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A Biosystematic Study on Polyploid Populations of the Genus *Spiraea* (Rosaceae) in Korea

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A total of 61 chromosome counts from 11 taxa of Korean Spiraea was made. Our counts are the first report for eight taxa; *S. blumei* (2n=18, 36), *S. chartacea* (2n=36), *S. chinensis* (2n=36), *S. fritschiana* (2n=27, 36), *S. microgyna* (2n=18), *S. prunifolia* var. *simpliciflora* (2n=18), *S. pseudocrenata* (2n=36), and *S. trichocarpa* (2n=18). A new chromosome number of 2n=36 (tetraploid) is reported for *S. pubescence*. Populations of three species including *S. blumei, S. pubescence* and *S. fritschiana*, show different ploidy levels; diploid and tetraploid populations are found in the former two species and triploid and tetraploid ones, in latter species. Multiplication of chromosome numbers contributes to increase in size of pollen and stomata in the three species. Populations with different ploidy levels in *S. blumei* occupy different regions; diploid populations in inland Korea and tetraploid ones in Ullung Island. Island tetraploid population of *S. blumei* might be originated from intra-island polyploidization through the introduction of diploid from inland Korea, considering the worldwide distribution of this species. Pollen fertilities of island populations of *S. blumei* are relatively low, and sometimes no pollen grain is produced in anther sacs; it suggests that tetraploid population of the island is gynodioecious which may serve reduction of inbreeding depression.

Keyword: Korean *Spiraea*, chromosome number, polyploidy, pollen and stomata, dioecy

Spiraea L. (Rosaceae) is a genus of about 70 species mainly distributed in temperate zones of the Northern Hemisphere including Manchuria and Siberia. Species of *Spiraea* also extend to Mexico and Himalaya (Nakai, 1916; Mabberley, 1990), and 11 species and two varieties are known to be distributed in Korea (Kim and Sun, 1996).

Chromosome numbers over 50 taxa of the genus *Spiraea* were reported (Darlington and Wylie, 1955; Cave, 1958-1965; Ornduff, 1967-1969; Fedorov, 1969; Moore, 1970-1977; Goldblatt, 1981-1988; Goldblatt and Johnson, 1990-1994), and the genus shows high levels of polyploidy as compared to other angiosperm taxa. In addition, interspecific hybrids are frequently reported in *Spiraea* because of incomplete genetic isolation (Schneider, 1905; Robertson, 1974). The high levels of polyploidy and the frequent occurrence of hybrids indicate that polyploidization and hybridization may play a very important role in speciation within this genus. McArthur and Sanderson (1985) in their cytotaxonomic study on the 4 subfamilies of the Rosaceae suggested that *Spiraea* is the most primitive genus within Rosaceae in terms of evolution of chromosome number. However, chromosome numbers of the Korean *Spiraea* has not been examined previously. The main objectives of this study are 1) to examine chromosome numbers of the Korean *Spiraea*, 2) to analyze morphological differentiation among different ploidy populations within each species, and 3) to discuss characteristics of polyploid population in Ullung Island.

MATERIALS AND METHODS

Plants materials were collected from various localities in Korea from May, 1989 to April, 1995 (Table 1), and the voucher specimens were deposited in the herbarium of Chonbuk National University (JNU).

For meiotic observation, flower buds were collected in the field from at least 10 individuals in each population. Young shoots or root tips were collected at the same time for mitotic observation. Pretreatment, slide preparation, and observation of chromosomes followed Sun *et al.* (1990).

For comparison of size of pollen grains and stoma-

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Table 1. Chromosome numbers of Genus *Spiraea* in Korea

Species Name	Chromosome number		Gametophyte
	Sporophyte	Voucher specimen	
Sect. *Chamaedryon*		18	Taebaek: Kim 1053
+ *S. blumei*	9		Yeongweol: Sun 102
			Danyang: Sun 110
			Songgye: Sun 118
			Mt. Wolak: Kim 71, Kim 76, Kim 77, Kim 85, Kim 88
			Hwangji: Kim 21, Kim 26, Kim 28, Kim 29, Kim 30
		36	Ullung Is.(Dodong): Sun 1015
	18		Ullung Is.(Dodong): Sun 1461, Sun 1462, Sun 3031, Sun 3032, Sun 3034
			Ullung Is.(Chusanri): Sun 3623, Sun 3624, Sun 3625, Sun 3629
		36	Huksan Is.: Kim 1040
+ *S. chartacea*			
S. chamaedryfolia			
var. *ulmifolia*		18	Mt. Chiri: Kim 1047
+ *S. chinensis*		36	Yemi: Kim 1068
+ *S. pseudocrenata*		36	Hwacheon: Kim 1078
S. pubescens	9	18	Mt. Weolak: Kim 1025
*	18		Mt. Wolak: Kim 96, Kim 98
			Mt. Naebyun: Kim 1038, Kim 104, Kim 105, Kim 106, Kim 107, Sun 213
			Mt. Solak: Kim 203, Kim 210, Kim 212, Kim 213, Kim 214, Kim 215, Kim 218
Sect. *Calospira*			
+ *S. fritschiana*	18		Mt. Wolak: Kim 1023, Kim 41, Kim 42, Kim 43, Kim 45.
			Mt. Solak: Kim 50, Kim 52, Kim 55.
		27	Mt. Bukhan: Kim 1020
		36	Hwacheon: Kim 1071
			Mt. Bukhan: Kim 1023, Sun 221, Sun 223, Sun 238
S. microgyna	9	18	Mt. Chiak: Kim 1007
Sect. *Glomerati*			
+*S. prunifolia* var. *simpliciflora*	9	18	Mt. Moak: Kim 1073
Sect. *Spiraria*			
S. salicifolia		36	Mt. Moak: Kim 1001
Sect. *Metachamaedryon*			
+*S. trichocarpa*		18	Taebaek: Kim 1070

+first report, *new chromosome level

ta and pollen fertility among populations with different chromosome numbers, four populations of *S. blumei* G. Don, two from Ullung Island (Do-dong and Chusan-ri) and two from continental Korea (Taebaek and Mt. Wolak), three populations of *S. pubescence* Turcz. (Mt. Naebyun, Mt. Wolak and Mt. Solak) and two populations of *S. fritschiana* C.K. Schneid. (Mt. Bukhan and Mt. Wolak) were selected.

Acetolysis of the pollen grains and slide preparations followed Ertdman (1972). Thirty pollen grains per individual were selected from 10 individuals at each population and equatorial length, polar diameter and aperture length were measured under light microscope. Mean and standard deviation were calculated from these measurements. In addition, pollens were stained with 1% anilinblue-lactophenol solution for 24 hours to measure pollen fertility. Fully-formed grains with deep nuclear staining under light microscope were assessed as fertile ones. Pollen fertilities were calculated by dividing the number of fertile pollen grains by total number of pollen grains observed at each population (Edward and Morrison, 1964).

About one cm^2 of adaxial leaf epidermis at the base of midrib was peeled off and length and width of stomata were measured under light microscope from 20 guard cells per individual. Mean and standard deviation were calculated from these measurements.

RESULT

Chromosome Number

Sixty one chromosome counts from 11 taxa of Korean *Spiraea* are listed in Table 1. First counts are reported for eight taxa: *S. blumei* (2n=18, 36), *S. chartacea* Nakai (2n=36), *S. chinensis* Max. (2n=36), *S. fritschiana* (2n=27, 36), *S. microgyna* Nakai (2n=18), *S. prunifolia* Siebold et Zucc. var. *simpliciflora* Nakai (2n=18), *S. pseudocrenata* Nakai (2n=36) and *S. trichocarpa* Nakai (2n=18). New chromosome number is reported for *S. pubescence* as 2n=36 of tetraploid here.

Among them, *S. chartacea, S. chinensis, S. pseudocrenata* and *S. salicifolia* L. are counted consistently as tetraploid of 2n=36. Whereas, *S. chamaedryfolia* L. var. *ulmifolia* (Scopoli) Max., *S. microgyna, S. prunifolia* var. *simpliciflora* and *S. trichocarpa* are counted consistently as diploid of 2n=18. However, three species, *S. blumei, S. pubescence* and *S. fritschiana* have different chromosome numbers among populations. For *S. blumei*, all the 14 counts from five populations in continental Korea (Kangwon Province: Taebaek and Yeongweol; Chungbuk Province: Danyang, Songye and Mt. Wolak) show always diploid as 2n=18, whereas all the seven counts from two populations in Ullung Island (Do-dong and Chusan-ri) show tetraploid as 2n=36. Three counts of *S. pubescence* from Mt. Wolak (Chungbuk Province) show diploid as 2n=18 whereas 13 counts from Mt. Naebyun (Chonbuk Province) and Mt. Solak (Kangwon Province) show tetraploid as 2n=36. For *S. fritschiana*, all the 13 counts from four populations (Chungbuk Province: Mt. Wolak; Kangwon Province: Mt. Solak and Hwacheon; Kyunggi Province; Mt. Bukhan) show consistently tetraploid as 2n=36 except one count from Mt. Bukhan with triploid as 2n=27.

Palynology

Measurements of equatorial length, polar diameter and aperture length of pollen grains between diploid and tetraploid populations of *S. blumei* and *S. pubescence* are listed in Table 2. In *S. blumei*, mean polar diameter of tetraploid population is 16.0 μm and those of two diploid populations are 12.4 and 13.3 μm, respectively. Mean equatorial length varies from 11.6~11.7 μm in diploid populations to 14.8 μm in tetraploid population, and mean aperture length varies from 9.8~10.6 μm in diploid populations to 12.4 μm in tetraploid population. This result indicates tetraploid population has much larger pollen than diploid populations. However, no significant difference is found between the two diploid populations. This tendency can be seen more clearly in two dimensional

Table 2. Measurement of pollen grains of *S. blumei* and *S. pubescence*

Species	Ploidy level	Population	Length (μm)			P/E
			Polar	Equatorial	Aperture	
S. blumei	Diploid	Mt. Wolak	12.38±0.79	11.71±0.68	9.79±0.37	1.09
	Tetraploid	Taebaek	13.31±0.49	11.59±0.72	10.56±0.40	1.16
		Ullung Is.	16.01±0.65	14.80±0.60	12.44±0.98	1.09
S. pubescence	Diploid	Mt. Wolak	14.37±1.14	13.06±0.49	10.57±0.51	1.11
	Tetraploid	Mt. Naebyun	15.29±0.61	14.42±0.58	11.35±0.44	1.07

Table 3. Measurement of pollen fertility of some polyploid complexes of *Spiraea* estimated by anilinblue-lactophenol staining

Species	Ploidy level	Population	Fertility (%)
S. blumei	Diploid	Mt. Wolak	93.4±5.5
		Taebaek	96.7±3.2
	Tetraploid	Ullung Is.	82.2±7.3
S. pubescence	Diploid	Mt. Wolak	90.6±9.7
	Tetraploid	Mt. Solak	97.0±2.1
		Mt. Naebyun	90.3±5.9
S. fritschiana	Tetraploid	Mt. Bukhan	93.8±4.2

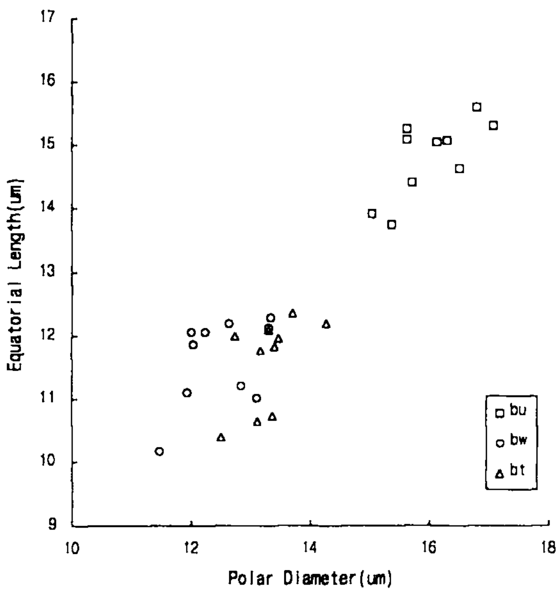

Fig. 1. Variation in equatorial length and polar diameter of pollen grains of polyploid complex of *S. blumei.* bu, tetraploid population of Ullung Island; bw, diploid population of Mt. Wolak; pt, diploid population of Taebaek.

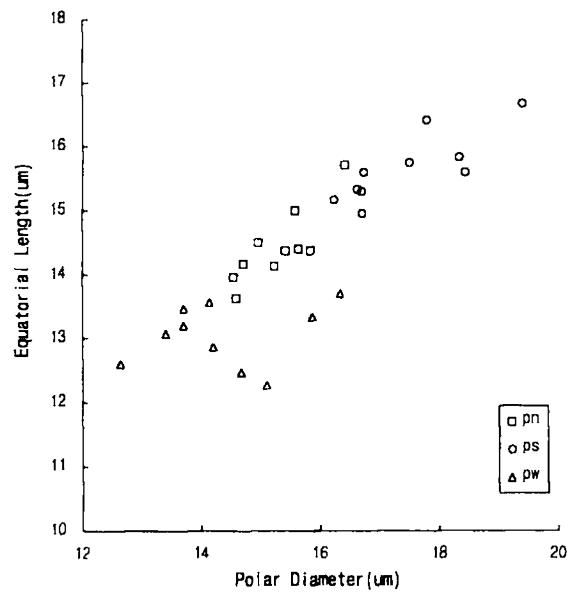

Fig. 2. Variation in equatorial length and polar diameter of pollen grains of polyploid complex of *S. pubescence*. pn, tetraploid population of Mt. Naebyun; ps, tetraploid population of Mt. Solak; pw, diploid population of Mt. Wolak.

plotting with equatorial length versus polar diameter (Fig. 1). In Fig. 1, the two diploid populations occupying in lower left mix together and stand apart from the tetraploid population. In the case of *S. pubescence,* similar to *S. blumei,* tetraploid populations have larger pollen than diploid population. Mean polar diameter, mean equatorial length and mean aperture length vary from 14.4, 13.1 and 10.6 μm in diploid population to 15.3~17.5, 14.4~15.7 and 11.4~13.1 μm in two tetraploid populations. Between the two tetraploid populations, however, Mt. Solak population has slightly larger pollen grains than Mt. Naebyun population which occupies intermediate region between diploid and Mt. Solak population as shown in Fig. 2. In contrast to quantitative characters, qualitative characters including striate sculpturing with pore in surface and shape of aperture are uniform throughout the populations. The P/E ratio is not significantly different among populations which varies from 1.09 to 1.16 in *S. blumei* and from 1.07 to 1.17 in *S. pubescence.*

Pollen Fertility

Measurements of pollen fertility estimated by staining with anilinblue-lactophenol are listed in Table 4. In *S. blumei,* the average fertility obtained from 16 individuals of the two diploid populations is 95.5% with small intraspecific variation from 91 to 98% except one individual with a value of 82%. However, considerable intraspecific variation occurs in two tetraploid populations in Ullung Island from 70.2 to 93.1%. The average fertility obtained from 10 individuals of the tetraploid populations is 82.2% and this value is low as compared to diploid populations.

Four individuals among them have less than 80% fertilities as 70.2, 73.9, 75.0 and 77.8%. Especially noteworthy is two very exceptional individuals are found (Sun 1448, Sun 1449, 14 May 1992. Do-dong, Ullung Is.). They produce no pollen grains in anther sac (These two individuals are not included in calculation of fertility of the population). In spite of intraspecific variation in amount of pollen produced, the morphology of anthers is the same. The data indicate, therefore, that *S. blumei* in Ullung Island might be gynodioecious or in the transition of dioecious condition. In contrast to *S. blumei*, the two different ploidy populations of *S. pubescence* have always more than 90% fertility and do not show significant variation among populations. The average fertilities are 90.6 in diploid population and 93.6% in two tetraploid populations, which indicate fertility is even higher in tetraploid populations than in diploid population. The two tetraploid populations of *S. fritschiana* also show average fertilities of 93.8 and 95.2%, similar to that of *S. pubescence*.

Stomata Size

Measurements of length and width of stomata among different ploidy populations of *S. blumei, S. pubescence* and *S. fritschiana* are listed in Table 4. In *S. blumei*, the average length and width of stomata are 8.3 and 3.7 μm in diploid population and 13.2 and 5.9 μm in tetraploid population. For *S. pubescence*, the average size of stomata is 9.2 in length and 4.5 μm in width in diploid population and 12.7 and 4.7 μm in tetraploid population. For *S. fritschiana*, the average length and width are 13.6 and 5.4 μm in diploid population and 17.0 and 5.7 μm in tetraploid population. Therefore, the data indicate that tetraploid populations have larger stomata in size than either diploid or triploid population. This tendency is more clear in length of stomata. The three measurements, equatorial length, polar diameter and aperture length are positively correlated with each other in all the populations of the three species.

DISCUSSION

Chromosome Number and Size of Pollen and Stomata

The result from sixty one chromosome counts from 11 taxa of Korean *Spiraea* is well consistent with previous report of the basic number of this genus as x=9 (Darlington and Wylie, 1955). A total of seven among 11 taxa of Korean *Spiraea* examined here are revealed as polyploids, which include triploid and tetraploid. This high level of incidence of polyploidy of Korean *Spiraea* is also consistent with the tendency of polyploidy of the genus as a whole inferred from previous reports of chromosome number of this genus. The level of polyploidy within sections of *Spiraea* referred from standard chromosomal indices (Darlington and Wylie, 1955; Cave, 1958-1965; Ornduff, 1967-1969; Fedorov, 1969; Moore, 1970-1977; Goldblatt, 1981-1988; Goldblatt and Johnson, 1990-1994) indicate that section *Glomerati* shows only diploid numbers, however, section *Spiraria* shows always polyploid numbers including tetraploid as well as octoploid. In accordance with this, our first report of *S. prunifolia* var. *simpliciflora* which belongs to sect. *Glomerati* is also counted as diploid and *S. salicifolia* belonging to section *Spiraria* is counted as tetraploid, which is consistent with previous report (Sax, 1936). More than 40% of the taxa previously counted in section *Chamaedryon* and *Calospira* is polyploid, and shows diverse ploidy levels from diploid to octoploid. Therefore, the data imply that polyploidization is probably correlated with the intra-sectional divergence within this genus. Peculiarly, two different ploidy populations of *S. fritschiana* occupy the same region but different elevations in Mt. Bukhan; tetraploid population are found near the summit, and triploid ones are found below the middle of the mountain. However, we failed to find diploid populations. In the case of *S. blumei*, two different ploidy populations occupy two different geographical systems, diploid populations in

Table 4. Measurements of length and width of stomata of some polyploid complexes of *Spiraea*

Species	Ploidy level	Length (μm)	Width (μm)
S. blumei	Diploid	6.00(8.34±0.85)10.00	2.00(3.67±1.57)8.00
	Tetraploid	6.00(13.24±2.24)24.00	2.00(5.91±1.86)10.00
S. pubescence	Diploid	8.00(9.21±1.54)14.00	2.00(4.47±0.94)6.00
	Tetraploid	8.00(12.67±1.53)16.00	4.00(4.66±1.12)8.00
S. fritschiana	Triploid	12.00(13.60±1.13)16.00	4.00(5.41±1.28)10.00
	Tetraploid	14.00(17.07±1.55)22.00	4.00(5.73±1.94)10.00

continental Korea and tetraploid ones in oceanic island (Ullung Island), which enables us to test general hypotheses of biological characteristics of oceanic island as discussed below. In comparison with pollen and stomata size between diploid and tetraploid populations of *S. blumei* and *S. pubescence*, multiplication of chromosome numbers contributes only to increase size of pollen and stomata, because external morphology of leaf, flower, trichome, and inflorescence does not show any differences between the populations.

Tetraploid of *S. blumei* in Ullung Island

Worldwide distribution of *S. blumei* is restricted to north of central Korea including Ullung Island, west of Kinki District of Honshu, Shikoku and Kyushu in Japan and southwestern China (Kim and Sun, 1996; Ohwi, 1984; Yü and Lu, 1974). Our first report of the chromosome number of 14 counts of seven inland populations of Korean Peninsula is consistently counted as diploid of 2n=18, and that of 10 counts of two populations of Ullung Island is counted as tetraploid of 2n=36. Chromosome numbers of Japanese and Chinese populations are still unknown. As for the origin of the island populations, it is more proper to consider that the island population is introduced from inland Korea rather than from Japan or China because of the geography and distance from the island. The island is located 150 km east of inland Korea and 300 km west of Japanese archipelagos. Considering the populations of inland Korea are counted consistently as diploid, island tetraploid populations might be originated by intra-island polyploidization through the introduction of diploid from inland Korea. Because the island population shows no morphological divergence from inland diploid population and no tendency toward any other species of this genus, island tetraploid population is more likely to be autotetraploid origin. This situation is interesting as compared with general tendency of changes of chromosome number within oceanic islands, where aneuploid or euploid changes are very rare (Carr, 1978; Sanders *et al.*, 1983). Sun *et al.* (1990) speculated that the reason for the tendency of chromosomal conservatism in oceanic islands may be related to the disruptive nature of chromosomal alterations in upsetting adaptive character complexes that are essential for survival in the microhabitats in the island. Unlike upsetting adaptive character complexes, the island population has no significant morphological divergence from inland diploid population. This may be one of the reason why island population could survive in spite of euploidic change of chromosome number.

As shown in Table 3, pollen fertility of island populations of *S. blumei* is relatively low as compared to inland populations. In addition, two very unusual individuals from island population which do not produce pollen grains in anther sacs are found. These facts suggest that tetraploid populations in the island is gynodioecious, which has been considered one of the possible steps to the evolution of dioecy (Richards, 1986). It may be also possible to suspect the low value of fertility of island populations may result from polyploidization, not from the tendency of dioecism. However, the tetraploid populations of *S. pubescence* and *S. fritschiana* in inland Korea show high and almost the same value with diploid ones. Hence, it seems to be more probable that the low value of fertility of island populations is resulted from the tendency of dioecy rather than from polyploidization.

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