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# Genetic Diversity and Population Structure of *Potentilla freyniana* in Korea

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The genetic diversity and population structure of *Potentilla freyniana* in Korea were determined using genetic variations at 19 allozyme loci. Thirteen of the 19 loci (68.4%) showed detectable polymorphism. Genetic diversity at the population level was high ($H_{EP}$ = 0.270). Total genetic diversity values ($H_T$) varied between 0.190 and 0.584, giving an average overall polymorphic loci of 0.371. The interlocus variation of genetic diversity within populations ($H_S$) was high (0.354). On a per locus basis, the proportion of total genetic variation due to differences among populations ($G_{ST}$) ranged from 0.008 for *Fe-2* to 0.310 for *Gpi* with a mean of 0.065, indicating that about 6.5% of the total allozyme variation was among populations. Wide geographic ranges, perennial herbaceous nature and the persistence of multiple generations are associated with the high level of genetic variation in *P. freyniana*. The estimate of gene flow based on $G_{ST}$ was high among Korean populations of *P. freyniana* ($Nm$ = 3.57). Although *P. freyniana* usually propagated by asexually-produced ramets, I could not rule out the possibility that sexual reproduction occurred at a low rate because each ramet may produce terminal flowers.

**Key words** – Allozyme, genetic diversity, population structure, *Potentilla freyniana*

## Introduction

Most plants, especially rhizomatous and stoloniferous species, have physical connections among ramets although the level of persistency is highly variable among species and habitats [18]. 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" [1,4]. Various studies have shown that asexually-reproducing plants can be much more genetically diverse than originally thought [5]. 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.

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The genus *Potentilla* includes about 300 species of medium to large sized herbs distributed throughout temperate and the arctic regions. *Potentilla freyniana* Bornmueller (Rosaceae) is herb (< 1m in height) that is distributed in natural habitats of fields and mountains. *P. freyniana* is diploid (2n=14) and predominantly insect-pollinated that blooms from April to June [14]. This species is a long-lived herb, which can reproduce extensively by vegetative rhizomes and potentially by sexually produced seeds. Rhizomes are generally prostate stems at the nodes.

Allozyme variation within plant populations often shows structured pattern in space, presumably reflecting kinship structure that has arisen through by distance due to restricted dispersals [10]. Once kinship structure is established, restricted seed and pollen dispersals lead to outcrossing between genetic relative, a phenomenon known as biparental inbreeding [21]. Thus, populations of obligate outcrossers may routinely experience some level of inbreeding [11]. In addition, selfing rate, inbreeding depression, and relative fecundity have been estimated at different life history stages [16].

The purposes of this study are to estimate how 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. freyniana*.

## Materials and Methods

### Sampling procedure

*P. freyniana* was collected from eight populations in Korea (Table 1). One leaf per plant was sampled during the period from 2005 to 2006. The distance between selected individuals was about 5 m in order to avoid including individuals with common lineage. Leaves gathered from natural populations were stored in plastic bags for several days in a refrigerator until electrophoresis was carried out.

### Enzyme electrophoresis

Homogenization, starch gel electrophoresis and enzyme assay procedures were followed according to the methods of Soltis et al. [19]. Leaves were homogenized by mechanical grinding to release enzymes from cell and organellar membranes with Tris-HCl grinding buffer-PVP solution. Enzyme electrophoresis was performed using 12.0% starch gels. Buffer systems and enzyme staining procedures of Soltis et al. [19] were used to assay eight enzyme systems; alcohol dehydrogenase (ADH), fluorescent esterase (FE), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), peroxidase (PER), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD). For enzymes resolved in more than one zone of activity, the most anodal isozyme was arbitrarily designated '1' and subsequent isozymes 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. freyniana* allozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most allozyme plant studies, as documented by Weeden and Wendel [22].

### Data analysis

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*) [8]. 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 [23]. These indices were tested for deviation from zero by $\chi^2$-statistics following Li and Horvitz [15]. 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 [17]. The $G_{ST}$ coefficient, in particular, estimates relative population differentiation. In addition, $\chi^2$-statistics were used to detect significant differences in allele frequencies among populations for each locus. Nei's genetic identity (I) was calculated for each pairwise combination of populations [17]. The genetic structure within and among populations was also evaluated using Wright's [24] *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 $\chi^2$-statistics [15]. An indirect estimate of gene flow was calculated. One estimate of *Nm* (the number of migrants per generation) was based on $G_{ST}$ [24]. A phenetic relationship was constructed by the neighborjoining (NJ) method using the NEIGHBOR program in PHYLIP version 3.57 [7].

## Results

Thirteen of the 19 loci (68.4%) showed detectable polymorphism in at least one population (Table 1). The remaining six loci (*Idh-1, Mdh-2, Mdh-3, Me-1, Me-2,* and *Pgm-1*) were monomorphic in all populations. An average of 57.2% of loci were polymorphic within populations, with individual population values ranging from 47.4% to 68.4% (Table 2). The majority of the polymorphic loci expressed two (*Fe-1, Gpi, Pgd-1, Pgd-2, Per-2, Per-3, Pgm-1,* and *Skd*) or three alleles (*Adh, Fe-2, Idh-2, Mdh-1,* and *Per-1*). The average number of alleles per locus (*A*) was 1.79 across populations, varying from 1.63 for the population with the lowest number of alleles and 1.95 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* = 1.46; *Aep* = 1.52). The mean genetic diversity within populations was 0.270. Population POT-2 had the highest expected diversity (0.303), while population POT-8 had the lowest (0.225). Genetic diversity at the species level was 0.254. In addition, the correlation between genetic distance and geographic distance was high ($r$ = 0.59), indicating that geographically-close populations tended to be genetically similar and about 65% ($1 - r^2$) of

Table 1. Numeric code, population location, and sample sizes of *Potentilla freyniana*

| Code | Location | Sample size(n) |
|---|---|---|
| POT-1 | Mt. Seorak, Yangyang Pref. | 42 |
| POT-2 | Mt. Odae, Myeongju Pref. | 36 |
| POT-3 | Mt. Worak, Mungyeong Pref. | 48 |
| POT-4 | Mt. Sobaek, Yeongpoong Pref. | 44 |
| POT-5 | Mt. Deogyu, Muju Pref. | 46 |
| POT-6 | Mt. Jiri, Sancheong Pref. | 45 |
| POT-7 | Mt. Weolchul, Youngam Pref. | 40 |
| POT-8 | Mt. Geumjeong, Busan Pref. | 38 |

Table 2. Measures of genetic variation for *P. freyniana*

| Population | Pp | *A* | $A_E$ | $H_{OP}$(SD) | $H_{EP}$(SD) |
|---|---|---|---|---|---|
| POT-1 | 63.2 | 1.89 | 2.42 | 0.190(0.017) | 0.298(0.054) |
| POT-2 | 68.4 | 1.95 | 2.38 | 0.193(0.017) | 0.303(0.050) |
| POT-3 | 63.2 | 1.84 | 2.33 | 0.184(0.017) | 0.271(0.051) |
| POT-4 | 57.9 | 1.79 | 2.36 | 0.161(0.016) | 0.260(0.052) |
| POT-5 | 52.6 | 1.74 | 2.40 | 0.164(0.016) | 0.268(0.055) |
| POT-6 | 52.6 | 1.74 | 2.40 | 0.158(0.015) | 0.276(0.056) |
| POT-7 | 52.6 | 1.74 | 2.40 | 0.149(0.015) | 0.257(0.054) |
| POT-8 | 47.4 | 1.63 | 2.33 | 0.111(0.014) | 0.225(0.052) |
| Mean | 57.2 | 1.79 | 2.38 | 0.164(0.006) | 0.270(0.019) |
| Species | 68.4 | 1.95 | 2.38 | - | 0.254 |

The percentage of polymorphism (Pp), mean number of alleles per locus (*A*), effective number of alleles per locus ($A_E$), observed heterozygosity ($H_{OP}$), and Hardy-Weinberg expected heterozygosity or genetic diversity ($H_{EP}$).

the variation in genetic distance was caused by unknown factors other than distance.

Total genetic diversity values ($H_T$) varied between 0.190 (*Fe-1*) and 0.584 (*Per-1*), giving an average overall polymorphic loci of 0.371 (Table 3). The interlocus variation of genetic diversity within populations ($H_S$) was high (0.354). On a per locus basis, the proportion of total genetic variation due to differences among populations ($G_{ST}$) ranged from 0.008 for *Fe-2* to 0.309 for *Gpi* with a mean of 0.065, indicating that about 6.5% of the total allozyme variation was among populations. The estimate of gene flow based on $G_{ST}$ was high among Korean populations of *P. freyniana* (*Nm* = 3.57). $F_{IS}$, a measure of the deviation from random mating within the eight populations, was 0.357, and ranged from -0.114 for *Skd* to 0.716 for *Gpi*. The observed significant and positive $F_{IS}$ value (0.357) 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. Wright's

Table 3. 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 *P. freyniana*.

| Locus | $H_T$ | $H_S$ | $F_{IT}$ | $F_{IS}$ | $G_{ST}$ |
|---|---|---|---|---|---|
| *Adh* | 0.412 | 0.411 | 0.327 | 0.329 | 0.003 |
| *Fe-1* | 0.190 | 0.169 | 0.339 | 0.411 | 0.109 |
| *Fe-2* | 0.556 | 0.551 | 0.520 | 0.524 | 0.008 |
| *Gpi* | 0.236 | 0.163 | 0.716 | 0.804 | 0.310 |
| *Idh-2* | 0.469 | 0.462 | 0.289 | 0.299 | 0.014 |
| *Mdh-1* | 0.514 | 0.505 | 0.323 | 0.335 | 0.017 |
| *Per-1* | 0.584 | 0.573 | 0.530 | 0.539 | 0.019 |
| *Per-2* | 0.285 | 0.283 | 0.182 | 0.189 | 0.009 |
| *Per-3* | 0.294 | 0.279 | 0.223 | 0.263 | 0.051 |
| *Pgd-1* | 0.230 | 0.208 | 0.406 | 0.465 | 0.098 |
| *Pgd-2* | 0.317 | 0.299 | 0.309 | 0.349 | 0.058 |
| *Pgm-2* | 0.492 | 0.482 | 0.595 | 0.603 | 0.021 |
| *Skd* | 0.250 | 0.216 | -0.114 | 0.034 | 0.133 |
| Mean | 0.371 | 0.354 | 0.357 | 0.396 | 0.065 |

Table 4. Genetic identity (upper diagonal) of *P. freyniana* and genetic distances (low diagonal) based on allozyme analysis.

| Pop. | POT-1 | POT-2 | POT-3 | POT-4 | POT-5 | POT-6 | POT-7 | POT-8 |
|---|---|---|---|---|---|---|---|---|
| POT-1 | - | 0.9900 | 0.9841 | 0.9754 | 0.9661 | 0.9632 | 0.9695 | 0.9610 |
| POT-2 | 0.0101 | - | 0.9903 | 0.9849 | 0.9788 | 0.9807 | 0.9861 | 0.9801 |
| POT-3 | 0.0160 | 0.0097 | - | 0.9969 | 0.9902 | 0.9896 | 0.9896 | 0.9841 |
| POT-4 | 0.0249 | 0.0152 | 0.0031 | - | 0.9901 | 0.9912 | 0.9884 | 0.9871 |
| POT-5 | 0.0345 | 0.0214 | 0.0099 | 0.0100 | - | 0.9943 | 0.9919 | 0.9887 |
| POT-6 | 0.0375 | 0.0195 | 0.0104 | 0.0089 | 0.0057 | - | 0.9940 | 0.9905 |
| POT-7 | 0.0310 | 0.0140 | 0.0105 | 0.0117 | 0.0081 | 0.0060 | - | 0.9942 |
| POT-8 | 0.0398 | 0.0201 | 0.0160 | 0.0130 | 0.0113 | 0.0096 | 0.0058 | - |

fixation indices for polymorphic loci were positive in most cases for eight natural populations (84/87), and 53.6% of those (45/84) departed significant from zero. Only three of indices were negative, indicating an excess of heterozygosity on *Skd* populations POT-1, POT-3, and POT-4, however, no one was departed significant from zero ($P < 0.05$).

Values of genetic distance (D) were below 0.040 (Table 4). Genetic identity values among pairs of populations range from 0.961 to 0.997. The similarity among *P. freyniana* populations can be seen in the UPGMA dendrogram, where total populations cluster below a genetic distance of 0.05 (Fig. 2).

## Discussion

Presence or absence of self-incompatibility mechanisms, availability of pollinators and their foraging behavior, and

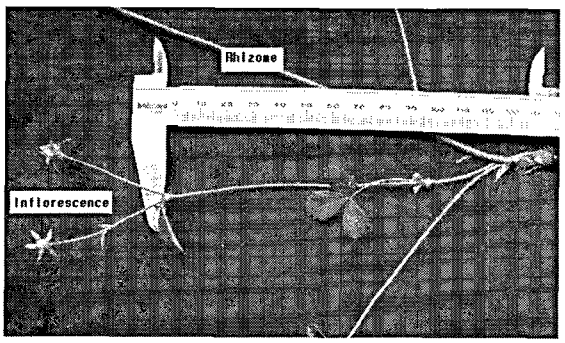


Fig. 1. Growth form of *Potentilla freyniana* with clonal reproduction.

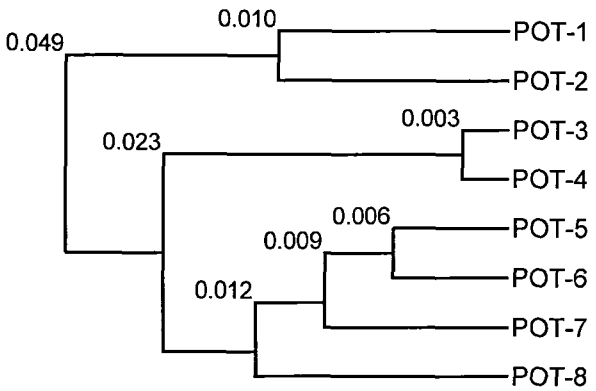


Fig. 2. A dendrogram showing the phylogenic relationships among the eight populations of *P. freyniana* based on data of genetic distance obtained by starch gel electrophoresis. Codes of populations are given in Table 1.

flower density and phonological variation are among the factors that affect the mating system [2,3].

The observed high, significant, and positive *F* value indicates that homozygotes were significantly in excess. If significant deficiencies of heterozygosity for each polymorphic locus are present, this indirectly indicates the existence of inbreeding. Generally, seedling stages are expected to have higher levels of inbreeding than found in adults [12]. These levels of inbreeding can result from a variety of causes because *P. freyniana* is mixed mating species; positive assortive mating (i.e., preferential mating among similar genotypes), selection for homozygotes, family structure within a restricted neighborhood, causing mating among relatives. The significant deficiency of heterozygotes found in many populations may partly be due to the fact that there has been selection favoring homozygotes among populations. This may suggest that selection against heterozygotes operated in the progeny populations throughout the life cycle. This allowed few inbred progenies to survive to the adult stage, resulting in more outcrossed adult plants. Selection in favor of heterozygotes typically occurs in more extreme environments. The reproductive strategy of *P. freyniana* could explain the observed inbreeding level. Because *P. freyniana* is polygamous species, it is expected that all of the inbreeding detected is due to consanguineous and self-mating. Nei et al. [17] have shown that the reduction in average heterozygosity per locus depends not only on the size of the population bottleneck, but also on the subsequent rate of population growth. If population growth is reduced, reduction in average heterozygosity is large.

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 [6,20]. 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 [4,20]. *P. freyniana* is no exception, and consists of repetitive units (ramets) which may be interconnected via rhizomes. The species flowers mainly in April or May, producing many inflorescences per ramet (cyme), although infrequently one inflorescence per ramet is produced. I also observed that fruits (achene) start to be visible in July. 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 [9] 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 [1]. *P. freyniana* usually propagates by asexually-produced rhizomes when several strong environmental disadvantages influence the habitat of this species. The species has physical connections among ramets (Fig. 1). Their asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to the immediate environment [13]. Although *P. freyniana* is able to reproduce by sexually-produced seeds, its ratio of asexual/sexual reproduction has not yet been studied. However, it cannot rule out the possibility that sexual reproduction occurs at a low rate.

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## 초록 : 한국내 세잎양지꽃의 유전적 다양성과 집단구조


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전분 젤 전기영동을 사용하여 한국내 분포하는 세잎양지꽃 8개 집단에서 유전적 다양성과 집단구조를 평가하였다. 종수준에서 효소내 다형현상을 나타내는 대립유전자좌위는 68.4%였다. 집단 수준에서 유전적 다양도는 유사한 생활사를 가진 초본류의 평균값에 비해 높았다. 전체 유전적 다양도는 조사한 8개 집단에 대해 0.190과 0.584사이에 있었으며 평균은 0.371이였다. 집단내 유전적 다양도는 0.354였다. 집단간 분화정도는 비교적 낮았다 ($G_{ST}$ = 0.065). 고정지수 분석 결과 많은 집단과 대립유전자좌위에서 이형접합체의 결핍이 있었다. 이는 세잎양지꽃은 줄기에서 꽃을 형성하여 종자번식을 하는 타가수분방식과 분지하여 새로운 개체를 형성하는 영양번식을 영위할 수 있는 다양한 번식법을 가지고 있는 클론 식물의 특성에 기인한 것으로 사료된다. 따라서 같은 집단에서 다양한 세대의 존재하여 내교잡(inbreeding)이 발생한 것으로 볼 수 있다.