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ORIGINAL ARTICLE

# Estimation of the relative DNA content in species of the genus *Spiraea*, sections *Chamaedryon* and *Glomerati* by flow cytometry

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The relative DNA content was studied in seven species of the genus *Spiraea* L., section *Chamaedryon* Ser., and in two species, section *Glomerati* Nakai, from 28 natural populations growing in Asian Russia. The cell nuclei were isolated from a leaf tissue. The relative intensity of fluorescence was measured using flow cytometry of propidium iodide-stained nuclei. The analysis was performed using a CyFlowSpace device (Germany, Sysmex Partec) with a laser radiation source of 532 nm. Fresh leaves of *Solanum lycopersicum* cv. 'Stupice' were used as an internal standard. Data on the relative DNA content are presented for the first time for *S. flexuosa* Fisch ex Cambess. (0.42–0.47 pg), *S. ussuriensis* Pojark. (0.49–0.52; 0.85 pg), *S. alpina*-Pall. (0.49–0.51 pg), *S. media* Schmidt. (0.45; 0.98–1.01 pg), *S. trilobata* L. (0.46 pg), S. *hypericifolia* L. (0.49–0.52 pg) and *S. aquilegifolia* Pall. (0.48–0.51 pg). Mesophytic species of the genus *Spiraea* growing in the forest zone (*S. chamaedryfolia* L. and *S. media*) exhibit a 2-fold higher relative DNA content (C-value) compared to xerophytic species (*S. hypericifolia*, *S. crenata* L., *S. aquilegifolia* and *S. trilobata*) and species confined only to mountain areas (*S. alpina*, *S. trilobata* and *S. aquilegifolia*).

Keywords: Spiraea; sections Chamaedryon and Glomerati; the relative DNA content; flow cytometry; DNA C-value

## Introduction

Studies on changes in the genome size of one species or closely related species of plants from different areas allow us to consider the evolutionary processes and their types as well as to predict next steps of evolution (Marda et al. 2008). Similar to chromosome number and morphological or anatomical characteristics, the genome size can be an informative marker in plant taxonomy at the species and subspecies level (Talluri & Murray 2009).

The genus *Spiraea* L. belongs to the subfamily *Spiroideae* Agardh., the most primitive in the family *Rosaceae* Juss (Takhtadzhyan, 1966). Representatives of the subfamily are highly decorative honey plants, which come in numerous forms and varieties and exhibit biological activity and other useful characteristics (Kostikova & Shaldaeva, 2017, Bespalov et al., 2018). The number of species in the genus *Spiraea* is not determined due to polymorphism of its species and different interpretations of the concept of species. High level of polyploidization and hybridization plays a very important role in speciation in the genus *Spiraea* (Sun et al., 1997; Zhang et al., 2002). The largest number of species of the genus *Spiraea* is concentrated in Asia: 70 species are indicated for China (Lu & Crinan, 2003), 9 species grow in Japan (Ikeda, 1995), and 8 species are confined to Mongolia (Grubov, 1955). In Russia and neighboring states, 20–25 *Spiraea* species grow under natural conditions, including 15–16 species from Siberia and 9–12 species from the Russian Far East (Poyarkova, 1939; Polozhiy, 1988; Yakubov, 1996).

We adhere to the system of the genius T.T. Yü & K.C. Kuan (1963) supplemented by T.I. Slavkina (1972). According to this system, the genus *Spiraea* L. falls into four sections: *Spiraria* Ser., *Calospira* C. Koch, *Chamaedryon* Ser. and *Glomerati* Nakai. Representatives of the largest section *Chamaedryon* Ser. differ from all other plants of the genus *Spiraea* in inflorescences – a short stalk is terminated by corymbose or umbellulate clusters of flowers. The section *Glomerati* includes species with a sessile umbel and is isolated from the section *Chamaedryon*. Representatives of these sections are common in Eurasia; however, they are not found in North America (Slavkina, 1972). The taxonomic rank of some species in the section *Chamaedryon*, such as *S. flexuosa* Fisch. ex Cambess., *S. ussuriensis* Pojark., *S. elegans* Pojark., *S. dahurica* (Rupr.) Maxim. and *S. sericea* Turcz., is often questioned and requires careful study and clarification using various methods. In Asian Russia, species of the section *Chamaedryofolia* L., *S. media* Schmidt., *S. pubescens* Turcz., *S. trilobata* L., *S. crenata* L., and

species of the section *Glomerati – S. hypericifolia* L. and *S. aquilegifolia* Pall should probably be considered the fully formed plants of the genus *Spiraea*. The relative DNA content of representatives of the genus *Spiraea*, sections *Chamaedryon* and *Glomerati*, growing in Asian Russia has not been previously studied.

The aim of the study was to compare the relative DNA content in species of the genus *Spiraea*, sections *Chamaedryon* and *Glomerati*.

# Methods

The materials for the study were fresh leaves of ten species of the genus *Spiraea*, sections *Chamaedryon* and *Glomerati*, collected in 2017 – 2019 from plants introduced in the experimental plot of the Central Siberian Botanical Garden SB RAS (CSBG) and from plants growing in native populations (Table 1). Almost all studied plants were brought from the native populations; some of them were grown from seeds collected also in the native populations. For each species, 3–5 plants from each of two populations were examined. All tests were carried out in 3 replicates. Some samples were investigated after drying in silica gel. Estimation of the relative DNA content in living plants and those dried in silica gel showed no differences.

The DNA content of plants was determined using flow cytometry of propidium iodide-stained nuclei. The analysis was carried out using a CyFlowSpace device (Germany, Sysmex Partec) with a laser radiation source of 532 nm. Fresh leaves of *Solanum lycopersicum* cv. 'Stupice' (2C DNA content = 1.96 pg) were used as an internal standard, and seeds were received from the Centre of the Plant Structural and Functional Genomics of the Institute of Experimental Botany AS CR, Olomouc, Czech Republic (Doležel et al., 1992).

Part of the fresh leaf 0.5 cm<sup>2</sup> in size was ground using an acute blade along with an appropriate amount of internal standard in 500  $\mu$ l of chilled Nuclei Extraction Buffer (Germany, Sysmex Partec) according to the manufacturer's protocol in plastic Petri dishes with addition of polyvinylpyrrolidone (MB ~ 29,000) (USA, Sigma-Aldrich). The samples incubation was carried out at room temperature for 2 minutes. The samples were filtered through the Partec CellTrics 50  $\mu$ m filter (Germany, Sysmex Partec). After that, 2 ml of staining solution containing another commercial Staining Buffer (Germany, Sysmex Partec), Pl (50  $\mu$ g/ml) and RNAse A (50  $\mu$ g/ml) were added. Staining was performed at room temperature in a dark place for 40 minutes. The prepared samples were stored in a refrigerator for not more than 4 hours. For each sample, 15,000 events were collected. The DNA 2C value content was calculated using a linear relationship between fluorescent signals from the stained nuclei of the internal standard and the studied samples.

The obtained data were processed using the Statistica 6.0. software (StatSoft, Inc. 1984–2001). The taxa differences in the relative DNA content were studied by nonparametric variance analysis (ANOVA) using the Kraskel-Wallis criterion.

#### **Results and Discussion**

The nuclear DNA content in plants of the genus *Spiraea*, sect. *Calospira*, growing in Asian Russia is determined for the first time by flow cytometry according to the fluorescence relative intensity (Table 1). The nuclear DNA content varies from 0.42 pg in *S. flexuosa* to 1.01 pg in *S. media*.

Figure 1 illustrates histograms of the relative DNA content generated by colored nuclei for the sample and the internal standard. Flow cytometry histograms show two dominant peaks corresponding to the nuclei at the  $G_0/G_1$  stage of the sample cell cycle and the internal standard, and the peak corresponding to the nuclei at the  $G_2$  stage of the standard or the sample.



**Figure 1.** Flow cytometry histograms: *S. chamaedryfolia* (1); *S. flexuosa* (2); *S. ussuriensis* (3). An internal standard is *Solanum lycopersicum* cv. "Stupice" (2C DNA content = 1.96 pg in Doležel et al., 1992)

Table 1. The nuclear DNA content in species of the genus *Spiraea*, sections *Chamaedryon* and *Glomerati*, from different populations (internal standard *Solanum lycopersicum* cv. "Stupice"(2C DNA content = 1.96 pg in Doležel et al., 1992)

Species	Sampling locality	Sampling locality (mean ± SD pg ***)				
Sect. <i>Chamaedryon</i>						
	Ser. <i>Trilobatae</i> Pojark. ex Yü					
S. trilobata	Altai Krai, Belokurikha city environs**	0.46 ± 0.01	224.94			
-//-	Altai Republic, Chemal village environs**	0.46 ± 0.00	224.94			
	Ser. <i>Mediae</i> Pojark. ex Yü					
	Republic of Buryatia,	0.54 . 0.00	2.42.22			
S. alpina	Moigoty village environs**	$0.51 \pm 0.00$	249.39			
-//-	Republic of Tuva, along Ak-Dovurak-Abakan road, N: 51°11.434'; E: 89°58.425'**	$0.49 \pm 0.00$	239.61			
S. media	Novosibirsk Oblast, Gorny settlement environs**	1.01 ± 0.01	493.89			
-//-	Krasnoyarsk Krai, Bolshaya Inya village environs**	1.01 ± 0.01	493.89			
-//-	Altai Krai, Navekalmanka villago environs**	1.00 ± 0.02	489,00			
	Republic of Yakutia,					
-//-	Aldan station environs**	$0.98 \pm 0.00$	479.22			
-//-	Amur Oblast, Mogot village environs**	0.45 ± 0.01	220.05			
	Ser. <i>Chamaedryfoliae</i> Pojark.					
S chamaadrufalia	Altai Krai,	0.88 ± 0.01	120 22			
S. Chamaeuryrona	Belokurikha city environs**	0.88 ± 0.01	430.32			
-//-	Altal Republic, Gorno-Altaysk city environs**	0.89 ± 0.01	435.21			
-//-	Altai Republic, Chemal village environs**	0.87 ± 0.01	425.43			
-//-	Krasnoyarsk Krai, shore of lake Ingol**	0.91 ± 0.01	444.99			
S. flexuosa	Krasnoyarsk Krai, Bolshaya Inya village environs**	0.46 ± 0.01	224.94			
-//-	Krasnoyarsk Oblast, Verkh-Miltyushi village environs**	$0.42 \pm 0.00$	195.60			
-//-	Irkutsk Oblast, Bolshoye Goloustnoye village environs**	0.47 ± 0.04	229.83			
S. ussuriensis	Khabarovsk Krai, Pivan village environs*	0.85 ± 0.02	415.65			
-//-	Primorsk Krai, Vladivostok city environs**	$0.49 \pm 0.00$	234.72			
-//-	Amur Oblast, Sergeevka village environs, Sergeevsky Utes**	0.50 ± 0.01	244.50			
-//-	Amur Oblast, Zeysky Nature Reserve**	0.52 ± 0.02	254.28			
	Ser. <i>Gemmatae</i> Yü					
S. crenata	Novosibirsk Oblast, Antonovo village environs **	0.45 ± 0.01	220.05			
-//-	Omsk Oblast, Zhukovka village environs**	0.48 ± 0.03	234.72			
-//-	Novosibirsk Oblast, 94 km of Karasuk-Ordynskoye highway (P382), N 53°56.358`E 78°32.947`** Sect. <i>Glomerati</i> Nakai	0.50 ± 0.01	244.50			

S. hypericifolia	Omsk Oblast Kalachinsk city environs**	0.49 ± 0.01	239.61
-//-	Novosibirsk Oblast, Gorny town environs**	0.52 ± 0.01	254.28
-//-	Novosibirsk Oblast, Steklyannoye village environs**	0.51 ± 0.00	249.39
S. aquilegifolia	Republic of Buryatia, Klyuchi village environs**	0.51 ± 0.00	249.39
-//-	Republic of Buryatia, Ust-Kyakhta village environs**	$0.48 \pm 0.00$	234.72

Notes: \* – plant grown from seeds; \*\* – natural material; \*\*\* SD – standard deviation (Std. Dev.); \*\*\*\* 1 pg of DNA = 978 Mbp (Doležel et al., 2003)

The relative DNA content (2C) in angiosperms varies from 0.06 to 254.80 pg (Leitch et al., 2005, Fleischmann et al., 2014). The variability of the C-value in the *Rosaceae* family is quite low: from 2C = 0.42 pg in *Physocarpus opulifolius* (L.) Maxim. to 2C = 3.11 pg in *Malus coronaria* (L.) Mill. Representatives of the subfamily *Spiraeoideae* exhibit the smallest genome size among angiosperms (Dickson et al., 1992). According to Leitch I.J. et al. (2005), relative DNA values in plants of the genus *Spiraea* can be referred to the category of "very small": *S. chamaedryfolia* 2C – 0.90 pg, *S. chinensis* – 0.40 pg; *S. crenata* – 0.45 pg; *S. pubescens* – 0.95 pg; *S. wilsonii* – 1.60 pg; *S. nipponica* – 1.75 pg; *S. sargentiana* – 1.85 pg; *S. betulifolia* – 0.91 and 1.01 pg; *S. beauverdiana* – 0.55 and 0.57 pg, and *S. aemiliana* (= *S. betulifolia* subsp. *aemiliana*) – 0.45 and 0.48 pg (Dickson et al., 1992, Siljak-Yakovlev et al., 2010, Bennett & Leitch, 2012, Kostikova et al., 2018). The values of the relative DNA content in the investigated plants of the genus *Spiraea*, sections *Chamaedryon* and *Glomerati*, are within the range of values previously recorded for *Spiraea* species (Table 1).

A conservative chromosome system is not inherent in plants of the genus *Spiraea*, which are capable of forming auto- and allopolyploids like other representatives of the family *Rosaceae*. The polyploidization in *Spiraea* often leads to pollen sterility (Sax, 1936). Triploids, tetraploids, hexaploids, and octoploids were found among *Spiraea* species (Zhukova, 1980, Oginuma et al., 2004, Polyakova & Muratova, 2015, Bennett & Leitch, 2012, Rice et al., 2015). For *Spiraea* species of the entire subfamily, the basic chromosomes number was found to be n = 9. *Spiraea* species, sect. *Chamaedryon*, are most often diploids with 2n = 18 (Table 2). Tetraploid forms with 2n = 36 are also encountered – *S. flexuosa, S. chamaedrifolia, S. media*, and *S. pubescens*, which were probably formed as a result of autopolyploidization (Oginuma et al., 2004). Triploid plants *S. media* with 2n = 27 were found. Isolated data on plants *S. alpina, S. chamaedrifolia*, and *S. media* with an atypical chromosome number 2n = 10; 20; 24; 32 (Table 2) are available. A linear relationship is often observed between the genome size and the number of chromosomes (Kechaykin et al., 2016).

*S. trilobata*, representative of Ser. *Trilobatae*, occurs in Russia – in Altai and the western part of the Western Sayan, and outside the country – in Central Asia, China and Korea (Koropachinskiy & Vstovskaya, 2002). The C-value content in species recorded from the Altai Republic and from Altai Krai is similar and equals 2C = 0.46 pg (Table 1). Diploids of *S. trilobata* are known from the Siberian part of the habitat only (Table 2).

*S. media* and *S. alpina*, ser. *Mediae Pojark*. ex Yü, have been investigated. *S. media*, a widespread and polymorphic species, exhibits the highest relative DNA content among *Spiraea* species studied, which varies from 0.45 to 1.01 pg (Table 1). Polymorphism is also characteristic of the somatic number of chromosomes in this species (Table 2). Apparently, diploidy is common in *S. media* (Probatova et al., 2007, Bennett & Leitch, 2012, Rice et al., 2015). One population with a genome size of 0.45 pg that grows in the Russian Far East is likely to have a diploid chromosome set. The relative DNA content in *S. media*, mainly from Siberian populations, is twice higher (0.98–1.01 pg). The relative DNA content in *S. alpina*, another less common species in this series, was 0.49–0.51 pg that indirectly indicates its diploid chromosome set. According to data available, only representatives of *S. alpina* are known to have 2n = 24 (Table 2).

S. chamaedryfolia, S. flexuosa and S. ussuriensis, ser. Chamaedryfoliae, are morphologically close to each other and exhibit numerous overlapping characteristics. The most evident distinguishing morphological characteristics include the shape of the shoot, inflorescence, leaf, and dentate of the leaf blade (Svyazeva, 1967). However, even these characteristics are sometimes unreliable due to intraspecific variability. Weak morphological differences, similar ecological and coenotic environment, and overlapping geographical areas confirm the previously stated idea that "these three species are isolated vicar species, and it would be more appropriate to consider them the subspecies of *S. chamaedryfolia*" (Svyazeva, 1967) The relative DNA content in *S. chamaedryfolia* recorded from different habitats ranged from 0.87 to 0.91 pg (Table 1), which is consistent with the values previously identified for this species – 2C = 0.90 pg (Siljak-Yakovlev et al., 2010). According to data available, diploids, tetraploids, and representatives with a cytotype of 2n = 32 are known for *S. chamaedryfolia* (Table 2). It is likely that we discovered tetraploid of this species. The genome size of *S. flexuosa* recorded from the Siberian region was 0.42–0.47 pg. The published data report diploids and tetraploids of S. flexuosa (Table 2). Apparently, we discovered diploids of this species. Spiraea ussuriensis (0.49-0.52 pg) is closer to *S. flexuosa* in genome size. The relative C-value content in species recorded from Khabarovsk Krai is close to that in S. chamaedryfolia and equals 0.85 pg. Only diploids of S. ussuriensis have been identified so far (Table 2). S. chamaedryfolia grows in Western and Eastern Siberia (Lower Yenisei basin), Central Asia (eastern Kazakhstan), Mongolia, and Europe. The eastern border of the species habitat can be characterized as rivalet, since the close species 5. flexuosa can be found to the east. S. ussuriensis occurs in Primorsk Krai and in the south of Khabarovsk Krai. Among the studied species, S.

*chamaedryfolia* exhibited the largest genome size, while the genome size of *S. flexuosa* and *S. ussuriensis* was half as large, except for plants recorded from Khabarovsk Krai (Table 1). To confirm the assumption that polyploidization occurred in certain types of habitat, it is necessary to analyze data from a larger number of plants throughout the habitat.

In Asian Russia, ser. *Gemmatae* Yü is represented by one mesoxerophytic species *S. crenata*. The relative DNA content in this species is 0.45–0.50 pg (Table 1), which is consistent with the DNA content previously identified for this species – 0.45 pg (Dickson et al., 1992). Only diploids of this species were found (Table 2).

The section *Glomerati* Nakai includes xerophytic shrubs *S. hypericifolia* and *S. aquilegifolia*. *S. hypericifolia* is widespread in Russia. *S. aquilegifolia* mainly grow in Mongolia and northern China; in Russia, it grows only in the steppes of Transbaikal (Koropachinskiy & Vstovskaya, 2002). *S. hypericifolia* (0.49–0.52 pg) and *S. aquilegifolia* (0.48–0.51 pg) have a similar genome size (Table 1). Data available report only diploids of *S. hypericifolia* (Table 2).

The genome size affects many aspects of the plant biology at the level of nuclei, cells and the entire plant, and ultimately affects the plant habitat and its survival rate under adverse environmental conditions (Greilhuber & Leitch, 2013, Suda et al., 2015). Species of sect. Chamaedryon grow in the steppe, forest-steppe and forest zones. Since the species of the genus Spiraea arose as mesophytes in Asia and in its eastern part, this area should be considered the center of origin of most and possibly all species of this genus (Svyazeva, 1967). More hygrophilous species of sect. Chamaedryon are probably older than xerophytic ones (Slavkina, 1972). The largest genome size among Spiraea species studied was revealed in S. chamaedryfolia and S. media – mesophytes growing in the forest zone. However, all xerophytic species (S. hypericifolia, S. crenata, S. aquilegifolia, and S. trilobata) have a genome size that is half as large (Table 1, Fig. 2). Spiraea alpina, S. trilobata and S. aquilegifolia that exhibit a small genome size are confined to mountain areas only. Other species grow both on the plain and in the mountains. A similar trend was observed for close species of the genus Spiraea, sect. Calospira. The genome size of S. beauverdiana, which grows in the northern tundra highlands, is half the genome size of the mesophytic plain forest species S. betulifolia (Kostikova & Polyakova, 2014, Kostikova et al., 2018). Individual genera and families of flowering plants with their own history of development responded differently to the conditions of highlands. Polyploid speciation was not observed for species of the family Rosaceae growing in highlands. Adaptation to conditions similar to those in the mountains occurred in this family at the diploid level (Krogulevich, 1971). Phenotypic and ecological spectra are much wider in plants with small genomes, which helps them to conquer different habitats (Simonin and Roddy, 2018). Extreme drought and salinity did not affect the size and complexity of the genome of *Reaumuria soongorica* (Pall.) Maxim. (Wang et al., 2011). In contrast to diploid species, polyploid species need a sufficient amount of N- and P-rich available nutrients (Pellicer et al., 2018). Xerophytic steppes or mountains are inhabited by species of the genus *Spiraea* with a small genome size. They are most likely diploids.

Due to the lack of reliable data on chromosomal numbers in the samples studied, the detected values of the relative DNA content cannot be used to establish ploidy in *Spiraea* species. In the future, we plan to count the number of chromosomes in the studied plant samples and to identify their relationship with the relative DNA content.



**Figure 2.** The relative nuclear DNA content (picogram) in species of the genus *Spiraea*, sect. *Chamaedryon* and sect. *Glomerati* (ecology): 1 – *S. trilobata* (xerophyte; mountain species); 2 – *S. alpina* (mesophyte; mountain species); 3 – *S. media* (mesophyte; forest species); 4 – *S. chamaedryfolia* (mesophyte; forest species); 5 – *S. flexuosa* (mesophyte); 6 – *S. ussuriensis* (mesophyte); 7 – *S. crenata* (xerophyte); 8 – *S. hypericifolia* (xerophyte); 9 – *S. aquilegifolia* (xerophyte, mountain species). Table 2. Number of chromosomes in the genus *Spiraea*, sect. *Chamaedryon* and *Glomerati* according to data available

Sc nur Species chror	matic nber of Study area nosomes, 2n	Reference
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Sect. Chamaedryon						
Ser. <i>Trilobatae</i>						
S. trilobata	18	Russia, Siberia	Rostovtseva, 1977; Polozhiy, 1988			
S. pubescens	18	USA	Sax, 1936 Dickson et al., 1992			
		Korea	Sun et al., 1997			
	36	Korea	Sun et al., 1997			
Ser. <i>Mediae</i>						
S. alpina	24	Russia, Siberia	Polozhiy, 1988			
<i>S. media</i>	10	Europe, Carpathians	Baksay, 1957			
	18	USA Russia, Siberia and RFE	Sax, 1936 Krogulevich, 1971; Krasnoborov et al., 1980; Polozhiy, 1988; Probatova et al., 2007; Probatova et al., 2009; Polyakova & Muratova, 2015, Ctepanov, 2018			
		Slovakia	Májovský et al., 2000			
		Russia, Moscow	Oginuma et al., 2004			
	20 27	Russia, Siberia Russia, RFE	Rostovtseva et al., 1981; Polozhiy, 1988 Probatova et al., 2007, Korobkov et al., 2014			
	36	Russia, Siberia	Krasnoborov et al., 1980; Polozhiy, 1988			
S. sericea	18	Russia, Siberia	Probatova et al., 2015			
		Ser. <i>Chamaedryfoliae</i>				
S. chamaedrifolia	18	USA	Sax, 1936			
			Delay, 1947			
	32	Austria	Wetschnig, 1998			
	36	Bulgaria	Česchmedziev, 1983			
		Russia, Moscow	Oginuma et al., 2004			
<i>S. chamaedryfolia</i> subsp. <i>ulmifolia</i>	36	Romania	Starlinger et al., 1994			
S. chamaedrifolia	18	Korea	Sun et al., 1997			
var. ulmifolia	36	USA	Sax, 1936			
S. flexuosa	18	Japan	Hara, 1952			
	36	Russia, Moscow	Oginuma et al., 2004			
		Russia, RFE	Polyakova & Muratova, 2015,			
S. ussuriensis	18	Russia, RFE	Polyakova & Muratova, 2015,			
Ser. <i>Gemmatae</i>						
S. crenata	18		Dickson et al., 1992			
Sect. Giomerati						
S. hypericifolia	18	USA	Sax, 1936			
		Kazakhstan	Rusanov, 1977			

#### Conclusion

The relative DNA content has been studied in seven species of the genus *Spiraea*, sect. *Chamaedryon* Ser., and in two species, sect. *Glomerati* Nakai, from 28 natural populations growing in Asian Russia. Data on the genome size are reported for the first time for S. *flexuosa, S. ussuriensis, S. alpina, S. media, S. trilobata, S. hypericifolia* and *S. aquilegifolia*. It is revealed that species of the genus *Spiraea* with a small genome size are confined to xerophytic conditions (*S. hypericifolia, S. crenata, S. aquilegifolia,* and *S. trilobata*) or mountain areas (*S. alpina, S. trilobata* and *S. aquilegifolia*). They are most likely diploids. In contrast, mesophytic species of the genus *Spiraea* growing in the forest zone (*S. chamaedryfolia* and *S. media*) exhibit a 2-fold higher relative DNA content, and they are probably polyploids.

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