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Population Genetic Structure of *Potentilla fragariodes* var. *major* (Rosaceae) in Korea

Man Kyu Huh[1]*, Hong Wook Huh[1], and Joo Soo Choi[2]
[1]Department of Biology Education, Pusan National University, Pusan 609-735, Korea
[2]Department of Biology, Dong Eui University, Pusan 614-714, Korea

The genetic diversity and population structure of eighteen *Potentilla fragariodes* var. *major* (*Rosaceae*) populations in Korea were determined using genetic variations at 22 allozyme loci. The percent of polymorphic loci within the enzymes was 66.7%. Genetic diversity at the species level and at the population level was high (Hes = 0.203; Hep = 0.185, respectively), whereas the extent of the population divergence was relatively low (G_{ST} = 0.069). F_{IS}, a measure of the deviation from random mating within the 18 populations, was 0.075. An indirect estimate of the number of migrants per generation (*Nm* = 3.36) indicated that gene flow was high among Korean populations of the species. In addition, analysis of fixation indices revealed a slight heterozygote deficiency in some populations and at some loci. Wide geographic ranges, perennial herbaceous nature and the persistence of multiple generations are associated with the high level of genetic variation. Although *P. fragariodes* var. *major* usually propagated by asexually-produced ramets, we could not rule out the possibility that sexual reproduction occurred at a low rate because each ramet may produce terminal flowers. Mean genetic identity between populations was 0.983. It is highly probable that directional movement toward genetic uniformity in a relatively homogeneous habitat operates among Korean populations of *P. fragariodes* var. *major*.

Keywords: allozyme, genetic diversity, population structure, *Potentilla fragariodes* var. *major*

Most plants, especially rhizomatous and stoloniferous species, have physical connections among ramets although the level of persistency is highly variable among species and habitats (Sobey and Barkhouse, 1977). Studies on the genetic structure of apomictic plant populations have received increased interest over the past decade with the advent of electrophoretic techniques, which allow us to better access 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 genetic variation data for conservation purposes and population genetic structure, detailed studies of the levels and distribution of genetic variation have not been performed on most species in Korea, and are particularly lacking for plants with both sexually - and asexually-reproductive ability (Chung et al., 1995; Huh and Huh, 1998).

The genus *Potentilla* is a large, diverse family (sometimes treated as many families) of 100-122 genera and 3000-3400 species, widely distributed but most common in temperate regions of the Northern Hemisphere. The genera with the most species is *Potentilla*, the cinquefoils (300 species) (Woodland, 1991). *Potentilla fragariodes* var. *major* (*Rosaceae*) BUNGE is generally distributed in fields and mountains. The species in Korea is typically found in low mountain regions where it grows at elevations as low as 500 m below sea level. The species is also found in North-east Asian regions such as Japan, central and north-east China. *P. fragariodes* var. *major* reproduces extensively by vegetative rhizomes and potentially by sexually-produced seed. It is a profusely flowering perennial, with yellow flowers that are occasionally visited by some insect species. The species is covered with many short fine silky hairs, and its fibrous root systems form extensive networks in the soil. It is important economically for medicine (root stock), as an ornamental shrub and as a protectant against washout. Recently, its anti-erosion properties have been used to create effective watersheds, stitching the soil together along fragile field embankments and in places prone to mud slides. In contrast to other ecologically and economically significant herbaceous species, the mode of allozyme inheritance has not yet been studied in the cinquefoils.

The purposes of this study were to estimate how

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*Corresponding author; fax +82-51-514-8576
e-mail hwhuh@hyowon.cc.pusan.ac.kr

much total genetic diversity is maintained in the species, to describe how genetic variation is distributed within and among its populations, and to assess the genetic structure of *P. fragariodes* var. *major.*

MATERIALS AND METHODS

Sampling Procedure

P. fragariodes var. *major* (*Rosaceae*) BUNGE was collected from eighteen populations in Korea (Fig. 1). One leaf per plant was sampled during the period from 1997 to 1998. The distance between selected individuals was about 5 m in order to avoid including individuals with common lineage. Twenty-eight to thirty 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.

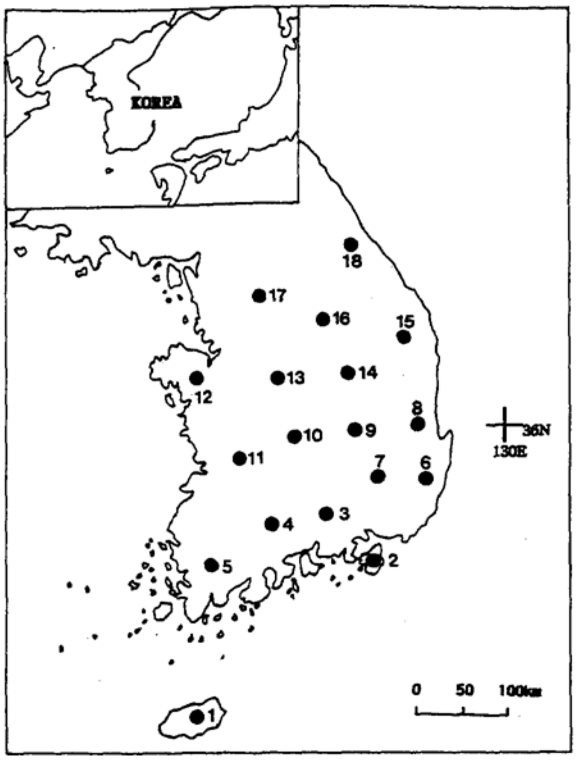

Figure 1. Collection localities for populations of *P. fragariodes* var. *major* as sources for allozyme analysis. **1:** Mt. Hanla, Cheju Island; **2:** Ayang-dong, Kyeje-ci; Gyeongsangnam-do; **3:** Chisu-myen, Chinju-ci, Gyeongsangnam-do; **4:** Acyang-myen, Hadong-gun, Gyeongsangnam-do; **5:** Kymjeong-myen, Youngam-gun, Chonllanam-do; **6:** Unyang-myen, Ulsan-ci; **7:** Daehap-myen, Changryeng-gun, Gyeongsang-nam-do; **8:** Hunghae-up, Pohang-ci, Gyeongsangbuk-do; **9:** Dongmeng-myen, Chilkok-gun, Gyeongsangbuk-do; **10:** Maekok-myen, Youngdong-gun, Chungchengbuk-do; **11:** Rongdam-myen, Chinan-gun, Chungchengbuk-do; **12:** Kwangchen-up, Hongseng-gun, Chungchengnam-do; **13:** Youngcheng-dong, Sang-gu, Chengju-ci; **14:** Dansan-myen, Youngpung-gun, Gyeongsangbuk-do; **15:** Dogae-up, Samcheck-ci, Kangwon-do; **16:** Woosan-dong, Wanju-ci, Kangwon-do; **17:** Choan-myen, Namyangju-ci, Kyonggi-do; **18:** Nohwa-dong, Sokcho-ci, Kangwon-do.

Enzyme Electrophoresis

Homogenization, starch gel electrophoresis and enzyme assay procedures were followed according to the methods of Soltis et al. (1983). Leaves were homogenized by mechanical grinding to release enzymes from cell and organellar membranes with Tris-HCl grinding buffer-PVP solution. Electrophoresis was performed using 10% starch gels, and a total of twelve enzyme systems were assayed for this study: fluorescent esterase (FE), glutamate oxaloacetate transaminase (GOT), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), peroxidase (PER), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), and superoxide dismutase (SOD).

For enzymes resolving in more than one zone of activity, the most anodal isozyme was arbitrarily designated '1' and subsequent isozymes were 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 *P. fragariodes* var. *major* allozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most allozyme plant studies, as documented by Weeden and Wendel (1989).

Analysis of Data

Four standard genetic parameters were estimated using a computer program developed by Loveless and 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). Subscripts refer to species (s) or population (p) level parameters. Observed heterozygosity (Ho) was compared with Hardy-Weinberg expected values 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). The G_{ST} coefficient, in particular, estimates relative population differentiation. 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. Deviations 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).

RESULTS

Fourteen of the 22 loci (66.7%) showed detectable polymorphism in at least one population (Table 1). The remaining seven loci (*Per-3, Idh-1, Mdh-2, Mdh-3, Lap, Pgm-2,* and *Me*) were monomorphic in all populations. An average of 46.0% of loci were polymorphic within populations, with individual population values ranging from 38.1% to 52.4%. The majority of the polymorphic loci expressed two (*Pgd-1, Pgd-2, Per-2, Fe-1, Per-2, Gpi-1, Pgm-1,* and *Skd*) or three alleles (*Fe-2, Per-1, Idh-2, Mdh-1,* and *Sod*). The average number of alleles per locus (A) was 1.67 across populations, varying from 1.52 for the population with the lowest number of alleles and 1.76 for the population with the highest number of alleles. The effective number of alleles per locus (Ae) was similar at the species and the population level (Aes = 1.39; Aep = 1.36). The mean genetic diversity within populations was 0.185. Population 16 had the highest expected diversity (0.243), while population 9 had the lowest (0.138). Genetic diversity at the species level was 0.203. In addition, the correlation between genetic distance and geographic distance was high ($r = 0.57$), indicating that geographically-close populations tended to be genetically similar and about 67% ($1 - r^2$) of the variation in genetic distance was caused by unknown factors other than distance.

Table 1. Allozyme variation within 18 populations of *P. fragariodes* var. *major.*

Pop[a].	N[b]	P	Ap	A	Ae	Hop	Hep
1	29	47.62	2.40	1.67	1.28	0.153	0.147
2	28	38.10	2.63	1.62	1.31	0.137	0.153
3	28	47.62	2.40	1.67	1.36	0.216	0.193
4	29	42.86	2.44	1.62	1.35	0.166	0.175
5	29	42.86	2.44	1.62	1.33	0.165	0.168
6	29	42.86	2.44	1.62	1.35	0.167	0.176
7	32	42.86	2.44	1.62	1.38	0.169	0.192
8	28	38.10	2.50	1.57	1.31	0.141	0.162
9	28	38.10	2.38	1.52	1.26	0.124	0.138
10	28	52.38	2.45	1.76	1.41	0.178	0.203
11	28	52.38	2.36	1.71	1.35	0.168	0.183
12	34	47.62	2.40	1.67	1.40	0.172	0.200
13	30	42.86	2.56	1.67	1.42	0.186	0.200
14	30	52.38	2.45	1.76	1.39	0.182	0.200
15	28	42.86	2.56	1.67	1.34	0.150	0.171
16	28	52.38	2.45	1.76	1.50	0.225	0.243
17	30	52.38	2.45	1.76	1.42	0.200	0.222
18	28	52.38	2.36	1.71	1.37	0.185	0.201
Mean	46.03	2.45	1.67	1.36	0.171	0.185	
SD	2.55	0.07	0.07	0.06	0.004	0.012	
Species	66.67	2.36	1.90	1.39	-	0.203	

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), and Hardy-Weinberg expected heterozygosity or genetic diversity (Hep).

[a] Abbreviation codes as in Figure 1.

[b] Number of individuals in each sample.

F_{IS}, a measure of the deviation from random mating within the 18 populations, was 0.075, and ranged from −0.160 for *Pgd-2* to 0.312 for *Skd* (Table 2). The observed significant and positive F_{IS} value (0.075) indicates that there was a significant deficit of heterozygotes in the populations. Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a slight deficiency of heterozygotes relative to Hardy-Weinberg expectations (data not shown). For example, 67.1% of fixation indices were positive (114 / 170), and 14 of those departed significantly from zero ($p < 0.05$). Forty-six indices were negative, indicating an excess of heterozygotes at *Pgd-1, Pgd-2,* and *Pgm-1.* Total genetic diversity values (H_T) varied between 0.0 (monomorphic loci) and 0.654 (*Fe-2*), giving an average overall polymorphic loci of 0.305. The interlocus variation of genetic

Table 2. Estimates of genetic diversity statistics and 13 polymorphic loci in *P. fragariodes* var. *major*.

ocus	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	G_{ST}
Sod	0.531	0.516	0.016	−0.071	−0.040	0.030
Gpi	0.006	0.006	0.000	−0.064	−0.003	0.057
Mdh-1	0.439	0.426	0.013	0.155	0.181	0.030
Idh-2	0.434	0.422	0.012	0.134	0.158	0.028
Pgd-1	0.407	0.343	0.063	0.084	0.227	0.156
Pgd-2	0.453	0.343	0.110	−0.161	0.121	0.242
Me-1	0.004	0.004	0.000	−0.042	−0.002	0.038
Got	0.007	0.006	0.000	−0.064	−0.003	0.057
Skd	0.126	0.113	0.013	0.312	0.385	0.105
Per-1	0.428	0.419	0.008	0.310	0.324	0.019
Per-2	0.203	0.193	0.011	0.224	0.264	0.052
Pgm-1	0.476	0.471	0.004	−0.079	−0.069	0.009
Fe-1	0.103	0.089	0.014	0.278	0.374	0.134
Fe-2	0.654	0.646	0.008	0.024	0.037	0.013
Mean	0.305	0.286	0.020	0.075	0.140	0.069

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

diversity within populations (H_S) was high (0.286). On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.013 for *Fe-2* to 0.242 for *Pgd-2* with a mean of 0.069, indicating that about 6.9% of the total allozyme variation was among populations. The estimate of gene flow based on G_{ST} was high among Korean populations of *P. fragariodes* var. *major* (*Nm* = 3.36). In contrast, the mean estimate of gene flow based on private alleles was 1.92. Values of genetic distance (D) were below 0.048 (data not shown). Genetic identity values among pairs of populations range from 0.953 to 0.997. The similarity among *P. fragariodes* var. *major* populations can be seen in the UPGMA dendrogram, where total populations cluster below a genetic distance of 0.18 (Fig. 2).

DISCUSSION

Genetic Diversity

P. fragariodes var. *major* maintains greater diversity in populations than the average plant species. Genetic diversity of *P. fragariodes* var. *major* (Hep = 0.203) is slightly higher than that of temperate-zone species (0.146), and species with sexual and asexual reproduction modes (0.138), but similar to long-lived perennial herbaceous species (0.205) and those with widespread geographic ranges (0.202) (Hamrick and Godt, 1989). The percent of polymorphic loci at the species level was 66.7%. This value is higher than for species with both sexual and asexual reproduction modes (43.8%), long-lived perennial herbaceous species (39.3%), temperature-zone species (48.5%), and species with widespread geographic ranges (58.9%) (Hamrick and Godt, 1989). The average number of alleles per locus was 1.90; this value is higher than that of species with both sexual and asexual reproduction modes (1.69) and long-lived perennial herbaceous species (1.42), but similar to temperate-zone species (1.91), and lower than species with widespread geographic ranges (2.29) (Hamrick and Godt, 1989). The same trend is observed at the population level.

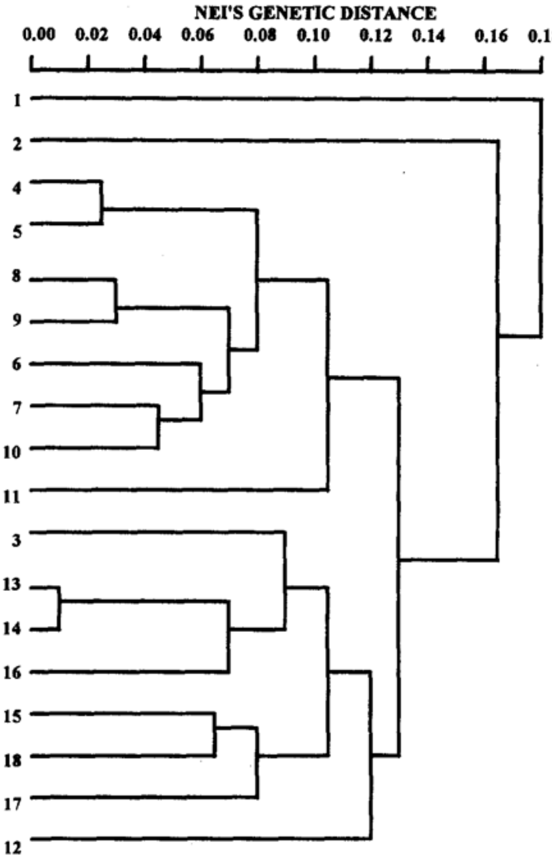

Figure 2. A dendrogram showing the genetic relationships among the 18 populations of *P. fragariodes* var. *major*, based on genetic distance data.

The relatively high level of genetic variation found in *P. fragariodes* var. *major* is consistent with several aspects of its biology. First, the species is found throughout Northeast Asia including Japan, China and Korea (Kim, 1996). Geographic range has been shown to be strongly associated with the level of variation maintained within populations and at the species level (Hamrick and Godt, 1989). Widely-distributed plant species tend to maintain more variation than more-narrowly-distributed species.

Second, long-lived perennial species like *P. fragariodes* var. *major* generally maintain relatively higher levels of variation than annuals and short-lived perennials (Loveless and Hamrick, 1984). As populations of *P. fragariodes* var. *major* live longer, there should be more opportunities for the accumulation of mutations (Ledig, 1986). In nature, clone genotypes may be long-lived; clones of some grasses routinely live for a few hundred years (Moore et al., 1995). Because *P. fragariodes* var. *major* can produce a few ramets per year (Kim, 1996), the persistence of multiple generations are associated with the high level of genetic variation. Finally, the reproduction type of *P. fragariodes* var. *major* has an important role in genetic variability. Factors contributing to the maintenance of this variation may be the persistence of multiple generations (maternal plants, ramets, and genets) within populations and large population sizes (Godt and Hamrick, 1993). 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 adds new clones to a population from time to time, clonal variation may be maintained.

Genetic Structure

In most species of *Potentilla*, especially perennial herbs, species consist of a series of internodes. Each ramet may produce only one terminal flower in the year it is formed (Eriksson, 1987; Eriksen, 1997; Eriksson et al., 1998; Spomer, 1999). Many plants have two reproductive strategies, sexual reproduction via seeds and clonal propagation via the development of vegetative rhizomes through the growth of a coordinated group of cells that form a meristem (Cook, 1983; Eriksson, 1987). *P. fragariodes* var. *major* is no exception, and consists of repetitive units (ramets) which may be interconnected via rhizomes (authors' observation). The species flowers mainly in March or April, producing many inflorescences per ramet (cyme), although infrequently one inflorescence per ramet is produced. We also observed that fruits (achene) start to be visible in May. Although genetic diversity among populations are high, there are only three rare alleles. In addition, three multiloci were local genotypes. These observations suggest that the present populations might have been founded from asexual fragmentation and dispersal of preexisting clones rather than from sexually-produced seed.

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). *P. fragariodes* var. *major* usually propagates by asexually-produced rhizomes when several strong environmental disadvantages influence the habitat of this species. The species has physical connections among ramets. Their asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to the immediate environment (Huh et al., 1998). Although *P. fragariodes* var. *major* is able to reproduce by sexually-produced seeds, its ratio of asexual/sexual reproduction has not yet been studied. In addition, we cannot rule out the possibility that sexual reproduction occurs at a low rate. Detailed breeding and demographic studies of *P. fragariodes* var. *major* are needed to confirm this speculation.

Genetic differentiation among populations is principally a function of gene flow among populations via pollen and seed dispersal (Loveless and Hamrick, 1984). The majority of genetic diversity observed at the polymorphic loci in *P. fragariodes* var. *major* occurred within populations (G_{ST} = 0.069). This low level of genetic differentiation also suggests that gene flow among population is high (*Nm* = 3.36). In addition, significant differences are found in allele frequencies between populations for all eleven polymorphic loci. Mean genetic identity between populations is somewhat high (I = 0.983), but it is unclear how the populations are genetically homogeneous. It is highly probable that directional movement toward genetic uniformity in a relatively homogeneous habitat (i.e. low mountain habitats, open ground, and a little swampy land) operates among the Korean populations of *P. fragariodes* var. *major*.

Heterozygote deficiency do not become an eyesore (F_{IS} = 0.075). If the number of genets is low due to limited numbers of founders, genetic drift after colonization, or the differential survival and spread of genotypes, populations of clonal species could consist of a few genotypes (Murawski and Hamrick, 1990). Considering the near-clonal propagation observed in *P. fragariodes* var. *major*, probable mating among relatives via localized pollinator behavior rather than self-pollinating occurs within these populations. Such structure can lead to biparental inbreeding, causing heterozygote deficiencies (Chung et al., 1995; Huh et al., 1995). In addition, this patch distribution of related individuals should generate a Wahlund effect (Huh et al., 1996). Our sampling included individuals from several patches per population, resulting in an overall deficiency of heterozygotes. It is probable that the combination of these factors may contribute to heterozygote deficiencies within these populations.

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