생명과학회지
Korean J. Life Science, 8(6), 687-694, 1998

Genetic Diversity and Population Structure of *Chimaphila japonica* in Southern Part of Korea

Joo-Soo Choi† and Man-Kyu Huh*

Department of Biology, Dong Eui University, Pusan 614-714, Korea
**Department of biology Education, Pusan Nation University, Pusan 609-753, Korea*

Abstract

Enzyme electrophoresis was used to estimate genetic diversity and population structure of *Chimaphila japonica* Miq. in Korea. The percent of polymorphic loci within the enzymes was 48.7%. Genetic diversity at the species level and at the population level was high (Hes=0.278 ; Hep=0.222, respectively), whereas the extent of the population divergence was relatively low (G_{ST}=0.079). F_{IS}, a measure of the deviation from random mating within the 7 populations, was 0.355. An indirect estimate of the number of migrants per generation (Nm=2.61) 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. Factors contributing to the high levels of genetic diversity found in the entire species of *C. japonica* include wide distribution, long-lived perennials, ability to regenerate due to rhizomatous spread, outcrossing induced by animal vectors, and occasional pollen dispersal by wind.

Key words : *Chimaphila japonica Miq.,genetic diversity,polymorphic loci*

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[1].

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[2]. Clearly, descriptive genetic work on both sexual and asexual plant populations is needed as well. Despite the importance of knowledge concerning genetic variation 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 Miq.(Pyrolaceae) is a widespread perennial herb occurring throughout boreal and temperate zones[3]. *C. japonica* is reproduced extensively by vegetative rhizomes and its seed is potentially produced sexually. The species in Korea is endemic to sever-

† Corresponding author

al 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[4]. 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 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 assess genetic structure of *C. japonica*.

MATERIAL AND METHODS

1. Sampling procedure

Chimaphila japonica was collected from seven natural populations in Korea (Table 1. Fig. 1). One leaf per plant was sampled during 1996 to 1997. More than 27 plants were collected from each population. Leaves gathered from natural populations were stored in plastic bags for several days in a refrigerator until electrophoresis was carried out.

2. Enzyme electrophoresis

Leaves were homogenized by mechanical grinding to

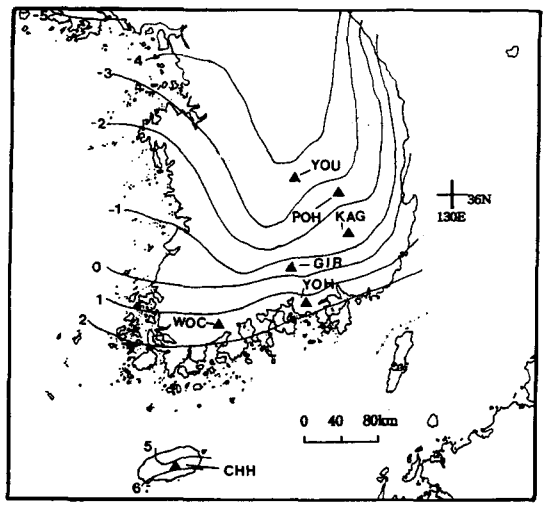

Fig. 1. Winter isotherms and collection localities for populations of *C. japonica* as source for isozyme analysis.

Table 1. 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 seven populations of *C. japonica*

Pop[a]	N[b]	P	Ap	A	Ae	Hop(SD)	Hep(SD)
CHH	35	40.91	2.67	1.68	1.39	0.133 (0.014)	0.185 (0.053)
GIR	31	50.00	2.82	1.91	1.46	0.126 (0.014)	0.227(0.053)
YOH	29	50.00	2.73	1.86	1.47	0.130 (0.014)	0.227 (0.054)
WOC	29	50.00	2.55	1.77	1.42	0.121 (0.013)	0.208 (0.052)
KAG	37	45.45	2.80	1.82	1.41	0.112 (0.013)	0.201 (0.053)
POH	31	50.00	2.82	1.91	1.59	0.137 (0.014)	0.251 (0.059)
YOU	32	54.55	2.75	1.95	1.59	0.151 (0.015)	0.253 (0.058)
MEAN		48.69	2.73	1.84	1.48	0.130(0.004)	0.222 (0.014)

[a] : Abbreviation codes as in Fig. 1. [b] : Number of individuals in the sample.

release enzymes from cell and organellar membranes with Tris-HCl grinding buffer-PVP solution described in Soltis et al.[5]. Electrophoresis was performed using 10% starch gel. Gel and electrode buffer systems and enzyme staining procedures from Soltis et al.[5] were used to assay five enzyme systems ; peroxidase (PER), 6-phosphogluconate dehydrogenase (PGD), acid phosphatase (ACP), and shikimate dehydrogenase (SKD). The procedures for horizontal starch gel electrophoresis were as reported by Soltis et al.[5]

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.

3. 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)[6]. 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[7]. These indices were tested for deviation from zero by x^2-statistics following Li and Horvitz[8].

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[9,10]. In addition, x^2-statistics were used to detect significant differences in allele frequencies among populations for each locus[11]. Nei's genetic identity (I) was calculated for each pairwise combination of populations[12]. We used the PC-SAS program[13]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 *F*-statistics[14] : 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 x^2-statistics[8]. Two indirect estimates of gene flow were calculated. One estimate of *Nm* (the number of migrants per generation) was based on G_{ST}[15] and the other estimate was based on the average frequency of "rare" alleles found in only one population[16,17]. The absolute population differentiation (Dm) was calculated using Nei's statistics[9]. Correlation between geographical and genetic distance was tested using Mantel's test[18] as advocated by Smouse et al.[19].

RESULTS

1. Genetic diversity

Four(*Per-1, Acp, Skd, and Pgd-1*) of the seven loci (57.1%) showed detectable polymorphism in at least two populations (Table 2). The remaining three loci (*Per-2, Per-3, and Pgd-2*) were monomorphic in all populations. An average of 48.7% of the loci were polymorphic within populations, with individual population values ranging from 40.9% to 54.6% (Table 1). The majority of the polymorphic loci (*Per-1, Skd, and Pgd-1*) expressed three alleles, while the remaining one expressed four alleles (*Acp*). The average number of alleles per locus (A) was 1.84 across populations, varying from 1.68 for the population with the lowest number of alleles and 1.95 for the population with the highest number of alleles. The number of alleles per polymorphic locus (Ap) was 2.73. The mean genetic diversity within populations was 0.222. Population YOU had the highest expected diversity (0.253), while population CHH had

Table 2. Total genetic diversity (H_T), genetic diversity within population (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 *C. japonica*

Locus	H_T	H_S	Dm	F_{IS}	F_{IT}	G_{ST}
Per-1	0.5726	0.5603	0.0132	0.3688	0.3825	0.0216
Acp	0.6343	0.6130	0.0227	0.3543	0.3760	0.0336
Skd	0.4343	0.3474	0.0927	0.4823	0.5859	0.2001
Pgd-1	0.4619	0.4290	0.0351	0.4055	0.4478	0.0712
Pgd-2	0.4698	0.4367	0.0354	0.1640	0.2231	0.0706
MEAN	0.5146	0.4857	0.0398	0.3549	0.4031	0.0794

the lowest (0.185). Genetic diversity at the species level was high, whereas the value at the population level was lower than that of species level (Hes=0.278 ; Hep=0.222).

2. Genetic structure

F_{IS}, a measure of the deviation from random mating within the 7 populations, was 0.355, and ranged from 0.164 for *Pgd-2* to 0.482 for *Skd*. The observed high, significant, and positive F_{IS} value (0.355) 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, all fixation indices were positive (31/31), and 19 of those departed significantly from zero ($p<0.05$). Total genetic diversity values (H_T) varied between 0.434 and 0.634, giving an average over all polymorphic loci of 0.515. The interlocus variation in within population genetic diversity (H_S) was high (0.486). On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.071 for *Pgd-2* to 0.200 for *Skd* with a mean of 0.079, indicating that about 8 % of the total allozyme variation was among populations. Values of genetic distance (D) were below 0.10, except in pairs involving population CHH. The estimate of gene flow based on G_{ST} was slightly high among Korean populations of *C. japonica* ($Nm=2.61$). The absolute measure of genetic differentiation among populations (Dm) was 0.040. Genetic identity values among pairs of populations range from 0.912 to 0.997. The similarity among *C. japonica* populations can be seen in the UPGMA dendrogram, where total populations cluster at a below genetic distance 0.812(Fig. 2). The UPGMA and correlation analysis show very strong correspondence between genetic distance and geographical distance. 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 ($r=-0.787$). With an r value -0.787 the most 61.9% of the variation in genetic diversity is explained by winter-time.

DISCUSSION

1. Comparison of genetic diversity

C. japonica maintains more diversity in populations than the average plant species. For example, its genetic diversity at 0.222 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)[20]. The percent

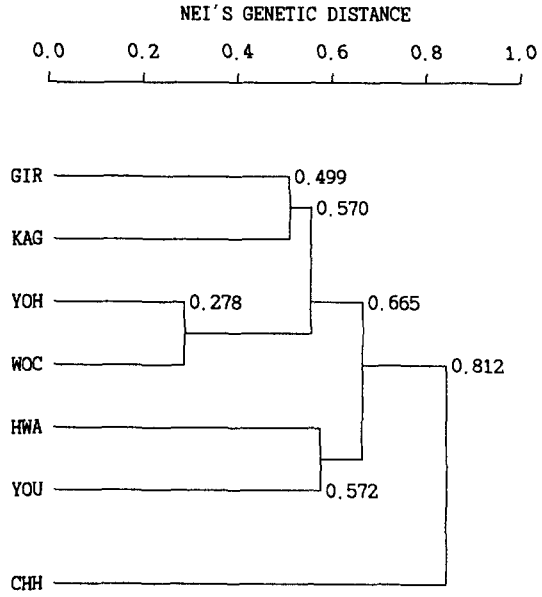

Fig. 2. A dendrogram showing the phylogenic relationships among the 7 populations of *C. japonica* based on data of genetic distance obtained by starch gel electrophoresis. Abbreviation of populations are given in Fig. 1.

polymorphic loci in the studied sample was 48.7%, which is higher than species with a reproduction mode that is sexual and asexual (43.8%), long-lived perennial herbaceous (39.3%), and temperature-zone species (48.5%), but it is lower than that of boreal temperate-zone species (64.5%)[20]. Its average number of alleles per locus was 1.84 ; 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)[20]. These comparisons suggest that genetic diversity levels of *C. japonica* are higher than that of its associates, the temperate-zone species. Ellstrand and Roose[2], 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 Roose2) about the levels of genetic diversity.

2. Reason for high level 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[20,21]. 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* is an outcrossing, insect/wind-pollinated species. This combination is well-known to be associated with high levels of allozyme variation[20,22,23]. In addition, vegetative reproduction and spread can also affect the genetic structure of populations[24]. Cook[25] 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[2]. Species with independent ramets could spread the risk of mortality among ramets, thus reducing the probability of genetic death and preserving genetic diversity. Hartnett and Bazzaz[26] have also argued that physiological independence among ramets may maintain genetic diversity by buffering clones against localized, patch specific selection forces. 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[27]. Fourth, the reproduction type of *C. japonica* is an important role of genetic variability. Sexual reproduction could act to enhance the genetic variation and asexual reproduction could maintain the enhanced genetic variation[28]. *C. japonica* commonly is reproduced by sexually produced seeds. But, *C. japonica* is usually propagated by asexually produced rhizomes when several strong environmental disadvantages influenced on the habitat of this species.

The species has physical connections among ramets. The asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to be the immediate environment[29]. Thus, in low temperature and snow environments, northern populations of *C. japonica* may posses 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 cluster analysis of Nei's genetic distances[12], the high value of *Nm* (2.61), and the low value of G_{ST} (0.079) also provide evidence for the existence of low differentiation of populations. Only population CHH aggregates correspond closely to broad geographical boundaries. Especially population CHH has more fixed alleles (two alleles) than other populations. The population is isolated from other populations by the surrounding the Pacific Ocean. Gene flow will therefore have restricted, genetic bottlenecks may have occurred, and selfing and /or consanguineous mating promoted.

In summary, the *Nm* (2.61) of *C. japonica* in Korea may also influence both the relatively high level of genetic diversity in this species and the relatively low population differentiation. Seed dispersal partly by wind and vegetative reproduction undoubtedly enhance gene flow with the species. *C. japonica* maintains higher levels of genetic diversity and exhibits lower levels of population divergence than expected on the basis of its life history characteristics.

REFERENCES

1. 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 borderregion.Canadian Field-Naturalist, **91**, 377-383.
2. Ellstrand, N. C. and Roose, M. L. 1987. Patterns of genotypic diversity in clonal plant species. Am. J. Bot. **74**, 123-131.
3. Woodland, D. W. 1991. Contemporary plant systematics. Prentice-Hall Inc., New Jersey.
4. Loveless, M. D. and Hamrick, J. L. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. **15**, 65-95.
5. Soltis, D. E., Haufler, C. H., Darrow, D. C., and Gastony, G. J. 1983. Starch gel electrophoresis of ferns : A complication of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern J. **73**, 9-27.
6. Hamrick, J. L., Godt, M. J. W. and Sherman-Broyles, S. L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests **6**, 95-124.
7. Wright, S. 1922. Coefficients of inbreeding and relationship. Am. Nat. **56**, 330-338.
8. Li, C. C. and Horvitz, D. G. 1953. Some methods of estimating the inbreeding coefficient. Am. J. Human Genet. **5**, 107-117.
9. Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**, 3321-3323.
10. Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Human Genet. **41**, 225-233.
11. Workman, P. L. and Niswander, J. D. 1970. Population studies on southern Indian tribes. II. local genetic differentiation in the Papago. Am. Human Genet. **22**, 24-49.
12. Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**, 282-292.
13. SAS Institute Inc. 1989. SAS/STAT user's guide, Ver. 6, 4th eds., Vol 1. SAS Institute, Cary, 943.
14. Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution **19**, 395-420.
15. Wright, S. 1951. The genetical structure of populations. Annuals of Eugenics, **15**, 313-354.
16. Slatkin, M. 1985. Rare alleles as indicators of gene

flow. Evolution, 39, 53−65.
17. Barton, N. H., Slatkin, M. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subpopulation. Heredity, 56, 409−415.
18. Mantel, N. A. 1967. The detection of disease clustering and generalized regression approach. Cancer Res. 27, 209−220.
19. Smouse, P. E., Long, J. C. and Sokal, R. R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35, 627−632.
20. 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. Clerg, A., L. Kahler, B. S. Weir), pp. 304−319. Sinauer Press, Sunderland, MA.
21. Hamrick, J. L., Linhart, Y. B. and Mitton, J. B. 19 79. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Eco. Syst. 10, 175−200.
22. Brown, A. D. H. 1979. Enzyme polymorphism in plant populations. Theoretical Population Biology, 15, 1−42.
23. Gottlieb, L. D. 1981. Electrophoretic evidence and plant populations. Prog. Phytochem. 7, 1−46.
24. 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.
25. Cook, R. E. 1983. Clonal plant populations. Am. Sci. 71, 244−253.
26. 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.
27. Ledig, F. T. 1986. Heterozygosity, heterosis, and fitness in outbreeding plants. In : Conservation biology (ed. : M.E. Soule), pp. 77−104, Sinauer Press, Sunderland.
28. Bayer, R. J. 1990. Patterns of clonal diversity in the Antennaria rosea (Asteraceae) polyploid agamic complex. Am. J. Bot. 77, 1313−1319.
29. 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.

초록 : 한국 남부지역의 매화노루발의 유전적 다양성과 집단구조

최주수† · 허만규*
(동의대학교 생물학과, *부산대학교 생물교육학과)

한국 남부지방의 매화노루발(*Chimaphila japonica* Miq.)의 유전적 다양성 및 집단구조를 조사하기 위해 전기영동을 실시하였다. 집단내 다형현상을 나타내는 대립유전자는 48.7%였다. 종 및 집단 수준에서 유전적 다양도는 각각 0.278, 0.222로 나타났고, 집단간 분화 정도는 비교적 낮았다(G_{ST}=0.079). 조사한 일곱 집단내 자가 수분계수는 0.355였다. G_{ST}로부터 간접적으로 구한 세대당 집단간 이동개체수는 약간 높게 나타나므로 한반도 남부 지방의 집단간 유전자 이동이 보편적으로 이루어 지고 있음을 시사한다 (Nm=2.61). 고정지수 분석에 의하면 이형접합자의 부족이 여러 집단 및 대립유전자좌위에서 나타났다. 이 종에서 유전적 다양도가 고양된 주된 이유는 광범위한 분포, 다년생, 영양번식, 곤충에 의한 타가수분, 그리고 바람에 의한 화분분산이 빈번하게 이루어진 것으로 사료된다.