Comparison of Reproductive Modes in Populations of *Potentilla freyniana*

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ABSTRACT: The mating systems of natural populations of *Potentilla freyniana* in Korea were determined using allozyme analysis. The result suggests that *P. freyniana* is outcrossing as well as employing vegetative reproduction by stolon (self-fertilization rate, $s < 0.5$). The values of the inbreeding coefficient of eight populations in Korea varied from 0.244 to 0.331, with an average value of 0.274. For eight natural populations, multi-locus estimates of outcrossing (t_m) was 0.603 across 13 polymorphic loci, with individual population values ranging from 0.530 to 0.652. The relatively low outcrossing rates of some populations could be attributed to extensive vegetative reproduction by stolon and the isolation of flowering mature plants. 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. Although heterozygote excess was observed in some natural populations, most populations exhibited varying degrees of inbreeding and a heterozygote deficit.

Key words: Allozyme analysis, Mating systems, *Potentilla freyniana*

INTRODUCTION

Most plants, especially rhizomatous and stoloniferous species, have physical connections among ramets, although the level of persistence is highly variable among species and habitats (Sobey and Barkhouse 1977). Studies on the genetic structure of clonal 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". However, 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. Despite the importance of genetic variation data for conservation purposes and for the understanding of 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 (Choung 1988, Min 2006).

The genus *Potentilla* includes about 300 species of medium- to large-sized herbs distributed throughout the temperate and arctic regions. *Potentilla freyniana* Bornmueller (Rosaceae) is distributed in fields and mountains. *P. freyniana* is a diploid (2n=14) and predominantly insect-pollinated herb (<1 m in height) that blooms from April to June. This species is long-lived, and can reproduce both by vegetative reproduction and by the sexual production of seeds. Rhizomes of *P. freyniana* are generally horizontal stems at the plant's nodes.

Allozyme variation within plant populations often shows structured patterns in space, presumably reflecting kinship structures that have arisen due to restricted dispersal (Heywood 1991, Kang and Lee 2006). Once kinship structure is established, restricted seed and pollen dispersal leads to sexual reproduction by genetic relatives, a phenomenon known as biparental inbreeding (Uyenoyama 1986). Thus, even populations of obligate outcrossers may routinely experience some level of inbreeding (Heywood 1993).

Brown (1990) classified plant mating systems into five major models: (1) predominant selfing (with outcrossing rate, $t < 0.1$); (2) predominant outcrossing (self-fertilization rate, $s < 0.5$); (3) mixed selfing and outcrossing; (4) facultative or obligate apomixis; and (5) intragametophytic or haploid selfing. Mating systems are dynamic and can vary in space and time (Liengsiri et al. 1998). For instance, Murawski et al. (1994) reported that population and individual outcrossing estimates were associated with tree density or degree of spatial isolation. In addition, selfing rates, inbreeding depression, and relative fecundity may differ at different life stages (Maki 1993). In this study I investigated the mating system of eight natural populations of *P. freyniana* in Korea using allozyme markers.

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MATERIALS AND METHODS

Sampling Procedure

P. freyniana was collected from eight populations which represent the geographical range of the plant in Korea and have large effective population sizes. Plants of this species were not distributed within communities but, rather, were clustered in distinct patches (Table 1). Quadrats were set up at 6~8 sites (subpopulations) per population. At each site, 11 contiguous 1 m × 1 m quadrats were established along an 11 m transect. One leaf per plant was sampled from 2005 to 2006. I selected individuals about 5 m apart for sampling to avoid including individuals from a 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

I conducted homogenization, starch gel electrophoresis and enzyme assays on the plant samples following 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. Enzyme electrophoresis was performed using 12.0% starch gels. The buffer systems and enzyme staining procedures of Soltis et al. (1983) 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 involved 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. freyniana* allozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most allozyme plant studies, as documented by Weeden and Wendel (1989).

Data Analysis

A locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. A multilocus mixed mating program (MLT) was used to estimate multilocus outcrossing rates (*tm*) based on the mixed mating model of Ritland (1990). The model assumptions are as follows: (1) each mating event involves a random outcrossing event (with probability *t*) or self-fertilization (with probability $s = 1 - t$), (2) the probability of an outcross is independent of maternal genotype, (3) outcross pollen allele frequencies are homogeneous among maternal genotypes, (4) selection does

not occur between fertilization and the assay of progeny genotypes, and (5) alleles at different loci segregate independently (for multilocus estimates) (Liengsiri et al. 1998).

The observed inbreeding coefficient (*F*) of Wright (1965) was calculated for natural populations as $F = 1 - (Ho/He)$, where *Ho* is the observed heterozygosity, $He = 1 - pi^2$ is the expected heterozygosity under random mating, and *pi* is the frequency of the ith allele (Liengsiri et al. 1998). The expected inbreeding coefficient at equilibrium (*Fe*) was calculated from the multilocus outcrossing rate (*tm*) using the equation of Fyfe and Bailey (1951): $Fe = (1 - tm)/(1 + tm)$. The observed heterozygosity was compared with Hardy-Weinberg expected values using Wright's fixation index (Wright 1922). These indices were tested for deviation from zero by χ^2 statistics following Li and Horvitz (1953).

RESULTS

Table 1 shows the number of ramets in quadrats at the sites. Although the mean number of ramets per site for the Mt. Geumjeong population was slightly greater than for the remainder of the populations, it was not significantly different ($F = 2.01$, $F_{0.05,55} = 2.21$). Thirteen of the 19 loci (68.4%) showed detectable polymorphism in at least one population. 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 remaining six loci (*Idh-1*, *Mdh-2*, *Mdh-3*, *Me-1*, *Me-2*, and *Pgm-1*) were monomorphic in all populations (Table 2). The fixation indices for 38.5% of polymorphic loci (5/13) departed significantly from zero. On average, 57.2% of loci were polymorphic within populations, with individual population values ranging from 47.4% to 68.4%.

Table 1. Numeric code, population location, and sample size of *P. freyniana*

Code	Location	Sample size (n)	No. of sites	Mean ramets/ site
POT-1	Mt. Seorak, Yangyang Pref.	42	8	40.0±6.90
POT-2	Mt. Odae, Myeongju Pref.	36	6	44.1±8.17
POT-3	Mt. Worak, Mungyeong Pref.	48	8	37.3±4.29
POT-4	Mt. Sobaek, Yeongpoong Pref.	44	7	41.7±4.68
POT-5	Mt. Deogyu, Muju Pref.	46	8	38.9±5.51
POT-6	Mt. Jiri, Sancheong Pref.	45	8	42.9±8.70
POT-7	Mt. Weolchul, Youngam Pref.	40	6	43.5±9.95
POT-8	Mt. Geumjeong, Busan Pref.	38	6	51.5±7.54

For the eight study populations, the mean inbreeding coefficient (F) was 0.274 across 13 polymorphic loci, with individual population values ranging from 0.244 (population POT-7) to 0.331 (population POT-2) (Table 3). The F values were significantly heterogeneous for the *Skd* locus. For example, 60% of F indices (3/5) were negative for the five polymorphic populations (POT-1, POT-2, POT-3, POT-4, and POT-5).

Multilocus outcrossing rates (tm) averaged 0.606, with individual population values ranging from 0.530 (population POT-2) to 0.652

Table 2. Chi-square test for allele frequencies and heterogeneity (DF) of 13 polymorphic loci

Locus	Value of chi-square	DF	p
Adh	17.254	14	ns
Fe-1	39.102	7	***
Fe-2	12.888	14	ns
Gpi	147.312	7	***
Idh-2	17.783	14	ns
Mdh-1	18.947	14	ns
Per-1	21.499	14	ns
Per-2	3.827	7	ns
Per-3	21.398	7	ns
Pgd-1	41.471	7	***
Pgd-2	33.352	7	***
Pgm-2	10.383	7	ns
Skd	51.951	7	***

ns and *** are non-significant at 5% and significant at 0.1% levels, respectively.

(population POT-7) (Table 4). The expected inbreeding coefficient at equilibrium (Fe) was 0.592.

Tests for deviations of mating systems from equilibrium revealed somewhat different results in each natural population (Table 5). 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 fixation indices for polymorphic loci were positive in most cases (84/87), and 53.6% of the positive indices (45/84) departed significant from zero. Only three indices were negative, indicating an excess of heterozygosity for the *Skd* locus in populations POT-1, POT-3, and POT-4. However, none of the negative indices differed significant from zero ($p<0.05$).

DISCUSSION

The presence or absence of self-incompatibility mechanisms, availability of pollinators and their foraging behavior, and flower density and phenological variation are among the factors that affect plant mating systems (Brown and Allard 1970, Clegg 1980, Charlesworth and Charlesworth 1987, Escalante et al. 1994). The mean value of tm of eight populations of *P. freyniana* in Korea was 0.603.

The observed significant and positive F values indicate that homozygotes were in excess for most populations and loci examined. Significant deficiencies of heterozygosity for each polymorphic locus indirectly indicate the existence of inbreeding. Generally, seedlings are expected to display higher levels of inbreeding than adults (Holsinger 1991, Cruzan et al. 1994). These high levels of inbreeding can result from a variety of causes in mixed mating species such as *P. freyniana*: positive assortative maing (i.e., preferential mating among similar genotypes), selection for homozygotes, and family structure within a restricted neighborhood, pro-

Table 3. Inbreeding coefficients (F) for eight populations of *P. freyniana*

Pop.	Adh	Fe-1	Fe-2	Gpi	Idh-2	Mdh-1	Per-1	Per-2	Per-3	Pgd-1	Pgd-2	Pgm-2	Skd	Mean
POT-1	0.145	0.291	0.475	0.818	0.248	0.382	0.518	0.127	0.193	0.000	0.341	0.507	−0.065	0.306
POT-2	0.299	0.157	0.505	0.689	0.392	0.223	0.491	0.382	0.278	0.289	0.080	0.476	0.036	0.331
POT-3	0.405	0.322	0.473	0.515	0.178	0.201	0.430	0.164	0.179	0.000	0.278	0.657	−0.333	0.267
POT-4	0.333	0.576	0.559	0.000	0.387	0.356	0.417	0.125	0.120	0.000	0.278	0.721	−0.267	0.277
POT-5	0.367	0.000	0.000	0.000	0.266	0.286	0.483	0.208	0.000	0.467	0.266	0.623	0.242	0.247
POT-6	0.352	0.000	0.000	0.000	0.305	0.354	0.586	0.151	0.201	0.400	0.505	0.515	0.000	0.259
POT-7	0.440	0.000	0.000	0.000	0.144	0.420	0.584	0.116	0.231	0.429	0.344	0.470	0.000	0.244
POT-8	0.236	0.000	0.000	0.000	0.422	0.306	0.690	0.246	0.328	0.429	0.000	0.777	0.000	0.264
Mean	0.322	0.168	0.251	0.253	0.293	0.316	0.525	0.190	0.191	0.252	0.262	0.593	−0.048	0.274

Table 4. Multilocus outcrossing rates (t_m) for eight populations of *P. freyniana*

Pop.	Adh	Fe-1	Fe-2	Gpi	Idh-2	Mdh-1	Per-1	Per-2	Per-3	Pgd-1	Pgd-2	Pgm-2	Skd	Mean
POT-1	0.746	0.549	0.356	0.100	0.602	0.447	0.318	0.775	0.676	1.000	0.491	0.328	1.000	0.568
POT-2	0.539	0.728	0.329	0.184	0.436	0.635	0.341	0.447	0.565	0.552	0.852	0.355	0.931	0.530
POT-3	0.424	0.512	0.358	0.320	0.698	0.665	0.399	0.719	0.696	1.000	0.565	0.207	1.000	0.582
POT-4	0.500	0.269	0.283	1.000	0.442	0.475	0.412	0.778	0.786	1.000	0.565	0.162	1.000	0.590
POT-5	0.463	1.000	1.000	1.000	0.580	0.555	0.348	0.655	1.000	0.363	0.580	0.232	0.610	0.645
POT-6	0.479	1.000	1.000	1.000	0.533	0.477	0.261	0.737	0.666	0.429	0.329	0.320	1.000	0.633
POT-7	0.389	1.000	1.000	1.000	0.748	0.409	0.263	0.792	0.625	0.399	0.488	0.360	1.000	0.652
POT-8	0.619	1.000	1.000	1.000	0.407	0.532	0.183	0.605	0.506	0.400	1.000	0.125	1.000	0.644
Mean	0.513	0.712	0.598	0.597	0.547	0.520	0.312	0.681	0.679	0.598	0.585	0.255	1.102	0.603

Table 5. Wright's fixation indices for eight populations of *P. freyniana*

Pop.	Adh	Fe-1	Fe-2	Gpi	Idh-2	Mdh-1	Per-1	Per-2	Per-3	Pgd-1	Pgd-2	Pgm-2	Skd
POT-1	0.157	0.304	0.482[**]	0.821[***]	0.260	0.390[*]	0.524[***]	0.136	0.208	–	0.353	0.515[**]	–0.136
POT-2	0.309	0.176	0.512[**]	0.693[***]	0.404[*]	0.237	0.499[**]	0.397	0.292	0.300	0.093	0.484[**]	0.054
POT-3	0.412[*]	0.336	0.480[**]	0.523[**]	0.192	0.214	0.439[**]	0.181	0.195	–	0.287	0.664[**]	–0.306
POT-4	0.342[*]	0.586[**]	0.566[***]	–	0.400[*]	0.366[*]	0.425[**]	0.141	0.136	–	0.286	0.726[***]	–0.233
POT-5	0.374[*]	–	0.475[**]	–	0.268	0.297	0.490[***]	0.222	–	0.477[*]	0.276	0.629[**]	0.258
POT-6	0.361[*]	–	0.589[***]	–	0.316	0.363[*]	0.592[***]	0.167	0.216	0.413[*]	0.511[***]	0.522[**]	–
POT-7	0.448[*]	–	0.544[***]	–	0.161	0.429[**]	0.589[***]	0.131	0.243	0.440[*]	0.355	0.478[**]	–
POT-8	0.253	–	0.566[***]	–	0.432[*]	0.316	0.694[***]	0.261	0.341	0.435[*]	–	0.780[***]	–

[*]$p<0.05$. [**]$p<0.01$, [***]$p<0.001$. Monomorphism for a population is indicated with a dash.

moting mating among relatives. The significant deficiency of heterozygotes found in many populations may be due in part to selection for homozygotes. Selection for heterozygotes typically occurs in more extreme environments. The reproductive strategy of *P. freyniana* could also explain the observed inbreeding level. Because *P. freyniana* is bisexual it is expected that the inbreeding detected is due to consanguineous and self-mating. Nei et al. (1975) have shown that the reduction in average heterozygosity per locus depends not only on the size of population bottlenecks, but also on the subsequent rate of population growth. If population growth is reduced, reduction in average heterozygosity is large, even given a small number of founders.

In most species of *Potentilla*, especially perennial herbs, plants consist of a series of internodes (Li et al 2003). A ramet may produce terminal flowers in the year it is formed (Eriksen 1997, Spomer 1999). Many plants have two reproductive strategies, sexual reproduction via production of seeds and clonal propagation via the

development of vegetative stolons through the growth of a coordinated group of cells that form a meristem (Cook 1985, Eriksson 1987). *P. freyniana* is no exception, and consists of repetitive units (ramets) which may be interconnected via rhizomes (Fig. 1). 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) first become visible in July. These observations suggest that the study populations might have been founded from asexual fragmentation and dispersal of preexisting clones rather than from sexually-produced seeds.

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 therefore
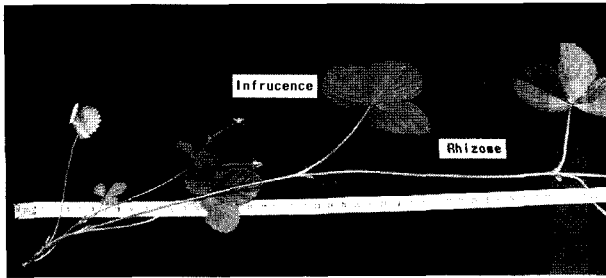
Fig. 1. Growth form of *P. freyniana* with clonal reproduction.

act to enhance genetic variation and asexual reproduction could then maintain the enhanced genetic variation (Bayer 1990). *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. Their asexual reproduction assures the stabilization and persistence of a phenotype that is well adapted to the immediate environment (Huh 2001). Although *P. freyniana* is able to reproduce by sexually-produced seeds, its ratio of asexual/sexual reproduction has not yet been studied.

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