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* 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 were 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 genus 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 *Spiraria* Ser. differ from all other plants of the genus *Spiraea* in inflorescences – a short stalk is terminated by corymbose or umbellate 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 *Chamaedryon* – *S. alpina* Pall., *S. chamaedryfolia* L., *S. media* Schmidt., *S. pubescens* Turcz., *S. trilobata* L., *S. crenata* L., and

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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$^2$ in size was ground using an acute blade along with an appropriate amount of internal standard in 500 μl of chilled Nuclei Extraction Buffer (Germany, Sysmex Partec) according to the manufacturer’s protocol in plastic Petri dishes with addition of polynvinlpyrrolidone (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 μm filter (Germany, Sysmex Partec). After that, 2 ml of staining solution containing another commercial Staining Buffer (Germany, Sysmex Partec), PI (50 μg/ml) and RNAse A (50 μg/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 G0/G1 stage of the sample cell cycle and the internal standard, and the peak corresponding to the nuclei at the G2 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 lycopersicums cv. ‘Stupice’ (2C DNA content = 1.96 pg in Doležel et al., 1992)](image)

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                                           | 2C DNA content (mean ± SD pg) | 1C**** (genome value, Mbp) |
|---------------------|-------------------------------------------------------------|-------------------------------|---------------------------|
| **S. trilobata**    | Altai Krai, Belokurikha city environs**                     | 0.46 ± 0.01                  | 224.94                    |
|                     | Altai Republic, Chemal village environs**                   | 0.46 ± 0.00                  | 224.94                    |
| **S. alpina**       | Republic of Buryatia, Moigoty village environs**            | 0.51 ± 0.00                  | 249.39                    |
|                     | Republic of Tuva, along Ak-Dovurak-Abakan road, N: 51°11.434', E: 89°58.425** | 0.49 ± 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, Novokalmanka village environs**                 | 1.00 ± 0.02                  | 489.00                    |
|                     | Republic of Yakutia, Aldan station environs**               | 0.98 ± 0.00                  | 479.22                    |
|                     | Amur Oblast, Mogot village environs**                       | 0.45 ± 0.01                  | 220.05                    |
| **S. chamaedryfolia**| Altai Krai, Belokurikha city environs**                     | 0.88 ± 0.01                  | 430.32                    |
|                     | Altai 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**     | Republic of Buryatia, Bolshaya Inya village environs**      | 0.46 ± 0.01                  | 224.94                    |
|                     | Krasnoyarsk Oblast, Verkh-Miltyushi village environs**      | 0.42 ± 0.00                  | 195.60                    |
|                     | Irkutsk Oblast, Bolshoye Goloustnoye village environs**     | 0.47 ± 0.04                  | 229.83                    |
| **S. ussuriensis**  | Novosibirsk Oblast, Khabarovsky Krai, Pivan village environs* | 0.85 ± 0.02                  | 415.65                    |
|                     | Primorsk Krai, Vladivostok city environs**                  | 0.49 ± 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                    |
| **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' ** | 0.50 ± 0.01                  | 244.50                    |
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, Ogimuna et al., 2004, Polyakova & Muratova, 2015, Bennett & Leitch, 2012, Rice et al., 2015). For Spiraea species of the entire subfamily, the basic chromosome 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 (Ogimuna 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 (Koropachinsky & Vstovskyaya, 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 rivulet, since the close species S. flexuosa can be found to the east. S. ussuriensis occurs in Primorsko 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 plants, it is necessary to analyze data from a larger number of plants throughout the habitat.

In Asian Russia, *ser. Gemmatae* Yu 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 (Kropachevsky & 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 (Kroglievich, 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 (Pelllicer 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.

### Table 2. Number of chromosomes in the genus *Spiraea*, sect. *Chamaedryon* and *Glomerati* according to data available

| Species | Somatic number of chromosomes, 2n | Study area | Reference |
|---------|----------------------------------|------------|-----------|
| *S. flexuosa* | 4 | Growing in highlands, Russia | (Dickson et al., 1992) |
| *S. crenata* | 4 | Growing in Transbaikal, Russia | (Kropachevsky & Vstovskaya, 2002) |
| *S. ussuriensis* | 4 | Growing in highlands, Russia | (Svyazeva, 1967) |
| *S. betulifolia* | 8 | Growing in tundra highlands, Russia | (Kostikova & Polyakova, 2014) |

**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).
### 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. aquilegifoila*. It is revealed that species of the genus *Spiraea* with a small genome size are confined to xerophytic conditions (*S. hypericifolia*, *S. crenata*, *S. aquilegifoila*, and *S. trilobata*) or mountain areas (*S. alpina*, *S. trilobata* and *S. aquilegifoila*). 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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