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Genome size evolution in Sapindaceae at subfamily level: a case study of independence in relation to karyological and palynological traits

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Sapindaceae *s.l.* is a moderately large family of trees, shrubs and lianas. Current knowledge on genome size and how it varies in this family is scarce. This research aims to characterize the DNA content in 39 species of Sapindaceae, mainly in tribe Paullinieae *s.s.*, by the analysis of the variation in genome size relative to karyotypic and palynological features. Nuclear DNA amount was measured by flow cytometry, and linear regression analyses were conducted to analyse the relationship between genome size variation and various karyotypic and palynological features. Genome size varied nine-fold among species, ranging from 1C = 0.305 pg (*Lophostigma plumosum*) to 2.710 pg (*Cardiospermum heringeri*). The low regression coefficients obtained suggest that genome size mainly varies independently of karyotypic and palynological features. With regard to karyotype evolution, the constant chromosome number but variable genome size in *Houssayanthus*, *Paullinia* and *Serjania* suggest that structural changes mainly caused by changes in the amounts of repetitive DNA are more important than numerical change. In contrast, in *Cardiospermum* and *Urvillea*, variation in chromosome number and genome size supports the suggestion that numerical and structural changes are important in the karyotype evolution of these genera. *Botanical Journal of the Linnean Society*, 2014, **174**, 589–600.

ADDITIONAL KEYWORDS: DNA content – flow cytometry – karyotypic evolution – pollen equatorial diameter – structural rearrangement.

INTRODUCTION

Sapindaceae *s.l.* is a moderately large family of trees, shrubs and lianas including *c.* 141 genera and *c.* 1900 species. Most have a tropical to subtropical distribution, although some genera extend into the temperate regions of Eurasia and North America. The first complete taxonomic treatment of the family *sensu stricto* was performed by Radlkofer (1931), who identified two subfamilies: Sapindoideae (= Eusapindaceae) and Dodonaeoideae (= Dyssapindaceae). Later, Buerki *et al.* (2009), using an evolutionary framework based on molecular phylogenetic analysis, detected that the infrafamilial groupings were paraphyletic, with the exception of tribe Paullinieae.

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As a result of these uncertainties in evolutionary relationships, this article follows the systems of Buerki *et al.* (2010) at the subfamilial level. For the lower levels of taxonomic classification, the informal phylogenetic group classification proposed by Buerki *et al.* (2009) is used. Although the latter is not ideal, at least it reflects the current understanding of evolutionary relationships within the family. With regard to these classifications, the *Paullinia* group belongs to subfamily Sapindoideae and is considered to be monophyletic (Harrington *et al.*, 2005; Buerki *et al.*, 2009). It comprises the tribes Paullinieae *s.s.* and Thouinieae *s.s.*, and the enigmatic *Sapindus oligophyllus* Merr. & Chun. This latter species has been synonymized, recombined and merged with different other taxa of the family (Rauschert, 1982; Xia & Gadek, 2007). The *Paullinia* group is one of the

largest in Sapindaceae, comprising more than one-quarter of all species. Most species in the *Paullinia* group are lianas that share the presence of tendrils (modified inflorescence axes) for climbing and stipules.

Paullinieae *s.s.* (Radlkofer, 1931) includes subtribes Paulliniinae and Thinouiinae. Thinouiinae includes only one genus, *Thinouia* Triana & Planch., whereas Paulliniinae comprises *Balsas* Jiménez Ram. & Vega, *Cardiospermum* L., *Houssayanthus* Hunz., *Lophostigma* Radlk., *Paullinia* L., *Serjania* Mill. and *Urvillea* Kunth. Members of Paulliniinae are characterized by zygomorphic flowers and schizocarpic or capsular fruits.

Chromosome counts in Sapindaceae *s.s.* (excluding Hippocastanoideae and Xanthoceroideae) are available for > 130 species, and karyotypes are described for about 40 species (Ferrucci, 1989, 2000a; Hemmer & Morawetz, 1990; Nogueira Zampieri *et al*., 1995; Ferrucci & Solís Neffa, 1997; Solís Neffa & Ferrucci, 1997, 1998; Lombello & Forni Martins, 1998; Urdampilleta, 2005, 2009; Urdampilleta *et al*., 2006; Urdampilleta, Ferrucci & Vanzela, 2007; Urdampilleta, Ferrucci & Forni-Martins, 2008; Coulleri, Dematteis & Ferrucci, 2012). The most frequent basic chromosome numbers as reported by Ferrucci (2000a) are $x = 15$, $x = 16$ and $x = 14$. Considering that $x = 7$ is likely to be the ancestral basic number for the family (Ferrucci, 1989), these higher base numbers are likely to have been derived by polyploidy and/or dysploidy. In Paullinieae *s.s.*, to date, the most common basic chromosome numbers are $x = 12$ (45% of the species with karyological data) and $x = 11$ (35% of the species with karyological data); no species have $x = 16$, although Paullinieae *s.s.* shows an almost complete dysploidy series, from $x = 7$ to $x = 14$ (Ferrucci, 2000a).

Both chromosome number and genome size vary tremendously across flowering plants; this has stimulated considerable speculation about the ancestral genome size and the base chromosome number of angiosperms, and has promoted hypotheses relating to trends in genome and chromosomal evolution (Soltis *et al*., 2003). The amount of DNA in an unreplicated gametic nuclear genome is referred to as the 1C-value (Bennett, Leitch & Hanson, 1998); and this value has been estimated in > 7500 species of angiosperms (Bennett & Leitch, 2012). Based on the modal genome size of angiosperms, Leitch, Chase & Bennett (1998) and Soltis *et al*. (2003) proposed a nomenclature to describe the DNA contents in plants. They defined species with 1C ≤ 1.4 pg as having very small genome sizes and those with 1C ≤ 3.5 pg as having small genome sizes. These authors also defined species with genome sizes between 3.51 and 13.9 pg as having intermediate genome sizes.

In Sapindaceae *s.s*., C-value data are scarce; the only data available in the literature are for species belonging to *Blighia* Kon., *Dodonaea* Mill., *Hemigyrosa* Blume, *Koelreuteria* Laxm., *Litchi* Sonn., *Sapindus* L. and *Schleichera* Willd., in which 1C-values vary from 0.45 to 1.17 pg (Ohri & Kumar, 1986; Ohri, 1996, 2002; Ohri, Bhargava & Chatterjee, 2004; Morgan & Westoby, 2005). Nearly all of these records belong to Old World species, with the exception of the cosmopolitan polymorphic species *Dodonaea viscosa* Jacq. The only New World species with data is *Paullinia cupana* Kunth var. *sorbilis* (Mart.) Ducke, with 1C = 11.4 pg (Vieira de Freitas *et al*., 2007). [Genome size data for *Acer* L. and *Aesculus* L. (members of Sapindaceae *sensu* APG III, 2009) are available, but they are excluded from our analyses as we focus on Sapindaceae *s.s*.].

The lack of knowledge about genome size in Sapindaceae *s.s.* and its relationship with other characteristics has encouraged us to determine the DNA contents of 39 species of the family, which are added to ten previous recorded DNA contents, to reach 18 genera representing Sapindoideae and Dodonaeoideae, with the following objectives: (1) to analyse the genome size variation in Sapindaceae, especially among genera of Paullinieae *s.s.*; (2) to study the relationship between DNA content variation and karyotype diversity; and (3) to analyse the relationship between DNA content variation and pollen size.

MATERIAL AND METHODS

Plant material

The material analysed is presented in Table 1; all the specimens used in this work were prepared from fresh, not silica gel-dried, material. Voucher specimens have been deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES).

Genome size estimates

Genome size was estimated using a PA flow cytometer (Partec GmbH, Germany). Each sample was measured three times. For nucleus extraction and release, a leaf portion was chopped with a sharp razor in 0.5 mL of extraction buffer (CyStain® UV Precise P); after the addition of staining buffer containing propidium iodide, the suspension of isolated nuclei was filtered through a nylon filter (pore size, 40 μm) and examined immediately. The internal standard for the analysis of our germplasm was *Paspalum intermedium* Munro ex Morong & Britton ($2n = 2x = 20 = 1.417$ pg) for most of the species, and *P. dilatatum* Chirú ($2n = 6x = 60 = 3.57$ pg) (Vaio *et al*., 2007) was used for *Cardiospermum*, *Lophostigma* and *Urvillea* spp. These standards were chosen as the flow cytometer was set up to

Table 1. Analysed phylogenetic groups, arranged *sensu* Buerki *et al*. (2009), species, collector's reference, 2C DNA content together with standard error (SE) and 1C-value (genome size)

Phylogenetic groups	Species	Collectors	2C ± SE (pg)	1C-value (pg)
Paullinia	*Cardiospermum bahianum* Ferrucci & Urdampilleta	Urdampilleta *et al*. 389	1.118 ± 0.190	0.519
	C. cristobaliae Ferrucci & Urdampilleta	Urdampilleta *et al*. 421	1.462 ± 0.0485	0.731
	C. grandiflorum Sw.	Coulleri & Ferrucci 270	4.22 ± 0.085	2.11
	C. halicacabum L.	Coulleri *et al*. 101	1.047 ± 0.128	0.524
	C. heringeri Ferrucci	Urdampilleta *et al*. 437	5.43 ± 0.074	2.715
	C. pterocarpum Radlk.	Coulleri *et al*. 102	2.02 ± 0.051	1.010
	C. urvilleoides (Radlk.) Ferrucci	Urdampilleta *et al*. 425	1.235 ± 0.029	0.618
	Houssayanthus incanus (Radlk.) Ferrucci	Ferrucci *et al*. 2710	4.02 ± 0.491	2.010
	Lophostigma plumosum Radlk.	Coulleri *et al*. 267	0.611 ± 0.110	0.305
	Paullinia coriacea Casar.	Obando *et al*. 293	1.815 ± 0.136	0.908
	P. elegans Cambess.	Coulleri *et al*. 95	4.122 ± 0.118	2.061
	P. pinnata L.	Coulleri *et al*. 94	3.935 ± 0.114	1.968
	P. racemosa Wawra	Urdampilleta *et al*. 593	2.095 ± 0.176	1.048
	P. rhomboidea Radlk.	Urdampilleta *et al*. 233	2.115 ± 0.186	1.057
	P. thalictrifolia Juss.	Urdampilleta *et al*. 372	2 ± 0.038	1.000
	P. uloptera Radlk.	Urdampilleta 436	2.015 ± 0.076	1.008
	P. weinmanniifolia Mart.	Urdampilleta *et al*. 318	2.116 ± 0.245	1.050
	Serjania altissima (Poepp.) Radlk.	Coulleri *et al*. 72	3.254 ± 0.0672	1.627
	S. caracasana (Jacq.) Willd.	Ferrucci 24	5.361 ± 0.148	2.681
	S. corrugata Radlk.	Urdampilleta *et al*. 369	4.526 ± 0.070	2.263
	S. fuscifolia Radlk.	Coulleri *et al. 52*	4.159 ± 0.653	2.080
	S. glabrata Kunth	López *et al*. 426	3.691 ± 0.185	1.845
	S. marginata Casar.	Coulleri *et al*. 43	1.948 ± 0.185	0.974
	S. paludosa Cambess.	Urdampilleta *et al* 391	2.545 ± 0.086	1.272
	S. pannifolia Radlk.	Coulleri *et al. 82*	3.604 ± 0.251	1.802
	S. perulacea Radlk.	Coulleri *et al*. 96	3.484 ± 0.263	1.742
	S. pinnatifolia Radlk.	Urdampilleta *et al*. 408	4.526 ± 0.144	2.260
	S. platycarpa Benth.	Coulleri *et al. 50*	2.633 ± 0.128	1.317
	S. sphaerococca Radlk.	Coulleri *et al. 21*	4.029 ± 0.159	2.015
	S. tripleuria Ferrucci	Coulleri *et al. 2*	4.903 ± 0.057	2.452
	Thinouia paraguayensis Britton	Ferrucci *et al*. 1915	1.268 ± 0.092	0.634
	U. andersonii Ferrucci	Urdampilleta *et al*. 345	1.908 ± 0.138	0.477
	Urvillea glabra Cambess.	Urdampilleta *et al*. 293	1.88 ± 0.095	0.481
	U. ulmacea Kunth	Urdampilleta *et al*. 340	1.936 ± 0.041	0.484
	Allophylus edulis (A.St.-Hil., A.Juss. & Cambess.) Hieron. ex Niederl.	Coulleri *et al*. 97	2.967 ± 0.081	1.483
Cupania	*Cupania vernalis* Cambess.	Ferrucci 26	2.945 ± 0.0801	1.472
Dodonaea	*Diplokeleba floribunda* N.E.Br.	Ferrucci & Vanni 804	2.056 ± 0.170	1.048
Melicoccus	*Melicoccus lepidopetalus* Radlk.	Ferrucci 69	4.113 ± 0.166	1.371
Koelreuteria	*Koelreuteria elegans* (Seem.) A.C.Sm. ssp. *formosana* (Hayata) F.G.Mey.	Ferrucci *et al*. 896	2.07 ± 0.08	1.035

analyse these species, and because these species have DNA contents which are suitable as calibration standards for comparison with the species measured here. A minimum of 5000 nuclei were measured in each sample, and the 2C DNA nuclear content of the unknown sample was calculated as follows:

Sample 2C DNA content =
(Sample peak mean/Standard peak mean)
× 2C DNA content of the standard

Mean and standard errors were determined in each set of measured tissues of each species.

Karyotypic features

Chromosome numbers, karyotype asymmetry indices A_1 and A_2 (Romero Zarco, 1986) and mean chromosome length (MCL) were taken from the literature.

Pollen size

The pollen equatorial diameters of 28 of the 39 species were compiled from various sources; nine of the remaining 11 species were measured for this work. Pollen grains were obtained from anthers of one collection of each species. Samples for light microscopy (LM) were acetolysed according to the procedure described by Erdtman (1966) and mounted in glycerine jelly. Permanent slides were deposited at the Palynological Laboratory of the Universidad Nacional del Nordeste, Corrientes, Argentina (PAL-CTES). The equatorial diameter was measured in 15–20 grains per specimen using a Leica DM LB2 microscope.

Statistical analyses

To understand the relationship between genome size and karyotype features in Paullinieae *s.s.*, four linear regression analyses were performed between the 1C-value and chromosome number, A_1 and A_2 (Romero Zarco, 1986) indices of asymmetry and MCL.

To test the relationship between the equatorial diameter of pollen (pollen size) and genome size, a linear regression was applied using the 1C-value as the independent variable and pollen size as the dependent variable. All statistical analyses were carried out using the software InfoStat Ver. 2011p (Di Rienzo *et al*., 2011).

RESULTS

Genome size

Genome size (1C-value) and DNA content (2C-value) with the standard error (SE) for the 39 species studied here are listed in Table 1, and representative flow histograms are shown in Figure 1. Genome sizes varied nine-fold among species, ranging from 1C = 0.305 pg (*Lophostigma plumosum* Radlk.) to 1C = 2.715 pg (*Cardiospermum heringeri* Ferrucci). However, the genome sizes tended to be small, as shown in Figure 2, which illustrates the generally narrow distribution of genome sizes across the species and groups studied.

Karyotype and genome size relationship

Relationships between available karyotype features and genome size (Table 2) were examined using linear regression analysis. The relationship between chromosome number and 1C-value was relatively high, but non-significant ($R^2 = 0.76$, $P = 0.0194$). Among the significant relationships (Fig. 3), that between the 1C-value and the A_2 asymmetry index was low ($R^2 = 0.316$, $P < 0.0001$) (Fig. 3C), whereas those between the 1C-value and MCL (Fig. 3A) and the A_1 asymmetry index (Fig. 3B) were very low ($R^2 = 0.03$, $P < 0.0001$ and $R^2 = 0.029$, $P < 0.0001$, respectively).

Relationship between pollen size and genome size

Pollen size varied 4.3-fold (Table 3), from 18.7 to 67.0 μm, with an average of 36.76 μm. Pollen size was largest in *Cardiospermum heringeri* and smallest in *Diplokeleba floribunda* N.E.Br. Linear regression analyses between pollen equatorial diameter and genome size (Fig. 4) showed a low, but significant, positive relationship ($R^2 = 0.24$, $P < 0.001$).

DISCUSSION

Variation in nuclear DNA content occurs at all taxonomic levels, even between closely related species (Jackson, 1971; Price, 1988). Based on the available genome size data, Sapindaceae *s.s*. is characterized by mainly possessing very small genomes (i.e. 1C < 1.4 pg) which are similar to those reconstructed for ancestral angiosperms by Leitch *et al*. (1998) and Soltis *et al*. (2003). Nevertheless, more recent studies (Vinogradov, 2003; Knight, Molinari & Petrov, 2005; Price *et al*., 2005) have emphasized the dynamic nature of genome size evolution, with some lineages possessing large genome sizes characterized by slower rates of diversification and disproportionately higher extinction rates than lineages with medium to very small genome sizes. Such observations and the fact that most angiosperms have small or very small genomes (Leitch *et al*., 1998; Soltis *et al*., 2003) suggest that natural selection may generally favour genome downsizing. Certainly, the available data for Sapindaceae *s.s*. show that it is mainly characterized by a conservative range of small and very small

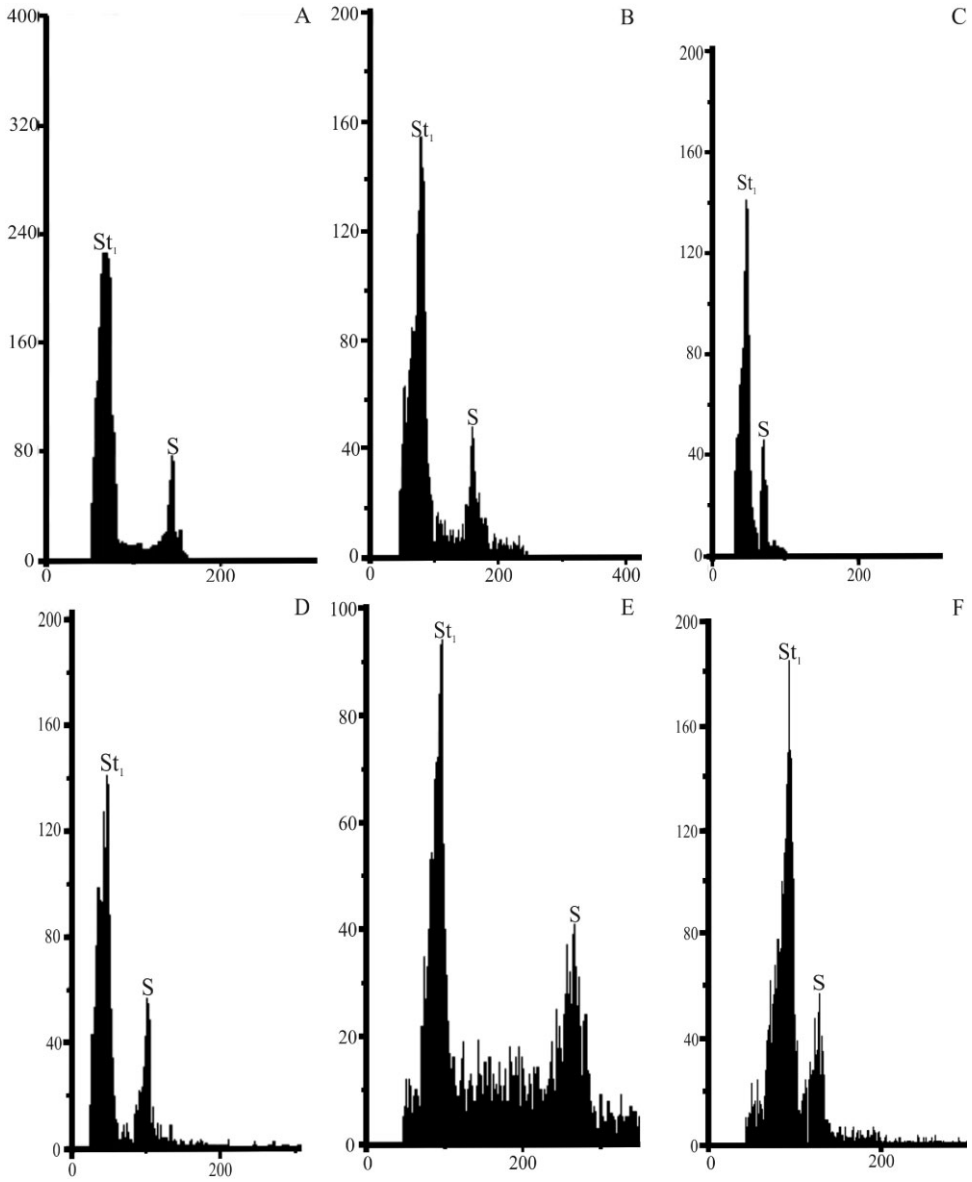

Figure 1. Flow cytometry plots. The *x* axis represents the propidium iodide (PI) fluorescence (i.e. relative DNA amount) and the *y* axis represents the number of nuclei measured (× 100). S, sample measured in each histogram; St_1, most used internal standard (*Paspalum intermedium*); St_2, less often used internal standard (*Paspalum dilatatum*). The histograms belong to: A, *Allophylus edulis*; B, *Cupania vernalis*; C, *Thinouia paraguayensis*; D, *Diplokeleba floribunda*; E, *Melicoccus lepidopetalus*; F, *Paullinia uloptera*.

genome sizes. Indeed, the mean genome sizes for most phylogenetic groups analysed show little variation: from 1.035 pg in the *Koelreuteria* group [*Koelreuteria elegans* (Seem.) A.C.Sm. ssp. *formosana* (Hayata) F.G.Mey.] to 1.472 pg in the *Cupania* group (*Cupania vernalis* Cambess.).

Nevertheless, more variability was detected in the *Paullinia* group, in which most of the studied species of *Serjania*, *Houssayanthus* and *Allophyllus* and some species of *Paullinia* and *Cardiospermum* had small and intermediate genome sizes, whereas *Lophostigma*, *Thinouia* and *Urvillea* species, and the remaining *Cardiospermum* and *Paullinia* species, had very small genome sizes.

In summary, based on the present genome size data (1C-values) and previous reports (Table 4), most of the species studied to date have a very small genome size, except for some species belonging to Paullinieae, which have small or intermediate DNA content.

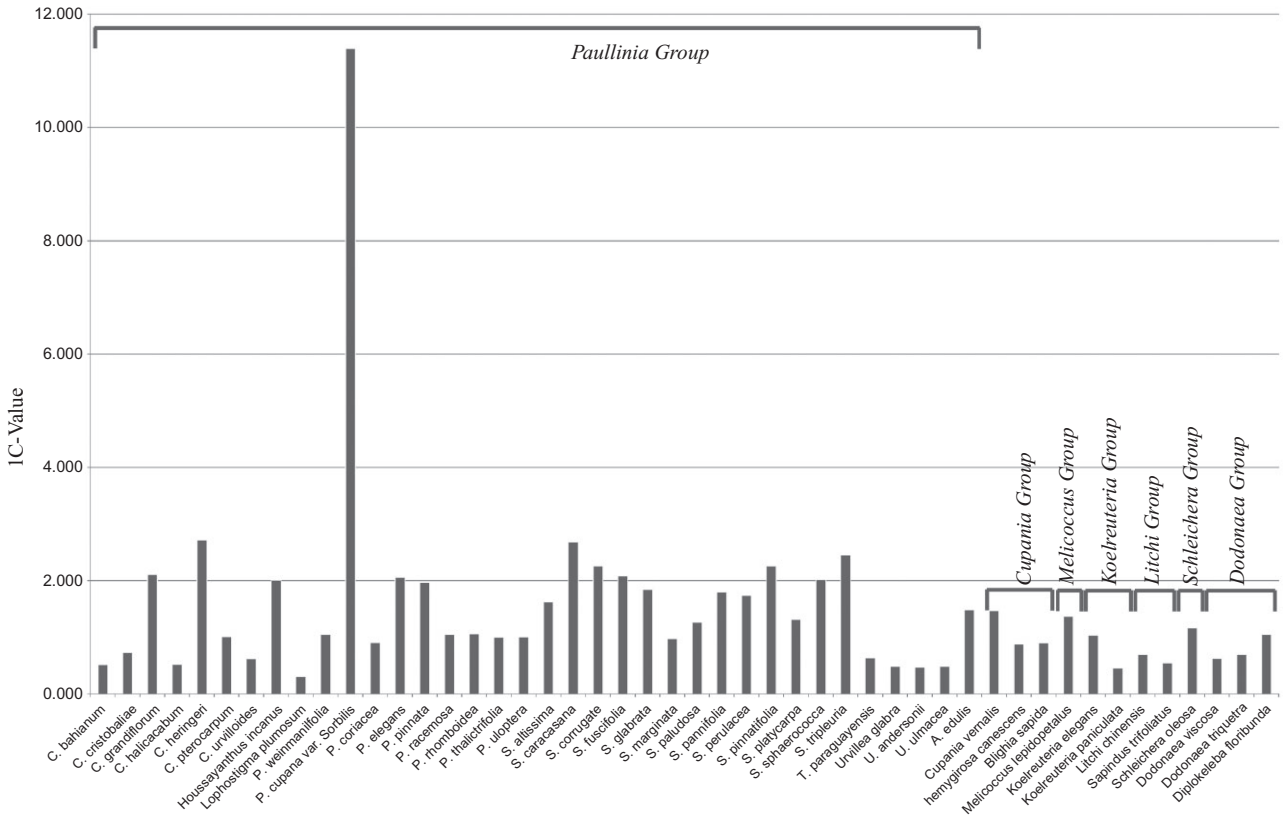

Figure 2. Distribution of genome size among the analysed species and previous records arranged *sensu* Buerki *et al*. (2009). *Paullinia cupana* var. *sorbilis* reaches a 1C-value of 3.5 pg; the remaining taxa are more conservative with a narrow range of small to very small genome sizes.

Genome size and karyotype evolution

The linear regression results suggest that variation in genome size is largely independent of chromosome number, although it may be slightly related to chromosome length, as demonstrated by the very low R^2 value of 0.03 for the analysis of 1C-value and MCL (Fig. 3A), and the low relationship (R^2 value of 0.316) between the 1C-value and A_2 asymmetry index (Fig. 3C). The interchromosomal asymmetry index A_2 estimates the variation in chromosome length, independent of chromosome number.

Karyotype symmetry was one of the parameters used to establish karyotypic evolution. Stebbins (1971) proposed the hypothesis that symmetrical karyotypes were more primitive than asymmetrical ones. In relation to this, for some species, changes in karyotype symmetry have been shown to be accompanied by increases or decreases in DNA content (e.g. Martínez & Azkue, 1987). In some genera, chromosomal complements with higher DNA contents tend to be more symmetrical than those with lower genome size (Rees, 1984), whereas, in other genera, exactly the opposite has been noted (e.g. Martínez & Ginzo, 1986). More recently, Poggio *et al*. (1986) and, Lavia & Fernández (2008) have reported cases in which changes in genome size are independent of changes in karyotypic symmetry. Such independence is found here in Sapindaceae *s.s*., with changes in genome size being only weakly correlated with changes in karyotype asymmetry. These results suggest that either increases or decreases in DNA content do not occur in all chromosomes of the complement, or that genome size changes do occur in all chromosomes, but unevenly, such that the distribution of DNA is not proportional to the length of each chromosome.

Concerning the species of Paullinieae *s.s.* studied in this work, in *Cardiospermum*, chromosome counts for 12 of the 16 species representing the three sections of the genus have been obtained (Urdampilleta *et al*., 2012). This genus shows four basic numbers: $x = 7$, $x = 9$, $x = 10$ and $x = 11$ (Ferrucci, 2000a). Section *Ceratadenia* Radlk. comprises the species with the largest genome size: *C. heringeri* ($2n = 2x = 14$) (Urdampilleta *et al*., 2012), with a 1C-value of 2.75 pg, and *C. grandiflorum* ($2n = 2x = 20$) (Ferrucci, 1981, 1989), with a 1C-value of 2.11 pg. In contrast, *C. bahianum*, a polyploid species belonging to

Table 2. Species with available karyotypic data, together with bibliographic reference of the karyotypic features, 2*n* chromosome number, mean chromosome length (MCL) and asymmetry indices A_1 and A_2 (Romero Zarco, 1986)

Species	Reference	Chromosome number	MCL (μm)	A_1	A_2
Cardiospermum bahianum	Urdampilleta (2009)	36	1.600	0.370	0.110
C. cristobaliae	Urdampilleta (2009)	24	3.000	0.460	0.250
C. grandiflorum	Urdampilleta (2009)	20	1.500	0,370	0,180
C. halicacabum	Urdampilleta (2009)	22	1.100	0.400	0.210
C. heringeri	Urdampilleta (2009)	14	3.200	0.390	0.230
C. pterocarpum	Urdampilleta (2009)	22	2.200	0.420	0.230
C. urvilleoides	Urdampilleta (2009)	24	4.600	0.390	0.200
Paullinia elegans	Urdampilleta *et al*. (2007)	24	1.820	0.480	0.240
P. pinnata	Urdampilleta *et al*. (2007)	24	2.530	0.470	0.200
P. rhomboidea	Urdampilleta *et al*. (2007)	24	1.450	0.460	0.290
Serjania altissima	Coulleri *et al*. (2012)	24	2.014	0.630	0.270
S. caracasana	Solís Neffa & Ferrucci (1997)	24	2.600	0.520	0.220
S. fuscifolia	Nogueira Zampieri *et al*. (1995)	24	2.250	0.480	0.240
S. glabrata	Solís Neffa & Ferrucci (1997)	24	2.390	0.490	0.230
S. marginata	Solís Neffa & Ferrucci (1997)	24	3.090	0.560	0.210
S. paludosa	Solís Neffa & Ferrucci (1997)	24	2.630	0.550	0.220
S. pannifolia	Coulleri *et al*. (2012)	24	2.750	0.510	0.310
S. perulacea	Solís Neffa & Ferrucci (1997)	24	2.280	0.540	0.220
S. platycarpa	Urdampilleta (2005)	24	1.850	0.300	0.250
S. sphaerococca	Coulleri *et al*. (2012)	24	3.900	0.240	0.330
S. tripleuria	Ferrucci (1985)	24	2.640	0.480	0.220
T. paraguayensis	Urdampilleta *et al*. (2008)	28	1.090	0.544	0.142

section *Carphospermum* Radlk. (Urdampilleta *et al*., 2012), paradoxically has the smallest genome size ($2n = 4x = 36$, 1C-value = 0.519 pg) of the genus. The remaining *Cardiospermum* spp. belong to section *Cardiospermum* [*C. cristobaliae* Ferrucci & Urdampilleta, *C. halicacabum* L., *C. pterocarpum* Radlk. and *C. urvilleoides* (Radlk.) Ferrucci]; 1C-values in these species range from 0.524 to 1.01 pg.

Urvillea possesses two basic numbers: $x = 11$ and $x = 12$; among the four species studied with $x = 11$, three ploidies have been reported previously: $2n = 2x = 22$, $2n = 4x = 44$ and $2n = 8x = 88$ (Ferrucci, 2000a; Urdampilleta *et al*., 2006). The widespread *U. ulmacea* Kunth is the only species with two known cytotypes: $2n = 2x = 22$ and $2n = 8x = 88$ (Urdampilleta *et al*., 2006). The present results and the reported presence of polyploidy in *Urvillea* suggest that karyotypic evolution may have been driven mainly by numerical changes, followed by structural rearrangements, as the DNA content was found to be relatively constant in the species analysed. However, as no chromosome counts were actually made for the plants analysed, the lack of variation in genome size between the *Urvillea* spp. studied could arise if only diploid cytotypes had been analysed.

Paullinia comprises *c*. 150 species, most of which are Neotropical; chromosome counts are available for 12 species. These counts belong to four of the 13 sections recognized in this genus (Mangenot & Mangenot, 1958; Semple, 1974; Ferrucci, 1981, 2000a; Guerra, 1986; Ferrucci & Solís Neffa, 1997 Urdampilleta *et al*., 2007; Vieira de Freitas *et al*., 2007). To date, the genome sizes for species belonging to three sections are known, two of which are reported in the present work. *Paullinia elegans* Cambess. and *P. pinnata* L., both belonging to section *Paullinia*, have 1C-values of 2.061 and 1.968 pg, respectively, whereas, in section *Phygoptilon* Radlk., represented by *P. coriacea* Casar., *P. racemosa* Wawra, *P. rhomboidea* Radlk., *P. thalictrifolia* Juss. and *P. uloptera* Radlk., 1C-values range from 0.908 to 1.057 pg. Thus, based on the studied species, sections *Paullinia* and *Phygoptilon*, both with $2n = 2x = 24$, show contrasting genome size profiles. In accordance with the karyotypic studies in *Paullinia* presented by Urdampilleta *et al*. (2007), the constancy in chromosome number and the variation in genome size suggest the predominance of structural rearrangements and differences in the amounts of repetitive DNA in the karyotypic evolution of these species. With regard to the only species with previously known karyological information, *P. cupana* var. *sorbilis* (section *Pleurotoechus* Radlk.), with $2n = 210$ and a genome size of 1C = 11.4 pg, the number and types of chromosomes

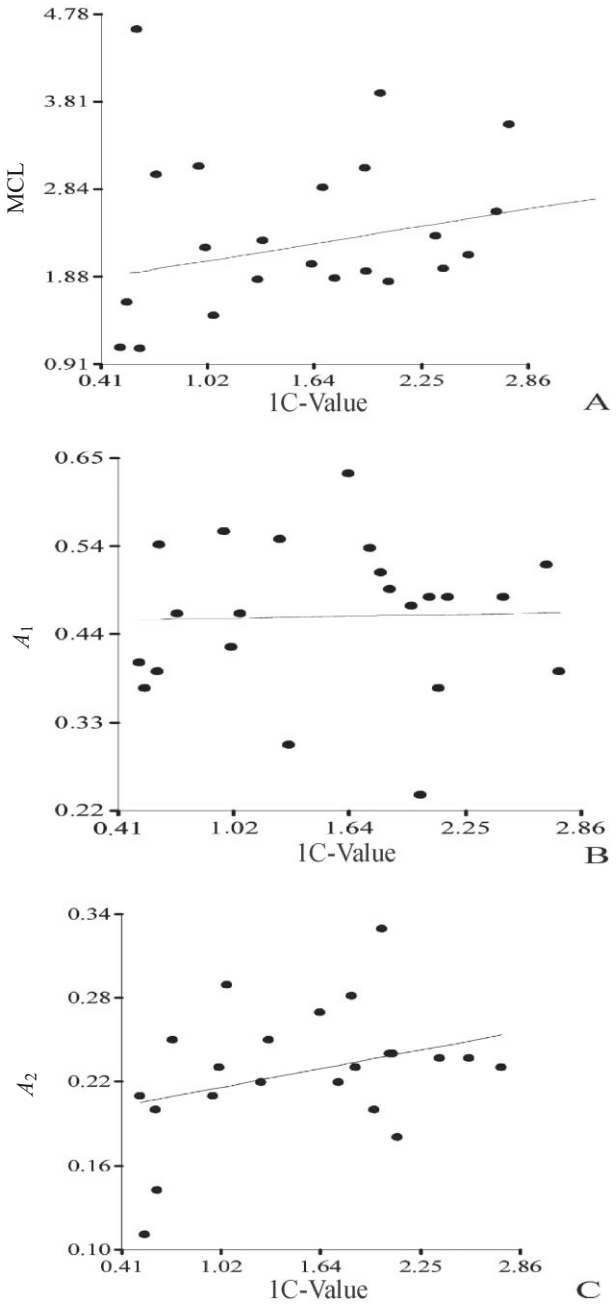

Figure 3. Relationship estimated by linear regression between the karyotypic features and the genome size. There is a weak but significant relationship between genome size and (A) mean chromosome length (MCL), (B) intrachromosomal asymmetry index A_1 and (C) interchromosomal asymmetry index A_2.

described suggest a complex origin of this karyotype (Vieira de Freitas *et al*., 2007).

Serjania with *c.* 230 species is the largest genus of Paullinieae. The cytological studies report karyotypes for 34 species, all with $2n = 2x = 24$ (Guervin, 1961; Fernández Casas & Fernández Piqueras, 1981; Ferrucci, 1981, 1985, 2000a; Sarkar *et al*., 1982; Maglio, Forni-Martins & Da Cruz, 1984; Hemmer & Morawetz, 1990; Nogueira Zampieri *et al*., 1995; Ferrucci & Solís Neffa, 1997; Solís Neffa & Ferrucci, 1997; Urdampilleta, 2005, 2009 and Coulleri *et al*., 2012). These studies also include chromosome counts for 45 species belonging to 11 of the 12 sections recognized in the genus. In the present work, the genome size of a few representatives belonging to seven of the 12 sections have been reported for the first time, showing variations in 1C-values from 0.974 pg in *S. marginata* Casar. to 2.681 pg in *S. caracasana* (Jacq.) Willd.

All the genera mentioned above belong to subtribe Paullinieae, whereas the genus *Thinouia*, considered to be a sister clade of Paullinieae, belongs to subtribe Thinouiinae. This genus comprises nine species (Ferrucci & Somner, 2008), but karyotypes for only three species are known, all with $2n = 28$. Chromosome size varies from very small to small (Urdampilleta *et al*., 2008). Chromosome number and size are similar to those of *Lophostigma*, perhaps suggesting a close evolutionary relationship between these genera, and supporting the possibility that *Lophostigma* may have evolved independently from the rest of the subtribe directly from *Thinouia*, whereas the remaining genera may have a common evolutionary track, as proposed by Ferrucci & Anzótegui (1993).

Relationship between pollen size and genome size

Based on the consistent, strong positive trend between cell size and genome size reported by Beaulieu *et al*. (2008), we speculated that genome size might be partly correlated with pollen size. In Sapindaceae, a pollen grain consists of a vegetative and a generative cell. For this analysis, we followed Knight *et al*. (2010), who referred to pollen as 'unicellular'. Our results showed only a weak relationship between genome size and pollen size (Fig. 4).

If a large genome size needs a large cell to contain it, why is the relationship between pollen size and genome size so weak? There are two possible explanations which may contribute to the observations. First, a cell with a large genome size is necessarily large because a large cytoplasm requires space for gene transcription and all the metabolic reactions needed for the survival of these cells (Beaulieu *et al*., 2008). However, according to Knight *et al*. (2010), pollen has a low metabolism until imbibition and germination, when it reaches the maximal volume at the pollen tube stage; it is possible that the weak correlation arose because the analysis was performed

Table 3. Species of Sapindaceae *s.s.* with data for the mean equatorial diameter (EqØ) of pollen grains, and the bibliographic reference for these data

Species	EqØ (μm)	Reference
Allophylus edulis	25.00	Anzótegui & Ferrucci (1998)
Cardiospermum bahianum	55.66	Ferrucci & Urdampilleta (2011a)
C. cristobaliae	54.09	Ferrucci & Urdampilleta (2011b)
C. grandiflorum	52.00	Anzótegui & Ferrucci (1998)
C. halicacabum	40.50	Ferrucci & Anzótegui (1993)
C. heringeri	67.00	Ferrucci (1993)
C. pterocarpum	30.00	Anzótegui & Ferrucci (1998)
C. urvilleoides	35.20	Ferrucci (2000b)
Cupania vernalis	23.00	Anzótegui & Ferrucci (1998)
Diplokeleba floribunda	18.70	Anzótegui & Ferrucci (1998)
Houssayanthus incanus	32.60	Anzótegui & Ferrucci (1998)
Koelreuteria elegans ssp. *formosana*	25.00	Meyer (1976)
Koelreuteria paniculata	21.00	Meyer (1976)
Litchi chinensis	10.00	Mustard *et al*. (1953)
Lophostigma plumosum	24.40	Ferrucci & Anzótegui (1993)
Melicoccus lepidopetalus	20.00	Anzótegui & Ferrucci (1998)
Paullinia coriacea	32.13	This work
P. elegans	30.80	Anzótegui & Ferrucci (1998)
P. pinnata	34.60	Anzótegui & Ferrucci (1998)
P. racemosa	30.35	This work
P. rhomboidea	32.40	This work
P. uloptera	32.79	This work
Serjania altissima	43.70	Van der Ham & Tomlik (1994)
S. caracasana	37.90	Ferrucci & Anzótegui (1993)
S. corrugata	43.76	This work
S. fuscifolia	30.30	Ferrucci & Anzótegui (1993)
S. glabrata	43.00	Anzótegui & Ferrucci (1998)
S. marginata	35.90	Ferrucci & Anzótegui (1993)
S. paludosa	39.68	This work
S. pannifolia	39.68	This work
S. perulacea	33.90	Anzótegui & Ferrucci (1998)
S. pinnatifolia	44.85	This work
S. platycarpa	51.10	Van der Ham & Tomlik (1994)
S. sphaerococca	38.20	Van der Ham & Tomlik (1994)
S. tripleuria	38.50	Ferrucci & Anzótegui (1993)
Thinouia paraguayensis	29.86	This work
Urvillea andersonii	44.00	Ferrucci (2000b)
U. ulmacea	32.90	Ferrucci (2000b)

at the wrong stage of pollen development. Perhaps a stronger correlation would have been uncovered if the pollen size had been measured during pollen growth. Second, small pollen may have a higher probability of transport to a receptive stigma, both by wind or insect vectors, than large pollen; thus, natural selection may strongly favour small pollen size in relation to dispersal strategies (Niklas, 1985; Hayter & Cresswell, 2006). Likewise, extremely large pollen is under strong selection pressures. Such strong natural selection for small pollen may therefore obscure any relationship between pollen size and genome size (Leitch *et al*., 2005).

CONCLUSIONS

The current knowledge of genome size variation in Sapindaceae *s.s*., including the present results, shows a low variability, which suggests a conservative nature in this trait. In addition, this variation appears to be relatively independent of karyotypic and palynological features.

Table 4. Previous reports of genome size for Sapindaceae *s.s.* taken from the literature, and arranged following the informal classification proposed by Buerki *et al.* (2009), species, bibliographic references and genome size (1C-value)

Phylogenetic groups	Species	Bibliographic references	1C-value (pg)
Paullinia	*Paullinia cupana* Kunth var. *sorbilis* (Mart.) Ducke	Vieira de Freitas *et al.* (2007)	11.4
Cupania	*Blighia sapida* K.D.Koenig	Ohri (1996)	0.9
	Hemigyrosa canescens Thwaites	Ohri & Kumar (1986)	0.88
Dodonaea	*Dodonaea triquetra* J.C.Wendl.	Morgan & Westoby (2005)	0.7
	Dodonaea viscosa Jacq.	Morgan & Westoby (2005)	0.63
Koelreuteria	*Koelreuteria paniculata* Laxm. (sub *K. paniculata* Rehder & E.H.Wilson)	Ohri *et al.* (2004)	0.45
Litchi	*Litchi chinensis* Sonn.	Ohri & Kumar (1986)	0.7
	Sapindus trifoliatus L.	Ohri (1996)	0.55
Schleichera	*Schleichera oleosa* (Lour.) Oken	Ohri (2002)	1.17

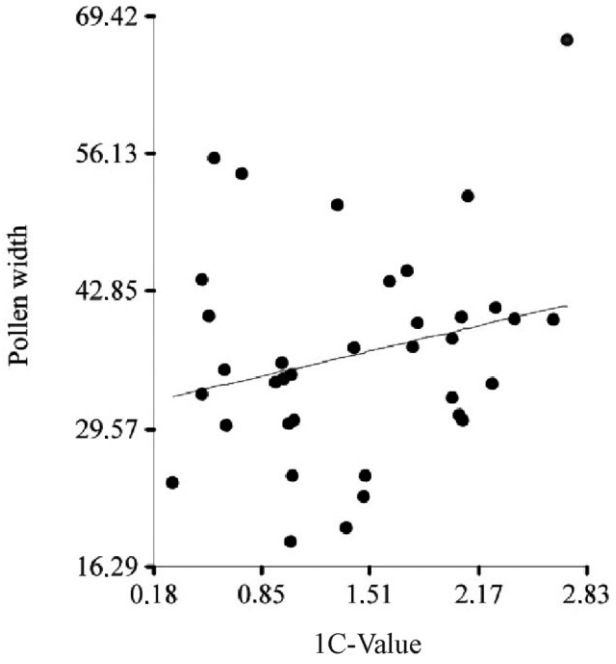

Figure 4. Linear regression analysis showing the weak but significant relationship between the mean equatorial diameter of pollen and the genome size of 38 species of Sapindaceae.

The weak relationship between karyotype and DNA content shows that any increase or decrease in DNA content is distributed unevenly across the chromosomal complement. Polyploidy and the amplification of repetitive DNA sequences are likely to be the two predominant processes responsible for increases in genome size (SanMiguel *et al.*, 1996; Vicient *et al.*, 1999), whereas decreases in DNA content probably arise from small deletions caused by unequal recombination or homologous and illegitimate recombination in regions rich in repetitive DNA (Bennetzen, Ma & Devos, 2005). Currently, the lack of phylogenetic resolution in Sapindaceae *s.s.* prevents insights into the direction of genome size changes, but this impediment is likely to be removed in the future as the understanding of evolutionary relationships improves.

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REFERENCES

Anzótegui SM, Ferrucci MS. 1998. Sapindaceae. In: Pires SM, Anzótegui LM, Cuadrado GA, eds. *Flora polínica del nordeste argentino*. Corrientes: EUDENE – UNNE, 95–110.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

Beaulieu JM, Leitch IJ, Patel S, Pendharkar A, Knight CA. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* **179:** 975–986.

Bennett MD, Leitch IJ. 2012. *Angiosperm DNA C-values database releases 8.0, December 2012*. Available at: http://www.rbgkew.org.uk/cval/homepage.html

Bennett MD, Leitch IJ, Hanson L. 1998. DNA amounts in two samples of angiosperm weeds. *Annals of Botany* **82** (Suppl A): 121–134.

Bennetzen JL, Ma J, Devos KM. 2005. Mechanisms of recent genome size variation in flowering plants. *Annals of Botany* **95:** 127–132.

Buerki S, Forest F, Acevedo-Rodríguez P, Callmander MW, Nylander JAA, Harrington M, Sanmartín I,

Küpfer P, Alvarez N. 2009. Plastid and nuclear DNA markers reveal intricate relationships at subfamilial and tribal levels in the soapberry family (Sapindaceae). *Molecular Phylogenetics and Evolution* **51:** 238–258.

Buerki S, Lowry PP II, Alvarez N, Razafimandimbison SG, Küpfer P, Callmander MW. 2010. Phylogeny and circumscription of Sapindaceae revisited: molecular sequence data, morphology and biogeography support recognition of a new family, Xanthoceraceae. *Plant Ecology and Evolution* **143:** 148–161.

Coulleri JP, Dematteis M, Ferrucci MS. 2012. A new insight into *Serjania* Mill. (Sapindaceae, Paullinieae) infrageneric classification: a cytogenetic approach. *Plant Systematics and Evolution* **298:** 1743–1753.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2011. *InfoStat versión 2011*. Cordoba, Argentina: Universidad Nacional de Córdoba. Available at: http://www.infostat.com.ar

Erdtman G. 1966. *Pollen morphology and plant taxonomy. Angiosperms*. New York: Hafner Publ. Co.

Fernández Casas J, Fernández Piqueras J. 1981. Estudio cariológico de algunas plantas bolivianas. *Anales del Jardín Botánico de Madrid* **38:** 149–152.

Ferrucci MS. 1981. Recuentos cromosómicos en Sapindáceas. *Bonplandia* **5:** 73–81.

Ferrucci MS. 1985. Recuentos cromosómicos en *Allophylus* y *Serjania* (Sapindaceae). *Boletín de la Sociedad Argentina de Botánica* **24:** 200–202.

Ferrucci MS. 1989. Cromosomas en *Cardiospermum* y *Diplokeleba* (Sapindaceae), significado taxonómico y evolutivo. *Bonplandia* **6:** 151–164.

Ferrucci MS. 1993. Una nueva especie y una nueva combinación en *Cardiospermum* (Sapindaceae). *Bonplandia* **6:** 245–259.

Ferrucci MS. 2000a. Cytotaxonomy of Sapindaceae with special reference to the tribe Paullinieae. *Genetics and Molecular Biology* **23:** 941–946.

Ferrucci MS. 2000b. Revisión de los géneros *Cardiospermum* y *Urvillea* para el Neotrópico (Sapindaceae). PhD Thesis, Universidad Nacional de Córdoba.

Ferrucci MS, Anzótegui LM. 1993. El polen de Paullinieae (*Sapindaceae*). *Bonplandia* **6:** 211–243.

Ferrucci MS, Solís Neffa VG. 1997. Citotaxonomía de Sapindaceae sudamericanas. *Boletín de la Sociedad Argentina de Botánica* **33:** 77–83.

Ferrucci MS, Somner G. 2008. *Thinouia restingae* (Sapindaceae: Paullinieae), una nueva especie de Brasil. *Brittonia* **60:** 371–376.

Ferrucci MS, Urdampilleta JD. 2011a. *Cardiospermum bahianum* (Sapindaceae: Paullinieae), a new species from Bahia, Brazil. *Systematic Botany* **36:** 950–956.

Ferrucci MS, Urdampilleta JD. 2011b. *Cardiospermum cristobaliae* (Sapindaceae, Paullinieae), una nueva especie de Minas Gerais, Brasil. *Brittonia* **63:** 478–483.

Guerra M dos S. 1986. Citogenética de angiospermas coletadas em Pernambuco. I. *Revista Brasileira de Genética* **9:** 21–40.

Guervin C. 1961. Contribution à l'étude cytotaxonomique des Sapindacées et caryologique des Mélianthacées et des Didiéréacées. *Revue de Cytologie et de Biologie Végétales* **23:** 49–87.

Harrington MG, Edwards KJ, Johnson SA, Chase MW, Gadek PA. 2005. Phylogenetic inference in Sapindaceae *sensu lato* using plastid *matK* and *rbcL* DNA sequences. *Systematic Botany* **30:** 366–382.

Hayter KE, Cresswell JE. 2006. The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural *Brassica napus*: implications for landscape-scale gene dispersal. *Journal of Applied Ecology* **43:** 1196–1202.

Hemmer W, Morawetz W. 1990. Karyological differentiation in Sapindaceae with special reference to *Serjania* and *Cardiospermum*. *Botanica Acta* **103:** 372–383.

Jackson RC. 1971. The karyotype in systematics. *Annual Review of Ecology and Systematics* **2:** 327–368.

Knight CA, Clancy RB, Götzenberger L, Dann L, Beaulieu JM. 2010. On the relationship between pollen size and genome size. *Journal of Botany* **2010:** 1–7.

Knight CA, Molinari NA, Petrov DA. 2005. The large genome constraint hypothesis: evolution, ecology and phenotype. *Annals of Botany* **95:** 177–190.

Lavia G, Fernández A. 2008. Genome size in wild and cultivated peanut germplasm. *Plant Systematics and Evolution* **272:** 1–10.

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

Leitch IJ, Soltis DE, Soltis PS, Bennet MS. 2005. Evolution of DNA amount across land plants (Embryophyta). *Annals of Botany* **95:** 207–217.

Lombello R, Forni Martins ER. 1998. Chromosomal studies in Sapindaceae. *Caryologia* **51:** 81–93.

Maglio CAFP, Forni-Martins ER, Da Cruz ND. 1984. In: Löve, A. ed. *Chromosome number reports LXXXIV*. *Taxon* **33:** 536.

Mangenot S, Mangenot G. 1958. Deuxième liste de nombres chromosomiques nouveaux chez diverses Dicotylédones et Monocotylédones d'Afrique occidentale. *Bulletin du Jardin Botanique de l'État* **28:** 315–329.

Martínez A, Azkue DD. 1987. Variación del contenido de ADN y su implicancia evolutiva. In: *Simposio Citología y Citotaxonomía. Anales del IV Congreso Latinoamericano de Botánica, Volumen III*, pp. 35–55. Available at: http://tropicos.org/Reference/9010813

Martínez A, Ginzo HD. 1986. DNA content in *Tradescantia*. *Canadian Journal of Genetics and Cytology* **27:** 766–775.

Meyer FG. 1976. A revision of the genus *Koelreuteria* (Sapindaceae). *Journal of the Arnold Arboretum* **57:** 129–166.

Morgan HD, Westoby M. 2005. The relationship between nuclear DNA content and leaf strategy in seed plants. *Annals of Botany* **96:** 1321–1330.

Mustard MJ, Liu S, Nelson RO. 1953. Observations of floral and fruit setting in lychee varieties. *Proceeding of Florida State Horticultural Society* **66:** 212–220.

Niklas KJ. 1985. The aerodynamics of wind pollination. *Botanical Review* **51:** 328–386.

Nogueira Zampieri C, Ruas PM, Ruas CF, Ferrucci MS. 1995. Karyotypic study of some species of *Serjania* and *Urvillea* (Sapindaceae, Tribe Paullinieae). *American Journal of Botany* **82:** 646–654.

Ohri D. 1996. Genome size and polyploidy variation in the tropical hardwood genus *Terminalia* (Combreataceae). *Plant Systematics and Evolution* **200:** 225–232.

Ohri D. 2002. *Reference 454*. Available at: http://data.kew.org/cvalues

Ohri D, Bhargava A, Chatterjee A. 2004. Nuclear DNA amounts in 112 species of tropical hardwoods – new estimates. *Plant Biology* **6:** 555–561.

Ohri D, Kumar A. 1986. Nuclear DNA amounts in some tropical hardwoods. *Caryologia* **39:** 303–307.

Poggio L, Wulff AF, Hunziquer JH. 1986. Chromosome size, nuclear volume and DNA content in *Bulnesia* (*Zygophyllaceae*). *Darwiniana* **27:** 25–38.

Price HJ. 1988. Nuclear DNA content variation within angiosperm species. *Evolutionary Trends in Plants* **2:** 53–60.

Price HK, Dillon SL, Hodnett G, Rooney WL, Ross L, Johnston JS. 2005. Genome evolution in the genus *Sorghum* (Poaceae). *Annals of Botany* **95:** 219–227.

Radlkofer L. 1931. Sapindaceae. In: Engler A, ed. *Das Planzenreich IV. 165(Heft 98a)*. Weinheim: Verlag von H. R. Engelmann (J. Cramer), 19–219.

Rauschert S. 1982. Nomina nova generica et combinationes novae Spermatophytorum et Pteridophytorum. *Taxon* **31:** 554–563.

Rees H. 1984. Nuclear DNA variation and the homology of chromosomes. In: Grant WF, ed. *Plant biosystematics*. Toronto, ON: Academic Press, 87–96.

Romero Zarco C. 1986. A new method for estimating karyotype asymmetry. *Taxon* **35:** 526–530.

SanMiguel PA, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL. 1996. Nested retrotransposons in the intergenic regions of the maize genome. *Science* **274:** 765–768.

Sarkar AK, Datta N, Chatterjee U, Hazra D. 1982. In: Löve A. ed. *IOPB Chromosome number reports LXXVI*. *Taxon* **31:** 578.

Semple JC. 1974. Chromosome numbers of phanerogams. 5. *Annals of the Missouri Botanical Garden* **61:** 902–903.

Solís Neffa VG, Ferrucci MS. 1997. Cariotipos de especies sudamericanas de *Serjania* (Sapindaceae, Paullinieae). *Bonplandia* **9:** 265–276.

Solís Neffa VG, Ferrucci MS. 1998. Cariotipos de Sapindaceae sudamericanas. *Boletín de la Sociedad Argentina de Botánica* **33:** 185–190.

Soltis DE, Soltis PS, Leitch IJ, Bennett MD. 2003. Evolution of genome size in the angiosperms. *American Journal of Botany* **90:** 1596–1603.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold, 125–189.

Urdampilleta JD. 2005. Estudo citogenetico em Paullinieae (Sapindaceae). Ms Thesis, Londrina, RGS.

Urdampilleta JD. 2009. Estudo citotaxônomico em espécies de Paullinieae (Sapindaceae). PhD Thesis, Campinas, SP.

Urdampilleta JD, Coulleri JP, Ferrucci MS, Forni-Martins ER. 2012. Karyotype evolution and phylogenetic analyses in the genus *Cardiospermum* L. (Paullinieae, Sapindaceae). *Plant Biology* **5:** 868–881.

Urdampilleta JD, Ferrucci MS, Forni-Martins ER. 2008. Chromosomes studies of some *Thinouia* species (Sapindaceae) and systematic implications. *Annales Botanici Fennici* **45:** 68–73.

Urdampilleta JD, Ferrucci MS, Torezan JMD, Vanzela ALL. 2006. Karyotype relationships among four South American species of *Urvillea* (Sapindaceae: Paullinieae). *Plant Systematics and Evolution* **258:** 85–95.

Urdampilleta JD, Ferrucci MS, Vanzela ALL. 2007. Cytogenetic studies of four South American species of *Paullinia* L. (Sapindaceae). *Botanical Journal of the Linnean Society* **154:** 313–320.

Vaio M, Mazzella C, Porro V, Speranza P, Lopez-Carro B, Estramil E, Folle GA. 2007. Nuclear DNA content in allopolyploid species and synthetic hybrids in the grass genus *Paspalum*. *Plant Systematics and Evolution* **265:** 109–121.

Van der Ham RWJM, Tomlik A. 1994. *Serjania* pollen and the origin of the tribe Paullinieae. *Review of Paleobotany and Palynology* **83:** 43–53.

Vicient CM, Suoniemi A, Anamthawat-Jónsson K, Tanskanen J, Beharav A, Nevo E, Schulman AH. 1999. Retrotransposon BARE-1 and its role in genome evolution in the genus *Hordeum*. *Plant Cell* **11:** 1769–1784.

Vieira de Freitas D, Carvalho CR, do Nascimento FJ (Filho), Astolfi S (Filho). 2007. Karyotype with 210 chromosomes in guaraná (*Paullinia cupana 'Sorbilis'*). *Journal of Plant Research* **120:** 399–404.

Vinogradov AE. 2003. Selfish DNA is maladaptive: evidence from the plant Red List. *Trends in Genetics* **19:** 609–614.

Xia N, Gadek PA. 2007. Hippocastanaceae. *Flora of China* **12:** 1–6. Available at: http://www.efloras.org