Number of nucleoli in diploids and polyploids of the genus *Achillea* L.

JANINA DĄBROWSKA

Department of General Botany, Institute of Botany, Wrocław University, Kanonia 6/8, 50-328
Wrocław, Poland

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Abstract

Nucleoli were counted in 9228 interphase nuclei of the apical root meristem of 40 *Achillea* L. taxa (di-, tetra-, hexa- and octoploids). It was established that the distribution of nucleoli number in an interphase nucleus can be used as a rough practical indicator to distinguish between diploids and polyploids. The highest number of nucleoli (12) was found in an octoploid *Achillea pannonica*, but only in a small percentage of the nuclei (0.3% out of 283 nuclei).

Key words: *Achillea* L., nucleolus number, chromosome number, polyploids

Some features of the polyploids are used for an indirect estimation of the degree of ploidy, especially in breeding but also in the systematic studies. In case of the genus *Achillea* L., which is a polyploid complex, attention was paid to pollen size (Ehrendorfer 1952, Háberová 1963, Oświecimska and Gawłowska 1967, Dąbrowska 1971a, Biste 1978, Savitsky 1984), size of stomata (Dąbrowska 1971a, Biste 1978, Ilinska 1984), of fruits (Dąbrowska 1977) as well as chloroplast number in stomata guard cells (Dąbrowska 1971b).

In my studies on the genus *Achillea* L. I have noticed a feature worth attention, which distinguishes diploids from polyploids. This was the number of nucleoli. I have not found any data in the available literature on the nucleolus numbers in the genus *Achillea* L. Relatively little studies have been carried out on the nucleoli in plants (Zenkter 1960).

I have noticed some differences as regards the number of nucleoli in the interphase nuclei of 40 *Achillea* L. taxa, observed in squashed samples from the

root growing apex, stained with the method of Feulgen. The samples were prepared for determination of DNA content. In order to check whether nucleoli counts could be used as an indirect method to determine the ploidy, observations were made on more numerous materials. Chromosome number was also determined in squashed samples. Whole small seedlings or roots were treated with saturated aquatic solution of alfa-bromonaphthalene before staining with orcein (Dąbrowska 1989a). On the other hand, seedlings of *A. ptarmica*, chromosomes of which are presented in Fig. 1, were treated for 5 hours with a mixture (1:1) of aquatic solutions of 0.005 M oxyquinoline and 0.25% colchicine before orcein staining (also squashes). Photographs were taken with Zeiss microscope NF, magn. K4 $\times$ 100.

Number of nucleoli was counted in 9228 interphase nuclei from apexes of 422 roots obtained from 40 taxa of *Achillea* L. (di-, tetra-, hexa- and octoploids). Distribution of the nucleoli numbers is presented in Table 1, in which number of nuclei is expressed in per cents. More nucleoli were present in polyploids than in diploids.

Diploids: (1–)2(–6) nucleoli. In as many as 22 diploids, 2 nucleoli represented the modal value. In most cases this distribution of the nucleoli could well be used to distinguish diploids from polyploids (Table 1). Taxa in which 2 nucleoli were most frequent had 33.8–55.3% of the nuclei with two nucleoli. Maximal number of 6 nucleoli was recorded in four species only.

Tetraploids: (1–)3(–10) nucleoli; 10 nucleoli were found in *A. asiatica* only, and only in small percentage (0.4% out of 211 nuclei).

Hexaploids: (1–)4(–11) nucleoli; 11 nucleoli were found only once, in *A. borealis*, a hexaploid from North America (0.7% out of 128 nuclei).

Octoploids: (1–)4(–12) nucleoli; 4 nucleoli were recorded in only 19% of the nuclei, but 6 nucleoli were almost as frequent, in 18.3% of the nuclei. The highest number of the nucleoli (12) was observed in small percentage of the nuclei (0.3% out of 283 nuclei).

Table 1 reveals that the modal value of the nucleoli numbers was found in less than 40% of polyploid nuclei (23.6–39.8% in tetraploids, 24.2–33.7% in hexaploids, 19% in octoploids).

Maximal number of nucleoli in particular taxa was rarely recorded, once or twice in a squash from the root apex. Maximal observed numbers of nucleoli were similar to the data given for other plants. According to Esau (1973) some

PLATE I

Fig. 1. *Achillea ptarmica*, $2n = 18$ (2x). Fig. 2. *A. clavenae*, $2n = 18$ (2x). Fig. 3. *A. ageratum*, $2n = 18$ (2x). Fig. 4. *A. abrotanoides*, $2n = 18$ (2x). Fig. 5. *A. lanulosa*, $2n = 36$ (4x). Fig. 6. *A. tanacetifolia*, $2n = 54$ (6x). Fig. 7. *A. nobilis*, $2n = 18$ (2x). Fig. 8. *A. ageratifolia*, $2n = 18$ (2x). Fig. 9. *A. stricta*, $2n = 54$ (6x). Fig. 10. *A. setacea*, $2n = 36$ (4x). Fig. 11. *A. crithmifolia*, $2n = 36$ (4x). Fig. 12. *A. millefolium* L. s. str., $2n = 54$ (6x). Fig. 13. *A. borealis*, $2n = 54$ (6x). Fig. 14. *A. crithmifolia*, $2n = 18$ (2x).

PLATE I

Figs. 1, 2, 7. Arrows point to SAT-chromosomes. Scale (10 μ m) is given in Fig. 2
 Figs. 3-6, 8-14. Nucleoli in the interphase nuclei. Scale (10 μ m) is given in Fig. 13

Table 1
Number of nucleoli in an interphase nucleus

Taxon	Number of nucleoli												Number of the observed nuclei	Number of the observed roots	Degree of ploidy
	1	2	3	4	5	6	7	8	9	10	11	12			
	Frequency of the observed nuclei in %														
<i>Achillea setacea</i>	47.6	39.8	9.7	2.7									256	10	2x
<i>A. salicifolia</i>	46.0	45.6	5.9	2.3									217	10	2x
<i>A. chamaemelifolia</i>	34.3	55.3	9.3	1.0									376	16	2x
<i>A. glaberrima</i>	27.7	52.2	13.3	6.6									90	8	2x
<i>A. macrophylla</i>	16.1	51.5	30.5	1.7									229	10	2x
<i>A. ptarmica</i>	36.4	51.3	11.2	0.9									222	10	2x
<i>A. compacta</i>	30.9	51.3	15.9	1.7									113	10	2x
<i>A. lingulata</i>	28.5	47.8	19.3	4.2									119	10	2x
<i>A. vermicularis</i>	39.4	46.5	11.7	2.2									307	10	2x
<i>A. impatiens</i>	28.9	43.6	22.8	4.5									197	10	2x
<i>A. filipendulina</i>	36.8	42.3	18.0	2.7									649	10	2x
<i>A. sulphurea</i>	32.6	42.2	20.0	5.0									279	10	2x
<i>A. tomentosa</i>	38.3	40.9	16.1	4.5									310	10	2x
<i>A. grandifolia</i>	31.6	33.8	26.0	8.4									142	10	2x
<i>A. teretifolia</i>	32.6	49.0	15.1	2.3	0.7								251	10	2x
<i>A. ageratum</i>	18.2	47.3	30.6	2.6	1.0								186	10	2x
<i>A. ligustica</i>	23.3	47.1	21.0	7.4	0.9								214	10	2x
<i>A. crithmifolia</i>	45.0	34.5	14.0	6.0	0.5								200	10	2x
<i>A. asplenifolia</i>	25.8	44.6	22.9	6.3	0.1								587	11	2x
<i>A. nobilis</i>	41.3	44.4	11.8	1.9	0.3								254	10	2x
<i>A. erba-rotta</i>	4.2	42.7	34.9	17.4	0.6								166	8	2x
<i>A. biebersteinii</i>	7.3	32.0	41.3	17.2	1.9								203	11	2x

<i>A. clavenae</i>	16.4	<u>37.9</u>	32.8	12.3	0.5								195	10	2x
<i>A. ochroleuca</i>	9.6	28.0	<u>44.7</u>	13.1	3.5	0.8							114	10	2x
<i>A. clypeolata</i>	35.0	<u>43.4</u>	17.1	4.0	—	0.2							368	13	2x
<i>A. abrotanoides</i>	25.9	<u>43.2</u>	19.1	11.1	—	0.6							162	10	2x
<i>A. ageratifolia</i>	4.4	<u>40.0</u>	25.5	22.2	5.5	2.2							90	10	2x
<i>A. sibirica</i>	14.2	<u>39.8</u>	30.4	14.0	1.3	—							364	10	4x
<i>A. collina</i>	8.1	<u>37.1</u>	32.0	15.7	2.5	4.4							159	7	4x
<i>A. lanulosa</i>	6.1	29.7	<u>31.6</u>	19.8	8.4	4.2							212	10	4x
<i>A. crithmifolia</i>	8.5	26.9	<u>30.2</u>	22.0	9.2	0.9	1.9						304	10	4x
<i>A. setacea</i> (Burgas)	12.7	8.4	10.1	21.1	11.0	<u>23.7</u>	5.9	4.2	2.5				118	10	4x
<i>A. asiatica</i>	4.7	14.6	<u>23.6</u>	23.2	9.9	15.1	4.2	1.8	1.8	0.4			211	15	4x
<i>A. stricta</i>	4.1	14.7	<u>33.7</u>	23.0	17.7	5.9	—	0.5	—	—			169	10	6x
<i>A. distans</i>	1.8	12.7	<u>22.4</u>	<u>24.2</u>	18.1	12.7	7.2	0.6	—	—			165	11	6x
<i>A. tanacetifolia</i>	2.3	17.7	<u>32.0</u>	25.3	13.8	5.2	2.3	0.4	0.4	—			209	10	6x
<i>A. millefolium</i> L. ssp. <i>sudetica</i>	2.5	16.0	21.7	<u>30.0</u>	11.3	13.4	2.0	2.0	0.5	—			193	10	6x
<i>A. millefolium</i> L. ssp. <i>millef.</i>	1.8	13.3	18.4	<u>25.8</u>	16.1	11.5	10.1	1.8	0.9	—			217	10	6x
<i>A. borealis</i>	1.5	8.5	15.6	24.2	<u>33.5</u>	14.8	0.7	—	—	—	0.7		128	11	6x
<i>A. pannonica</i> (Ligota Dolna)	2.4	7.4	10.9	<u>19.0</u>	14.8	18.3	13.7	7.4	4.5	0.7	—	0.3	283	21	8x
													Sum	9228	422

Modal values underlined. From among 40 taxa there were 2 species occurring in two forms 2x and 4x, i.e. *Archillea crithmifolia* and *A. setacea* (Dąbrowska 1989a), in this a tetraploid form of *A. setacea* which by author was found in Burgas, Bulgaria. Origin of the taxa under study has been given elsewhere (Dąbrowska 1989a, b). Information on an octoploid *A. pannonica* a refers to Ligota Dolna, Opole District.

plants may contain as many as 10 nucleoli. 6-13 Nucleoli were observed in tulips (Woods 1937, after Busch and Smetana 1970).

After division of the nucleus, the nucleoli appear in an even number, but later they can fuse (Hejnowicz 1980). Most probably, this phenomenon took place in the observed interphase nuclei. Synergism or fusion reduced the number of nucleoli in cells of *Drosophila*, *Pisum*, some spermatogonia and Sertoli cells, and in meiosis in many species (Kopac and Mateyko 1964, after Busch and Smetana 1970). In some species, the nucleolus does not disappear in course of mitosis; mutants have been observed with persistent nucleoli among the species in which the nucleolus normally disappeared. This suggests that the nucleolus cycle is not necessary for normal cell cycle (Hejnowicz 1980).

In view of the observations on the number of nucleoli, a question arises what number of SAT-chromosomes characterizes *Achillea* L. taxa?

During my inspection of a number of squashes made of root apices and stained with orcein (for chromosome determinations) I have discovered that SAT-chromosomes in *Achillea* L. taxa were often not seen. Satellites were very small. SAT-chromosomes in an even number were found in four diploids (Figs. 1, 2, 7, Table 2). Table 2 presents my own observations, with the exception of the number of SAT-chromosomes in *A. collina*.

Table 2
Number of SAT-chromosomes in taxa of *Achillea* L.

Taxon and degree of ploidy		SAT-chromosome numbers	Nucleolus number	
			range	modal value
<i>Achillea ptarmica</i>	2x	4 (Fig. 1)	1-4	2
<i>A. nobilis</i>	2x	4 (Fig. 7)	1-5	2
<i>A. clavenae</i>	2x	6 (Fig. 2)	1-5	2
<i>A. setacea</i>	2x	4 —	1-4	1
<i>A. collina</i>	4x	4 (Háberová 1963)	1-6	2

It was expected that *A. collina* would be characterized by high number of SAT-chromosomes, but Háberová 1963) found only four. Ehrendorfer (1958) showed experimentally that a tetraploid species *A. collina* originated from two diploids: *A. asplenifolia* and *A. setacea*. I have recorded four SAT-chromosomes in the latter species (the diploid form).

Table 2 shows that the modal number of nucleoli in an interphase nucleus is lower than the number of SAT-chromosomes. This observation conforms with the literature data. In most instances the number nucleoli in cells is smaller than the number of organizers which apparently represent a maximum

number (Busch and Smetana 1970). In some cells, there are as many nucleoli as there are constrictions or organizers; included in this group are cells of hyacinths, salamanders, axolotls, gerbils and others (Kopac and Mateyko 1964, after, Busch and Smetana 1970).

SUMMARY

Observations on the number of nucleoli in the interphase nucleus can be used as an indirect method of determining the ploidy of *Achillea* L. taxa, especially of distinguishing between diploids and polyploids.

Attention should be given to the fact that the number of nucleoli is differentiated among 27 diploids of *Achillea* L. (Table 1). Significant differences were also observed in the DNA content in the same diploids, and partly in the same squashes or in particular squashes from the same series (Dąbrowska 1989b). Variability of diploids in the polyploid complex of the genus *Achillea* L. is most interesting and calls for further studies in order to gain better knowledge on the history of this complex.

Data on nucleoli may be helpful in studies on the origin of polyploids. Use of nucleoli in the analysis of the origin of polyploidy is excellently reviewed by Gates (1942, after Lin 1955).

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Liczba jąder u diploidów i poliploidów rodzaju Achillea L.

Streszczenie

W merystemie wierzchołkowym korzeni 40 taksonów *Achillea* L. (di-, tetra-, heksa- i oktoploidów) liczono jąderka w 9228 jądrach interfazowych. Rozkład liczby jąder w jądrze interfazowym uznano za praktyczny orientacyjny wskaźnik, pozwalający na odróżnienie diploidów od poliploidów. Najwięcej jąder (12) stwierdzono u oktoploidu *Achillea pannonica*, lecz w niewielkim procencie jąder (0.3% z 283 jąder).