Research Article

New Record Holders for Maximum Genome Size in Eudicots and Monocots

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This paper presents the largest genome sizes reported so far for angiosperms: for the monocots *Trillium hageae* (Melanthiaceae) $2C = 264.9$ pg and for the eudicots *Viscum album* (Santalaceae) with $2C = 205.8$ pg. They were found during ongoing measurements of nuclear DNA content in more than 4000 accessions. Moreover, it is demonstrated that both of the values for the largest genome size so far for the monocots of *Fritillaria assyriaca* and for the eudicots of *Viscum cruciatum* need to be corrected.

1. Introduction

Until recently, genome size in angiosperms varied from 0.321 pg in *Arabidopsis thaliana* (Brassicaceae) [1] to 254.8 pg in *Fritillaria assyriaca* (Liliaceae) [2], an 800-fold range. This genome size is not related to the number of genes (polyploids excluded) but is due to large amounts of repetitive DNA. Also the number of chromosomes is not very relevant as some plants with very high chromosome numbers have low amount of nuclear DNA like *Sedum suaveolens* with about $2n = 640$ and 18.3 pg of DNA (Table 2). This is called the C-value paradox or the C-value enigma [3]. Genome size is not very useful at higher taxonomic levels, but is especially of value at the species level [4–9]. The amount of nuclear DNA is positively correlated with nuclear and cellular volume, whereby mainly cell size is subjected to selection [10].

The smallest angiosperm genomes are found in the Lentibulariaceae with *Genlisea margaretae* with a $2C$ DNA value of only 0.129 pg [11]. This is less than half the size of the genome of *Arabidopsis thaliana* with $2C = 0.321$ pg [1] and seems to increase the total range from 800 to 1968-fold. At the other end of the scale always the monocot *Fritillaria assyriaca* with $2C = 254.8$ pg is named as the species with the largest genome size for the angiosperms [2]. However, this is based on the wrong assumption [2], as will be demonstrated below, that *Fritillaria assyriaca* has the largest genome size. The supposed largest eudicot is *Viscum cruciatum* with $2C = 158.6$ pg. The present study presents

the highest values reported so far. They were found during ongoing measurements of nuclear DNA content in more than 4000 accessions [4–9]. They represent a new record at the higher end of the scale of angiosperm C-values. This emphasizes the need for a proper explanation of these very large genome sizes.

2. Material and Methods

2.1. Plant Material. *Trillium hageae* material was obtained from the collection of C. Denton, UK. *Viscum crassulae* was obtained from E. van Jaarsveld of Kirstenbosch Botanical Garden RSA and *Viscum minimum* of C. Grootsholten Succulent Nursery, The Netherlands. *Viscum album* was obtained from the Botanical Garden Wageningen, The Netherlands, and also from Arboretum Trompenburg Rotterdam, The Netherlands, and collected in south Limburg, The Netherlands; Pyrenees, France, and southern Greece. Live material of *Fritillaria* was contributed by P. Christian, J. de Groot, Hortus Bulborum Limmen, Potterton and Martin, W. Kletzing and M. de Jaeger. Where possible, material of known wild origin was used, and care was taken to ensure correct identification of all materials.

2.2. Flow Cytometric Measurement of Nuclear DNA Content. For the isolation of nuclei, about 1 cm² of adult leaf tissue was chopped together with a piece of *Haemantus albidiflos* (Amaryllidaceae) as an internal standard (see below).

TABLE 1: Genome sizes (2C) of *Fritillaria* species, standard deviation (SD), chromosome numbers, and number of accessions (#), compared with McLeish and LaCour [1].

Species	2C in pg	S.D.	2n *	#	McLeish and LaCour 2C in pg	Difference in pg
<i>Fritillaria verticillata</i>	84.9	1.9	24	4	96.5	11.6
<i>Fritillaria pallidiflora</i>	87.4	1.3	24	2	***116.7	29.3
<i>Fritillaria raddeana</i>	91.1	0.7	24	2	119.5	28.4
<i>Fritillaria liliacea</i>	89.6	1.8	24	1	121.4	31.8
<i>Fritillaria pyrenaica</i>	104.9	3.1	24	3	127.4	22.5
<i>Fritillaria meleagris</i>	112.4	3.0	24	4	141.4	29.0
<i>Fritillaria imperialis</i>	102.5	1.3	24	5	145.0	42.5
<i>Fritillaria olivieri</i>	117.1	0.4	24	3	145.6	28.5
<i>Fritillaria acmopetala</i>	132.5	1.9	24	4	151.1	18.6
<i>Fritillaria glauca</i>	111.7	0.5	24	1	155.3	43.6
<i>Fritillaria crassifolia</i>	133.1	3.5	24	5	160.8	27.7
<i>Fritillaria tuntasia</i>	130.4	1.5	24	2	163.2	32.8
<i>Fritillaria pinardii</i>	131.1	1.6	24	4	164.4	33.3
<i>Fritillaria rhodocanakis</i>	134.9	3.1	24	1	172.3	37.4
<i>Fritillaria davisii</i>	138.9	2.3	24	4	179.0	40.1
<i>Fritillaria assyriaca**</i>	203.9	1.4	48	3	254.8	50.9
<i>Fritillaria assyriaca</i>	134.0	4.3	24	4		

* from literature. ** = uva-vulpis, [15]. *** Bennett and Smith [16].

The chopping was done with a new razor blade in a Petri dish in 0.25 mL nuclei isolation buffer to which 0.25 mg RNase/mL was added [8]. After adding 1.5 mL propidium iodide (PI) solution (50 mg PI/l in isolation buffer) the suspension with nuclei was filtered through a 30 μ m nylon filter. The fluorescence of the nuclei was measured half an hour and one hour after addition of PI, using a Partec CA-II flow cytometer. The optical path contained an HBO mercury lamp, filters KG1, BG12, dichroic mirror TK500, filter OG570, and a Leitz 50 $\times$ 1 water immersion objective. Data were analyzed by means of DPAC software (Partec GmbH). If available, three different samples, and at least 3000 nuclei, were measured twice for each clone. Most histograms revealed a Coefficient of Variation (CV) of less than 5%.

2.3. Internal Standard and Absolute DNA Content Values. When measuring nuclear DNA content by means of flow cytometry, it is necessary to chop tissue from the plant of interest together with an internal standard: this standard must be as close as possible to the plants of interest. In this way, variation in signal intensities due to staining kinetics, to light absorption and quenching by sample components, as well as to instrument and other variables, is reduced to a minimum. *Haemanthus albiflos* was chosen as internal standard, because it has a convenient amount of DNA relative to plants with very high DNA values. Moreover, it is available year-round, does not mind several weeks without water, and it can serve numerous determinations. It also has a low background in PI measurements and shows a single G₀ peak, almost lacking G₂ arrest. Fresh male human leucocytes (2C = 7.0 pg [12]) (1 picogram = 0.978×10^9 base pairs [13]) were chosen as primary standard. This yields 2C = 15.9 pg for nuclei of *Agave americana*. This in turn was used to measure *Haemanthus albiflos* and resulted in 2C = 76.0 pg.

3. Results

3.1. The Monocots: *Fritillaria*, *Trillium*. The value of 2C = 254.8 pg for *Fritillaria assyriaca* is usually presented as the highest genome size in articles using flow cytometrical methods (this volume!). In the list of plant DNA C-values [2], 19 out of 24 species of *Fritillaria* are by McLeish and LaCour. If these are compared with the present results (Table 1), they are all higher in 2C DNA value with an average increase of 32 pg. It runs from 11 pg higher in *F. verticillata* (96.5 pg.) to 50 pg higher in *F. assyriaca* (254.8 pg). If the present value of 2C = 203.9 pg for *F. assyriaca* hort. (= *F. uva-vulpis*) is used instead of 254.8 pg of McLeish and LaCour, then several other species come in the same league like *F. imperialis* “*Maxima*” with 200.2 pg, and *F. elwesii* with 207.3 pg (Table 1). For real high values one must look to *Trillium* (*Melanthiaceae*). Especially the *trilliums* of Asia have high C-values due to their usually high ploidy [14]. The new record holder presented here is *T. hageae* with 264.9 pg (2n = 30). It is an allohexaploid of *T. camchatcense* (2n = 10) and *T. tschonoskii* (2n = 20). Another high DNA 2C-value is the Asiatic *T. apetalon* (2n = 20) with 190.0 pg [5].

3.2. The Eudicots: *Viscum*. *Viscum cruciatum* is with 2C = 158.7 pg [17] presented in the list of plant DNA C-values [2] as the eudicot with the largest genome size. For *V. album* a value of 2C = 152.0 pg is chosen [18]. Clearly it is chosen as “prime estimate” as it is intermediate between the value of Nagl et al. for *V. album* with 2C = 107.0 pg [19] and those of Ulrich et al. with 2C = 181.5 pg [17]. It would have been better to choose both values from a single laboratory. Ulrich et al. [17] found the 2C-value of *V. album* to be higher than those of *V. cruciatum* and that is confirmed here. Ulrich et al.

TABLE 2: Comparison of genome sizes (2C in pg) in this paper. # = number of accessions.

Species		2C in pg	S.D.	2n *	#	Nagl et al. [19]	Marie and Brown [18]	Ulrich et al. [17]
<i>Trillium</i>	<i>hagae</i>	264.9	3.2	30	1			
<i>Trillium</i>	<i>apetalon</i>	190.0	4.1	20	2			
<i>Viscum</i>	<i>album</i>	205.8	3.9	20	6	107.0	**152.0	181.6
<i>Viscum</i>	<i>cruciatum</i>	175.8	2.6	20	2			**158.7
<i>Viscum</i>	<i>crassulae</i>	164.0	3.6	24	1			
<i>Viscum</i>	<i>minimum</i>	124.6	3.2	28	2			
<i>Fritillaria</i>	<i>elwesii</i>	207.3	2.3	48	3			
<i>Fritillaria</i>	<i>imp.Maxima</i>	200.2	0.3	48	2			
<i>Fritillaria</i>	<i>assyriaca</i>	134.0	4.3	24	4			
<i>Haemanthus</i>	<i>albiflos</i>	76.0	2.1	32	6			
<i>Loranthus</i>	<i>europaeus</i> [20]	16.5						
<i>Orobanchae</i>	<i>hederae</i> [4]	5.5	0.4	38	2			
<i>Sedum</i>	<i>suaveolens</i>	18.3	2.4	c640	4			

* from literature. ** "Prime values" [2].

used several both A-T and G-C specific dyes and from these results calculated the "absolute DNA content." They also used Feulgen for *V. album* and obtained nearly the same result (184.4 pg). The present results for these species are 2C = 205.8 pg for *V. album* (2n = 20), and 2C = 175.8 pg for *V. cruciatum* (2n = 20) (Table 2). Also *V. crassulae* with 2C = 164.0 pg (2n = 24) and *V. minimum* with 2C = 124.6 pg (2n = 28) are here presented as new DNA 2C-values (Table 2).

4. Discussion

Most of the values of *Fritillaria* in the list of plant DNA C-values are from McLeish and LaCour from 1971 [2]. They were measured with Feulgen cytometry and based on a personal communication. The standard species is unknown and they might not have used "best practice" as advocated for the Feulgen method [20]. The increasing difference of 11–50 pg going from the lowest to the highest value in *Fritillaria* as presented by McLeish and LaCour compared with the present results (Table 1) suggest problems with the linearity of their measurements. Actually a 2C DNA value of 134 pg was found for four accessions of the diploid *F. assyriaca* (Table 1). It cannot be excluded that McLeish and LaCour had a tetraploid *F. assyriaca*. However, it is more likely, they used the plant that is in the trade as *F. assyriaca*, but actually is the tetraploid *F. uva-vulpis* with 2C = 203.9 pg. Moreover, Rix (1974) [15] has shown that up to that time all plants in commerce as *F. assyriaca* were a separate species *F. uva-vulpis*. The high values of McLeish and LaCour can be explained by the fact that they were obtained after recalibrating their values against *F. pallidiflora* with 116.7 pg [16]. The present results show *F. pallidiflora* to have only 87.4 pg (Table 1).

The combined results of the 2C DNA values of 0.129 pg for *Genlisea margaretae* [11] with those presented here with 264.9 pg for *Trillium hagae* increase the range of genome sizes for angiosperms to 2056-fold.

It may be wondered whether the high value found for *V. album* has anything to do with its parasitic nature. This seems

not likely as the related tree parasite *Loranthus europaeus* has 16.5 pg [18] and *Orobanchae hederae* has 5.5 pg [5]. The large genome size seems also not an impediment to growth rate. *V. album* on an apple tree in my garden reached football size and bear fruits in three years from seed.

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