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Genetic diversity assessment of wild populations of *Paeonia lactiflora* Pall. in Gyeongju National Park, Korea

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경주국립공원 내 야생 작약(*Paeonia lactiflora* Pall.) 집단의 유전다양성 분석

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ABSTRACT: *Paeonia lactiflora* is a valuable natural resource for horticulture and traditional Chinese medicine. To propose conservation strategy and future utility of the wild *Paeonia lactiflora* populations recently found around the Gyeongju National Park, genetic diversity analysis using microsatellite markers were performed. Three populations in and near the Gyeongju N.P. and one population from Jilin, China were analyzed for five microsatellite markers, producing 61 alleles with mean observed heterozygosity(H_o) of 0.452. F_{ST} value (0.11642) suggested moderate level of genetic differentiation among the populations, and hierarchical AMOVA suggested most of the genetic variation resides within/among the individuals rather than among-population. While AMOVA with F_{ST} suggested lack of genetic differentiation between the regional (Korean vs. Chinese) populations, AMOVA with R_{ST}, which incorporates the allele sizes, suggested considerable differentiation between them, but without significant statistical support. STRUCTURE analysis also suggested segregation of regional populations with presence of gene flow among the three Gyeongju N.P. populations. Considering small population size and scarcity of mature individuals, further protection and long-term monitoring are needed.

Keywords: *Paeonia lactiflora*, Gyeongju National Park, Genetic diversity, microsatellite marker, conservation

적 요: 작약은 원예 및 전통한약으로 중요한 자연자원이다. 경주국립공원 일대에서 발견된 야생 작약 집단에 대한 보전 및 활용 방안 마련을 위해 마이크로새틀라이트 마커를 활용한 유전적 다양성 분석을 수행하였다. 경주국립공원 일대 3개 집단과 중국 연변 1개 집단 등 4개 집단을 대상으로 유전적 다양성 분석을 수행한 결과, 5개의 마이크로새틀라이트 마커로부터 61개의 대립유전자를 확인하였으며, 평균 이형접합성(H_o)은 0.452로 나타났다. 집단 간의 유전적 분화는 F_{ST}=0.116로 볼 때 비교적 낮은 수준인 것으로 나타났으며, 계층적 AMOVA 분석 결과 유전적 변이가 집단 간보다는 집단 내 개체사이에 분포하는 것으로 확인되었다. 그러나 F_{ST}값 대신 대립유전자의 크기를 고려한 R_{ST} 값을 사용한 AMOVA 분석 결과에서는 중국 집단과 국내 집단 사이에 두드러진 차이가 나타났다. 이러한 양상은 STRUCTURE 분석에서도 확인되었다. 한편, 경주국립공원 일대 3개 집단 사이에는 지속적인 유전자 흐름이 일어나고 있는 것으로 확인되었으며, 작은 집단 크기와 성숙한 개체가 적은 것으로 볼 때, 추가적인 보호 및 장기 모니터링이 필요할 것으로 판단된다.

주요어: 작약, 경주국립공원, 유전적 다양성, 마이크로새틀라이트, 보전

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Paeoniaceae is consisted of monotypic genus *Paeonia*, whose species are distributed mainly in temperate East Asia and Europe (30-40 species), and western North America (1-2 species; Hong et al., 2001; Hong, 2010, 2011). Paeoniaceae had been traditionally placed in Ranunculaceae, Ranunculales, but recent molecular phylogenetic studies placed it within Saxifragales, core eudicots (The Angiosperm Phylogeny Group, 2009). Genus *Paeonia* is subdivided into 3 sections - sect. *Moutan* which includes woody species, and sect. *Onepia* and sect. *Paeonia* which include herbaceous species (Hong, 2010). There are 15 species reported for China, four species for Korea, and two species in Japan, respectively, including cultivated species

Four *Paeonia* species in Korea are *P. suffruticosa* Anders. of sect. *Moutan*, *P. obovata* Maxim., *P. japonica* Miyabe & Takeda, and *P. lactiflora* Pall. of sect. *Paeonia*. Out of the four, *P. suffruticosa* Anders. and *P. lactiflora* var. *hortensis* Makino (horticultural variety of *P. lactiflora*) have long been cultivated in Korea for traditional Chinese medicine and gardening, probably imported from China more than a thousand years ago.

In case of wild *P. lactiflora*, there are three varieties reported for Korean flora - var. *lactiflora*, var. *hirta* Regel, and var. *trichocarpa* (Bunge) Stern, but research on their identity has been limited due to lack of herbarium specimens and available samples in Korea. Also, there has been different opinion about the taxonomic status of these varieties. Especially Hong et al. (2001) and Hong (2010) treated these varieties of *P. lactiflora* as belonging to a same taxon, since those morphological characters such as color of petal, density and distribution of trichomes on leaf surface, which has long been recognized as key morphological characters distinguishing varieties, were unreliable and showed wide range of variation. Also, DNA sequence analysis on the Korean *Paeonia* species using variable regions of nuclear, chloroplast and mitochondrial genomes couldn't distinguish the varieties of *Paeonia* species (Lim and Won, unpublished data).

Although *P. lactiflora* has been one of the four major garden flowers of China and a major horticultural resource for breeding, not much research has been done for the species due to the lack of available wild populations. Since 2008, however, wild populations of *P. lactiflora* var. *trichocarpa* have been discovered from Youngwol and Samcheok of Gangwon Prov., and Pohang of Gyungbuk Prov. These discoveries suggest that there may be more wild populations of *P. lactiflora* exist in Korea. Still, the number of flowering/fruiting individuals and individuals per population, number of populations reported have been pretty much limited, which made morphological, taxonomical and genetic study of the species difficult.

Here we are presenting the results of the genetic diversity assessment of those wild *P. lactiflora* populations discovered in/near the Gyeongju National Park for the first time. We have applied microsatellite markers to estimate the genetic diversity and genetic relationships within/among the wild populations and are suggesting conservation strategy and utilization of the populations. This will enhance our understanding on the evolution, diversification of the group and further utilization of their genetic resources in horticulture.

Materials and Methods

Sampling: DNA samples of wild *P. lactiflora* populations were collected during June - August 2012, from 2 sites located within the Gyeongju National Park, Gyeongju and 1 site in Pohang, Gyungbuk (Table 1). In addition to the three populations, we have collected one population from Jilin, China for comparison as outgroup (Table 1). Although those in Korea have sometimes been treated as var. *trichocarpa* and those in Jilin as var. *hirta* (Lee, 2006), we treated them as conspecific *P. lactiflora* following Hong's opinion (2010). The distance between Pohang and two Gyeongju populations are 32 km and 38 km, respectively, and the distance between the two Gyeongju populations are 18 km apart, straightly. DNA samples were obtained by collecting part of terminal leaf section (ca. 2×5 cm), and obtained leaf materials were stored

Table 1. Localities of wild *Paeonia lactiflora* populations analyzed in the study.

Popn.	n	Locality	GPS coordinates*	Altitude
Pohang	16	Korea. Gyungbuk, Pohang	N36° 02' **.*" E129° 31' **.*"	144 m
Toham	23	Korea. Gyungbuk, Mt. Toham	N35° 48' **.*" E129° 19' **.*"	438 m
Gumi	19	Korea. Gyungbuk, Mt. Gumi	N35° 53' **.*" E129° 08' **.*"	469 m
Jilin	23	China. Jilin, Wangqing	N43° 32' **.*" E129° 39' **.*"	369 m
Total	81			

*For the protection of the populations, detailed GPS coordinates are omitted. The information is available upon request to the corresponding author.

in silica-gel or stored fresh in refrigerator until DNA extraction.

Extraction of Total DNA: DNA was extracted using DNeasy Plant mini Kit(QIAGEN, Germany), after removing leaf veins from the leaf sample. We used 50-100 mg of fresh leaf tissue or 20-40 mg of dried leaf tissue. Quantity of the extracted DNA was measured by comparing the strength of fluorescence with the Lambda DNA/Hind plus marker or 100 bp DNA Ladder under the UV trans-illuminator, after Ethidium Bromide (EtBr) stained 1.0% agarose gel electrophoresis running.

Microsatellite marker selection and amplification: Microsatellite markers for *P. lactiflora* have been developed by Sun et al. (2011) and Li et al.(2011) for wild populations. For this study, we tested the ten microsatellite markers developed by Sun et al. (2011) for those Korean populations. For marker selection, we tested the microsatellite markers' amplification and validity of alleles amplified, using representative DNAs of four populations. We applied Schuelke's (2000) method for microsatellite amplification. That is, we used both the M13-linked forward primer and the fluorescent dye(VIC, PET, FAM)-linked M13 primer to amplify the microsatellite fragments together with the reverse primer. Once amplified, the amplified microsatellite fragments were run with 2% agarose gel to check amplification and size. We successfully amplified Pmg117, Pmg153, Pmg155, Pmg164, Pmg165, Pmg180, Pmg183, and Pmg209, while failed in Pmg50 and Pmg196 of Sun et al. (2011). Amplified microsatellite fragments were sent to Biomedic Ltd. (Bucheon, Gyunggi, Korea) for genotyping. We found that six microsatellite markers except Pmg183 and Pmg209 were useful, and applied those six microsatellite markers to all the samples. For multiplex-running, we used FAM for Pmg117 and Pmg153, PET for Pmg155 and Pmg165, and VIC for Pmg164 and Pmg180, respectively, and mixed three amplified microsatellite markers together for genotyping at Biomedic Ltd.

Analysis of genetic diversity using microsatellite markers: Six microsatellite markers, Pmg117, Pmg153, Pmg155, Pmg164, Pmg165, and Pmg180, were amplified and genotyped for all the *P. lactiflora* samples obtained to analyze their genetic diversity, following the protocols explained previously. Pmg180 were excluded from the analysis due to many null alleles, and the remaining five microsatellite markers were coded. Fragment size data were converted into input data file using Convert program (Glaubitz, 2004). Using GENEPOP (v 4.0; Raymond and Rousset, 1995; http://genepop.curtin.edu.au/) and FSTAT (v 2.9.3.2; Goudet 2001), Arlequin (ver. 3.0; Excoffier et al., 2005), we estimated the *NA* (number of alleles), *AR* (allelic richness), H_O (observed heterozygosity), and H_E (expected heterozygosity under H-W equilibrium), F_{IS} (inbreeding coefficient; Weir and Cockerham, 1984) and F_{ST} (the effect of subpopulations (S) compared to the total population (T); Weir and Cockerham, 1984), and Slatkin's R_{ST} (Slatkin, 1995) based on stepwise mutation model. Hardy-Weinberg equilibrium (HWE) test using Markov Chain were done with GENEPOP with exact test (Guo and Thompson, 1992). The MICROCHECKER program (Van Oosterhout et al. 2004) was used to check for null alleles and scoring errors due to stuttering or large allele drop-out.

To estimate the pairwise genetic differentiation among the populations, we estimated the population pairwise F_{ST}'s and pairwise R_{ST}'s using Arlequin. We used STRUCTURE (ver. 2.0; Pritchard et al., 2000) for model-based clustering of genotypes. We applied admixture model, which clusters each individual based on its genetic information disregarding its original grouping, by running Monte Carlo Markov chain for 60,000 iteration with 30,000 initial burn-in with Bayesian statistics model. We tested K values from 2 to 6, with 10 iteration per each K value for best fit likelihood value cluster model. Also, we performed a hierarchical AMOVA (analysis of molecular variance) using F_{ST} and R_{ST} values obtained from Alrequin. For the test of the existence of regional groups, we subdivided the Korean and Chinese populations as two separate regional groups.

Results

We were able to amplify five microsatellite markers out of ten developed by Sun et al., (2011) from wild *P. lactiflora* populations. We obtained 2-23 alleles per locus and 61 alleles in total (Table 2). 23 alleles were detected from Pmg117, while there are only two alleles from Pmg164. Pmg153, Pmg155, and Pmg165 presented significantly smaller H_O compared to

Table 2. Allelic variability observed at five microsatellite loci of the four wild *Paeonia lactiflora* populations.

Microsatellite loci		Population(n)				
		Pohang (16)	Toham (23)	Gumi (19)	Jilin (23)	Total (81)
Pmg117						
	GD	0.688	0.914	0.803	0.886	0.823
	NA	7	14	8	14	23
	AR	6.87	12.05	7.5	11.33	13.77
	H_E	0.690	0.911	0.798	0.874	0.925
	H_O	0.750	0.913*	0.632**	1.000	0.840*
	F_{IS}	−0.091	0.001	0.213	−0.128	−0.005

Table 2. Continued.

Microsatellite loci		Population(n) Pohang (16)	Toham (23)	Gumi (19)	Jilin (23)	Total (81)
Pmg153						
	GD	0.844	0.800	0.522	0.834	0.750
	NA	7	6	3	7	11
	AR	6.88	5.85	2.99	6.59	8.73
	H_E	0.827	0.791	0.526	0.818	0.818
	H_O	0.313**	0.391**	0.647**	0.773	0.538*
	F_{IS}	0.630*	0.511*	−0.239	0.074	0.290
Pmg155						
	GD	0.788	0.565	0.426	0.335	0.531
	NA	6	4	4	7	10
	AR	6.00	3.88	3.75	5.54	6.23
	H_E	0.756	0.559	0.431	0.330	0.537
	H_O	0.267**	0.304*	0.263**	0.182*	0.253*
	F_{IS}	0.662*	0.462*	0.396	0.458*	0.506
Pmg164						
	GD	0.063	0	0	0.231	0.074
	NA	2	1	1	2	2
	AR	1.94	1.00	1.00	2.00	1.77
	H_E	0.063	N/A	N/A	0.232	0.083
	H_O	0.063	N/A	N/A	0.261	0.086
	F_{IS}	0	N/A	N/A	−0.128	−0.108
Pmg165						
	GD	0.740	0.709	0.523	0.640	0.653
	NA	7	5	5	5	15
	AR	6.87	4.87	4.92	4.53	8.39
	H_E	0.740	0.704	0.518	0.639	0.737
	H_O	0.876	0.478*	0.316**	0.565	0.543*
	F_{IS}	−0.183	0.326*	0.397*	0.117	0.167
All loci						
	GD	0.625	0.598	0.457	0.585	0.566
	NA	29	30	21	35	61
	AR	28.56	27.65	20.16	29.97	38.88
	H_E	0.615	0.742	0.568	0.579	0.620
	H_O	0.453	0.522	0.464	0.556	0.452
	F_{IS}	0.274	0.302	0.187	0.050	0.186

n, sample size; GD, gene diversity per locus and population; NA, number of alleles per locus; AR, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient of Weir & Cockerham (1984).
* indicates P<0.01 ** indicates P<0.001

Table 3. Pairwise F_{ST} and R_{ST} values estimated between the wild *Paeonia lactiflora* populations. Above the diagonal is R_{ST}, below is F_{ST}.

Popn.	Pohang	Toham	Gumi	Jilin
Pohang	-	0.18083**	0.15280*	0.50070**
Toham	0.10188**	-	−0.01706	0.31214**
Gumi	0.16465**	0.11484**	-	0.29293**
Jilin	0.15212**	0.09132**	0.11971**	-

* indicates P<0.01; ** indicates P<0.001

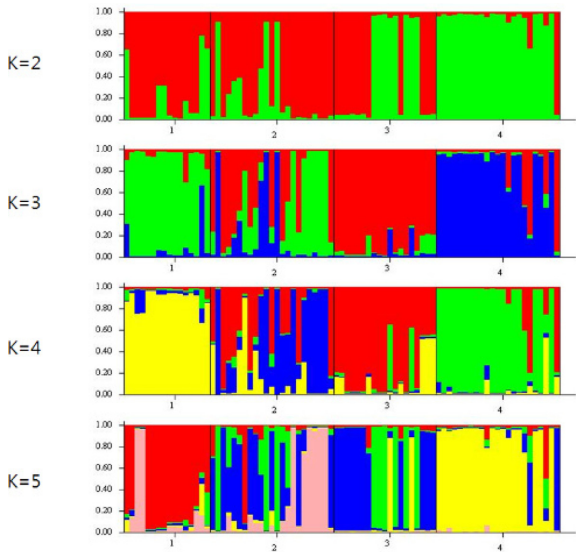

Fig. 1. Estimated genetic subsamples of four wild *Paeonia lactiflora* populations generated by STRUCTURE program. Each color represents a different genetic cluster and black lines separate the populations. Numbers beneath the cluster diagrams refer to Pohang (1), Toham (2), Gumi (3), and Jilin (4) populations, respectively.

H_E under H-W equilibrium (P<0.001), which may have been caused by homozygote excess according to the result of Micro-Checker. These loci also presented higher F_{IS} value than other loci. By the way, Pmg164 showed lowest number of alleles and heterozygosity than other loci. Chinese population (Jilin population) showed the highest H_O of 0.556, and Toham (0.522), Gumi (0.464), and Pohang (0.453), respectively, without noticeable difference among populations. H_E under H-W equilibrium is highest in Toham (0.742), Pohang (0.615), Jilin (0.579), and Gumi (0.568), respectively. *Nm* (number of migrants) was estimated as 1.257 after correction for population size.

Overall F_{ST} value of 0.11642 (P<0.001) suggests moderate genetic differentiation among the populations according to Wright (1978). Population pairwise F_{ST} is highest between

Table 4. Result of the hierarchical AMOVA (analysis of molecular variance) applying the F_{ST} values.

Source	d.f.	S.S.	Variance	% Var	Statistics	P-value
No grouping						
Among populations	3	26.711	0.18091	11.64	F_{ST} = 0.11642	<0.001
Within populations	77	125.405	0.25569	16.45	F_{IS} = 0.18623	<0.001
Within individuals	81	90.500	1.11728	71.90	F_{IT} = 0.28097	<0.001
Regional grouping (S Korea vs. China)						
Among group	1	9.386	−0.00555	−0.36	F_{CT} = −0.00358	0.753
Among populations within groups	2	17.325	0.18394	11.86	F_{SC} = 0.11814	<0.001
Within populations	77	125.406	0.25569	16.48	F_{IS} = 0.18623	<0.001
Within individuals	81	90.500	1.11728	72.02	F_{IT} = 0.27980	<0.001

Table 5. Result of the hierarchical AMOVA (analysis of molecular variance) applying the R_{ST} values.

Source	d.f.	S.S.	Variance	% Var	Statistics	P-value
No grouping						
Among populations	3	2659.739	20.29005	28.03	R_{ST} = 0.28033	<0.001
Within populations	77	5438.953	18.54627	25.62	R_{IS} = 0.35605	<0.001
Within individuals	81	2717.000	33.54321	46.34	R_{IT} = 0.53656	<0.001
Regional grouping (S Korea vs. China)						
Among group	1	2172.606	28.87643	33.78	R_{CT} = 0.33778	0.246
Among populations within groups	2	487.132	4.52208	5.29	R_{SC} = 0.07988	0.003
Within populations	77	5438.953	18.54627	21.69	R_{IS} = 0.35605	<0.001
Within individuals	81	2717.000	33.54321	39.24	R_{IT} = 0.60763	<0.001

Pohang and Gumi (0.16465; P<0.001; Table 3), while Jilin and Toham is lowest (0.09132; P<0.001). While the pairwise F_{ST} values do not discriminate between the Korean and Chinese population, pairwise R_{ST} values significantly distinguish between them - the pairwise R_{ST} values between the Korean and Chinese populations are 0.50070-0.29293, which is significantly larger than those among Korean populations ranging 0.18083-0.15280. There is no difference in pairwise R_{ST} value between Gumi and Toham populations (P = 0.892).

Clustering using STRUCTURE program presents consistent genetic structure at K = 3 and K = 4 (Fig. 1). In each case, individuals of Pohang, Gumi, and Jilin populations form distinct clusters while those of Toham are allocated into 2 or more clusters which belong to Pohang or Gumi. Individuals of Jilin population, especially, always form a distinct cluster from Korean clusters. These results correspond with the results from the pairwise population R_{ST} values where Jilin population shows distinct genetic constituents from Korean populations.

Results of hierarchical AMOVA using pairwise F_{ST} value and pairwise R_{ST} value were presented in Table 4 and 5, respectively. In the case of AMOVA results by F_{ST} value, 11.64% of the total variance has originated from the difference among the populations, while 16.45% by among individuals within population and 71.90% by within individual difference. These indicate that there is no significant genetic differentiation among the populations. When we tested with regional grouping by separate Korean and Chinese populations, no significant regional difference was also detected (Table 4). However, AMOVA by R_{ST} value suggested 28.03% of total variance was caused by the difference among the populations, while 25.62% by among individuals within population and 46.34% by within individual difference (Table 5). When we tested with regional grouping, 33.78% were represented by the difference between the regional groups (Korea vs. China), although not significant statistically (P = 0.246; Table 5).

Discussion

Current microsatellite marker analyses present distinct results from what Sun et al. (2011) presented, in the number of alleles and heterozygosity of each locus. For example, we only found two alleles from Pmg164, while there were more than four in Sun et al.'s result. Also its heterozygosities are much lower compared to those of Sun et al. (0.063-0.261 vs. 0.6534-0.7932). Such inconsistencies are also observed from other loci (Pmg117, Pmg153, Pmg155, Pmg165). If it is not caused by experimental bias, it may have been caused by genetic differences of the populations analyzed. Sun et al. (2011) analyzed populations of Hebei and Shanxi, China, which are at least 1,200-1,600 km apart from Jilin population, and Jilin population is at least 850 km apart from Gyeongju populations. This strongly suggests that gene flow is limited by distance and these populations may be geographically isolated. However, Jilin and Gyeongju populations share many of the alleles with low genetic differentiation estimated by pairwise F_{ST} value, although results from pairwise *Rst* value segregate the regional populations. This suggests that *P. lactiflora* populations in NE China may be genealogically closer to Korean populations than the western Chinese populations. Or, this may represent genetic differences between the varieties of *P. lactiflora*. But the current sampling is not enough to test the idea and more populations are required for conclusive results.

The genetic differentiation of Gyeongju and nearby *P. lactiflora* populations are estimated as moderate level (pairwise F_{ST} = 0.10188-0.16465), and this indicates that these local populations are genetically differentiated but there exists consistent gene flow among the populations. Also the estimated number of migrants (Nm = 1.257) and results of STRUCTURE analysis support the idea. Especially, Toham population is consisted of genetic types of both Gumi and Pohang populations, having highest heterozygosity and allele numbers out of the three populations. As the Toham population is located in the middle of Gumi and Pohang populations, Toham population may function as a stepping stone or founder. The pairwise F_{ST} value corroborates the idea since the values between Toham and Gumi, Toham and Pohang are smaller than between Gumi and Pohang. In the case of the former scenario (stepping stone), Toham population may have been established from individuals contributed from both Gumi and Pohang populations. In the case of the latter scenario (founder), Gumi and Pohang populations may have been established by individuals originated from Toham population (source). Considering the results of STRUCTURE analysis, the latter seems more reasonable, but further research applying more microsatellite markers will be necessary. Comparable genetic diversity measure has been done for *Paeonia rockii* populations in Qinling Mountains (Yuan et al., 2012), where average F_{ST} = 0.302 and a significant molecular variation among the group (11.3%) and populations (21.2%) were estimated. The results support that geographic barriers in the Qinling Mountains and adjacent areas caused the regional and population differentiation.

There has been discrepancy in the estimates of population differentiation (F_{ST} & R_{ST}). While R_{ST} discriminated Chinese population from Korean population, F_{ST} didn't. As R_{ST} counts for stepwise evolution of microsatellite repeat units (Slatkin, 1995), existence of alleles detected uniquely from Jilin population which tends to be longer or shorter than those from Korean population explains the discrepancy. This also influenced the hierarchical AMOVA, where regional genetic variance is partly accounted for when using R_{ST}, though statistically insignificant. The presence of those alleles also affects the STRUCTURE analysis, where individuals of Jilin populations were allocated in a distinct cluster.

The significant deviation of H_O from H_E, homozygote excess, high positive F_{IS} value in loci Pmg153 and Pmg155 may have been caused by strong natural selection acting on those loci, Wahlund effect, self-fertilization, or unrecognized null alleles. As *P. jishanensis* T. Hong & W. Z. Zhao (Zhou et al., 1999) and *P. delavayi* (Li et al., 2013) are self-incompatible and *P. lactiflora* also undergo cross-pollination (Hong and Yu, 2006), self-fertilization seems unlikely cause. Also, the Wahlund effect seems unlikely, since H_O and H_E of the pooled data were 0.446 and 0.281, and 0.799 and 0.601, respectively for Pmg153 and Pmg155, not much deviating from the overall mean value.

The current study populations are consisted mainly of young individuals with fully mature individuals flowering/fruiting less than 10% of the total population. This was probably caused by lack of available light, since these populations are under shady forest and take long time for sexual maturation. Although no life history dynamics on *P. lactiflora* has been studied so far, long-term monitoring and demographic study on *P. lactiflora* are necessary for their conservation. Although these populations are distributed discontinuously, these populations need to be kept healthy, since they are interconnected through consistent gene flow. Through long-term effort and assessment, their population size and distribution range can be expanded naturally. Also, other wild populations in Korea and nearby areas need to be included for future study to clarify population dynamics as a natural resource for breeding.

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Literature Cited

Excoffier, L., G. Laval and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics. 1: 47-50.

Glaubitz, J. C. 2004. Convert: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecololgy Notes 4: 309-310.

Goudet J., 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485-486.

Guo, S. W. and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-372.

Hong, D. Y. 2010. Peonies of the World: Taxonomy and Phytogeography. Kew Publishing, London.

Hong, D. Y. 2011. Peonies of the World: Polymorphism and Diversity. Kew Publishing, London.

Hong, D. Y., K. Y. Pan and N. J. Turland. 2001. Paeoniaceae. *In* Flora of China. Vol. 6. Wu, Z. Y. and P. H. Raven (eds.), Science Press and Missouri Botanic Garden Press. Pp. 127-132.

Hong, Y. and Q. Liu. 2006. A study on pollination biology of *Paeonia lactiflora* Pall. Guihaia 26: 120-124.

Lee, Y.N. 2006. New Flora of Korea. Kyohaksa Publishing Co., Ltd. Seoul, Korea. (in Korean)

Li, K., B.Q. Zheng, Y. Wang and X. Guo. 2013. Study on pollination biology of *Paeonia delavayi* (Paeoniaceae). Acta Horticulturae 977: 175-181.

Li, L., F. Cheng and Q. Zhang. 2011. Microsatellite markers for the Chinese herbaceous peony *Paeonia lactiflora* (Paeoniaceae). American Journal of Botany 98: e1-e3.

Pritchard, J.K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233-234.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462.

Sun, J., J. Yuan, B. Wang, J. Pan and D. Zhang. 2011. Development and characterization of 10 microsatellite loci in *Paeonia lactiflora* (Paeoniaceae). American Journal of Botany 98: e242-e243.

The Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of Linnean Society 161: 105-121.

Van Oosterhout, C., W.F. Hutchinson, D.P.M. Willis and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Wright, S. 1978. Evolution and the Genetics of Populations. Vol. 4. Variability within and among Natural Populations. University of Chicago Press, Chicago.

Yuan, J.-H., F-Y. Cheng and S.-L. Zhou. 2012. Genetic structure of the tree peony (*Paeonia rockii*) and the Qinling Mountains as a geographic barrier driving the fragmentation of a large population. PLoS One 7: e34955.

Zhou, S.L., D.Y. Hong and K.Y. Pan. 1999. Pollination biology of *Paeonia jishanensis* T. Hong & W.Z. Zhao (Paeoniaceae), with special emphasis on pollen and stigma biology. Botanical Journal of Linnean Society 130: 43-52.