

Genetic Diversity and Relationship of Genus *Spiraea* by Random Amplified Polymorphic DNA Markers

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Genus *Spiraea* is a woody species primarily distributed throughout Asia. Many species of this genus are important plants medicinally and ecologically. I evaluated a representative sample of the sixteen taxa with random amplified polymorphic DNA (RAPD) markers to estimate genetic relationships within genus *Spiraea*. In addition, RAPD analysis was also conducted to estimate the genetic diversity and population structure of these species. As the typical populations of *Spiraea* were small, isolated, and patchily distributed for natural populations, they maintained a low level of genetic diversity for polymorphic primers. The mean H was 0.117 across species. The Korean endemic species (*S. chartacea*) and patchily distributed species (*S. betulifolia*) showed fewer alleles per locus (mean 1.240 vs. 1.297), lower percent polymorphic locus (24.0 vs. 29.7), and lower diversity (0.092 vs. 0.121) than a relatively widely spread species. An assessment of the proportion of diversity present within species, $H_{\text{POP}}/H_{\text{SP}}$, indicated that about 87.8% the total genetic diversity was among species. Thus, the majority of genetic variation (87.8%) resided within species. The phylogenetic tree showed three distinct groups. One clade includes *S. prunifolia* for. *simpliciflora*, *S. thunbergii*, *S. chamaedryfolia* var. *ulmifolia*, *S. media*, and *S. cantoniensis*. Another clade includes *S. blumei*, *S. pubescens*, *S. chartacea*, and *S. chinensis*. The other clade is the remaining seven species.

Key words : Genus *Spiraea*, genetic diversity, RAPD, phylogenetic tree

Introduction

Spiraea (also known as Meadowsweet) is a genus of about 80~100 species of shrubs in the Rosaceae, subfamily Spiraeoideae [21]. The genus *Spiraea* is native to the temperate Northern Hemisphere, with the greatest diversity in eastern Asia [23]. For example, *Spiraea prunifolia* Siebold & Zucc. forma *simpliciflora* Nakai occurs in mountains of Korea. This species is also reported from China and Taiwan [13].

Spiraea contains methyl salicylate and other salicylates, compounds with similar medicinal properties of aspirin. Unlike aspirin, meadowsweet is effective in treating stomach disorders in minute amounts. The salicylates in this plant are a highly effective analgesic, anti-inflammatory, and fever reducer, without the side effects attributed to aspirin [18].

Asian regions such as China, Korea, Japan and Russia are well known for giving various *Spiraea* species [9]. The genus *Spiraea* is comprised of about 10~14 species in Korea. The taxonomy of *Spiraea* has processed mainly through morphological characteristics [8]. However morphological character-

istics are restricted their resolving power mainly because of the small number of variables available. The development of molecular makers has provided powerful tools that may overcome such limitations [3].

The random amplified polymorphic DNA (RAPD) markers have popular means for identification and authentication of plant and animal species because these marker techniques may generate relatively high numbers of DNA markers per sample and are technically simple [14]. This molecular marker is based on the PCR amplification of random locations in the genome of the plant [16]. With this technique, a single oligonucleotide is used to prime the amplification of genomic DNA. Because these primers are 10 nucleotides long, they have the possibility of annealing at a number of locations in the genome [4]. For amplification products to occur, the binding must be to inverted repeats sequences generally 150~4,000 base pairs apart. The number of amplification products is directly related to the number and orientation of the sequences that are complementary to the primer in the genome. The methods have been used extensively in genetic analysis of prokaryotes and eukaryotes though the marker system has certain disadvantages such as reproducibility [17].

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Although it is important to gain knowledge of the genetic variation for conservation purposes, detailed information on the levels and distribution of this variation as well as population structure are not available for most woody taxa in Korea. The aims of this study were: 1) to estimate how much total genetic diversity is maintained in the *Spiraea* species, 2) to describe how genetic variation is distributed within and among species, and to elucidate the suitability and efficiency of the RAPD analyses assess the phylogenetic relationships among the related species in Korea. In addition, we compared amounts of genetic variation of species between endemic group and relative widespread group. Although randomly amplified polymorphic DNA (RAPD) marker has disadvantage of low reproduction and of dominant segregation, it is a preferable approach for *Spiraea* species.

This study was carried out to examine several populations of *Spiraea* species in order to evaluate genetic diversity and

population structure in this species and genus.

Materials and Methods

Plant materials

All of the 30 populations of sixteen taxa were collected from *Spiraea* populations in Korea (Table 1). They are *Spiraea prunifolia* for. *simpliciflora* NAKAI, *S. cantoniensis* LOUR., *S. chamaedryfolia* var. *ulmifolia* (SCOP.) MAXIM., *S. blumei* G. DON, *S. thunbergii* SIEBID., *S. miyabei* KOIDZ, *S. trichocarpa* NAKAI, *S. pubescens* TURCZ., *S. chartacea* NAKAI, *S. chinensis* MAXIM., *S. salicifolia* L., *S. japonica* L., *S. betulifolia* PALLAS, *S. fritschiana* SCHNEID, *S. media* SCHMIDT., and *S. microgyna* NAKAI. Samples of the germplasmic *S. betulifolia* were obtained from the Korea Forest Research Institute. One young leaf per mature tree (≥ 5 yr) was sampled. Twelve plants were randomly collected from each

Table 1. Code and locations of the genus *Spiraea* and the outgroup in this study

Species	Code	Locality of populations
1. <i>S. betulifolia</i>	SFO101	Mt. Bukhan, Dongdaemun-gu, Seoul
2. <i>S. blumei</i>	SBL101	Sema-dong, Osan-si, Gyeonggi-do
	SBL102	Mt. Chiak, Wonju-si, Kangwon-do
3. <i>S. cantoniensis</i>	SCN101	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do
	SCN102	Gwonseon-gu, Suwon-si, Gyeonggi-do
4. <i>S. chamaedryfolia</i> var. <i>ulmifolia</i>	SCU101	Sohol-eup, Pocheon-si, Gyeonggi-do
	SCU102	Seorak-san, Yangyang-gun, Gangwon-do
5. <i>S. chartacea</i>	SCA101	Daeheuksan Island, Sinan-gun, Jeollanam-do
	SCA102	Hong Island, Sinan-gun, Jeollanam-do
6. <i>S. chinensis</i>	SCI101	Mt. Bukhan, Dongdaemun-gu, Seoul
	SCI102	Mt. Soback, Yeongju-si, Gyeongsangbuk-do
7. <i>S. fritschiana</i>	SFR101	Mt. Nayeon, Pohang-si, Gyeongsangbuk-do
	SFR102	Mt. Giri, Sancheong-gun, Gyeongnam-do
8. <i>S. japonica</i>	SJP101	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do
	SJP102	Mt. Odae, Gangneung-si, Kangwon-do
9. <i>S. media</i>	SME101	Punggok-ri, Samcheok-si, Gangwon-do
10. <i>S. microgyna</i>	SMI101	Mt. Odae, Gangneung-si, Kangwon-do
11. <i>S. miyabei</i>	SMI101	Sema-dong, Osan-si, Gyeonggi-do
	SMI102	Mt. Daemo, Gangnam-gu, Seoul
12. <i>S. prunifolia</i> for. <i>simpliciflora</i>	SPS101	Ilbanseong-myeon, Jinju-si, Gyeongsangnam-do
	SPS102	Mt. Maebong, Jeongseon-gun, Gangwon-do
13. <i>S. pubescens</i>	SPU101	Mt. Gaya, Hapcheon-gun, Gyeongsangnam-do
	SPU102	Mt. Giri, Sancheong-gun, Gyeongsangnam-do
14. <i>S. thunbergii</i>	STH101	Sohol-eup, Pocheon-si, Gyeonggi-do
	STH102	Mt. Seorak, Yangyang-gun, Kangwon-do
15. <i>S. salicifolia</i>	SSA101	Sohol-eup, Pocheon-si, Gyeonggi-do
	SSA102	Mt. Seorak, Yangyang-gun, Kangwon-do
16. <i>S. trichocarpa</i>	STR101	Sema-dong, Osan-si, Gyeonggi-do
	STR102	Mt. Odae, Gangneung-si, Kangwon-do
<i>Physocarpus amurensis</i>	AUC100	Gwonseon-gu, Suwon-si, Gyeonggi-do

population. In addition, the species of same family Rosaceae, *Physocarpus amurensis* was provided for the outgroup and used to compare the phenetic relationship.

DNA extraction and RAPD analysis

The genomic DNA of the 342 samples including outgroup was extracted from fresh leaves using the plant DNA Zol Kit (Life Technologies Inc., Grand Island, New York, USA) according to the manufacturer's protocol.

RAPD primers were obtained from Operon Technologies Inc. (USA). All the reactions were repeated twice and only reproducible bands were scored for analyses. Of 40 primers (OPC01-OPC20 and OPD01-OPD20) used for a preliminary RAPD analysis, 12 primers produced good amplification products both in quality, reproducibility, and variability (Table 2).

Amplification reactions were performed in 0.6 ml tubes containing 2.5 μ l of the reaction buffer, 10 mM Tris-HCl (pH 8.8), 1.25 mM each of dATP, dCTP, dGTP, dTTP, 5.0 pM primer, 2.5 units Taq DNA polymerase, and 25 ng of genomic DNA. A 100 bp ladder DNA marker (Pharmacia) was used in the end of for the estimation of fragment size. The amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light using Alpha Image TM (Alpha Innotech Co., USA).

Statistical analyses

All RAPD bands were scored by eye and only unambiguously scored bands were used in the analyses. Because RAPDs are dominant markers, they were assumed that each band corresponded to a single character with 2 alleles, presence (1) and absence (0) of the band, respectively.

The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh et al. [20]: the percentage of polymorphic loci (P_p), mean numbers of alleles per locus (A), effective number of alleles per locus (A_E) and gene diversity (H) [11].

The phenotypic frequency of each band was calculated and used in estimating total genetic diversity (H_T), genetic diversity within populations (H_S) proportion of total genetic diversity partitioned among populations (G_{ST}), and gene flow (N_m) [10,19].

The degree of polymorphism was quantified using Shannon's index of phenotypic diversity [2]:

$$H_0 = -S \sum p_i \log p_i$$

where p_i is the frequency of a particular phenotype i . H_0 can be calculated and compared for different populations [12]. Let

$$H_{POP} = 1/n \sum H_i$$

be the average diversity over the different species and let

$$H_{SP} = -S \sum p \log p$$

be the diversity calculated from the phenotypic frequencies p in all species considered together. Then the proportion of diversity present within species, H_{POP}/H_{SP} , can be compared with that of between species (G_{ST}), $(H_{SP}-H_{POP})/H_{SP}$.

The estimation of genetic similarity (GS) between genotypes was based on the probability that an amplified fragment from one individual will also be present in another [11]. GS was converted to genetic distance (1-GS). Homogeneity of variance among populations was tested by Bartlett's statistics [1].

A phenetic relationship was constructed by the neighbor-joining (NJ) method [15] using the NEIGHBOR program in PHYLIP version 3.57 [5]. Relative support for clades was assessed using phylogenetic bootstrapping with 1000 replicates [5].

Results

From the 40 decamer primers used for a preliminary RAPD analysis, 12 primers produced good amplification products both in quality and variability. Overall, 93 fragments were generated among the tested *Spiraea* array (Table 2). The fragments ranged from 4~12 per primer.

In a simple measure of intraspecies variability by the per-

Table 2. List of decamer oligonucleotide utilized as RAPD primers, their sequences, and associated polymorphic fragments amplified in the *Spiraea* taxa

Primer	Sequence (5'~3')	No. of fragments detected
OPC-04	CCGCATCTAC	9
OPC-05	GATGACCGCC	7
OPC-09	CTCACCGTCC	8
OPC-15	GACGGATCAG	5
OPC-20	ACTTCGCCAC	4
OPD-02	GGACCCAACC	7
OPD-05	TGAGCGGACA	6
OPD-07	TTGGCACGGG	10
OPD-08	GTGTGCCCCA	8
OPD-14	CTTCCCAAG	8
OPD-16	AGGGCGTAAG	9
OPD-18	GAGAGCCAAC	12

centage of polymorphic bands, the *S. chartacea* exhibited the lowest variation (21.9%) (Table 3). The *S. chamaedryfolia* var. *ulmifolia* showed the highest (37.5%). Mean number of alleles per locus (A) ranged from 1.219~1.375 with a mean of 1.290. The effective number of alleles per locus (A_E) ranged from 1.139~1.295.

The phenotypic frequency of each band was calculated and used in estimating genetic diversity (H) within populations. As the typical populations of *Spiraea* were small, isolated, and patchily distributed for natural populations, they maintained a low level of genetic diversity for polymorphic primers. The mean H was 0.117 across species. In particular, *S. pubescens* had the highest expected diversity (0.154); *S. chartacea*, the lowest (0.081).

Shannon's index of phenotypic diversity (H_0) of *S. pubescens* (0.216) was highest of all species and *S. miyabei* was the second (0.210).

As one Korean endemic species (*S. chartacea*) was isolated at only two small islands (the Daeheuksan Island and the Hong Island) and narrow restricted species (*S. betulifolia*) was patchily distributed at only one small region (the middle parts of Korea), they were found to have fewer alleles per locus (mean 1.240 vs. 1.297), lower percent polymorphic locus (24.0 vs. 29.7), and lower diversity (0.092 vs. 0.121) than

relatively widespread species (Table 3). The both groups showed significant difference for measures of genetic variability except A_E (paired t test).

On a per locus basis, total genetic diversity (H_T) varied between 0.080 for *S. chartacea* and 0.372 for *S. cantoniensis* and *S. fritschiana* (Table 4). The interlocus variation of population genetic diversity (H_S) was low (0.113). The average number of individuals exchanged between populations per generation (N_m) was estimated to be 0.458. Korean endemic species (*S. chartacea*) was found to have lower H_T (0.080 vs. 0.329), lower H_S (0.058 vs. 0.117), and lower G_{ST} (0.285 vs. 0.638) than relatively widespread species.

An assessment of the proportion of diversity present within species, H_{POP}/H_{SP} , indicated that about 87.8% the total genetic diversity was among species. Thus, the majority of genetic variation (87.8%) resided within species (Table 5).

A similarity matrix based on the proportion of shared fragments (GS) was used to evaluate relatedness among species. The estimate of GS ranged from 0.535~0.999 (Table 6).

Clustering of species, using the NJ algorithm, was performed based on the matrix of calculated distances (Fig. 1). The phylogenetic tree showed three distinct groups. One clade includes *S. prunifolia* for. *simpliciflora*, *S. thunbergii*, *S. chamaedryfolia* var. *ulmifolia*, *S. media*, and *S. cantoniensis*. Another

Table 3. Measures of genetic variability for RAPDs generated among *Spiraea* taxa

Species	N_P	P_P	A	A_E	H	H_0
Endemic species						
<i>S. betulifolia</i>	25	26.0	1.260	1.189	0.103	0.150
<i>S. chartacea</i>	21	21.9	1.219	1.140	0.081	0.121
Mean	23.0	24.0	1.240	1.165	0.092	0.136
Widespread species						
<i>S. blumei</i>	27	28.1	1.281	1.230	0.123	0.176
<i>S. cantoniensis</i>	31	32.3	1.323	1.223	0.124	0.181
<i>S. chamaedryfolia</i> var. <i>ulmifolia</i>	36	37.5	1.375	1.233	0.133	0.198
<i>S. chinensis</i>	28	29.2	1.292	1.246	0.130	0.185
<i>S. fritschiana</i>	30	31.3	1.313	1.239	0.130	0.188
<i>S. japonica</i>	24	25.0	1.250	1.202	0.109	0.156
<i>S. media</i>	28	29.2	1.292	1.229	0.124	0.179
<i>S. microgyna</i>	27	28.1	1.281	1.207	0.114	0.166
<i>S. miyabei</i>	30	31.3	1.313	1.293	0.150	0.210
<i>S. prunifolia</i> for. <i>simpliciflora</i>	29	30.2	1.302	1.139	0.087	0.136
<i>S. pubescens</i>	31	32.3	1.323	1.295	0.154	0.216
<i>S. salicifolia</i>	25	26.0	1.260	1.211	0.113	0.162
<i>S. trichocarpa</i>	26	27.1	1.271	1.208	0.113	0.163
<i>S. thunbergii</i>	27	28.1	1.281	1.152	0.092	0.140
Mean	28.5	29.7	1.297	1.222	0.121	0.175
Total mean	27.8	29.0	1.290	1.215	0.117	0.170

N_P : The number of polymorphic loci, P_P : The percentage of polymorphic loci, A : Mean numbers of alleles per locus, A_E : Effective number of alleles per locus, H : Gene diversity, H_0 : Shannon's index of phenotypic diversity.

Table 4. Measures of genetic variability for RAPDs generated among *Spiraea* taxa

Species	H_T	H_S	G_{ST}	N_m
Endemic species				
<i>S. chartacea</i>	0.080	0.058	0.285	1.255
Widespread species				
<i>S. blumei</i>	0.364	0.158	0.586	0.454
<i>S. chamaedryfolia</i> var. <i>ulmifolia</i>	0.365	0.113	0.683	0.428
<i>S. cantoniensis</i>	0.372	0.118	0.668	0.303
<i>S. chinensis</i>	0.312	0.111	0.589	0.474
<i>S. fritschiana</i>	0.372	0.166	0.552	0.476
<i>S. japonica</i>	0.303	0.121	0.641	0.453
<i>S. miyabei</i>	0.309	0.126	0.602	0.398
<i>S. prunifolia</i> for. <i>simpliciflora</i>	0.368	0.110	0.661	0.311
<i>S. pubescens</i>	0.255	0.069	0.711	0.311
<i>S. salicifolia</i>	0.301	0.115	0.594	0.441
<i>S. trichocarpa</i>	0.313	0.112	0.640	0.380
<i>S. thunbergii</i>	0.312	0.090	0.724	0.272
Mean	0.329	0.117	0.638	0.392
Total	0.310	0.113	0.610	0.458

S. betulifolia, *S. media*, and *S. microgyna* are not listed because one population is not obtained Wright's F -statistics.

clade includes *S. blumei*, *S. pubescens*, *S. chartacea*, and *S. chinensis*. The other clade is the remainder seven species.

Discussion

Genetic diversity and population structure

In RAPD analysis, eleven species, two varieties, and one form belonging to genus *Spiraea* maintain a lower than average level of genetic diversity compared with other plant species, although there is the difference in methodology (e.g., dominant marker and co-dominant marker) that may pre-

clude meaningful comparisons. For example, its genetic diversity of 0.117 is lower than that for temperate-zone species (0.146), dicots (0.136), species with a sexual reproduction mode (0.151), and those with a long-lived woody habit (0.177) [6]. The percentage of polymorphic loci at the species level for *Spiraea* is 29.0%, which is also lower than that for species with temperature-zone distributions (48.5%), dicots (44.8%), species with a sexual reproduction mode (51.6%), and long-lived and woody (64.7%).

Geographic range has been shown to be strongly associated with the level of variation maintained both within populations and at the species level [7]. Widely distributed species tend to maintain more variation than more narrowly distributed species level [6]. For all Korean *Spiraea* taxa where number of alleles per polymorphic loci was calculated, relatively widespread species had more alleles than restricted species (*S. chartacea* and *S. betulifolia*). In addition, effective population sizes are also important roles maintaining genetic diversity. Korean *Spiraea* has been saved from artificial distribution. Periodical cutting of branches and stems have often been moved from hillside to nearby farmhouse for the purpose of firewood or medicine during the past several decades of years. Small populations tend to have fewer multilocus genotypes and genetic diversity than large populations. Smaller populations probably exhibit more selfing than larger populations by virtue of the limited opportunities for outcrossing during any flowering season. Two introduced species, *S. chinensis* and *S. cantoniensis* were not maintained high level of genetic diversity (Table 3). It is likely that some of the secondary growth of virgin trees I sampled were from a restricted parent pool and thus are

Table 5. Partitioning of the genetic diversity into within and among *Spiraea* taxa using RAPD markers

Primer	H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp} - H_{pop})/H_{sp}$
OPC-04	1.552	1.851	0.839	0.161
OPC-05	1.663	2.054	0.810	0.190
OPC-09	1.693	1.990	0.851	0.149
OPC-15	1.769	2.509	0.705	0.295
OPC-20	1.125	1.369	0.821	0.179
OPD-02	1.732	1.886	0.919	0.081
OPD-05	1.588	1.782	0.891	0.109
OPD-07	2.128	2.248	0.946	0.054
OPD-08	1.865	2.022	0.922	0.078
OPD-14	2.120	2.253	0.941	0.059
OPD-16	2.085	2.188	0.953	0.047
OPD-18	2.284	2.442	0.935	0.065
Mean	1.800	2.049	0.878	0.122

H_{pop} : Phenotypic diversity for different populations, H_{sp} : The average diversity over the different species, H_{pop}/H_{sp} : The proportion of diversity within species, $(H_{sp} - H_{pop})/H_{sp}$: The proportion of diversity between species.

Table 6. Genetic identity (upper diagonal) among 16 taxa of genus *Spiraea* and genetic distances (low diagonal) based on RAPD analysis

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	-	0.700	0.934	0.614	0.956	0.667	0.556	0.683	0.673	0.616	0.616	0.631	0.656	0.648	0.647	0.642
2	0.357	-	0.664	0.651	0.708	0.755	0.639	0.667	0.642	0.592	0.588	0.594	0.618	0.624	0.624	0.616
3	0.069	0.409	-	0.635	0.947	0.674	0.535	0.666	0.652	0.586	0.585	0.602	0.619	0.631	0.634	0.620
4	0.489	0.430	0.454	-	0.660	0.801	0.748	0.959	0.773	0.778	0.799	0.771	0.770	0.803	0.801	0.810
5	0.045	0.345	0.055	0.416	-	0.694	0.577	0.702	0.693	0.641	0.631	0.642	0.661	0.660	0.658	0.656
6	0.405	0.281	0.395	0.221	0.365	-	0.907	0.752	0.736	0.706	0.733	0.717	0.745	0.757	0.759	0.754
7	0.588	0.448	0.625	0.290	0.551	0.098	-	0.832	0.815	0.837	0.834	0.808	0.810	0.821	0.820	0.827
8	0.381	0.405	0.406	0.042	0.354	0.285	0.184	-	0.798	0.916	0.897	0.813	0.893	0.897	0.894	0.897
9	0.396	0.443	0.428	0.257	0.367	0.307	0.205	0.226	-	0.945	0.803	0.808	0.898	0.890	0.888	0.896
10	0.486	0.525	0.535	0.251	0.444	0.348	0.178	0.088	0.057	-	0.889	0.893	0.917	0.898	0.895	0.910
11	0.485	0.532	0.536	0.224	0.460	0.310	0.182	0.109	0.219	0.118	-	0.953	0.915	0.903	0.900	0.915
12	0.460	0.520	0.508	0.260	0.443	0.332	0.213	0.207	0.213	0.113	0.048	-	0.914	0.905	0.902	0.917
13	0.422	0.482	0.480	0.261	0.414	0.295	0.211	0.113	0.108	0.087	0.089	0.090	-	0.942	0.911	0.910
14	0.433	0.472	0.460	0.220	0.416	0.278	0.197	0.109	0.117	0.107	0.102	0.099	0.060	-	0.999	0.989
15	0.436	0.471	0.456	0.222	0.418	0.275	0.199	0.112	0.119	0.111	0.105	0.104	0.099	0.001	-	0.989
16	0.443	0.484	0.479	0.211	0.422	0.282	0.190	0.109	0.110	0.094	0.089	0.087	0.095	0.011	0.011	-

Numbers of taxa are same as the order from the upside to below of Table 1.

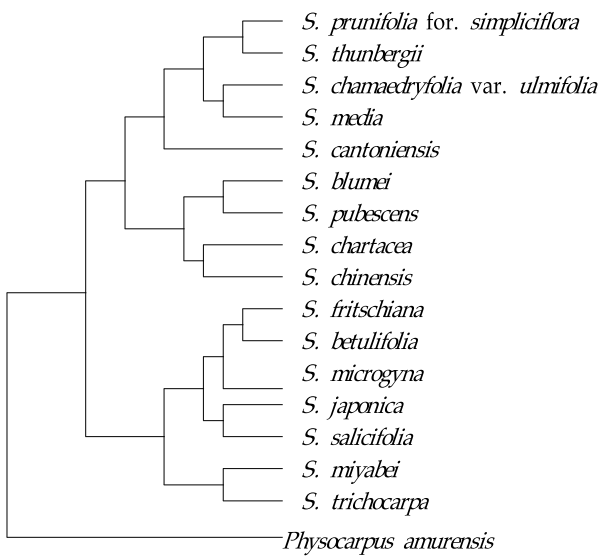


Fig. 1. A phenetic tree for 16 *Spiraea* taxa and one outgroup based on RAPD analysis.

more similar in genetic constitution than those stands found on less distributed populations or sites.

The correlation coefficient between genetic distance and geographic distance using Mantel's test for all populations in Korea were 0.54 ($r^2=0.29$). Most of the variation (46.0%) in genetic distance seemed to be caused by unknown factors such as genetic drift, natural selection, and limited gene flow other than geographic distance. Thus the hypothesis of the isolation speciation from wild populations of widespread species needs to be tested by future work.

Phenetic relationship within *Spiraea*

At present, the phenetic positions of these species shown in Fig. 1 seem to be agreed partly with results of morphological and molecular data [9]. Although *S. chartacea* has many hairs in leaves and *S. pubescens* is hairless, they are same clade and show little intra-specific variation. It is different to distinguish among *Spiraea* complex because of the minglement of variety specific characteristics. The distinct taxonomic treatment among *Spiraea* is not also evidenced by nuclear ribosomal DNA internal transcribed spacer sequences (ITS) data.

S. betulifolia can be classified as a narrow distribution as it grows only at middle parts of Korea such as Seoul. *S. chartacea* was also endemic to the populations from the southern parts of islands of the Korean Peninsula. Although I did not analyze further subdivision of a local population, it may be inferred that RAPD variation that resided mainly within *S. chartacea* populations is maintained in patchily distributed subpopulations or demes, either by random drift of neutral alleles or micro-environmental selection for adaptive alleles according to geographic isolation. Zhang et al. [22] also reported that the specific phylogeny of *Spiraea* is closely linked to the general floristic evolution and historical environmental changes in East Asia.

In a phenetic tree based on RAPD variability, the position of the taxa in the NJ tree and their morphological data did not almost completely matched in the Korean *Spiraea*.

However, it is relevant to stress that RAPD markers which

used allowed us to discriminate among all populations, even those that could not be distinguished on the basis of ITS analysis. Thus, RAPD markers are very effective in classifying wild populations of genus *Spiraea* in Korea.

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초록 : 조팝나무속 분류군의 RAPD에 의한 유전적 다양성과 관련성

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조팝나무속 식물은 주로 아시아에 많이 분포하는 목본으로 생태 및 약용으로 중요하다. 이 속내 16종, 29집단에 대해 RAPD (random amplified polymorphic DNA) 마커로 이들 집단에 대한 유전적 변이와 집단구조를 조사하였다. 이들 집단은 작고 격리되어 있어 낮은 유전적 다형성을 나타내었다. 전체 유전적 다양도는 종 수준에서 0.117이었다. 국지적 분포를 보이는 종(*S. chartacea*)은 광범위하게 분포하는 종에 비해 유전자 좌위당 대립유전자의 수는 적었고(평균 1.240:1.297), 다형성을 나타내는 유전자 좌위 %(24.0:29.7), 낮은 다형성(0.092 vs. 0.121)을 나타내었다. 종내 다양성의 비율(H_{OP}/H_S)은 전체 변이중 87.8%가 종간에 있었고 전체 변이의 12.2%는 종내에 있었다. 계통도에서 세 그룹으로 나타났다. 한 분지군은 조팝나무, 가는잎조팝나무, 인가목조팝나무, 긴조팝나무, 공조팝나무이었다. 또한 분지군은 산조팝나무, 아구장나무, 떡잎조팝나무, 당조팝나무였다. 나머지 분지군은 7종을 포함하고 있었다.