Population Genetic Structure of *Potentilla discolor* Bunge, Rosaceae in Korea

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The genetic diversity and population structure of fifteen *Potentilla discolor* Bunge populations in Korea were determined using genetic variations at 19 allozyme loci. Fourteen of the 19 loci (73.7%) showed detectable polymorphism. Genetic diversity at the species level and at the population level was high (H_{ES} = 0.215, H_{EP} = 0.196, respectively), whereas the extent of the population divergence was relatively low (G_{ST} = 0.069). Total genetic diversity values (H_T) varied between 0.0 and 0.656, giving an average overall polymorphic loci of 0.292. The interlocus variation of genetic diversity within populations (H_S) was high (0.274). On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.010 for *Pgm-2* to 0.261 for *Pgd-2* with a mean of 0.069, indicating that about 6.9% 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. discolor*. The estimate of gene flow based on G_{ST} was high among Korean populations of *P. discolor* (Nm = 3.36).

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

Introduction

The amount and distribution of genetic variation within species is a subject of considerable interest because of its evolutionary importance. Many plant species have been surveyed using electrophoresis, prompting several attempts to elucidate relationships between ecological features of plants and the amount and patterns of genetic variation [3,14]. For example, study comparing closely related widespread and endemic species pairs showed that endemic species generally have less allozyme diversity [8,9].

The genus *Potentilla* is a large, diverse family (sometimes treated as many families) of 100-122 genera and 3,000-3,400 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) [23]. *Potentilla discolor* BUNGE, Rosaceae is generally distributed in fields and mountains. The species is also found in Northeast Asian regions such as Japan, central and northeast China. *P. discolor* 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, as an ornamental, 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 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. discolor*. In addition, the results are compared with plant species having similar life-history characteristics.

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Materials and Methods

Sampling procedure and enzyme electrophoresis

P. discolor was collected from fifteen populations in Korea (Fig. 1). One leaf per plant was sampled during the period from 2002 to 2005. The distance between selected individuals was about 5 m in order to avoid including individuals with common lineage. Twenty-five to thirty-six individuals were collected from each population. Leaves gathered from natural populations were stored in plastic bags for several days in a refrigerator until electrophoresis

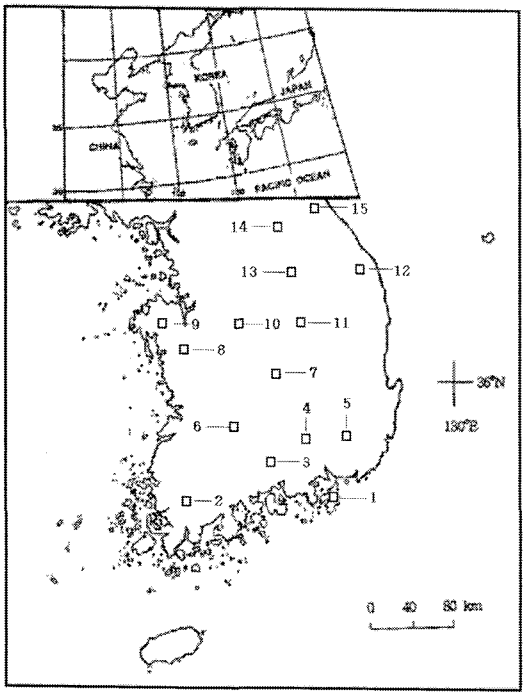

Fig. 1. Collection localities for populations of *P. discolor* as sources for allozyme analysis.
1: Koheun-ci, Gyeongsangnam-do; 2: Youngam-gun, Chonlanam-do; 3: Hadong-gun, Gyeongsangnam-do; 4: Euireong-gun, Gyeongsangnam-do; 5: Miyang-ci, Gyeongsangnam-do; 6: Chinan-gun, Chonlabuk-do; 7: Youngdong-gun, Chungchengbuk-do; 8: Cheongyang -gun, Chungcheongnam-do; 9: Seosan-ci, Chungcheong nam-do; 10: Cheongwon-gun, Chungcheongbuk-do; 11: Munkeong-ci, Gyeongsangbuk-do; 12: Samcheok-ci, Kangwon-do; 13: Woanju-ci, Kangwon- do; 14: Chuncheon-ci, Kangwon-do; 15: Goseong-gun, Kangwon-do.

was carried out.

Homogenization, starch gel electrophoresis and enzyme assay procedures were followed according to the methods of Soltis *et al.* [20]. Electrophoresis was performed using 11.0% starch gels, and a total of ten enzyme systems were assayed for this study: fluorescent esterase (FE), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), 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. discolor* allozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most allozyme plant studies, as documented by Weeden and Wendel[22].

Analysis of data

To provide information on genetic diversity of populations used in this study, we calculated the following genetic standard measures: percent polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (A_E), and gene diversity (H_E) [9]. Subscripts refer to species (s) or population (p) level parameters. Observed heterozygosity (H_O) was compared with Hardy-Weinberg expected values using Wright's fixation index (F) or inbreeding coefficients [25]. 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 [16,17]. 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 [24]. Nei's genetic identity (I) was calculated for each pairwise combination of populations [16]. A phylogenetic tree was constructed by the neighbor-joining (NJ) method using the NEIGHBOR program in PHYLIP version 3.57[18]. The genetic structure within and among populations was also evaluated using Wright's 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 [25]. Deviations of F_{IT} and F_{IS} from zero were tested using χ^2-statistics [13]. Two indirect estimates of gene flow were calculated. One estimate of Nm (the number of migrants per generation) was based on G_{ST} [25] and the other estimate was based on the average frequency of "rare" alleles found in only one population [19].

Results

Fourteen of the 19 loci (73.7%) showed detectable polymorphism in at least one population (Table 1). The remaining five loci (*Idh-1*, *Mdh-2*, *Mdh-3*, *Me*, and *Pgm-1*) were monomorphic in all populations. An average of 49.5% of loci were polymorphic within populations, with individual population values ranging from 38.1% to 52.4%. The average number of alleles per locus (A) was 1.72 across populations, varying from 1.58 for the population with the

Table 1. Allozyme variation within 15 populations of *P. discolor*

Pop.[a]	P_P	A_P	A	A_E	H_{OP}	H_{EP}
1	52.6	2.40	1.74	1.31	0.167	0.160
2	42.1	2.63	1.68	1.34	0.152	0.169
3	52.6	2.40	1.74	1.40	0.239	0.213
4	47.4	2.44	1.68	1.39	0.183	0.193
5	47.4	2.44	1.68	1.36	0.182	0.186
6	47.4	2.44	1.68	1.39	0.185	0.194
7	47.4	2.44	1.68	1.42	0.186	0.212
8	42.1	2.50	1.63	1.34	0.156	0.179
9	42.1	2.38	1.58	1.29	0.137	0.153
10	57.9	2.45	1.84	1.45	0.197	0.225
11	57.9	2.36	1.79	1.39	0.186	0.202
12	52.6	2.40	1.74	1.44	0.190	0.220
13	47.4	2.56	1.74	1.46	0.205	0.220
14	57.9	2.45	1.84	1.43	0.201	0.221
15	47.4	2.56	1.74	1.38	0.166	0.190
Mean	49.5	2.46	1.72	1.39	0.182	0.196
SD	2.94	0.07	0.07	0.05	0.005	0.014

Percentage of polymorphic loci (P), mean number of alleles per polymorphic population (A_P), 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}).

[a] : Abbreviation codes as in Figure 1.

Table 2. Estimates of genetic diversity statistics and 13 polymorphic loci in *P. discolor*

Pop.[a]	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	G_{ST}
Sod	0.525	0.513	0.012	-0.075	-0.050	0.023
Gpi	0.007	0.007	0.004	-0.064	-0.004	0.057
Mdh-1	0.441	0.428	0.013	0.154	0.178	0.029
Idh-2	0.449	0.438	0.012	0.133	0.155	0.026
Pgd-1	0.379	0.318	0.061	0.156	0.293	0.162
Pgd-2	0.423	0.312	0.111	-0.130	0.165	0.261
Me-1	0.005	0.005	0.000	-0.042	-0.003	0.037
Skd	0.008	0.008	0.000	-0.064	-0.004	0.056
Per-1	0.084	0.076	0.009	0.399	0.461	0.103
Per-2	0.411	0.404	0.007	0.310	0.322	0.018
Per-3	0.171	0.164	0.008	0.181	0.219	0.046
Pgm-2	0.478	0.473	0.005	-0.122	-0.110	0.010
Fe-1	0.054	0.047	0.007	0.269	-0.363	0.129
Fe-2	0.656	0.648	0.008	0.026	0.038	0.012
Mean	0.293	0.274	0.018	0.081	0.145	0.069

Total genetic diversity (H_T), geneticdiversity 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}).

lowest number of alleles and 1.84 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 level (A_{ES} = 1.42; A_{EP} = 1.39). The mean genetic diversity within populations was 0.196. Population 10 had the highest expected diversity (0.225), while population 9 had the lowest (0.153). Genetic diversity at the species level was 0.215. In addition, the correlation between genetic distance and geographic distance was high (r = 0.53), indicating that geographically-close populations tended to be genetically similar and about 72% (1 - r^2) of the variation in genetic distance was caused by unknown factors other than distance.

F_{IS}, a measure of the deviation from random mating within the 15 populations, was 0.081, and ranged from -0.130 for *Pgd-2* to 0.399 for *Per-1* (Table 2). The observed significant and positive F_{IS} value (0.081) 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).

Total genetic diversity values (H_T) varied between 0.0 (monomorphic loci) and 0.656 (*Fe-2*), giving an average overall polymorphic loci of 0.292. The interlocus variation of genetic diversity within populations (H_S) was high (0.274). On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.010 for *Pgm-2* to 0.261 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. discolor* (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 0.039 (data not shown). Genetic identity values among pairs of populations range from 0.962 to 0.997. The similarity among *P. discolor* populations can be seen in the dendrogram, where total populations cluster below a genetic distance of 0.40 (Fig. 2). Although some populations have exception, geographically close populations situate close positions in the phylogenetic tree.

Discussion

Genetic diversity

In allozyme analysis, natural populations belonging to *P. discolor* maintain a higher than average level of genetic

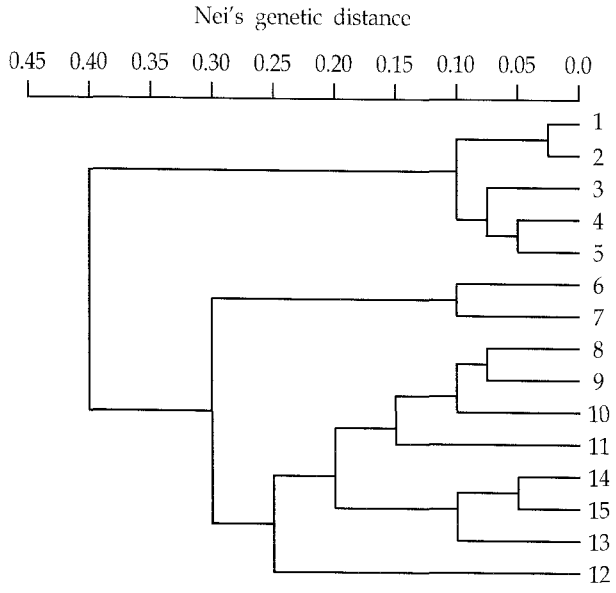

Fig. 2. A dendrogram showing the genetic relationships among the 15 populations of *P. discolor*, based on genetic distance data.

diversity compared with other plant species. For example, its genetic diversity of 0.215 is higher than that of temperate-zone species (0.146), species with sexual and asexual reproduction modes (0.138), similar to long-lived perennial herbaceous species (0.205), and those with wide spread geographic ranges (0.202) [8]. The percent of polymorphic loci at the species level was 73.7%. 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%) [8]. 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) [8]. The same trend is observed at the population level.

The relatively high level of genetic variation found in *P. discolor* is consistent with several aspects of its biology. First, the species is found throughout Northeast Asia including Japan, China and Korea [11]. Geographic range has been shown to be strongly associated with the level of variation maintained within populations and at the species level [8]. Widely-distributed plant species tend to maintain more variation than more-narrowly-distributed species. Second, long-lived perennial species like *P. discolor* generally maintain relatively higher levels of variation than annuals and short-lived perennials [14]. As populations of *P. discolor* live longer, there should be more opportunities for the accumulation of mutations [12]. In nature, clone genotypes may be long-lived clones of some grasses routinely live for a few hundred years [5]. Because *P. discolor* can produce a few ramets per year [11], the persistence of multiple generations are associated with the high level of genetic variation. Finally, the reproduction type of *P. discolor* 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 [7]. Vegetative reproduction and spread can also affect the genetic structure of populations [15]. Cook [2] 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. 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 [10] 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. discolor* usually propagates by asexually-produced rhizomes when several strong environmental disadvantages influence the habitat of this species.

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 [4,5,6,21]. 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 [2,4]. *P. discolor* is no exception, and consists of repetitive units (ramets) which may be interconnected via rhizomes. The species flowers mainly in March or April, 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 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.

Genetic differentiation among populations is principally a function of gene flow among populations via pollen and seed dispersal [14]. The majority of genetic diversity observed at the polymorphic loci in *P. discolor* 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. discolor*.

Heterozygote deficiency do not become an eyesore (F_{IS} = 0.081). 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 [15]. Considering the near-clonal propagation observed in *P. discolor*, 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. In addition, this patch distribution of related individuals should generate a Wahlund effect. The 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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초록 : 한국내 솜양지꽃의 집단 유전 구조

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한국내 분포하는 장미과의 솜양지꽃(*Potentilla discolor* Bunge) 15집단에 대한 19 알로자임 대립유전자좌위에서 유전적 다양성과 집단구조를 분석하였다. 조사한 좌위에 대해 약 73.7%가 다형성을 나타내었다. 종과 집단 수준에서 유전적 다양도는 각각 0.215, 0.196이었으며, 집단간 분화정도는 낮았다(G_{ST} = 0.069). 전체 유전적 다양성는 0~0.656이며 평균 0.292였다. 유전적 다양도 중 집단내 변이는 높았다(H_S = 0.274). 전체 유전적 변이에서 집단간 차이는 *Pgm-2*에서 0.010, *Pgd-2*에서 0.261로 평균 0.069였다. 이는 전체 알로자임 변이 중 약 6.9%가 집단간에 있음을 의미한다. 솜양지꽃의 특성으로 광범위한 분포, 다년생 초본, 여러 세대의 존재 등이 높은 유전적 다양성을 나타내는데 기여하는 것으로 설명된다. 조사한 솜양지꽃 집단에서 세대당 이주하는 개체수는 3.36으로 평가되었다.