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REGULAR PAPER

Nuclear DNA content in some species of *Lessingianthus* (Vernonieae, Asteraceae) by flow cytometry

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Abstract The nuclear DNA content was determined for the first time in 25 species of the South American genus *Lessingianthus* H.Rob. (Vernonieae, Asteraceae) by flow cytometry. This analysis constitutes the first estimation of the genome size for the Vernonieae tribe. The 2C- and 1Cx-values were calculated in all the species. The 2C-value ranged from 2.04 to 14.34 pg. The 1Cx-value ranged from 0.995 to 1.43 pg. The general tendency indicated a decrease in the 1Cx-value with increasing ploidy level, with some exceptions, in some species the 1Cx-value increased with the ploidy increase. The measuring of DNA content allowed reporting a new cytotype for *L. polyphyllus* (Sch.Bip.) H.Rob.

Keywords Asteraceae · Polyploidy · Vernonieae · 1Cx-value · 2C-value

Introduction

The tribe Vernonieae Cass. is one of the largest groups of Asteraceae with about 1,700 species distributed in the tropical regions of Asia, Africa and America. It presents two major centers of diversification, the central region of Africa and southern Brazil (Robinson 1999). Members of the tribe are grouped into seven subtribes based on inflorescence pattern, persistence of the phyllaries, pollen morphology, chemical composition and chromosome number (Keeley and Robinson 2009). The subtribe Lepidaploinae Keeley & H.Rob. constitutes the largest group within the Vernonieae, including more than 300 species. Almost all these taxa were previously placed into the huge section *Lepidaploa* (Cass.) DC. of the genus *Vernonia* Schreb., which has been separated into different genera by Robinson (1988a, b, c, 1992).

The genus *Lessingianthus* H.Rob. was established to recognise the species originally arranged under *Vernonia* Schreb. sect. *Lepidaploa* (Cass.) DC. subsect. *Macrocephalae* Benth. & Hook. (Baker 1873). As currently delimited, the genus comprises more than 120 species that are widely distributed in tropical South America, with a concentration in eastern Brazil. The species are perennial herbs or shrubs with xylopodia, having medium-or large-sized heads and seriate-cymose inflorescences (Robinson 1988a). Among other features, *Lessingianthus* can be distinguished from the remaining genera of the tribe Vernonieae by its pollen type, anther appendages, chromosome number and the shape of the achene wall crystals (Dematteis 2006).

The base number of *Lessingianthus* is $x = 16$ (Angulo and Dematteis 2009a). Chromosome numbers reported for the genus range from $2n = 32$ (diploid) to $2n = 176$ (endecaploid). Besides, cytological studies have shown that polyploidy is common within the genus with the greatest proportion of polyploids known (82.5 % of a total of 39 analyzed taxa) in the Vernonieae tribe (Angulo and Dematteis 2012).The chromosomes are widely variable in number and morphology within *Lessingianthus,* which suggests that they can be used in taxonomic and evolutionary studies (Angulo and Dematteis 2009a, b, 2012; Dematteis 1996, 1997, 1998, 2002, Dematteis et al. 2007; Oliveira et al. 2007; Ruas et al. 1991). The variations in chromosome number of the genus can lead to some variation in DNA content. These changes in genome size in relation to variation number of chromosome have been well documented in many taxa (Soltis et al. 2009).

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Several mechanisms have been proposed to explain changes in genome size and it has been observed increases and decreases in DNA amount. The increases of DNA content may occur through repeated cycles of polyploidization (Leitch and Bennett 1997, 2004; Otto and Whitton 2000; Soltis and Soltis 1999) or the activation of transposable elements (Bennetzen 2000; Soltis et al. 2003). Bennetzen and Kellogg (1997) indicate that plants have a one way ticket to the increase in genome size ("genome obesity"). The larger genomes of several groups of angiosperms (Liliaceae, Triticaceae, Santalales) would support this hypothesis (Kellogg and Bennetzen 2004; Leitch et al. 1998).

On the other hand, the genome contraction is believed to operate several phenomena such as inequal recombination, illegitimate recombination and other deleterious processes (Bennetzen 2000; Kellogg and Bennetzen 2004). The loss of DNA occurs in polyploid species. These changes in genome sizes are common after the polyploid formation. This phenomenon called "genome downsizing" by Leitch and Bennett (2004) can occur by the elimination of specific DNA sequences or homologous recombination.

The genome size is a character used in taxonomy and evolutionary studies. It has been used in different groups of plants to establish the origin of different basic number (Lavia and Fernández 2008), changes in the karyotypes (Poggio et al. 2007), and the relationships in a species complex (López et al. 2011). In *Lessingianthus* species there are no reported measurements of DNA content, but genome size variation has been explored in some genera of Asteraceae. This parameter (2C-values) ranged from 2.26 to 23.52 pg in *Microseris* D. Don. (Price et al. 1981), 1.80 to 31.30 pg in *Crepis* L. (Bennett and Smith 1976), 1.15–38.60 pg in *Senecio* L. (Lafuma et al. 2003; Zonneveld et al. 2005) and 5.20 to 25.90 pg in *Helianthus* L. (Price et al. 2000; Sims and Price 1985);. A full list of DNA content in species of the Asteraceae family has been recently provided by Garnatje et al. (2010).

As mentioned above, the genome size of the genus *Lessingianthus* was never analyzed. In this work, we used flow cytometry to estimate for the first time the nuclear DNA content in some species of the genus. The results are discussed in relation to the chromosomal data available. These results could be important for the interpretation of evolutionary trends of the genus.

Materials and methods

Plant material

The specimens studied were obtained from natural populations growing at different locations of Argentina, Bolivia, Paraguay and Uruguay. The source of the examined specimens is presented in Table 1. Voucher specimens are deposited at the herbarium of the Instituto de Botánica del Nordeste (CTES).

Flow cytometry

Young leaves were used to estimate nuclear DNA content (in picograms) in all the analyzed taxa. The measurements were calculated from three replicates per individuals. In total we analyzed three individuals per species. The leaves of *Paspalum intermedium* Munro ex Morong. accession Sch 28857 (diploid, 2C = 1.417 pg, Vaio et al. 2007) were used as internal standard for diploid (2*x*) entities of genus. While, *P. dilatatum* Poir. ssp. *flavescens* Roseng., B.R.Arrill. & Izag. (tetraploid, 2C = 2.43 pg, Vaio et al. 2007), was used as the standard for all tetraploid (4*x*) and hexaploid (6*x*) species. For octoploid (8*x*) taxon, leaves of *Secale cereale* L. 'Daňkovské' (2C = 16.19 pg, Doležel et al. 2007) were used as standard, while *P. dilatatum* Chirú biotype (hexaploid, 2C = 3.57 pg; Vaio et al. 2007) was used to measure the DNA content of decaploid (10*x*) species. Finally, *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg, Doležel et al. 2007) was a standard for endecaploid (11*x*) taxon of *Lessingianthus*.

Different species of *Paspalum* were used, because they are the common standard used in the Laboratory of genetics where the flow cytometer is located (IBONE, Corrientes, Argentina). In a few cases (taxa 8*x* and 11*x*), when the peaks of the examined species and the internal standard overlapped, we selected *Pisum sativum* and *Secale cereale* as internal standard.

The DNA content measurements were made using the technique of flow cytometry and the suspensions of intact nuclei were prepared according to López et al. (2011).

All the samples were analyzed with a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) with the detector operating at 355 nm. At least 5,000 nuclei were counted per sample. The PA-II Partec FloMax software was used for the data analysis.

The absolute 2C-value of DNA content of each sample was calculated according to Doležel and Bartoš (2005) employing the following formula:

$$\text{Sample 2C DNA content} = \frac{\text{sample G1 peak mean}}{\text{standard G1 peak mean}} \times \text{standard 2C DNA content (pg)}.$$

The monoploid genome size (1Cx) was calculated dividing the 2C-value by the ploidy level (Greilhuber et al. 2005). The factor for conversion of pg to Mbp is 978 (Doležel et al. 2003).

We calculated the coefficients of variation (HPCV: standard deviation divided by the mean of peaks) of the

Table 1 Species, voucher, locality, chromosome number (2*n*), ploidy level, 2C-value (pg) ± SE, coefficient of variation (HPCV), 1C-value in Mbp and 1Cx-value (pg) of all the *Lessingianthus* studied

Species	Voucher specimens	Chromosome number (2*n*)	Ploidy	2C	HPCV (%) of samples	HPCV (%) of standard[b]	1C (in Mbp)[a]	1Cx
L. brevifolius (Less.) H.Rob.	Argentina. Corrientes. Dept. Mercedes. 11 km S of Mercedes, old road to Curuzú Cuatiá. *M. Dematteis* et al. *2468* (CTES)	32	2*x*	2.41 (±0.07)	2.79	1.15	1174	1.20
L. coriaceus (Less.) H.Rob.	Bolivia. Dept. La Paz, Nor Yungas Province. Climb to Coroico, 1.4 km SE of the city. *M. Dematteis* et al. *4062* (CTES)	32	2*x*	2.61 (±0.05)	3.86	0.90	1271	1.30
L. polyphyllus (Sch. Bip. ex Baker) H. Rob	Argentina. Misiones. Dept. San Ignacio, 4 km S of San Ignacio, road to Teyú Cuaré. *M. Dematteis 2752* (CTES)	32	2*x*	2.31 (±0.03)	2.81	1.12	1125	1.15
L. pusillus (Dematt.) M. B. Angulo	Argentina. Corrientes. Dept. Capital, Perichón. *M. Dematteis* et al. *2769* (CTES)	32	2*x*	2.04 (±0.01)	1.17	0.90	997	1.02
L. rubricaulis (Humb. & Bompl.) H.Rob.	Argentina. Corrientes. Dept. San Roque. Route 12, 2 km N of route 123. *M. Dematteis* et al. *2756* (CTES)	32	2*x*	2.12 (±0.07)	1.30	0.99	1037	1.06
L. sellowii (Less.) H.Rob.	Uruguay. Dept. Tacuarembó, Gruta de los Helechos, 10 km NW of Tacuarembó. *M. Dematteis* et al. *3760* (CTES)	32	2*x*	2.05 (±0.06)	3.31	1.15	997	1.02
L. varroniifolius (DC.) H.Rob.	Bolivia. Dept. Santa Cruz, Velasco Province 67 km E of Concepción, on the road to San Ignacio. *M. Dematteis* et al. *3860* (CTES)	32	2*x*	2.51 (±0.02)	1.89	0.92	1222	1.25
L. cataractarum (Hieron.) H.Rob.	Argentina. Misiones. Dept. San Pedro. Parque Nacional Moconá. *M. Dematteis* et al. *3096* (CTES)	64	4*x*	4.82 (±0.09)	3.46	1.10	2357	1.20
L. intermedius (DC.) Dematt.	Uruguay. Dept. Maldonado. Piriapolis, San Antonio Mount. *M. Dematteis* et al. *3807* (CTES, SI)	64	4*x*	4.05 (±0.03)	1.41	0.89	1975	1.01
L. laniferus (Cristóbal & Dematt.) M. B. Angulo	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Américo. M. *Dematteis* et al. *3076* (CTES)	64	4*x*	4.08 (±0.15)	0.90	1.00	1995	1.02
L. mollissimus (D. Don ex Hook. & Arn.) H.Rob.	Argentina. Corrientes. Dept. Santo Tomé, 12.7 km S of Virasoro, on the road to Santo Tomé. *M. Dematteis* et al. *4136* (CTES)	64	4*x*	3.99 (±0.01)	4.29	0.99	1946	0.997
L. plantaginoides (Kuntze) H.Rob.	Argentina. Dept. San Martín. Tres Cerros, 27 km W of La Cruz. *M. Dematteis* et al. *4140* (CTES)	64	4*x*	4.44 (±0.09)	4.77	0.98	2171	1.11
L. pseudoincanus (Hieron.) Dematt.	Argentina. Misiones. Dept. Capital. Arroyo Zaiman. *M. B. Angulo 19* (CTES.)	64	4*x*	3.98 (±0.04)	0.80	1.02	1946	0.995
L. rubricaulis (Humb. & Bonpl.) H.Rob.	Bolivia. Dept. Santa Cruz, Chiquitos Province. 25 km N of San José, road to San Rafael. *M. Dematteis* et al. *3567* (CTES)	64	4*x*	4.50 (±0.06)	2.53	1.05	2200	1.12
L. saltensis (Hieron.) H.Rob.	Argentina. Jujuy. Dept. Ledesma, 19 km of San Francisco, on the road to Valle Grande. *M. Dematteis* et al. *2952* (CTES)	64	4*x*	4.60 (±0.10)	0.70	1.10	2543	1.30
L. sellowii (Less.) H.Rob.	Argentina. Misiones. Dept. Concepción, road to Puerto Azara, 6 km E of Azara. *M. Dematteis* et al. *3315* (CTES)	64	4*x*	4.58 (±0.09)	3.64	1.11	2240	1.14
L. argenteus (Less.) H.Rob.	Paraguay. Dept. Amambay. Chirigüelo. *M. Dematteis* et al. *3396* (CTES)	96	6*x*	8.63 (±0.15)	3.20	0.81	4215	1.43
L. centauropsideus (Hieron.) Dematt.	Argentina. Salta. Dept. Santa Victoria. 6 km S of Los Toldos, road to Lipeo. *M. Dematteis* et al. *2937* (CTES)	96	6*x*	6.84 (±0.07)	1.93	1.10	3345	1.14

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Table 1 continued

Species	Voucher specimens	Chromosome number (2*n*)	Ploidy	2C	HPCV (%) of samples	HPCV (%) of standard[b]	1C (in Mbp)[a]	1Cx
L. niederleinii (Hieron.) H.Rob.	Argentina Misiones. Dept. General Manuel Belgrano. Campina de Américo. *M. Dematteis* et al. *3052* (CTES)	96	6*x*	7.02 (±0.02)	0.70	0.98	3433	1.17
L. sp. *nov.*	Bolivia. Dept. Santa Cruz, Chiquitos Province. Natural Reserve of Tuca Vaca Valley. *M. Dematteis* et al. *3930* (CTES)	96	6*x*	8.05 (±0.06)	1.46	0.90	3931	1.34
L. profusus (Dematt. & Cabrera) M. B. Angulo	Paraguay. Dept. Canindeyú. 3.4 km N of Igatimí on the road to Ypé-Jhú. *M. Dematteis* et al. *2843* (CTES)	96	6*x*	6.60 (±0.10)	2.50	0.99	3227	1.10
L. scabrifoliatus (Hieron.) H.Rob.	Bolivia. Dept. Santa Cruz. Velasco Province 67 km E of Concepción, on the road to San Ignacio. *M. Dematteis* et al. *3856* (CTES)	128	8*x*	9.79 (±0.08)	1.45	1.03	4782	1.22
L. teyucuarensis (Cabrera) Dematt.	Argentina. Misiones. Dept. San Ignacio. Teyú Cuaré. *M. Dematteis* et al. *3049* (CTES)	160	10*x*	10.64 (±0.03)	4.34	1.11	5203	1.06
L. robustus (Rusby) H.Rob.	Bolivia. Dept. La Paz. Nor Yungas Province. Climb to Coroico, 1.4 km SE of the city. *M. Dematteis* et al. *4063* (CTES)	160	10*x*	11.65 (±0.09)	1.23	0.90	5692	1.16
L. macrocephalus (Less.) H.Rob.	Uruguay. Dept. Rivera, road from Tranqueras to Paso Ataques, 4 km of route 30. *M. Dematteis* et al. *3731* (CTES)	176	11*x*	14.34 (±0.16)	1.65	0.98	7012	1.30

[a] 1 pg DNA = 978 Mbp according to Doležel et al. (2003)

[b] Coefficient of variation of the internal standard

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samples and the standards. Additionally, we performed the dispersion diagrams to evaluate the relationship among the 2C-values, 1Cx-values and the chromosome numbers (2*n*) of species. The analysis was carried out with the Infostat software, version 2009 (Di Rienzo et al. 2009).

Results

The DNA content of a total of 25 species belonging to the genus *Lessingianthus* were analyzed (Table 1). In all cases, the coefficients of variation (HPCV) were lower than 5 % (Table 1), supporting the reliability of the flow cytometric assessments. Holoploid (2C) genome size in *Lessingianthus* species ranged from 2.04 pg in *L. pusillus* (2*x*) to 14.64 pg in *L. macrocephalus* (11*x*). The dispersion diagram (Fig. 1) which relates the 2C-value and chromosome number (2*n*) shows groups well differentiated: group 1 included all diploid species (2C = 2.04–2.61 pg), group 2 consisted of tetraploid taxa (2C = 3.98–4.82 pg) and the hexaploid entities composed group 3 (2C = 6.84–8.63 pg). *Lessingianthus scabrifoliatus* was isolated from the other entities and formed group 4 (2C = 9.79 pg), while the two decaploid species formed group 5 (2C = 10.64 to 11.65 pg) and finally, group 6 included the endecaploid taxon (2C = 14.34 pg).

The analysis of the relationship between the 1Cx-value and chromosome number (2*n*) (Fig. 2), shows that tetraploid taxa had similar or lesser 1Cx-values than the diploid species. Among them, *L. saltensis* (4*x*) presented the highest value (1Cx = 1.30), which is similar to the value obtained in *L. coriaceus* (2*x*), while the smallest 1Cx-value was found in *L. pseudoincanus* (4*x*). The hexaploid species presented, in general, greater 1Cx-values when they were compared to the remaining analyzed entities. Among the 6*x* species, *L. argenteus* was the taxon with the highest 1Cx-value (1.43 pg). *Lessingianthus scabrifoliatus* (8*x*) presented a similar 1Cx-value to *L. cataractarum* (4*x*) and the decaploid entities showed 1Cx-values similar to the diploid taxa. Finally, *L. macrocephalus* (11*x*) had the same 1Cx-value than *L. coriaceus* (2*x*) and *L. saltensis* (4*x*).

Discussion

The DNA contents of 25 species of *Lessingianthus* are published here for the first time. Also, this work constitutes the first analysis of genome size for the Vernonieae. The results obtained in this study showed that increases in the 2C-value are related to increases of ploidy level (see Fig. 1), while, the 1Cx-values in most *Lessingianthus* species decrease in relation to the increases of the ploidy (see Fig. 2). These results agree with the proposal by Leitch and Bennett (2004) and Bennett and Leitch (2005), who pointed out that opposite to expectations, the 1Cx-value decreases with the increase of the ploidy.

Downsizing of the genome after polyploidization appears to be a general trend in angiosperms (Kellogg and Bennetzen 2004; Leitch and Bennett 2004). The 1Cx-values obtained here also reflect the genome downsizing process. Therefore, the loss of DNA in polyploids or genome downsizing occurs in some polyploid taxa of *Lessingianthus* (see Fig. 2). Mechanisms leading to a decrease in genome size include unequal crossing over (Wendel et al. 2002), illegitimate crossing over (Devos et al. 2002), a higher overall rate of deletions than insertions (Petrov

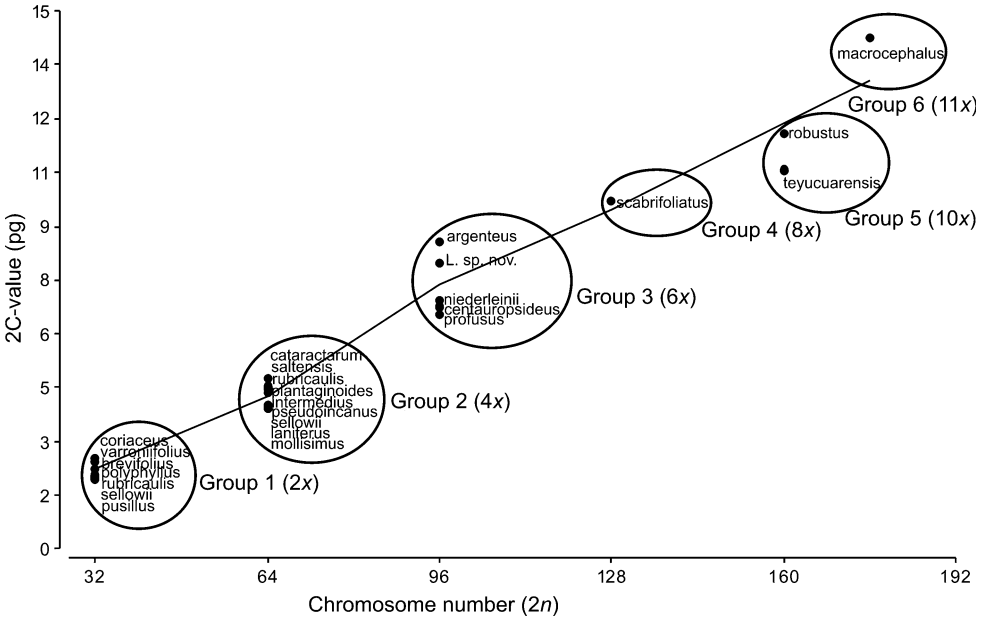

Fig. 1 Dispersion diagram representing relationship between 2C-value and chromosome number (2*n*). The *line* denote the observed tendency between the variables (using Lowess: locally weighted scatterplot smoothing). The *circles* show the grouped species by the DNA content

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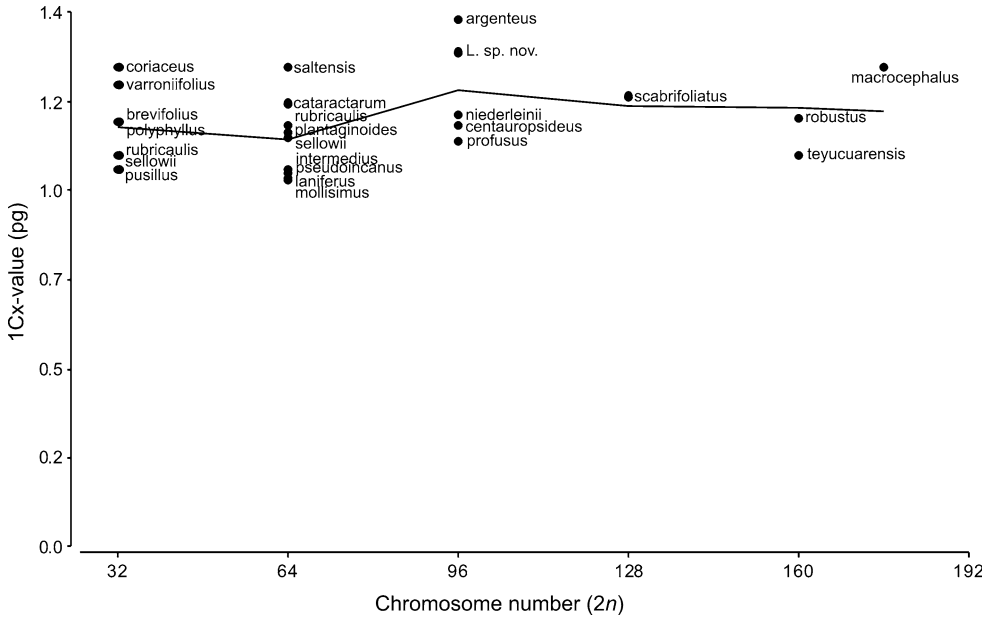

Fig. 2 Dispersion diagram between 1Cx-value and chromosome number (2*n*) showing the observed tendency (*line*) between variables (Lowess)

2001), and selection against transposable elements (Wright and Schoen 1999; Morgan 2001). Ozkan et al. (2003) suggested that this slight reduction in DNA content with the increase of the ploidy level could be an adaptation necessary for the formation and stabilization of polyploid genomes.

In some entities of *Lessingianthus* there is an increase of 1Cx-values with respect to the ploidy. If we compare the mean 1Cx-values of the diploid species (mean 1Cx = 1.14 pg) with the values of the remaining examined entities, it can be observed that polyploids present similar values to the diploid species (Table 1). The tetraploid species *L. mollisimus* (D. Don ex Hook. & Arn.) H.Rob. and *L. pseudoincanus* (Hieron.) Dematt., showed lower values of monoploid genome size with respect to the diploid taxa (1Cx = 0.995 and 0.994 pg, respectively). But, an exception to this downsizing pattern was observed in the hexaploid species *L. argenteus* (Less.) H.Rob. (1Cx = 1.43 pg) and *L.* sp. *nov*. (1Cx = 1.34 pg) with higher values of 1Cx with regards to 2*x* species. Also, in *L. rubricaulis* (Humb. & Bonpl.) H.Rob. and *L. sellowii* (Less.) H. Rob an increase in 1Cx-value in relation to the ploidy level was observed. The tetraploid cytotypes of both entities had higher 1Cx-values (*L. rubricaulis* 1Cx = 1.12 pg and *L. sellowii* 1Cx = 1.14 pg) than the diploid cytotypes (*L. rubricaulis* 1Cx = 1.06 pg and *L. sellowii* 1Cx = 1.02 pg).

A possible autopolyploid origin would be assumed to occur to *L. rubricaulis* and *L. sellowii*. In both species, contrary to expectations, the 4*x* cytotypes had higher 1Cx-values in relation to the 2*x* cytotypes. Several causes may be attributed to the increase of the genome size. Bennetzen and Kellogg (1997) suggest the possibility of "genome obesity" in plants. This increase in size could obey to the need of mechanisms to get rid of superfluous DNA. Another alternative might be that polyploidization in these species has occurred relatively recently and that there has not yet been time for substantial reductions in nuclear DNA-content. Alternatively, mechanisms causing major reductions in other polyploids might not be active in this species or only to a minor extent (Jacob et al. 2004).

Therefore, in the genus *Lessingianthus* there are increases and decreases of the genome size. In Asteraceae, a similar situation was documented in several genera of the family, such as *Artemisia* L. (Pellicer et al. 2007), *Centaurea* L. (Bancheva and Greilhuber 2006) and *Hieracium* L. (Chrtek et al. 2009). The downsizing of genome was found in most of the examined species and upsizing in a few species.

The measuring of DNA content in the genus allowed reporting a new cytotype for an Argentinian population of *L. polyphyllus* (Sch. Bip. ex Baker) H.Rob. (population not studied chromosomically). This population had 2C = 2.32 pg, a lower value than expected for a tetraploid taxon (2C = 3.98–4.82 pg). Cytological studies previously conducted on other samples of Argentina of this species indicate that it is tetraploid with 2*n* = 4*x* = 64 (Dematteis 2002). However, the 2C-value (2C = 2.32 pg) presented in the population studied suggests that it would be diploid because this value is consistent with the values obtained for the 2*x* species of the genus (2C = 2.04–2.80 pg). Chromosome counts are necessary; however, to confirm this cytotype for the species.

The DNA content of *L.* sp. *nov.* allowed distinguishing it from *L. scabrifoliatus* (Hieron.) H.Rob. Both species are closely related but can be separated by leaves margin, sizes of heads and the trichomes of the fruits, among other

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features. Besides, the entities also have different chromosome number and DNA content. *Lessingianthus* sp. *nov*. is hexaploid with $2n = 96$ and has 1Cx = 1.34 pg, while *L. scabrifoliatus* is octoploid with $2n = 128$ (Angulo and Dematteis 2012) and has 1Cx = 1.22 pg. On the other hand, *L. intermedius* (DC.) Dematt. and *L. plantaginoides* (Kuntze) H.Rob. are closely related species that show the same chromosome number ($2n = 4x = 64$). Nevertheless, both taxa have different DNA content, because *L. intermedius* has 1Cx = 1.01 pg, while *L. plantaginoides* presents 1Cx = 1.11 pg.

In conclusion, the results of this study suggest that both increases and decreases in DNA content have occurred during the evolution of genome size in *Lessingianthus*. Besides, the measurements of DNA content allowed reporting a new cytotype for one of the species of the genus. The DNA content knowledge of *Lessigianthus* is still limited and additional studies on other species of the genus are necessary to understand the evolution of the genus.

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References

Angulo MB, Dematteis M (2009a) Caryological analysis of South American species of *Vernonia* (Vernonieae, Asteraceae). Plant Biosyst 143:20–24

Angulo MB, Dematteis M (2009b) Karyotype analysis in eight species of *Vernonia* (Vernonieae, Asteraceae) from South America. Caryologia 62:81–88

Angulo MB, Dematteis M (2012) Cytotaxonomy of some species of the South American genus *Lessingianthus* (Asteraceae, Vernonieae). Plant Syst Evol 298:277–285

Baker JG (1873) Compositae. I. Vernoniaceae. In: Martius C (ed) Flora Brasiliensis, vol 6, pp 1–179

Bancheva S, Greilhuber J (2006) Genome size in Bulgarian *Centaurea* s.l. (Asteraceae). Plant Syst Evol 257:95–117

Bennett MD, Leitch IJ (2005) Nuclear DNA amounts in Angiosperms: progress, problems and prospects. Ann Bot 95:45–90

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc B 274:227–274

Bennetzen JL (2000) Transposable element contributions to plant gene and genome evolution. Plant Mol Biol 42:251–269

Bennetzen JL, Kellogg EA (1997) Do plants have a one-way ticket to genomic obesity? Plant Cell 9:1509–1514

Chrtek J Jr, Zahradníček J, Krak K, Fehrer J (2009) Genome size in *Hieracium* subgenus *Hieracium* (Asteraceae) is strongly correlated with major phylogenetic groups. Ann Bot 104:161–178

Dematteis M (1996) Estudios cromosómicos en especies argentinas de *Vernonia* (Asteraceae). Bonplandia 9:103–110 (in Spanish)

Dematteis M (1997) Números cromosómicos y cariotipos de algunas especies de *Vernonia* (Asteraceae). Bol Soc Argent Bot 33:85–90

Dematteis M (1998) Karyotype analysis in some *Vernonia* species (Asteraceae) from South America. Caryologia 51:279–288

Dematteis M (2002) Cytotaxonomic analysis of South American species of *Vernonia* (Vernonieae: Asteraceae). Bot J Linn Soc 139:401–408

Dematteis M (2006) Two new species of *Lessingianthus* (Vernonieae, Asteraceae) from the Brazilian highlands. Bot J Linn Soc 150:487–493

Dematteis M, Molero J, Angulo MB, Rovira A (2007) Chromosome studies of some Asteraceae from South America. Bot J Linn Soc 153:221–230

Devos K, Brown J, Bennetzen J (2002) Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. Genome Res 12:1075–1079

Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW (2009) InfoStat version 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Doležel J, Bartoš J (2005) Plant flow cytometry and estimation of Nuclear Genome size. Ann Bot 95:99–110

Doležel J, Bartoš J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51:127–128

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc 2:233–2244

Garnatje T, Canela MA, Garcia S, Hidalgo O, Pellicer J, Sánchez-Jiménez I, Siljak-Yakovlev S, Vitales D, Vallès J (2010) GSAD: A genome size database in the Asteraceae. Release 1.0, July (http://www.asteraceaegenomesize.com, database environment by Gálvez F.)

Greilhuber J, Doležel J, Lysak MA, Bennett MD (2005) Evolution and proposed stabilization of the terms 'Genome Size' and 'C-Value' to describe nuclear DNA contents. Ann Bot 95:255–260

Jacob SS, Meister A, Blatner FR (2004) The considerable genome size variation of *Hordeum* species (Poaceae) is linked to phylogeny, life form, ecology, and speciation rates. Mol Biol Evol 21:860–869

Keeley SC, Robinson H (2009) Vernonieae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ (eds) Systematics, evolution and biogeography of Compositae. IAPT, Wien, pp 439–469

Kellogg EA, Bennetzen JL (2004) The evolution of nuclear genome structure in plants. Am J Bot 91:1709–1725

Lafuma L, Balkwill K, Imbert E, Verlaque R, Maurice S (2003) Ploidy level and origin of the European invasive weed *Senecio inaequidens* (Asteraceae). Plant Syst Evol 243:59–72

Lavia GI, Fernández A (2008) Genome size in wild and cultivated peanut germplasm. Plant Syst Evol 272:1–10

Leitch IJ, Bennett MD (1997) Polyploidy in angiosperms. Trends Plant Sci 2:470–476

Leitch IJ, Bennett MD (2004) Genome downsizing in polyploid plants. Biol J Linn Soc 82:651–663

Leitch IJ, Chase MW, Bennett MD (1998) Phylogenetic analysis of DNA C-values provides evidence for small ancestral genome size in flowering plants. Ann Bot 82:85–94

López A, Panseri AF, Poggio L, Fernández A (2011) Nuclear DNA content in the polyploid complex *Turnera ulmifolia* (*Turnera* L., Passifloraceae). Plant Syst Evol 296:225–230

Morgan M (2001) Transposable element number in mixed mating populations. Genet Res 77:261–275

Oliveira VM, Forni-Martins ER, Semir J (2007) Cytotaxonomic studies in six species of *Vernonia* (Asteraceae: Vernonieae). Caryologia 60:37–47

Otto SP, Whitton J (2000) Polyploid incidence and evolution. Annu Rev Genet 34:401–437

Ozkan H, Tua M, Arumuganathan K (2003) Nonadditive changes in genome size during allopolyploidization in the wheal (Aegilops–Triticum) group. J Hered 94:260–264

Pellicer J, Garcia S, Garnatje T, Dariima S, Korobkov AA, Vallès J (2007) Chromosome numbers in some *Artemisia* (Asteraceae,

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Anthemideae) species and genome size variation in its subgenus *Dracunculus*: karyological, systematic and phylogenetic implications. Chromosome Bot 2:45–53

Petrov DA (2001) Evolution of genome size: new approaches to an old problem. Trends Genet 17:23–28

Poggio L, González G, Naranjo CA (2007) Chromosome Studies in *Hippeastrum* (Amaryllidaceae): variation in genome size. Bot J Linn Soc 155:171–178

Price J, Chambers KL, Bachmann K (1981) Geographic and ecological distribution of genomic DNA content variation in *Microseris douglasii* (Asteraceae). Bot Gaz 142:415–426

Price HJ, Hodnett G, Johnston JS (2000) Sunflower (*Helianthus annus*) leaves contain compounds that reduce nuclear propidium iodide fluorescence. Ann Bot 86:929–934

Robinson H (1988a) Studies in the *Lepidaploa* complex (Vernonieae: Asteraceae). IV. The new genus *Lessingianthus*. Proc Biol Soc Wash 100:929–951

Robinson H (1988b) Studies in the *Lepidaploa* complex (Vernonieae: Asteraceae). V. The new genus *Chrysolaena*. Proc Biol Soc Wash 101:952–958

Robinson H (1988c) Studies in the *Lepidaploa* complex (Vernonieae: Asteraceae). VI. The new genus *Aynia*. Proc Biol Soc Wash 100:959–965

Robinson H (1992) *Mesanthophora*, a new genus of Vernonieae (Asteraceae) from Paraguay. Novon 2:169–172

Robinson H (1999) Generic and subtribal classification of American Vernonieae. Smithson Contribution Bot 89:1–116

Ruas PM, Ruas CF, Vieira AOS, Matzenbacher NI, Martins NS (1991) Cytogenetics of genus *Vernonia* Schreber (Compositae). Cytologia 56:239–247

Sims LE, Price HJ (1985) Nuclear DNA content variation in *Helianthus* (Asteraceae). Am J Bot 72:1213–1219

Soltis DE, Soltis PS (1999) Polyploidy: recurrent formation and genome evolution. Trends Ecol Evol 14:348–352

Soltis DE, Soltis PS, Tate JA (2003) Advances in the study of polyploidy since Plant Speciation. New Phytol 161:173–191

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng Ch, Sankoff D, de Pamphilis CW, Wall PK, Soltis PS (2009) Polyploidy and angiosperm diversification. Am J Bot 96: 336–348

Vaio M, Mazzella C, Porro V, Speranza PS, López-Carro B, Estramil E, Folle GA (2007) Nuclear DNA content in allopolyploid species and synthetic hybrids in the grass genus *Paspalum*. Plant Syst Evol 265:109–121

Wendel JF, Cronn RC, Johnston JS, Price HJ (2002) Feast and famine in plant genomes. Genetica 115:37–47

Wright SI, Schoen DJ (1999) Transposon dynamics and the breeding system. Genetica 107:139–148

Zonneveld BJM, Leitch IJ, Bennett MD (2005) First nuclear amounts in more than 300 angiosperms. Ann Bot 96:229–244