	b	0.815	0.704	0.630	0.648	0.852	0.768	0.714	0.742	0.607	0.692	0.600	0.672	0.788	0.552	0.724	0.696
<i>Pgd-2</i>	c	0.185	0.222	0.315	0.241	0.000	0.000	0.000	0.000	0.214	0.000	0.000	0.259	0.000	0.258	0.000	0.000
	a	0.167	0.222	0.167	0.204	0.444	0.464	0.407	0.431	0.317	0.556	0.567	0.286	0.333	0.464	0.464	0.500
	b	0.833	0.778	0.833	0.796	0.556	0.536	0.593	0.569	0.683	0.444	0.433	0.714	0.667	0.536	0.536	0.500
<i>Idh-2</i>	a	0.222	0.185	0.167	0.167	0.130	0.000	0.000	0.186	0.038	0.241	0.290	0.250	0.214	0.196	0.278	0.286
	b	0.482	0.722	0.685	0.500	0.815	1.000	0.907	0.614	0.962	0.586	0.532	0.607	0.643	0.804	0.667	0.571
	c	0.296	0.093	0.148	0.333	0.056	0.000	0.093	0.200	0.000	0.172	0.178	0.143	0.143	0.000	0.055	0.143
<i>Mdh-2</i>	a	0.000	0.000	0.000	0.000	0.017	0.107	0.222	0.229	0.000	0.077	0.143	0.000	0.000	0.000	0.222	0.143
	b	0.685	0.759	0.707	0.759	0.724	0.679	0.648	0.586	0.750	0.846	0.857	0.696	0.815	0.714	0.778	0.768
	c	0.315	0.241	0.293	0.241	0.259	0.214	0.130	0.186	0.250	0.077	0.000	0.304	0.185	0.286	0.000	0.089
<i>Got-2</i>	a	0.093	0.111	0.130	0.130	0.148	0.161	0.185	0.147	0.133	0.173	0.217	0.232	0.089	0.250	0.196	0.224
	b	0.704	0.759	0.704	0.630	0.667	0.625	0.593	0.603	0.700	0.673	0.533	0.679	0.786	0.625	0.643	0.603
	c	0.204	0.130	0.168	0.241	0.185	0.214	0.222	0.250	0.167	0.154	0.250	0.089	0.125	0.125	0.161	0.172
<i>Fe-1</i>	a	0.196	0.000	0.000	0.111	0.063	0.000	0.000	0.130	0.139	0.056	0.000	0.000	0.000	0.000	0.077	0.071
	b	0.500	0.611	0.518	0.722	0.625	0.421	0.204	0.815	0.500	0.352	0.379	0.536	0.442	1.000	0.481	0.768
	c	0.232	0.279	0.278	0.167	0.250	0.290	0.389	0.055	0.361	0.389	0.318	0.357	0.365	0.000	0.442	0.161
<i>Fe-2</i>	d	0.071	0.111	0.204	0.000	0.063	0.224	0.296	0.000	0.000	0.148	0.167	0.107	0.192	0.000	0.000	0.000
	e	0.000	0.000	0.000	0.000	0.000	0.066	0.111	0.000	0.000	0.056	0.136	0.000	0.000	0.000	0.000	0.000
	a	0.259	0.093	0.052	0.000	0.130	0.196	0.204	0.222	0.161	0.000	0.000	0.000	0.167	0.096	0.148	0.000
<i>Pgm-1</i>	b	0.611	0.796	0.793	0.926	0.870	0.804	0.796	0.778	0.839	1.000	1.000	0.875	0.722	0.904	0.778	1.000
	c	0.130	0.111	0.155	0.074	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.111	0.000	0.074	0.000
	a	0.000	0.185	0.148	0.000	0.000	0.000	0.000	0.077	0.000	0.000	0.196	0.000	0.000	0.000	0.096	0.180
<i>Pgm-2</i>	b	1.000	0.815	0.852	1.000	1.000	1.000	1.000	0.769	1.000	1.000	0.804	1.000	1.000	0.804	0.827	0.820
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.154	0.000	0.000	0.000	0.000	0.000	0.196	0.077	0.000
	a	0.000	0.074	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.056	0.000	0.000	0.000	0.000	0.096	0.100
<i>Me</i>	b	1.000	0.241	0.304	0.533	0.224	0.241	0.140	0.197	0.426	0.296	0.352	0.400	0.482	0.426	0.538	0.483
	c	0.000	0.444	0.518	0.267	0.552	0.630	0.740	0.591	0.574	0.574	0.481	0.433	0.407	0.574	0.327	0.300
	d	0.000	0.241	0.179	0.200	0.224	0.129	0.120	0.182	0.000	0.074	0.167	0.167	0.111	0.000	0.039	0.117
<i>Pgi</i>	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.096	0.000	0.111	0.241	0.000	0.000	0.000	0.148	0.173
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.904	1.000	0.889	0.759	1.000	1.000	1.000	0.852	0.827
	a	1.000	1.000	1.000	0.963	1.000	0.944	0.907	1.000	0.833	1.000	0.796	1.000	0.926	1.000	0.815	0.796
<i>Pgi</i>	b	0.000	0.000	0.000	0.037	0.000	0.056	0.093	0.000	0.167	0.000	0.204	0.000	0.074	0.000	0.185	0.204

Abbreviations for localities given in Table 1.

Table 3. Percentage of polymorphic loci (P), mean number of alleles per polymorphic population (Ap), mean number of alleles per locus (A), effective number of alleles per locus (Ae), observed heterozygosity (Hop), Hardy-Weinberg expected heterozygosity or genetic diversity (Hep) for sixteen populations of *C. japonica*

Pop ^a	N ^b	P	Ap	A	Ae	Hop (SD)	Hep (SD)
CHH	35	42.86	2.67	1.71	1.41	0.139 (0.014)	0.194 (0.055)
GIR	31	52.38	2.82	1.95	1.49	0.132 (0.014)	0.238 (0.054)
YOH	29	52.38	2.73	1.90	1.49	0.136 (0.014)	0.237 (0.056)
WOC	29	52.38	2.55	1.81	1.44	0.126 (0.014)	0.218 (0.054)
KAG	37	52.38	2.82	1.95	1.48	0.128 (0.014)	0.233 (0.055)
HWA	31	52.38	2.91	2.00	1.65	0.152 (0.015)	0.272 (0.061)
YOU	32	57.14	2.75	2.00	1.62	0.158 (0.015)	0.265 (0.059)
POH	30	61.90	2.85	2.14	1.67	0.192 (0.016)	0.299 (0.058)
CHL	27	57.14	2.50	1.86	1.50	0.151 (0.014)	0.237 (0.053)
WOL	40	52.38	3.09	2.10	1.63	0.165 (0.016)	0.268 (0.061)
SOL	37	61.90	2.69	2.05	1.77	0.192 (0.016)	0.314 (0.061)
SAW	30	47.62	2.70	1.81	1.48	0.134 (0.014)	0.226 (0.057)
DAE	30	57.14	2.67	1.95	1.52	0.158 (0.015)	0.247 (0.055)
TIL	29	52.38	2.45	1.76	1.46	0.155 (0.015)	0.229 (0.054)
OAD	29	66.67	2.79	2.19	1.66	0.214 (0.017)	0.310 (0.054)
HAO	40	61.90	2.77	2.10	1.67	0.211 (0.017)	0.304 (0.057)
Mean		55.06	2.73	1.96	1.56	0.159 (0.004)	0.256 (0.014)

^a Abbreviation codes as in Table 1.

^b Number of individuals in the sample.

high, significant, and positive F_{IS} value (0.374) indicates that there was a significantly in deficit of heterozygotes in the populations. Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a substantial deficiency of heterozygotes relative to Hardy-Weinberg expectations. For example, 97.3% of fixation indices were positive (182/187), and 102 of those departed significantly from zero ($p < 0.05$). Only five of indices were negative, indicating an excess of heterozygotes at *Idh-2* (population CHL), *Pgd-2* (populations WOL and OAD), and *Pgi* (populations WOC and HWA). Total genetic diversity values (H_T) varied between 0.090 and 0.639, giving an average over all polymorphic loci of 0.418. The interlocus variation in within population genetic diversity (H_S) was high (0.385). On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.017 for *Got-2* to 0.178 for *Skd* with a mean of 0.086, indicating that about 9% of the total allozyme variation was among populations. Values of genetic distance (D) were below 0.097. The estimate of gene flow based on G_{ST} was slightly high among Korean populations of *C. japonica* ($N_m = 2.65$). The absolute measure of genetic differentiation among populations (D_m) was 0.035. Genetic identity values among pairs of populations range from 0.907 to 0.996. The similarity among *C. japonica* populations can be seen in the UPGMA dendrogram, where total populations cluster at a below genetic distance 1.463. Genetic distance was correlated with geographical distance between populations ($r = 0.718$). Only one most isolated population CHH was distantly

related to the other populations. There was a significant positive and near-linear relationship between genetic diversity and mean temperature in the winter ($r = 0.787$).

DISCUSSION

Genetic diversity. *C. japonica* maintains more diversity in populations than the average plant species. For example, its genetic diversity at 0.256 is slightly higher than that of temperate-zone species (0.146), species with a reproduction mode that is sexual and asexual (0.138), species with a long-lived perennial herbaceous (0.205), and that of widespread geographic ranges (0.202) (Hamrick and Godt, 1989). The percent polymorphic loci in the studied sample was 66.7%, which is higher than species with a reproduction mode that is sexual and asexual (43.8%), long-lived perennial herbaceous (39.3%), temperate-zone species (48.5%), and species with widespread geographic ranges (58.9%), but it is similar to boreal temperate-zone species (64.5%) (Hamrick and Godt, 1989). Its average number of alleles per locus was 1.96; this value is higher than that of species with a reproduction mode that is sexual and asexual (1.69) and long-lived perennial herbaceous (1.42), but it is lower than that of species with widespread geographic ranges (2.29). It is almost alike to temperate-zone species (1.91) (Hamrick and Godt, 1989). These comparisons suggest that genetic diversity levels of *C. japonica* are higher than that of its associates, the temperate-zone species. Ellstrand and Roose (1987), in a review of studies of population genetic structure of primarily obligate clonal

plant species, concluded that clonal plant species tend to have intermediate levels of genetic diversity. The results of the present study are not consistent with the general conclusion of Ellstrand and Roose (1987) about the levels of genetic diversity. The relatively high level of genetic variation found in *C. japonica* is consistent with several aspects of its biology. First, geographic range has been shown to be strongly associated with the level of variation maintained within populations and at the species level (Hamrick et al., 1979; Hamrick and Godt, 1989). Widely distributed plant species tend to maintain more variation than more narrowly distributed species. Although *C. japonica* in Korea is distributed patchily, the species is wide geographic ranges of the Northern Hemisphere including East Asia. Second, the breeding system of a species is an important determinant of variability at both the species and population levels. *C. japonica* has a short style and tall stamens (almost thrum type). Therefore, this species appears to be predominantly by insects. Third, long-lived perennial species, like *C. japonica*, generally maintains relatively higher levels of variation than annuals and short-lived perennials. As populations of *C. japonica* are older, opportunities for the accumulation of mutations should be high (Ledig, 1986). Fourth, the reproduction type of *C. japonica* is an important role of genetic variability. In addition, vegetative reproduction and spread can also affect the genetic structure of populations (Murawski and Hamrick, 1990). Cook (1983) argued that clonal growth could act to retard the loss of genetic diversity within populations. If a small amount of gene flow and/or mutation add new clones to a population from time to time, clonal variation may be maintained. Thus, if clonalization occurs by multiple genotypes, the ephemeral nature of woody populations may preclude significant loss of genetic variation while those populations are extant (Ellstrand and Roose, 1987).

Genetic structure. Species with independent ramets could spread the risk of mortality among ramets, thus reducing the probability of genet death and preserving genetic diversity. Hartnett and Bazzaz (1985) have also argued that physiological independence among ramets may maintain genetic diversity by buffering clones against localized, patch specific selection forces. Sexual reproduction could act to enhance the genetic variation and asexual reproduction could maintain the enhanced genetic variation (Bayer, 1990). *C. japonica* commonly reproduces by sexually produced seeds. But, *C. japonica* usually propagates by asexually produced rhizomes when several strong environmental disadvantages influenced on the habitat of this species. It is likely that there are robust differences in genetic features between sexually reproducing species and asexually reproducing ones (Akiyama, 1994). The species has physical connections among ramets. There asexual reproduction assures the stabili-

zation and persistence of a phenotype that is well adapted to be the immediate environment (Huh et al., 1998). Thus, in low temperature and snow environments, northern populations of *C. japonica* may possess a selective disadvantage over southern populations which are mainly grown vegetative elongation than reproduction under warm winter. We can consider that reproductive system in *C. japonica* is more important than other factors.

The considerable amount of heterozygote deficiencies is notable ($F_{IS} = 0.374$). Although the species has many seeds, the dispersal distance seems to be short under rich pine (*Pinus densiflora* and *P. rigida*) forests, which may favor the establishment of clusters of related individuals. Consider the near self-incompatibility observed in *C. japonica*, probable mating among relatives via localized pollinator behavior rather than selfing could occur within populations. Such structure could lead to biparental inbreeding, causing heterozygote deficiencies. In addition, this patch distribution of related individuals should generate a Wahlund effect. Our sampling included individuals from several patches per population, resulting in an overall deficiency of heterozygotes. It is probably that the combination of these factors may contribute to high levels of heterozygote deficiencies within populations.

In addition, significant differences were found in allele frequencies between populations for all fourteen polymorphic loci. Mean genetic identity between populations ($I = 0.973$) was somewhat above the mean identity ($I = 0.945$) reported by Gottlieb (1981) for 22 species. It is unclear how the populations are genetically homogeneous. It is highly probable that directional toward genetic uniformity in a relatively the homogeneous habitat (i.e. mountain habitats, shaded ground) is thought to be operated among Korean populations of *C. japonica*.

REFERENCES

- Akiyama, H. (1994) Allozyme variability within and among populations of the epiphytic moss leucodon (Leucodontaceae: Musci). *Am. J. Bot.* **81**, 1280–1287.
- Aspinwall, N., and Christian, T. (1992) Clonal structures, genotypic diversity, and seed production in populations of *Filipenula rubra* (Roseaceae) from the northcentral United States. *Am. J. Bot.* **79**, 294–299.
- Barton, N. H., and Slatkin, M. (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subpopulation. *Heredity* **56**, 409–415.
- Bayer, R. J. (1990) Patterns of clonal diversity in the *Antennaria rosea* (Asteraceae) polyploid agamic complex. *Am. J. Bot.* **77**, 1313–1319.
- Cook, R. E. (1983) Clonal plant populations. *Am. Sci.* **71**, 244–253.
- Ellstrand, N. C., and Roose, M. L. (1987) Patterns of genotypic diversity in clonal plant species. *Am. J. Bot.* **74**, 123–131.
- Hamrick, J. L., and Godt, M. J. W. (1989) Allozyme diversity in plant species. In: *Plant Population Genetics, Breeding and Genetic Resources* (eds.: A. D. H. Brown, M. T. Clegg, A., L. Kahler, and B. S. Weir), pp. 304–319. Sinauer Associ., Inc.,

- Sunderland, MA.
- Hamrick, J. L., Godt, M. J. W., and Sherman-Broyles, S. L. (1992) Factors influencing levels of genetic diversity in woody plant species. *New For.* **6**, 95–124.
- Hamrick, J. L., Linhart, Y. B., and Mitton, J. B. (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annu. Rev. Ecol. Syst.* **10**, 175–200.
- Hartnett, D. C., and Bazzaz, F. A. (1985) The regulation of leaf, ramet and gene densities in experimental populations of the rizomatous perennial *Solidago canadensis*. *J. Ecol.* **73**, 429–443.
- Huh, M. K., Chung, S. D., and Huh, H. W. (1998) Allozyme variation and population structure of *Pyrola japonica* in Korea. *Bull. Acad. Bot. Bull. Acad. Sin.* **39**, 107–112.
- Ledig, F. T. (1986) Heterozygosity, heterosis, and fitness in outbreeding plants. In: *Conservation Biology* (ed.: M. E. Soule), pp. 77–104. Sinauer Associ., Inc., Sunderland, MA.
- Li, C. C., and Horvitz, D. G. (1953) Some methods of estimating the inbreeding coefficient. *Am. J. Hum. Genet.* **5**, 107–117.
- Loveless, M. D., and Hamrick, J. L. (1984) Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.* **15**, 65–95.
- Murawski, D. A., and Hamrick, J. L. (1990) Local genetic and clonal structure in the tropical terrestrial bromelid, *Aechmea magdalenae*. *Am. J. Bot.* **77**, 1201–1208.
- Nei, M. (1972) Genetic distance between populations. *Am. Nat.* **106**, 282–292.
- 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. Hum. Genet.* **41**, 225–233.
- Odasz, A. M., and Savolainen, O. (1996) Genetic variation in populations of the Arctic perennial *Pedicularis dasyantha* (Scrophulariaceae), on Svalbard, Norway. *Am. J. Bot.* **83**, 1379–1385.
- SAS Institute Inc. (1989) SAS/STAT User's Guide, Ver. 6, 4th eds., vol 1. SAS Institute, Cary, 943
- Slatkin, M. (1985) Rare alleles as indicators of gene flow. *Evolution* **39**, 53–65.
- Sobey, D. G., and Barkhouse, P. (1977) The structure and rate growth of the rhizome of some forest herbs and dwarf herbs of the New Brunswick-Nova Scotia border region. *Can. Field-Nat.* **91**, 377–383.
- Soltis, D. E., Haufler, C. H., Darrow, D. C., and Gastony, G. J. (1983) Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* **73**, 9–27.
- Weeden, N. F., and Wendel, J. F. (1989) Genetics of plant isozymes. In: *Isozymes in Plant Biology* (eds.: D. E. Soltis, and P. S. Soltis), pp. 42–72. Dioscorides Press, Portland, OR.
- Woodland, D. W. (1991) *Contemporary Plant Systematics*. Prentice-Hall Inc., New Jersey.
- Workman, P. L., and Niswander, J. D. (1970) Population studies on southern Indian tribes. II. local genetic differentiation in the Papago. *Am. Hum. Genet.* **22**, 24–49.
- Wright, S. (1922) Coefficients of inbreeding and relationship. *Am. Nat.* **56**, 330–338.
- Wright, S. (1951) The genetical structure of populations. *Annu. 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.