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REGULAR PAPER

A novel indicator of karyotype evolution in the tribe Leucocoryneae (Allioideae, Amaryllidaceae)

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Abstract The tribe Leucocoryneae is taxonomically and cytogenetically complex, mainly due to its extraordinary morphological and karyological variation. Robertsonian translocations had long been recognized as a central factor contributing to karyotype diversity within the Leucocoryneae, but so far no major tendency prevailing on the observed complexity of karyotype formula among species has been identified. The assessment of nuclear DNA contents by flow cytometry using propidium iodide in 23 species, representing all genera within the tribe, showed a monoploid genome size variation of 1Cx = 9.07–30.46 pg denoting a threefolds fluctuation. A highly significant linear association between the average DNA content per chromosome arm (2C/FN) and the monoploid genome size (1Cx) is reported for the first time and identified as a novel indicator of a trend governing karyotype diversity within Leucocoryneae. This trend shows that a reduction in DNA content per chromosome arm is influencing and has shaped karyotype evolution of different monophyletic groups within the tribe despite the complex karyotype diversity and apparently contrasting patterns of genome sizes.

Keywords Flow cytometry · Fundamental number · Genome size · Robertsonian translocations

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Introduction

Chromosomal diversity is a key factor contributing to genetic, phenotypic and ecological evolution in Angiosperms. Chromosomal diversity can be expressed in a wide range of numerical, morphological and molecular features [e.g. chromosome number, dysploidy, aneuploidy, polyploidy, chromosome size, karyotype length and symmetry, genome size, etc. (Weiss-Schneeweiss and Schneeweiss 2013)]. In particular, monocots contain the widest range of genome size variation (0.6–152.2 pg) observed among the main angiosperm groups, yet the large genomes are confined to the orders Asparagales and Liliales (Leitch and Leitch 2013; Schubert and Vu 2016). Within the family Amaryllidaceae (Asparagales) the largest monoploid genome size belongs to *Galanthus lagodechianus* Kem.-Nath. (82.2 pg; Bennett and Leitch 2012; Leitch and Leitch 2013). Patterns of karyotype evolution present in different angiosperm groups display both tendencies towards an overall increment or reduction in DNA contents. However, no association between an increase in genome size and organismal complexity have been found yet (Schubert and Vu 2016), and little is known about the role of natural selection or other evolutionary forces acting upon variation in genome sizes (Wolf et al. 2014). Since changes in the DNA content not always reflect changes in karyotype formula (e.g. Bennetzen et al. 2005; Poggio et al. 2014), mechanisms responsible for genome size variation are independent of those leading to changes in chromosome numbers.

Tribe Leucocoryneae represents a small group within the subfamily Allioideae (Amaryllidaceae), composed by six South American genera comprising ca. 100 species (Sassone et al. 2014a): *Beauverdia* Herter (4 spp, Sassone et al. 2014b), *Ipheion* Raf. (3 spp), *Latace* Phil. (2 spp, Sassone et al. 2015), *Leucocoryne* Lindl. (15 spp), *Nothoscordum*

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Kunth (ca. 80 spp) and *Tristagma* Poepp. (13 spp, Arroyo-Leuenberger and Sassone 2016). The only exception is *Nothoscordum bivalve* (L.) Britton, which extends farther into North America (Guaglianone 1972). Cytogenetic surveys have been carried out in species of Leucocoryneae since the 1970s (further details below); hitherto, only a few reports on DNA content had been published (but see Pellicer et al. 2017). Different authors have reported a high variation in cytogenetic parameters among genera and species in the Leucocoryneae (e.g. Araneda et al. 2004; Crosa 1972, 1974, 1975a, b, 1981, 2004; Jara-Arancio et al. 2012; Montes and Nuciari 1987; Nuñez et al. 1974; Nuñez 1990; Souza et al. 2009, 2010, 2012, 2015, 2016a, b). Such parameters comprise base chromosome numbers ranging from $x=4$ to $x=12$, ploidy levels including $2x$, $3x$, $4x$ and $6x$ chromosome sets, fundamental numbers (FN) varying from 14 to 48, and a karyotype formula showing different associations of metacentric, submetacentric and acrocentric chromosomes. Chromosomal rearrangements have been identified to have a preponderant role in speciation events, and most cases were accompanied by karyotypic changes (White 1978). As mentioned before, even when changes in the amount of DNA are not necessarily in correspondence with karyotype formula variation, due to the high frequency of polyploidy in plants the number of chromosome are usually positively associated to DNA content variations (Soltis et al. 2009). However, it is not yet clear how increments and reductions in DNA amounts are distributed among chromosomes in a given complement set of stables karyotypes (Chalup et al. 2014). DNA content can be differentially distributed among chromosomes or chromosome arms and thus, lead to important changes in karyotype attributes (Peruzzi et al. 2009). Hence, considering the complexity of Leucocoryneae karyotype evolution, it is expected that ordinary DNA content parameters do not always reflect karyotype evolution within the tribe.

Phylogenetic relationships within subfamily Allioideae, resolved the tribe Leucocoryneae as monophyletic (Pellicer et al. 2017; Sassone 2017; Souza et al. 2016a). Based on ancestral state reconstruction of cytogenetic and molecular data, Souza et al. (2016a) and Pellicer et al. (2017) independently inferred the basic chromosome number $x=5$ ($3M+2A$, metacentric and acrocentric chromosomes, respectively) as being the ancestral state of tribe Leucocoryneae and they hypothesized that new karyotype combinations may well be the basis for the origin of most lineages. As support to this view, bimodal karyotypes found in most of the tribe genera are explained, to a great extent, as a consequence of independent events of Robertsonian translocations (RT) (Crosa 1981; Jones 1998; Pellicer et al. 2017; Pires et al. 2006; Souza et al. 2010, 2016a, b; Tamura 1995).

Here we present a comprehensive study of genome size variation in a wide number of species of the tribe Leucocoryneae to highlight and better understand evolutionary patterns and to identify possible trends linked to changes in karyotype compositions among species. Thus, we aim at (1) estimate the genome sizes of different species, (2) recognize patterns of genome size variation, (3) assess DNA content variation in relation with known karyotypes, and (4) identify potential drivers of karyotype evolution within the tribe. As a whole, we also propose a novel indicator of karyotype evolution.

Materials and methods

Data collection

An exhaustive review of literature was undertaken in order to assemble all relevant information on the cytogenetic knowledge of species within Leucocoryneae, concerning chromosome numbers, karyotypes, ploidy levels and genome sizes. A first check on Goldblatt and Johnson's plant chromosome number indexes series allowed us to identify relevant papers from which we gathered information: Araneda et al. (2004); Crosa (1972, 1974, 1975a, b, 1981, 1988); Crosa and Marchesi (2002); Jara-Arancio et al. (2012); Jones (1998); Meric and Dane (2005); Montes and Nuciari (1987); Nassar and Aguiar (1978); Nuñez et al. (1974); Nuñez (1990); Palomino et al. (1992); Pellicer et al. (2017); Souza et al. (2009, 2010, 2012, 2015, 2016a, b) and Souza (2012). Furthermore, Plant DNA C-values database (Bennett and Leitch 2012) and Chromosome Counts Database [CCDB, version 1.45, Rice et al. (2014)] were consulted.

Plant material

A total of 25 taxa (23 species) belonging to the 6 genera of tribe Leucocoryneae (Sassone et al. 2014a) were analyzed. Field trips were carried out in Central and South Chile, South and East Uruguay, and the Argentine provinces of Buenos Aires, Entre Ríos, Mendoza, Neuquén, Río Negro and Santa Cruz. Also, some specimens of commercially cultivated value were included. Plants were collected and then cultivated at the greenhouse in the "Darwinion" Institute of Botany; fresh material was recovered for the analyses from 3 to 5 individuals per species. Voucher specimens per locality were deposited at SI; acronyms follow Thiers (2017). The package 'raster' (Hijmans and Elith 2016), available in the R statistical package 3.2.2 (R Development Core Team 2016), was employed to plot specimens in a distribution map. Altitude as represented in the map has been obtained from WorldClim-Global Climate Data (http://www.worldclim.org/). Data for the analyzed species of *Leucocoryne* were obtained from literature.

Nuclear DNA measurements and analyses

DNA contents were estimated by flow cytometry using fresh or silica gel dried young leaves. For each species, a mean value of genome size (2C) was obtained from measurements of at least three individuals. A diploid genotype of *Ipheion uniflorum* (Graham) Raf. (2C = 19.3 pg, Zonneveld et al. 2005) was used as internal standard. DNA content of the standard was validated using *Allium cepa* L [2C = 34.98 pg, Doležel et al. (1998)]. Suspensions of intact nuclei were prepared according to Otto (1990). Briefly, plant tissue of each sample + internal standard were chopped with a razor blade in a Petri dish containing 0.5 ml of Otto I buffer (0.1 M citric acid and 0.5% Tween 20). The chopped material was filtered through a 30 µm nylon mesh, incubated with 2 ml of Otto II buffer (0.4 M Na_2HPO_4.12 H_2O, with 1 µg/l µl of propidium iodide, $C_{27}H_{34}I_2N_4$), and then analyzed using a Partec PA II flow cytometer (Sysmex Partec GmbH, Münster, Germany) located at Floriculture Institute (INTA Castelar, Buenos Aires, Argentina). For each sample, histograms with relative fluorescence intensity from around 5000 nuclei were analyzed, CV value of 8% was accepted for each sample peak (G_0/G_1peak).

All cytometric parameters (chromatogram peaks, mean values, and coefficient of variation) were calculated using FloMax® software (Sysmex Partec GmbH, Münster, Germany). Nuclear genome size of each sample was calculated using the formula:

$$\text{Sample 2C DNA content} = \frac{\text{sample } G_1 \text{ peak mean}}{\text{standard } G_1 \text{ peak mean}} \times \text{standard 2C DNA content (pg DNA)}.$$

Differences in DNA content between species were tested by one-way analysis of variance (ANOVA) at a significance level of 5%. Linear regression between monoploid genome size (1C*x*) vs. (1) ploidy levels, (2) karyotype formula, (3) base chromosome number, (4) FN, and (5) average of DNA content per chromosome arm values (in pg) were performed. The latter measure was estimated as: 2C (total DNA content in pg)/FN (number of chromosome arms in a somatic cell). All statistical analyses were completed using the Info-Stat software version 2012 (Di Rienzo et al. 2012).

Genome size and phylogenetic framework

Genome sizes and other cytogenetic parameters including 2C/FN values per species were mapped in a phylogenetic tree to track patterns and trends in karyotype evolution. Tree topology has been adapted from a previous molecular phylogenetic inference based on three molecular markers (Sassone 2017). The topology is in concordance with previous phylogenetic inferences and karyotype formulas are represented as reconstructed by Souza et al. (2016a) and Pellicer et al. (2017).

Results

Karyotype diversity in the tribe Leucocoryneae

A comprehensive bibliographic search summarizing previous cytogenetic information was performed, and presented in Table 1. *Tristagma* and some species of *Nothoscordum* sect. *Nothoscordum* present the smallest basic chromosome number, $x = 4$. However, these taxa do not share the same karyotype formula. Within *Tristagma*, the species are characterized by having 3M + 1A. Some species within *N.* sect. *Nothoscordum* are the only ones for which acrocentric chromosomes had not been reported; hence the karyotype formula is 4M (e.g. *N. gaudichaudianum* Kunth, *N. montevidense* Beauverd var. *montevidense*). Although, other species of *N.* sect. *Nothoscordum* exhibit also acrocentric chromosomes (e.g. *N. bivalve, N. bonariense* Beauverd). Species from *Nothoscordum* sect. *Inodorum, Leucocoryne* and *Beauverdia* all share the same basic chromosome number $x = 5$ and a karyotype formula constituted by 3M + 2A. As for the fundamental number, FN = 16 is the modal value within the tribe (*Nothoscordum, Beauverdia* and *Leucocoryne*). Despite having different basic chromosome number and karyotype formula, all reported species within *Tristagma* ($x = 4$; 3M + 1A) and *Ipheion* ($x = 5$, 6, 7; 1SM + 4A, 1SM + 5A, 7A, respectively) share the fundamental number FN = 14, with the exception of *Ipheion sessile* (Phil.) Traub. [= *Ipheion recurvifolium* (C.H.Wright) Traub], exhibiting a FN = 24 (Table 1).

Latace and some of the tetraploid species of *Nothoscordum* and *Leucocoryne* (e.g. *Leucocoryne ixioides* Lindl., *N.bivalve, N. montevidense*) share a high fundamental number (FN = 32). However, when analyzing genus *Latace*, some incongruences are found in bibliography. Based on chromosome counts of both species of *Latace* (under *Zoellneralium*), Crosa (2004) assumed that the species were tetraploid carrying $2n = 4x = 24$ chromosomes, and deduced a basic chromosome number of $x = 6$ (2M + 4A). Such conclusions differ with the recent inference made by Souza et al. (2016a) and Pellicer et al. (2017) who concluded the basic chromosome number to be $x = 12$ (4M + 8A; Table 1).

In particular, *Nothoscordum bonariense* present the highest fundamental number (FN = 47, 48) within the tribe $2n = 26$ (22M + 4A) due to its assumed allohexaploid origin with parental genomes of *N. bivalve* and *N. gaudichaudianum* (Crosa 1974; Nuñez 1990; Jones 1998; Souza 2012).

Concerning chromosome morphology, a stasis in karyotype constitution is observed within and among species in *Nothoscordum* sect. *Inodorum, Tristagma* spp., and

Table 1 Cytogenetic parameters as previously reported in literature

Species	*x*	2*n*	Karyotype formula	FN
Beauverdia dialystemon	5	10	6M + 4A	16
Beauverdia hirtella subsp. *hirtella*	5	10	6*M* + 4*A*	16
Beauverdia hirtella subsp. *lorentzii*	Unknown			
Beauverdia sellowiana	5	10	6*M* + 4*A*	16
Beauverdia vittata	5	10	6*M* + 4*A*	16
Ipheion sessile	5	20	4*SM* + 16*A*	24
Ipheion tweedieanum	7	14	14*A*	14
Ipheion uniflorum	6	12/24	2*SM* + 10*A*/4*SM* + 20*A*	14/28
Latace andina	6	24	8*M* + 16*A*	32
	12	24	8*M* + 16*A*	32
Latace serenense	6	24	8*M* + 16*A*	32
	12	24	8*M* + 16*A*	32
Leucocoryne coquimbensis	5	10/18	6*M* + 4*A*/14*M* + 4*A*	16/32
Leucocoryne ixioides	5	18	14*M* + 4*A*	32
Leucocoryne pauciflora	5	10	6*M* + 4*A*	16
Leucocoryne purpurea	5	14/18	10*M* + 4*A*/14*M* + 4*A*	24/32
*Nothoscordum andicolum**	5	18	14*M* + 4*A*	32
Nothoscordum bivalve+	5	10/18	6*M* + 4*A*/14*M* + 4*A*	16/32
Nothoscordum bonariense+	?	26	21*M* + 5*A*/22*M* + 4*A*	47/48
Nothoscordum gaudichaudianum+	4	8/16	8*M*/16*M*	16/32
*Nothoscordum gracile**	5	18/19/20	14*M* + 4*A*/13*M* + 6*A*/12*M* + 8*A*	32
Nothoscordum montevidense var. *minarum*$^{+}$	(4) 5	10 (16)	6*M* + 4*A*	32
Nothoscordum montevidense var. *montevidense*$^{+}$	4	8/16	8*M* /16M	16/32
Nothoscordum nudicaule*	5	10/18/19	6*M* + 4*A*/14*M* + 4*A* /13*M* + 6*A*	16/32
Tristagma sp	4	24	18*M* + 6*A*	42
Tristagma bivalve	4	8	6*M* + 2*A*	14
Tristagma circinatum	Unknown			
Tristagma gracile	Unknown			
Tristagma graminifolium	4	8	6*M* + 2*A*	14
Tristagma nivale	4	16	12*M* + 4*A*	28
Tristagma patagonicum	4	8	6*M* + 2*A*	14
Tristagma violaceum	Unknown			

x base chromosome number, 2*n* diploid number, karyotype formula, *FN* fundamental number (number of chromosome arms in a somatic cell)

$^{+}$Indicates species belonging to *Nothoscordum* sect. *Nothoscordum*;

*Indicates species belonging to *Nothoscordum* sect. *Inodorum*

Beauverdia spp. In contrast, species of *Ipheion* display high variation in the number of acrocentric chromosomes and, within *N.* sect. *Nothoscordum* different karyotypes are observed (Table 1), whereas *Latace* and *Ipheion* present the highest proportion of acrocentric/submetacentric or metacentric chromosomes among the tribe. Submetacentric chromosomes are exclusively found in species of *Ipheion*, though a few acrocentric chromosomes in *Leucocoryne* had been categorized as submetacentric or subtelocentric chromosomes (Jara-Arancio et al. 2012; Pellicer et al. 2017).

Analysis of genome sizes variation at genera and species levels

DNA content was measured on 78 specimens from 42 accessions of the tribe Leucocoryneae, representing its entire distribution in South America (Table 2; Fig. 1). DNA content analyses using an internal standard revealed clear and well-defined peaks, and a coefficient of variation below 8%. The 2C nuclear DNA amount of the species varied from 18.72 pg [*Ipheion tweedieanum* (Baker) Traub] to 121.84 pg [*Leucocoryne coquimbensis* F.Phil.] representing 6.5-fold of variation in total DNA content

Table 2 List of the studied Leucocoryneae species including collector & number, geographic location and coordinates. All the specimens are stored at SI

Species	Collector	No.	Country	Province	Longitude	Latitude
Beauverdia dialystemon	Giussani, L.M	500	Argentina	Buenos Aires	−58.018	−34.887
Beauverdia dialystemon	Giussani, L.M	501	Argentina	Buenos Aires	−58.018	−34.887
Beauverdia hirtella subsp. *hirtella*	Giussani, L.M	468	Uruguay	Lavalleja	−57.286	−34.372
Beauverdia hirtella subsp. *hirtella*	Giussani, L.M	482	Uruguay	Lavalleja	−55.247	−34.302
Beauverdia hirtella subsp. *lorentzii*	Giussani, L.M	490	Argentina	Entre Ríos	−58.293	−32.451
Beauverdia sellowiana	Giussani, L.M	465	Uruguay	Lavalleja	−57.286	−34.372
Beauverdia sellowiana	Giussani, L.M	466	Uruguay	Lavalleja	−57.286	−34.3725
Beauverdia vittata	Giussani, L.M	425	Argentina	Entre Ríos	−58.293	−32.451
Beauverdia vittata	Giussani, L.M	481	Uruguay	Lavalleja	−55.247	−34.302
Beauverdia vittata	Giussani, L.M	491	Argentina	Entre Ríos	−58.293	−32.451
Ipheion sessile	Giussani, L.M	469	Uruguay	Lavalleja	−57.286	−34.3725
Ipheion sessile	Giussani, L.M	487	Uruguay	San José	−56.760	−33.86
Ipheion sessile	Giussani, L.M	508	From	cultivar	Unknown	
Ipheion tweedieanum	Giussani, L.M	420	Argentina	Entre Ríos	−58.592	−32.966
Ipheion tweedieanum	Giussani, L.M	488	Uruguay	San José	−58.007	−32.643
Ipheion uniflorum	Giussani, L.M	496	Argentina	Buenos Aires	−58.537	−34.488
Ipheion uniflorum	Giussani, L.M	655	Argentina	Buenos Aires	−59.170	−37.355
Ipheion uniflorum	Giussani, L.M	656	Argentina	Buenos Aires	−57.769	−37.945
Ipheion uniflorum	Morrone, O	6250	Argentina	Buenos Aires	−59.202	−37.327
Latace andina	Giussani, L.M	604	Argentina	Mendoza	−69.838	−32.848
Latace andina	Sassone, A	24	Argentina	Mendoza	−69.334	−32.995
Nothoscordum bonariense+	Giussani, L.M	450	Argentina	Buenos Aires	−57.4461	−35.148
*Nothoscordum gracile**	Giussani, L.M	568	Argentina	Buenos Aires	−58.0144	−34.994
Nothoscordum montevidense var. *montevidense*[+]	Villamil	11,687	Argentina	Buenos Aires	−62.18	−38.570
Nothoscordum montevidense var. *minarum*+	Giussani, L.M	s.n	Argentina	Entre Ríos		
Nothoscordum montevidense var. *montevidense*+	Giussani, L.M	449	Argentina	Buenos Aires	−57.446	−35.148
Nothoscordum montevidense var. *montevidense*+	Morrone, O	6312	Uruguay	Lavalleja	−55.271	−34.271
Nothoscordum montevidense var. *montevidense*+	Urtubey, E	878	Uruguay	Colonia	−57.673	−33.829
*Nothoscordum nudicaule**	Giussani, L.M	506	Argentina	Buenos Aires	−58.014	−34.994
Tristagma bivalve	Giussani, L.M	624	Chile	Región Metropolitana	−70.32	−33.355
Tristagma bivalve	Giussani, L.M	629	Chile	Región Metropolitana	−70.082	−33.828
Tristagma bivalve	Giussani, L.M	631	Chile	Región Metropolitana	−70.32	−33.355
Tristagma bivalve	Giussani, L.M	645	Chile	VIII Región del Biobío	−71.513	−36.911
Tristagma bivalve	Giussani, L.M	646	Chile	VIII Región del Biobío	−71.431	−36.916
Tristagma circinatum	Sassone, A	34	Argentina	Mendoza	−70.125	−35.091
Tristagma gracile	Giussani, L.M	650	Chile	VIII Región del Biobío	−71.615	−36.761
Tristagma graminifolum	Giussani, L.M	637	Chile	Región Metropolitana	−70.7172	−33.398
Tristagma nivale	Humano, G	s.n	Argentina	Santa Cruz		
Tristagma patagonicum	Sassone, A	25	Argentina	Mendoza	−69.353	−32.9858
Tristagma patagonicum	Sassone, A	28	Argentina	Mendoza	−70.059	−34.77
Tristagma patagonicum	Sassone, A	s.n	Argentina	Neuquen	−70.23	−39
Tristagma violaceum	Giussani, L.M	652	Chile	VIII Región del Biobío	−71.579	−36.921

[+]Indicates species belonging to *Nothoscordum* sect. *Nothoscordum*;

*Indicates species belonging to *Nothoscordum* sect. *Inodorum*

(Table 3). When considering the 1C*x* by genera, *Ipheion* and *Latace* presented the lowest values (9.3 pg; Table 3; Fig. 2), and *Leucocoryne* exhibited the highest value (30.46 pg; Table 3; Fig. 2). Statistical analyses revealed significant differences in genome size variation among genera ($P < 0.0001$).

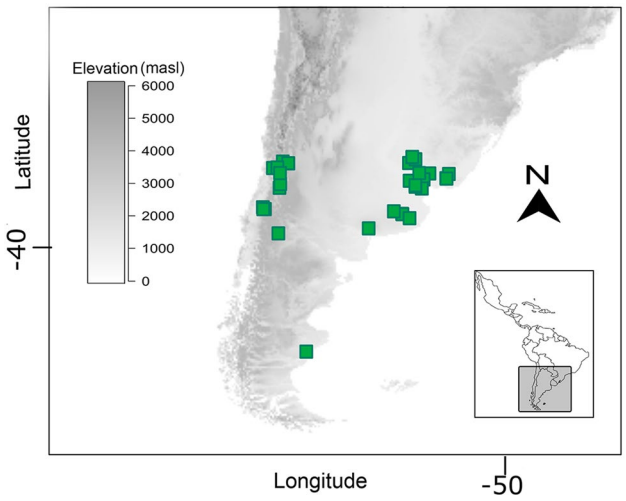

Fig. 1 Geographic distribution of the samples used in this study is represented by green squares. For location details see Table 2

Monoploid genome sizes and their parallel to cytogenetic attributes of species

When analyzing the relationship between monoploid genome sizes (1C*x*) vs. (1) ploidy levels, (2) karyotype formula, (3) base chromosome numbers and (4) fundamental numbers, linear regression model showed no significant correlations. However, when comparing monoploid genome sizes to the average DNA content per chromosome arm values (2C/FN), a strong linear association was found ($F = 601{,}51$, $P < 0.0001$; Fig. 3), and a clear trend depicting a positive arithmetic growth was observed for all 23 studied species. *Latace andina* showed the lowest 2C/FN value (1.17 pg per chromosome arm), while *Leucocoryne* species showed the highest 2C/FN value (3.6 pg per chromosome arm) (Fig. 3; Table 3).

Nothoscordum bonariense is a particular case within species of *Nothoscordum*, showing a 1C*x* = 11.83 pg and an average DNA content per chromosome arm (2C/FN = 1.49 pg) lower than other *Nothoscordum* species. In spite of this, the comparison of 1C*x* vs. 2C/FN values fits perfectly the positive trend in the linear regression modeling. Regarding total DNA content, *Beauverdia dialystemon*, is another particular case within genus *Beauverdia*, due to the presence of a 1C*x* = 28.54 pg and average DNA content per chromosome arm of 3.5 pg. (Fig. 3; Table 3).

Monoploid genome size variation and their phylogenetic framework

Monoploid genome size, when mapped into a phylogenetic tree, depicted an interesting pattern of genome size variation for the different clades within Leucocoryneae. Following the character state reconstruction by Pellicer et al. (2017), the estimated genome size for the common ancestral karyotype of Leucocoryneae (CAKL) would have been 24 pg. This observation nicely correlates with the ancestral monoploid genome size for the clades *Leucocoryne* + *Latace*, and [*N.* sect. *Inodorum* + (*N.* sect *Nothoscordum* / *Beauverdia*)], which are estimated to be 1C*x* = 24–26 pg (Pellicer et al. 2017). On the other hand, in the *Tristagma* + *Ipheion* clade at least two rounds of genome downsizing compared to the genome size of the ancestor are predicted. First, a reduction from 1C*x* = 24 pg in the common ancestor of the CAKL to the estimated genome size of 1C*x* = 18 pg for the *Tristagma* + *Ipheion* clade that we found to be conserved in extant species of *Tristagma* (Fig. 4). Second, a substantial reduction from the observed genome size of 1C*x* ~ 18 pg in *Tristagma* to the one in *Ipheion* of 1C*x* ~ 9 pg.

Within the clades, a drastic DNA loss and genome size reduction is observed in *Latace*, from 26 pg estimated in the ancestor to the observed 1C*x* = 9.3 pg. Meanwhile, in the analyzed species of *N.* sect. *Inodorum* a monoploid genome size of 1C*x* = 18.7 pg is observed; and the clade including *Beauverdia* and *N.* sect *Nothoscordum* presents 1C*x* ~ 17.8 pg.

Concerning the amount of DNA per chromosome arm, *Leucocoryne* species exhibit the highest observed content per chromosome arm (*x* = 5, 3M + 2A; 2C/FN = 3.6 pg; Fig. 4; Table 3). In contrast, *Latace*, the sister genus of *Leucocoryne*, presents the lowest DNA content per chromosome arm (*x* = 6, 2M + 4A; 2C/FN = 1.2 pg; Fig. 4; Table 3). Our findings indicates that the redistribution of DNA content along chromosomes is rather similar in *Tristagma* (*x* = 4, 3M + 1A, 2C/FN ~ 2.4 pg), *N.* sect. *Inodorum* (x = 5, 3M + 2A, 2C/FN ~ 2.3 pg) and the analyzed species of *N.* sect. *Nothoscordum* (*x* = 4, 4M, 2C/FN ~ 2.6 pg). However, in genera like *Ipheion* (*x* = 5, 6, 7; 1SM + 4A, 2SM + 2A, 7A, respectively; 2C/FN ~ 1.4 pg) and *Latace* (*x* = 6, 2M + 4A; 2C/FN = 1.17 pg), independent evolution is evident from the wide variation in chromosome sizes and karyotype diversity displayed among the species, wherein the apparent loss of DNA per chromosome arm is the only common factor (Fig. 4; Table 3).

Discussion

The diversification success of Angiosperms has been related with approximately 2000-fold variation in genome size (Puttick et al. 2015). The modal genome size for this group has been reported to be 5.9 pg (Leitch and Leitch 2013); larger genome sizes are rarely found among flowering plants, and transitions to very large genomes have occurred in only a few groups (Soltis et al. 2003). Monocot species within

Table 3 Cytogenetic data of the analyzed species

Taxa	Ploidy	2C (pg) ± SD (*n*)	1C*x* (pg)	2C/FN (pg)
Ipheion tweedieanum	2*x*	18.72 ± 3.75 (3)	9.36	1.37
Ipheion sessile	4*x*	36.29 ± 1.22 (5)	9.07	1.51
Ipheion uniflorum	2*x*	19.3 ± 0.15 (5)	9.65	1.38
Ipheion uniflorum	4*x*	37.49 ± 1.5 (2)	9.37	1.34
Beauverdia sellowiana	2*x*	30.82 ± 0.16 (3)	15.41	1.93
Beauverdia vittata	2*x*	29.22 ± 3.14 (4)	14.61	1.82
Beauverdia hirtella	2*x*	35.87 ± 1. 44 (3)	17.94	2.24
Beauverdia hirtella subsp. *lorentzii*	4*x*	52.24 ± 0.07 (3)	13.06	1.63
Beauverdia dialystemon	2*x*	57.07 ± 2.21 (4)	28.54	3.56
Latace andina	4*x*	37.33 ± 1.38 (5)	9.33	1.17
Leucocoryne coquimbensis×	2*x*	56.12	28.06	3.81
Leucocoryne coquimbensis×	4*x*	121.84	30.46	3.79
Leucocoryne pauciflora×	2*x*	56.53	28.17	3.53
Leucocoryne purpurea×	4*x*	114.96	28.74	3.38
Leucocoryne purpurea×	3*x*	86.88	28.96	3.62
Leucocoryne ixioides×	4*x*	115.64	28.91	3.40
Nothoscordum montevidense var. *montevidense*+	2*x*	44.24 ± 9.24 (3)	22.12	2.76
Nothoscordum montevidense var. *minarum*+	4*x*	77.55 ± 1.2 (3)	19.38	2.42
Nothoscordum bonariense+	6*x*	70.97 ± 1.5 (3)	11.83	1.49
*Nothoscordum nudicaule**	4*x*	74.55 ± 3.10 (3)	18.63	2.33
*Nothoscordum gracile**	4*x*	75.42 ± 3.2 (2)	18.85	2.41
Tristagma circinatum	2*x*	30.56 ± 0.09 (3)	15.28	2.18
Tristagma bivalve	2*x*	33.00 ± 1.46 (6)	16.50	2.35
Tristagma gracile	2*x*	34.58 ± 2.01(3)	17.29	2.47
Tristagma graminifolium	2*x*	35.55 ± 0.34 (3)	17.77	2.54
Tristagma patagonicum	2*x*	33.16 ± 1.94 (6)	16.58	2.37
Tristagma nivale	2*x*	33.53 ± 0.27 (3)	16.77	2.39
Tristagma violaceum	4*x*	66.48 ± 2.63 (3)	16.62	2.37

2C DNA content, *SD* standard deviation, *n* number of analyzed specimens, C*x* monoploid genome size, *2C/FN* average DNA content per chromosome arm

+Indicates species belonging to *Nothoscordum* sect. *Nothoscordum*

*Indicates species belonging to *Nothoscordum* sect. *Inodorum*

×Estimated in/from Pellicer et al. (2017)

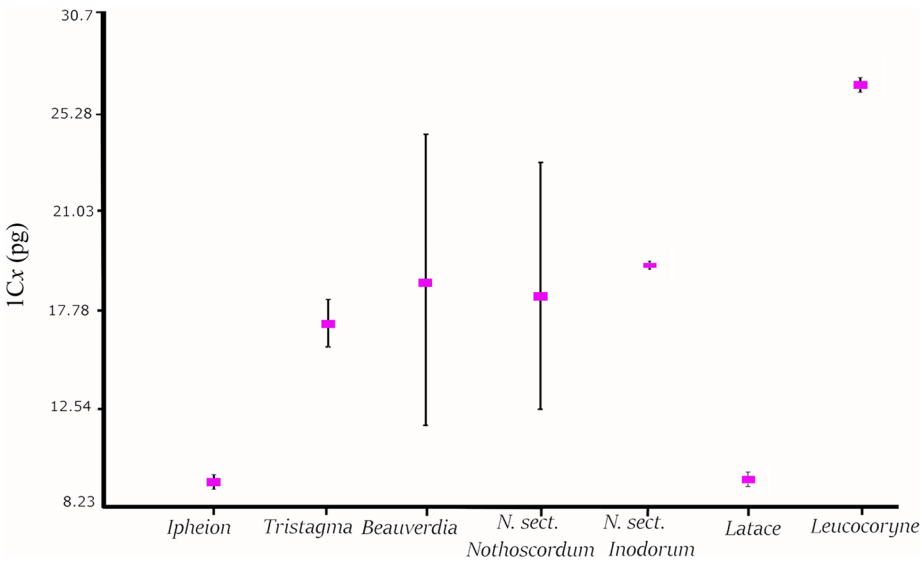

Fig. 2 Box plot of 1C*x* values (pg) of Leucocoryneae genera. For this analysis, sections of *Nothoscordum* were treated as independent groups based on their differences in basic chromosome numbers (see Tables 1, 2)

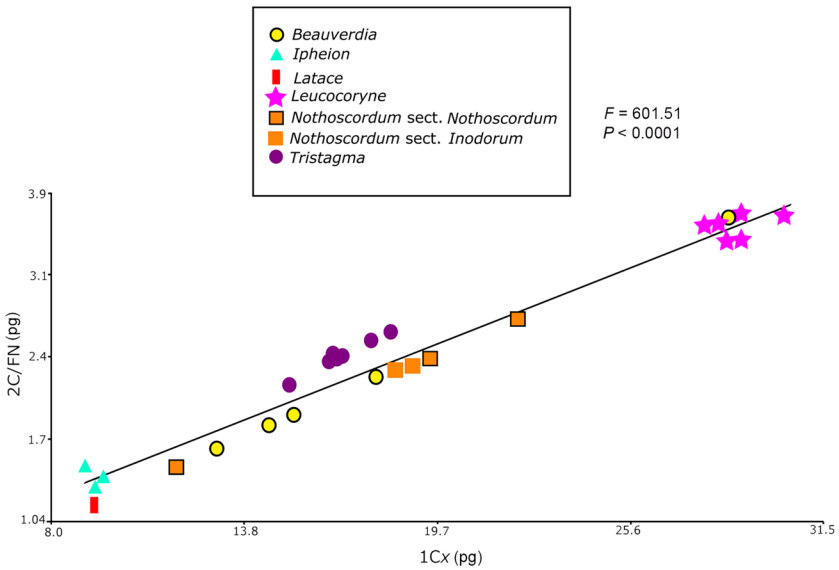

Fig. 3 Linear regression between 1C*x* value and the average DNA content per chromosome arm

the orders Asparagales, Commelinales and Liliales are the plants with the largest recorded genomes (Leitch and Leitch 2013; Soltis et al. 2003), and specimens analyzed in this work are not the exception. Genome size variation within Leucocoryneae is in concordance with the huge diversity of cytogenetic parameters. Among the studied species, we found a remarkable variation in total DNA content ranging from 18.72 to 121.84 pg, representing 6.5-fold of variation in the tribe. While, monoploid genome sizes varied from 1C*x* = 9.07–30.46 pg, thus being 1.5 to fivefolds higher than the average for Angiosperms. It is also noticeable, within the tribe Leucocoryneae, an internal variation of threefolds. This remarkable genome size variation can only be explained by plasticity of the genome (Leitch and Leitch 2008).

Karyotype diversity and associated genome size variations

The conspicuous genome size differences observed within Leucocoryneae are mainly related to phylogenetic groups. The tribe Leucocoryneae includes the monophyletic genera *Ipheion* [*x* = 5 (4SM + 1A), 6 (1SM + 5A), 7 (7A)], *Latace* [*x* = 6 or 12 (2M + 4A or 4M + 8A)], *Leucocoryne* [*x* = 5 (3M + 2A)], and *Tristagma* [*x* = 4 (3M + 1A)], plus the paraphyletic genus *Nothoscordum*, composed by the monophyletic section *N.* sect. *Inodorum* [*x* = 5 (3M + 2A)] and, *N.* sect. *Nothoscordum* [*x* = 4 (4M), *x* = 5 (3M + 2A)] with all species of *Beauverdia* [*x* = 5 (3M + 2A)]. While certain plant groups are characterized by a stasis of karyotype formula, asymmetry indexes and genome features that can be accompanied by morphological radiation (e.g. *Iris* spp., Samad et al. 2016), other groups might show very little morphological differentiation associated to important chromosomal change and karyotype diversification (e.g. *Prospero* spp., Jang et al. 2013). Within Leucocoryneae the chromosomal variation and karyotype evolution found among the species are correlated to a wide range of morphological changes (Sassone 2017; Sassone et al. 2014a, 2015). Thus, not only the karyotype formula differs among species and genera of Leucocoryneae (Table 1), but a wide variation is also found from different cytogenetic attributes, including genome sizes, that support the observed morphological variation and suggest a predisposition of the group for chromosomal rearrangements and genomic flexibility to tolerate DNA losses during evolution. In this tribe, monoploid genome size values seem to be a suitable indicator of the emergence of monophyletic groups. Based on the interpretation of DNA content and previous reports (Table 1), we proposed an evolutionary hypothesis where different cytogenetic events may have led to the formation of the current karyotype arrangements (Fig. 4).

Genome size variation in *Latace* + *Leucocoryne* [*La* + *Le*]

According with Pellicer et al. (2017), the common ancestor of [*La* + *Le*] clade conserves the karyotype formula and similar genome size of the CAKL. Although, no change in base chromosome number or karyotype formula is observed, some species of *Leucocoryne* exhibit an increase in the total genome size (e.g. *L. coquimbensis*). In our analysis, *Leucocoryne* species present the highest value of genome size per average chromosome arm (2C/FN = 3.6 pg), a condition almost certainly associated to the occurrence of large chromosomes (reaching > 20 µm in length in some species). Following Souza et al. (2015), the presence of chromosomes with such length in *Leucocoryne* can be explained both by pericentric inversions as by amplification of pericentromeric repetitive sequences. In *Latace*, however, the evolution of

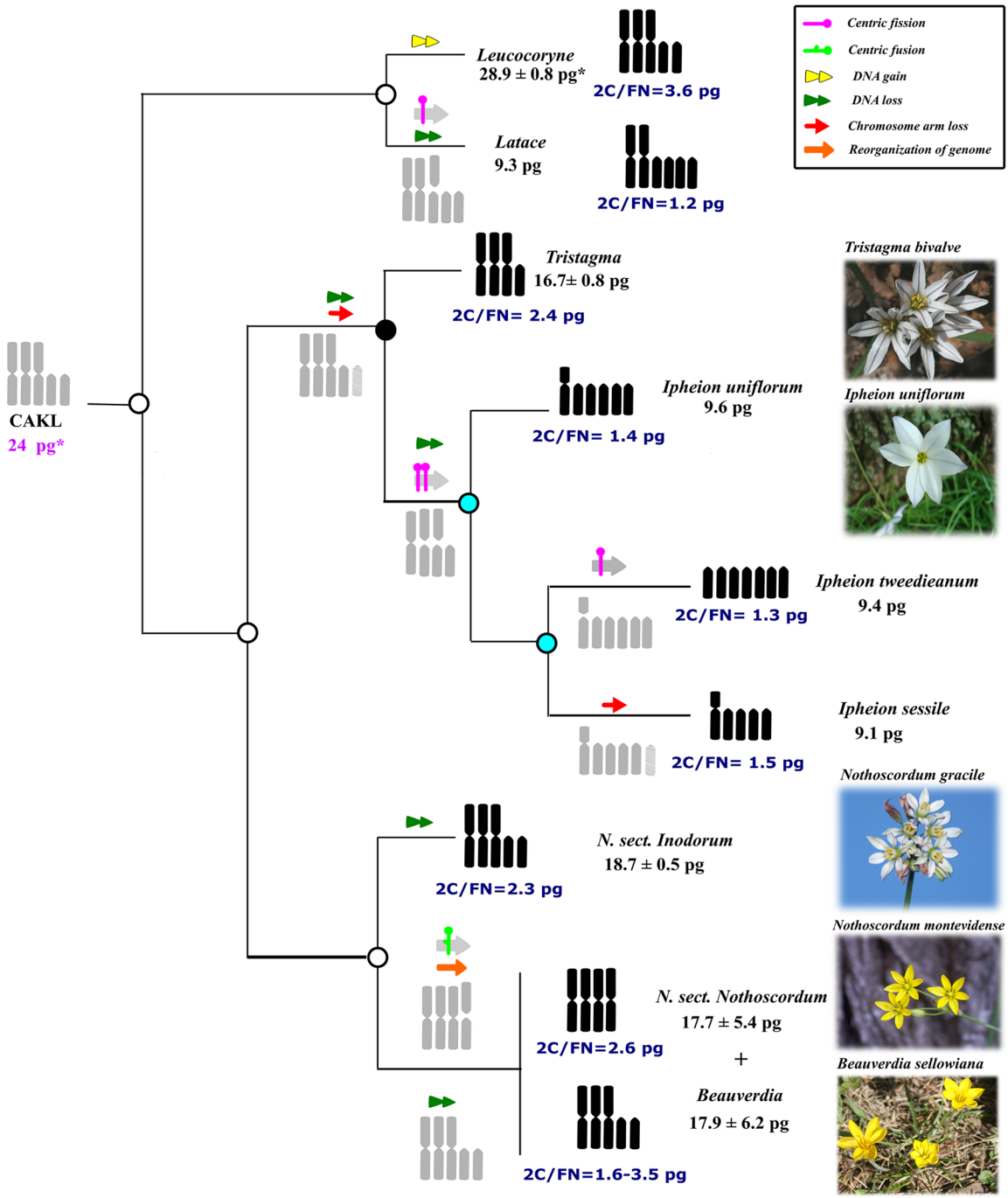

Fig. 4 Hypothetical origin of karyotypes in the tribe Leucocoryneae. Tree topology has been adapted from Sassone (2017); karyotype formulas follow Souza et al. (2016a). Nodes' colors indicate different karyotype formula. Photographs were taken by Sassone and Giussani. *Estimation made by Pellicer et al. (2017)

the karyotype from the [*La* + *Le*] ancestor happened via centric fissions (Fig. 4). Remarkably, we found this event to be associated with a significant loss of DNA depicted by the observed reduction of monoploid genome size in *Latace andina* (from ~ 24 to 9.3 pg) which is connected to an extensive drop of the amount of DNA per chromosome arm and the consequent reduction of the parameter 2C/FN (from 3.6 to 1.2 pg). When comparing chromosomal lengths, we found clearly shorter chromosomes in *Latace* species (4.3–11 µm, Crosa 2004; Souza et al. 2016a) as those of *Leucocoryne* species (11–29 µm, Jara Arancio et al. 2012), suggesting that the reduction in the observed amount of DNA must

necessarily be explained by a dramatic loss of DNA during the RT event. Unfortunately, we have not had the chance to evaluate DNA content in *Leucocoryne* species, but monoploid genome size values were taken from the literature (Pellicer et al. 2017).

Genome size variation in *Tristagma* + *Ipheion* [T + I]

The loss of an acrocentric chromosome from the CAKL gave place to an ancestor of the [T + I] clade with $x = 4$ (3M + 1A) and derived in a decrease of total DNA content (from 24 to 18 pg, Pellicer et al. 2017) with a concomitant change of the plesiomorphic fundamental number from FN = 16 to 14 (Fig. 4). This reduction of DNA content can be explained by the loss of a chromosome arm. While *Tristagma* species show a karyotype stasis (Crosa 1981) that seems to have conserved the karyotype of the ancestor of the [T + I] clade, the evolution of the karyotype formula in *Ipheion* has been less stationary (Pellicer et al. 2017), involving several chromosomal changes mainly associated with RT, and also with hybridization events at least in the case of *Ipheion sessile* (Souza et al. 2016a). In this regard, our analysis further shows that these changes and their consequences on the fundamental number of chromosome arms have had an important impact on the DNA contents found on each species. The 2C/FN values are radically reduced in *Ipheion* compared with *Tristagma* (from 2.4 to 1.4 pg) after two events of centric fissions and a big deletion in one chromosome that likely transformed one ancestral metacentric chromosome into submetacentric (Fig. 4; Table 1). *Ipheion uniflorum* ($2n = 2x = 12$, FN = 14) is the only species that has apparently conserved the ancestral karyotype of the group, from which one centric fission may have originated the reported karyotype of *I. tweedieanum* ($2n = 2x = 14$, FN = 14). The karyotype of the tetraploid species *I. sessile* ($2n = 4x = 20$, FN = 24) evolved after a loss of an acrocentric chromosome, accompanied by DNA loss. Polyploidy is a key factor for diversification and evolution in higher plants, and together with chromosomal rearrangements, play a central role creating variation in plant genome sizes (e.g. Soltis et al. 2015). The occurrence of duplicated chromosome sets ($2n = 20$) as well as duplicated 5S rDNA sites and other cytogenetic features (Souza et al. 2010, 2016a) in the origin of *I. sessile* further stress the relevance of chromosomal rearrangements and polyploidization events in the evolution of the group.

Genome size variation in *Nothoscordum* + *Beauverdia*

In the *Nothoscordum* + *Beauverdia* clade, the analyzed species evolved from the ancestor by downsizing the monoploid genome size (from 1Cx = 24 pg to 1Cx ~ 18 pg) and a complex pattern of chromosomal rearrangements and karyotype diversification [see Souza et al. (2016a) and references therein]. While *Nothoscordum* sect. *Inodorum* and *Beauverdia* have conserved the ancestral tribal karyotype (CAKL, see Fig. 4), some species included in *Nothoscordum* sect. *Nothoscordum* present a derived karyotype with an extra metacentric chromosome gained after a fusion event between two acrocentric chromosomes (e.g. *Nothoscordum gaudichaudianum, N. montevidense* var. *montevidense*). In spite of the observed stasis in chromosome morphology and general conservation of karyotypes in species of *Nothoscordum* sect. *Inodorum* and *Beauverdia*, the clade exhibit variable levels of DNA loss among species, being seemingly more drastic in some species of *Beauverdia*. Thus, in *Beauverdia dialystemon*, a species recently characterized as diploid by Pellicer et al. (2017), we found a genome size of 2C = 57 pg representing a monoploid genome size of 1Cx = 28.54 pg of DNA, almost double the amount of DNA found in cell nuclei of closely related species (e.g. diploid *B. vittata* 2C = 29.2 pg, Cx = 14.6 pg) and per chromosome arm (2C/FN = 1.82 pg in *B. vittata* vs. 3.56 pg in *B. dialystemon*). This increase in DNA content can be attributed to the accumulation of repetitive DNA, as hypothesized for *Leucocoryne* species (Souza et al. 2015). However, all other diploid species studied exhibit genomes with half the size estimated for *B. dialystemon*. Since the accessions analyzed are different to those studied by Pellicer et al. (2017), a cytogenetic evaluation will be needed before resolving this inconsistency between the suggested ploidy for this species and the estimated genome size. Overall, the observed occurrence of karyotype constancy accompanied by a differential reduction of genome sizes among species has been previously reported for other plant groups (for example see reports on the genus *Hippeastrum*, Poggio et al. 2014), and suggests the simultaneous action of selective mechanisms that preserve karyotype morphology without restricting downsizing processes.

Similar to our observations in *Latace* and *Ipheion* showing that centromere fissions during RT events cause extensive loss of DNA and reduction in monoploid genome sizes, our analysis in species of *Nothoscordum* sect. *Nothoscordum* show that centromere fusion events may be also associated with genome downsizing and reduction of 1Cx values (Fig. 4).

A trend towards an increment of acrocentric chromosomes carrying less DNA per chromosome

A positive linear regression between the monoploid genome size (1Cx) and the average of DNA content per chromosome arm (2C/FN) was found for the first time among species and genera of Leucocoryneae (Fig. 3). Our findings indicate that changes in karyotype formula caused by RT generate reductions in the monoploid genome size in Leucocoryneae are linked to a drop in the average DNA content per chromosome arm (Fig. 4). Species of *Leucocoryne* with a relative

karyotype stasis that conserved the chromosomal morphology of the CAKL ancestor (*i.e.* without chromosome rearrangement events like RT) show the highest values of DNA content per chromosome arm, while species like *Ipheion* and *Latace* with a relative less constant karyotypes (i.e. high incidence of chromosome rearrangements events and RT) and with the major proportion of acrocentric chromosomes, show the lowest values; supporting the idea that karyotype asymmetry and genomic size are highly correlated as previously proposed (Peruzzi et al. 2009). In other words, species with similar FN numbers but higher RT events contain less DNA per chromosome arm, meaning that centric fission–fusion are associated with drastic losses of DNA. This observation can be useful, for example, helping to resolve cases with observed discrepancy on basic chromosome numbers, like *Latace*. By plotting *Latace andina* average value to our linear regression model stand that the only basic number for the genus that fits in the observed trend expected for species with variable FN and Cx is $x = 6$ (Fig. 3). On the one hand, considering *Latace* with $x = 6$ add support to the hypothesis proposed by Crosa (2004) and help us to resolve the previous disparity on chromosomal evolution within this genus; on the other hand, it works as a test of the accuracy of the evolutionary trend within the tribe that we are presenting here.

Our results are in concordance and add support to the findings of Pellicer et al. (2017) in the tribes Gilliesieae and Leucocoryneae who pointed out the utility of FN to understand the evolution of the tribe, and suggested that the preservation or loss of chromosome arms (as a consequence of RT) together with polyploidization events are the most important driving forces that cause lineage divergence. Our findings link the positive association between 1Cx and 2C/FN that shed light on the cytogenetic forces that are shaping genome sizes and the evolution and diversification of karyotypes within the tribe Leucocoryneae.

Conclusion

It has been proposed that the high rate of diversification in Angiosperms is related with the ability to take profit from changes in genome size, like polyploidy and other genome rearrangements (e.g. RT) (Puttick et al. 2015). Our findings support the tribe Leucocoryneae as a good example of this hypothesis; it is clearly observed that changes in genome size are linked with the diversification of lineages. The observed trend in the distribution of DNA content per chromosome arm (2C/FN) reinforces the hypothesis that RT events played a central role not only as a major mechanism promoting karyotype diversification and the emergence of monophyletic genera in Leucocoryneae (e.g. *Ipheion, Tristagma, Latace*), but also as leading mechanism shaping genome size variation and monoploid DNA content in particular genera (e.g. *Nothoscordum, Latace, Ipheion*). Additionally, ancestral hybridization and polyploidization (e.g. *Ipheion sessile, Latace andina, Nothoscordum bonariense, N. gracile*, among others) events have provided other sources of variation promoting karyotype diversification within the tribe. The ratio between total genome size and number of chromosome arms (2C/FN) exhibit a clear trend and is considered here as a cytogenetic parameter to explain how monoploid genome size and RT events interrelate, and helps to elucidate the complex patterns of karyotype evolution within Leucocoryneae. Due to its anticipated independence of chromosomal morphology, such parameter standardizes DNA content and paves the way to comparing unrelated genera and species and therefore could be used as a diagnostic feature in other plant and animal groups.

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