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ORIGINAL ARTICLE

Karyological relationships among some South American species of *Solanum* (Solanaceae) based on fluorochrome banding and nuclear DNA amount

M. Cristina Acosta · Marcelo Guerra · Eduardo A. Moscone

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Abstract Fluorescent chromosome banding and measurements of nuclear DNA content by image cytometry of Feulgen-stained cells were performed in one sample each of eight diploid ($2n = 24$) species of *Solanum*: *S. endoadenium*, *S. argentinum*, *S. pseudocapsicum*, *S. atropurpureum*, *S. elaeagnifolium*, *S. sisymbriifolium*, *S. chenopodioides*, and *S. palustre*. The species studied could be distinguished by heterochromatin amount, banding patterns, and genome size. They exhibited only GC-rich heterochromatin and showed a comparatively low heterochromatin amount (expressed as percentage of haplotype karyotype length), ranging from 2.10 in *S. argentinum* to 8.37 in *S. chenopodioides*. Genome size displayed significant variation between species, with 1C-values ranging from 0.75 pg (735 Mbp) in *S. palustre* to 1.79 pg (1,754 Mbp) in *S. sisymbriifolium*. No significant correlation between genome size and heterochromatin amount was observed, but intrachromosomal asymmetry index (A_1) was negative and significantly correlated with heterochromatin amount. DNA content was positively and significantly correlated with karyotype length. DNA C-value distribution in the genus as well as karyotype affinities and relationships between species are discussed in relation to different infrageneric classifications of *Solanum*.

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Introduction

Solanum L., with more than 1,000 known species, mostly native to South America, is the largest genus of Solanaceae and one of the largest and most diverse genera of angiosperms (Hunziker 2001). It is an extremely important taxon economically, as several species are cultivated for their edible tubers, fruits or leaves [e.g., *S. tuberosum* L. (potato), the world's fourth most important crop, *S. lycopersicum* L. (tomato), *S. melongena* L. (eggplant), *S. muricatum* Aiton (pepino), and *S. quitoense* Lam. (lulo)]. Moreover, the genus has pharmaceutical and ornamental uses (Hawkes 1999; Barboza et al. 2006). Because of its large size and complexity, infrageneric classification of *Solanum* has been difficult. The subgenus system proposed by D'Arcy (1972) and followed by Hunziker (2001) which divides *Solanum* into seven subgenera is now replaced by the phylogenetic system that breaks *Solanum* into 12–15 major clades (Bohs 2005; Weese and Bohs 2007).

Up to now, the karyology of less than half of the *Solanum* species has been studied (Hunziker 2001). Further, most of the cytological information is limited to chromosome number reports, meiotic chromosome studies, or karyotypic composition without qualitative or quantitative analyses. Karyotype measurements have been performed for species of *Solanum* sections *Basarthrum* (Bitter) Bitter, *Pachyphylla* (Dunal) Dunal, *Lasiocarpa* (Dunal) D'Arcy, *Lycopersicon* (Mill.) Wettst., and subgenus *Leptostemonum* (Dunal) Bitter (Bernardello and

Anderson 1990; Pringle and Murray 1991; Bernardello et al. 1994; Chiarini and Bernardello 2006; Brasileiro-Vidal et al. 2009), and several other species from different subgenera (Acosta et al. 2005; Rego et al. 2009) sensu D'Arcy (1972). Moreover, karyotype data with constitutive heterochromatin banding patterns analyzed by C-banding or fluorescence banding have been obtained in 46 species in the genus (Pijnacker and Ferwerda 1984; Pringle and Murray 1993; Sultana and Alam 2007; Rego et al. 2009; Brasileiro-Vidal et al. 2009; Melo et al. 2011; Miguel et al. 2012). No extensive DNA content measurements exist for species of *Solanum*, as only very few species (51) have been analyzed, most of them by Feulgen densitometry (cf. Bennett and Leitch 2010). It is well known that nuclear DNA content, being a specific karyological feature, is a very useful tool for addressing systematic and evolutionary questions (Bennett and Leitch 2011).

In this work, fluorochrome chromosome banding and measurements of nuclear DNA content were performed in eight wild *Solanum* species from Argentina, aiming to cytologically characterize these species and explore their heterochromatin constitution and DNA content variation. The ultimate aim of the ongoing research is to contribute to species delimitation and infrageneric classification in *Solanum* and to examine the evolution of karyological features in the genus.

Materials and methods

The provenance of the plant material studied is presented in Table 1, organized according to the system proposed by Bohs (2005). The respective voucher specimens were identified by A.T. Hunziker, G.E. Barboza, and M. Matesevach, and deposited in the herbarium of the Botanical Museum of Córdoba, Argentina (CORD).

For chromosome analysis, root tips of germinated seeds were pretreated with 2 mM 8-hydroxyquinoline for 16 h at 4 °C, and fixed in 3:1 ethanol:acetic acid mixture for a minimum of 12 h. The maceration procedure was performed using an enzymatic solution of 2 % cellulase (w/v) plus 1 % pectinase (v/v) at 37 °C for 1 h. The meristems were macerated in a drop of 45 % acetic acid, and the coverslip removed in liquid nitrogen. The cells were double stained with chromomycin A3 and 4′,6-diamidino-2-phenylindole (CMA/DAPI) following Barros e Silva and Guerra (2010). Enhanced or reduced fluorescence of a chromosome segment is indicated in the text by a "+" or "–" signal, respectively.

A total of 29 individuals from eight species were analyzed by fluorochrome banding, and 4–30 cells per species were examined (Table 1). Photomicrographs of 2–6 fluorochrome banded metaphase plates from 2–4 individuals of each species were used for chromosome measurements. For each metaphase plate, the absolute and relative lengths of short (p) and long (q) chromosome arms, chromosomes and bands (data not shown), and the absolute length of the karyotype were calculated (for relative values, haploid karyotype length = 100 %). The terminology for centromere position and satellites is the same as in Acosta et al. (2005). For each chromosome, the centromere position was calculated by the index r (arm ratio) = q/p (Levan et al. 1964). The satellite lengths were added to the length of the corresponding arms. Lengths of the secondary constrictions [nucleolar organizing regions (NORs)] were excluded. Different lengths of the same arm or band from homologous chromosomes were combined to compute mean values. The position of intercalary bands was calculated by the index: $di = d \times 100/a$ (d = distance of band center from the centromere, a = length of corresponding chromosome arm), according to Greilhuber and Speta (1976). Data on band length were added to idiograms previously calculated from measurements after conventional techniques (Acosta et al. 2005). In the idiograms, chromosomes were arranged first into groups according to their increasing arm ratio (from m to st), and then according to decreasing length within each group. Chromosome markers allowed positive identification of several chromosome pairs. As certain chromosomes showed great similarity, some homologies were tentatively established, whereas the remaining chromosomes were grouped in the idiograms.

Nuclear DNA measurements with Feulgen staining were performed according to the procedure described by Greilhuber and Ebert (1994) with slight modifications. Root-tip meristems from primary roots 0.5–1 cm long were fixed with neutral 4 % formaldehyde in Sorensen's phosphate buffer (pH 7.0) for 1.5 h, then rinsed in methanol:glacial acetic acid (3:1) and stored in 96 % ethanol at –20 °C until use. Meristems were hydrolyzed in 5 N HCl for 60 min at room temperature, stained with Schiff′s reagent for 2 h, rinsed in SO_2 water for 30 min, and squashed in 45 % acetic acid. The preparations were frozen in liquid nitrogen and air-dried. *Vigna radiata* (L.) R. Wilczek cv. Berken, with 1C-values = 0.53 pg, was used as internal standard for calculations of absolute genome size, as suggested by Bennett and Leitch (1995). Specimens and standards were processed strictly in parallel. DNA measurements were carried out in 26–30 Feulgen-stained early telophase nuclei (2C) per slide, and three plants of each sample were analyzed.

A Leica DMLB microscope equipped with a Cohu video camera was used to take photomicrographs of single metaphase plates and interphase and early telophase nuclei. Leica Q-FISH and QPloidy software packages were used to process the images and to estimate the relative amount of nuclear DNA, respectively. Calculations of mean and

Table 1 List of *Solanum* species studied, code, provenance, and voucher specimen

Taxon	Code	Provenance and voucher specimen
Dulcamaroid clade		
S. endoadenium Bitter (4, 8)	end	Argentina: Prov. Tucumán, Dept. Tafí, El Molle; *ATH*, *GEB*, *EAM 24875*
Geminata clade		
S. argentinum Bitter and Lillo (2, 5)	arg	Argentina: Prov. Córdoba, Dept. Capital, Córdoba; *MCA 20*
S. pseudocapsicum L. (5, 22)	pse	Argentina: Prov. Córdoba, Dept. Río Cuarto, Alpa Corral; *EAM 242*
Leptostemonum clade		
S. atropurpureum Schrank (3, 5)	atr	Argentina: Prov. Misiones, Dept. Iguazú, Puerto Bossetti; *EAM*, *JRD 221*
S. elaeagnifolium Cav. (2, 8)	ela	Argentina: Prov. San Juan, Dept. Ullum, between Ullum and Loma de las Tapias; *AAC*, *ANS 1014*
S. sisymbriifolium Lam. (2, 4)	sis	Argentina: Prov. Córdoba, Dept. Río Cuarto, Alpa Corral; *EAM 238*
Morelloid clade		
S. chenopodioides Lam. (8, 30)	che	Argentina: Prov. Córdoba, Dept. Calamuchita, Villa Quillinzo; *MCA 11*
Potato clade		
S. palustre Schltdl. (3, 5)	pal	Argentina: Prov. Neuquén, Dept. Lácar, Quila Quina; *GB*, *EAM 573*

In parentheses are the numbers of seedlings and somatic metaphases analyzed per species using fluorescent chromosome banding

Prov. province, *Dept.* department, *MCA* M.C. Acosta, *GEB* G.E. Barboza, *GB* G. Bernardello, *AAC* A.A. Cocucci, *JRD* J.R. Daviña, *ATH* A.T. Hunziker, *EAM* E.A. Moscone, *ANS* A.N. Sérsic

standard deviation of absolute 1C-values were done according to Greilhuber and Ebert (1994).

Single classification analysis of variance (ANOVA) and correlation analysis were performed using INFOSTAT version 1.1 (Infostat Group 2002). Correlations between heterochromatin amount, DNA content values, and karyotype length were evaluated using the nonparametric Spearman test (Sokal and Rohlf 1995).

Results

The somatic chromosome number $2n = 24$ was found in all taxa examined. The haploid karyotype length (HKL) for individual species ranged from 16.30 μm in *S. palustre* to 38.75 μm in *S. sisymbriifolium* (Table 2). All species displayed one chromosome pair carrying a NOR plus an attached satellite of variable size in the short arms. The NOR-bearing pair can be submetacentric (sm) (six species) or subtelocentric (st) (two species). In general, karyotypes were symmetrical considering both centromere position and chromosome size variation (Fig. 4). All species had a majority of metacentric (m) chromosome pairs in their diploid complements. The karyotypes of *S. endoadenium*, *S. argentinum*, and *S. palustre* with 4–5 sm or st pairs were the most asymmetrical, whereas the remaining species, displaying only 2–3 sm or st pairs, were more symmetrical.

The *Solanum* species here studied showed a comparatively low heterochromatin amount (expressed as percentage of HKL), ranging from 2.10 in *S. argentinum* to 8.37 in *S. chenopodioides* (Table 2). They exhibited CMA^+/$DAPI^-$ (chromomycin positive and DAPI negative) constitutive heterochromatin (Fig. 2a, b) at the satellites, at chromosomal termini in *S. chenopodioides* (Fig. 1a), *S. pseudocapsicum* (Fig. 1c), *S. sisymbriifolium* (Fig. 2a), and *S. elaeagnifolium* (Fig. 2c), and at the intercalary region (nearly subterminal, $di = 62.28$) in the short arm of chromosome pair no. 3 in *S. endoadenium* (Fig. 1d). *Solanum argentinum* (Fig. 1b), *S. palustre* (Fig. 1e), and *S. atropurpureum* (Fig. 2d) showed the simplest fluorescence banding pattern, with only one chromosome pair with satellites on the short arms. Centromeric heterochromatin was not found in any species examined. The only heteromorphisms observed in some metaphase of different individuals were the presence or absence of minute bands. The number and size of fluorochrome-stained chromocenters in interphase nuclei (Fig. 3) corresponded with the number and size of bands on metaphase chromosomes. The most conspicuous band was always associated with the NOR. Some m chromosomes (pairs 2 in *S. chenopodioides* and 1 and 3 in *S. pseudocapsicum*) have more prominent terminal bands on short arms. The NOR-associated heterochromatin comprised the distal satellite and, usually, a minute band proximal to the NOR. NORs were always placed on short arms and appeared as constrictions or gaps in the DAPI-stained chromosomes, sometimes with enhanced CMA fluorescence. Curiously, in all species studied here, the chromosomes bearing satellites lacked terminal bands in the long arms (Fig. 4).

Nuclear DNA measurements by image cytometry in one accession each of eight diploid *Solanum* species resulted in a maximum difference of 2.39-fold between species (Table 2). The absolute 1C-value ranged from 0.75 pg (735 Mbp) in *S. palustre* to 1.79 pg (1,754 Mbp) in *S. sisymbriifolium*, with an average 1C-value of 1.02 pg

Table 2 Karyotype features of *Solanum* species studied, all with $2n = 24$, ordered as in Table 1

Species	Haploid karyotype formula	NOR-bearing pair	HKL[a] (μm) *x* (sd)	A_1[a]	Heterochromatin amount[b]		Maximum no. of bands per haploid complement	Maximum no. of pairs with bands	1C DNA in pg (sd)	CV (%)	Genome size (Mbp)
					Total	NOR-assoc.					
S. endoadenium	8 m + 4 sm	9 (sm)	26.92 (3.08)	0.29	2.90	2.64	2	2	0.83 (0.06)	6.32	813
S. argentinum	8 m + 3 sm + 1 st	12 (st)	23.40 (2.94)	0.27	2.10	2.10	1	1	0.94 (0.06)	5.52	921
S. pseudocapsicum	9 m + 2 sm + 1 st	12 (st)	20.64 (3.06)	0.22	7.41	1.70	18	12	1.06 (0.07)	7.12	1,039
S. atropurpureum	10 m + 2 sm	12 (sm)	19.12 (3.44)	0.19	2.56	2.56	1	1	0.80 (0.07)	8.73	784
S. elaeagnifolium	10 m + 2 sm	11 (sm)	20.83 (1.28)	0.21	8.26	3.55	20	12	1.10 (0.06)	8.80	1,078
S. sisymbriifolium	9 m + 3 sm	12 (sm)	38.75 (5.61)	0.24	6.94	1.86	21	12	1.79 (0.09)	6.05	1,754
S. chenopodioides	9 m + 3 sm	10 (sm)	16.56 (0.36)	0.22	8.37	2.29	15	12	0.87 (0.09)	7.88	853
S. palustre	7 m + 4 sm + 1 st	8 (sm)	16.30 (0.64)	0.31	3.86	3.86	1	1	0.75 (0.09)	5.61	735

m metacentric, *sm* submetacentric, *st* subtelocentric, *NOR* nucleolus organizing region, *HKL* haploid karyotype length, *x* mean value, *sd* standard deviation, A_1 intrachromosomal asymmetry index. Genome size was calculated from the equivalence 1 pg = 980 Mbp; *CV%* coefficient of variation as a percentage of mean, with reference to the number of nuclei of the test sample

[a] Acosta et al. (2005)

[b] Heterochromatin amount expressed as percentage of HKL, *NOR-assoc.* NOR-associated heterochromatin

(1,000 Mbp). Analysis of variance for all the data showed a highly significant difference between species ($P < 0.0001$).

DNA content was positively correlated with karyotype length ($r = 0.64$; $P = 0.09$) (Table 2; Fig. 5), except in *S. endoadenium*, which has a comparatively small genome and large haploid karyotype length. This single lack of correlation may be due to the lower condensation level of the metaphases utilized for chromosome measurements. On the other hand, heterochromatin amount was significantly correlated with neither karyotype length ($r = -0.24$; $P = 0.53$) nor DNA content ($r = 0.40$; $P = 0.28$), but it was negatively correlated with the intrachromosomal asymmetry index (A_1) ($r = -0.78$; $P = 0.06$), except for *S. atropurpureum* (Table 2; Fig. 6).

Discussion

The species of the Solanaceae family are notable by their karyotype stability, including low percentage of polyploidy and low rate of chromosomal changes (Wu and Tanksley 2010). The *Solanum* species examined are diploids with $2n = 24$, the chromosome number present in more than half of the species studied in the family up to now (Hunziker 2001), and have small chromosomes, as is the rule in *Solanum* (see, for example, Acosta et al. 2005; Chiarini and Bernardello 2006; Rego et al. 2009; Melo et al. 2011), except in the Cyphomandra clade (Pringle and Murray 1991; Moscone 1992; Miguel et al. 2012). Five of eight species studied here (*S. argentinum*, *S. chenopodioides*, *S. elaeagnifolium*, *S. endoadenium*, and *S. palustre*) were examined for the first time using fluorochromes. They have GC-rich heterochromatin, exhibiting CMA-positive and DAPI-negative bands, mainly associated with the NOR heterochromatin, as observed in most *Solanum* species (cf. Sultana and Alam 2007; Rego et al. 2009; Brasileiro-Vidal et al. 2009; Melo et al. 2011; Miguel et al. 2012).

Nuclear DNA content values for *S. argentinum*, *S. chenopodioides*, *S. elaeagnifolium*, *S. endoadenium*, and *S. sisymbriifolium* are given here for the first time. Our DNA 1C-values obtained for *S. atropurpureum* (0.80 pg) and *S. pseudocapsicum* (1.06 pg) were about 29 and 22 % lower than reported by Bennett and Smith (1991) for these species (1.13 pg and 1.35 pg, respectively). Such differences were not expected because, as in the present work, these authors also used Feulgen cytophotometry and *Vigna radiata* cv. Berken as internal control. The possibility of taxon misidentification could not be discarded since these authors did not indicate the vouchers of the plants investigated. In addition, one sample registered in Bennett and Leitch (2010) as *S. tucumanense* Griseb. (mean 1C-value = 0.88 pg) is now recognized as *S. pseudocapsicum* by the Planetary Biodiversity Inventories (PBI) *Solanum*

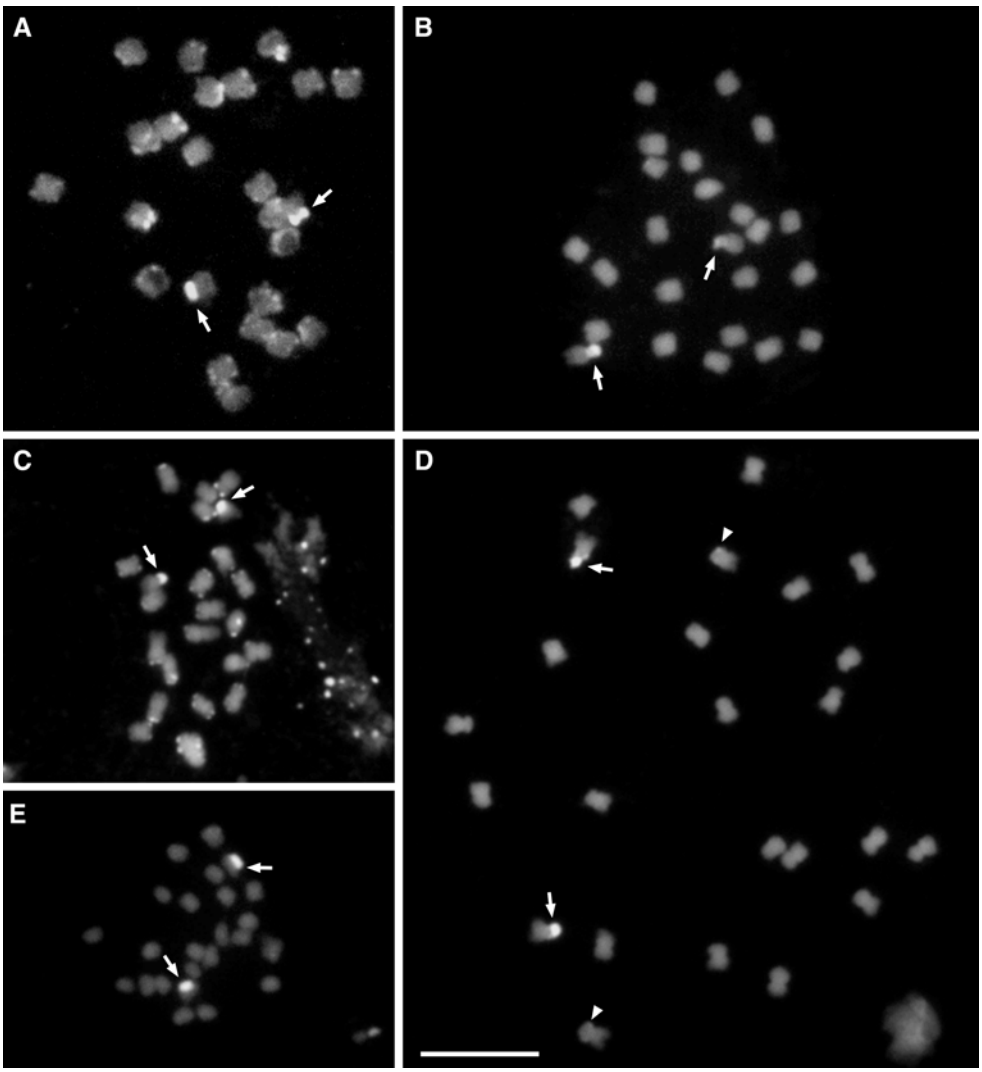

Fig. 1 Somatic metaphases of *Solanum* species ($2n = 24$) double-stained with CMA/DAPI (only CMA fluorescence is shown). **a** *S. chenopodioides*. **b** *S. argentinum*. **c** *S. pseudocapsicum*. **d** *S. endoadenium*. **e** *S. palustre*. *Arrows* mark $CMA^+/DAPI^-$ NOR-associated heterochromatin; *arrowheads* point out $CMA^+/DAPI^-$ intercalary bands. *Scale bar* corresponds to 10 μm

project (2012); probably this taxon has great variability in DNA 1C-values. On the other hand, using flow cytometry and chicken erythrocytes, Valkonen (1994) found only a 1 % higher mean 1C-value (0.76 pg) than the one observed here for *S. palustre* (sub nom. *S. brevidens* Phil.).

Surveys among angiosperm species have shown that the variation in DNA content is mainly due to the amount and proportion of repeated DNA sequences in the nuclear genome, especially tandem repeats or satellite DNAs that make up heterochromatic C-bands on the chromosomes (Heslop-Harrison and Schwarzacher 2011). In the *Solanum* species studied here, no correlation between genome size and heterochromatin amount has been observed, in agreement with previous findings in Cyphomandra clade (Pringle and Murray 1993) but in contrast to *Capsicum* L. (Moscone et al. 2003) where a clear correlation between both parameters was found. In *Solanum* only small amounts of highly repeated DNA sequences could be located in well-defined heterochromatic bands of the chromosomes, and the mass of the repeated sequences fraction must be spread throughout the complement, mainly in the region proximal to the centromere (Zhu et al. 2008; Brasileiro-Vidal et al. 2009).

Distribution of DNA C-values in *Solanum*

Eight out of the 13 major clades of *Solanum* (Bohs 2005) have been investigated with respect to DNA content, i.e., the Brevantherum, Cyphomandra, Dulcamaroid, Geminata, Leptostemonum, Morelloid, and Potato clades, with $x = 12$ and the Archaesolanum clade with $x = 23$ (cf. Bennett and Leitch 2010; PBI *Solanum* project 2012). The

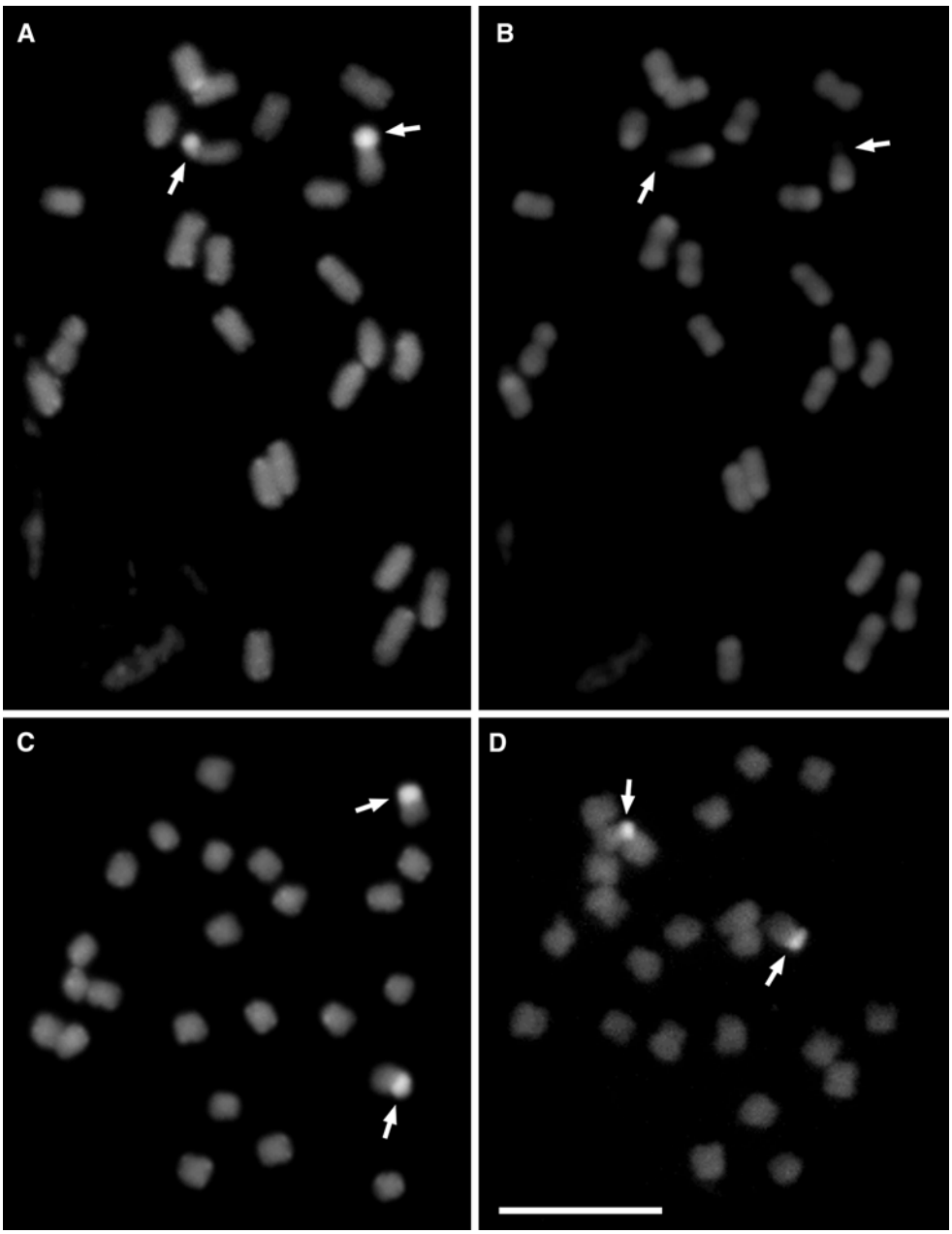

Fig. 2 Somatic metaphases of *Solanum* species ($2n = 24$) double-stained with CMA/DAPI. **a**–**b** *S. sisymbriifolium*. **c** *S. elaeagnifolium*. **d** *S. atropurpureum*. **a**, **c**, **d** CMA fluorescence. **b** DAPI fluorescence. *Arrows mark* $CMA^+/DAPI^-$ NOR-associated heterochromatin. *Scale bar* corresponds to 10 μm

diploid species showed an average 1C-value content estimation of 10.604 ± 5.087 in the Cyphomandra clade [ranging between 6.75 pg in *S. corymbiflorum* (Sendtn.) Bohs and 24.80 pg in *S. circinatum* Bohs], 1.611 ± 0.830 pg in the Leptostemonum clade (from 0.80 pg in *S. atropurpureum* to 3.65 pg in *S. grandiflorum* Ruiz & Pav.), 1.019 ± 0.349 pg in the Potato clade (from 0.63 pg in *S. chacoense* Bitter to 2.55 pg in *S. lycopersicum*), 0.975 ± 0.295 in the Morelloid clade (from 0.65 pg in *S. tripartitum* Dunal to 1.35 pg in *S. villosum* Mill.), and 0.935 ± 0.152 in the Dulcamaroid clade (from 0.80 to 1.13 pg in *S. dulcamara* L.). The remaining major clades have been poorly studied, with DNA content known for only one entity of the Brevantherum clade [1.53 pg for *S. abutiloides* (Griseb.) Bitter and Lillo], and two species of the Geminata (0.88–1.35 in *S. pseudocapsicum* and 0.94 pg in *S. argentinum*) and Archaesolanum clades (0.90 pg for *S. aviculare* G. Forst. and 1.78 pg for the tetraploid *S. laciniatum* Aiton). In general, tetraploid and hexaploid species of *Solanum* show 1C-values lower than expected based on the mean diploid value in the subgenera (Bennett and Leitch 2010).

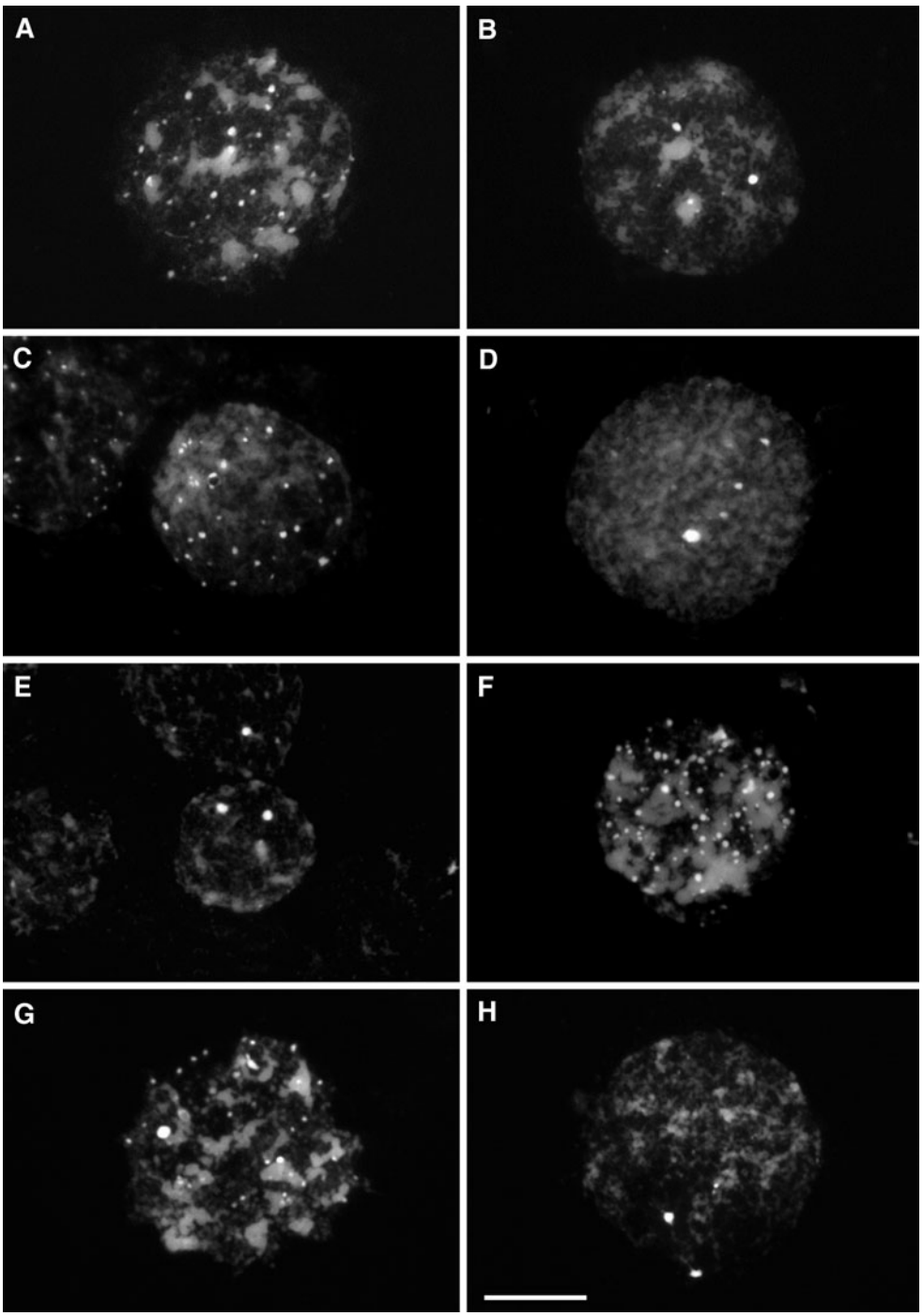

Fig. 3 CMA-stained interphase nuclei of *Solanum* species showing CMA^+ chromocenters. **a** *S. chenopodioides*. **b** *S. argentinum*. **c** *S. pseudocapsicum*. **d** *S. endoadenium*. **e** *S. palustre*. **f** *S. sisymbriifolium*. **g** *S. elaeagnifolium*. **h** *S. atropurpureum*. *Scale bar* corresponds to 10 μm

Karyotype comparison and systematic considerations

In spite of the general karyotype conservation, *Solanum* species can be distinguished by a combination of karyotype formula, the position of NORs in particular chromosome pairs, the intrachromosomal asymmetry index (A_1), heterochromatin amount, banding patterns, and DNA content.

The two species belonging to the Geminata clade (*S. argentinum* and *S. pseudocapsicum*) exhibited the same NOR position, with slight differences in karyotype formula and length and 1C-values of DNA content (Acosta et al. 2005 and present work). However, they differ in A_1 karyotype asymmetry index estimates, heterochromatin amount, and heterochromatic banding pattern since

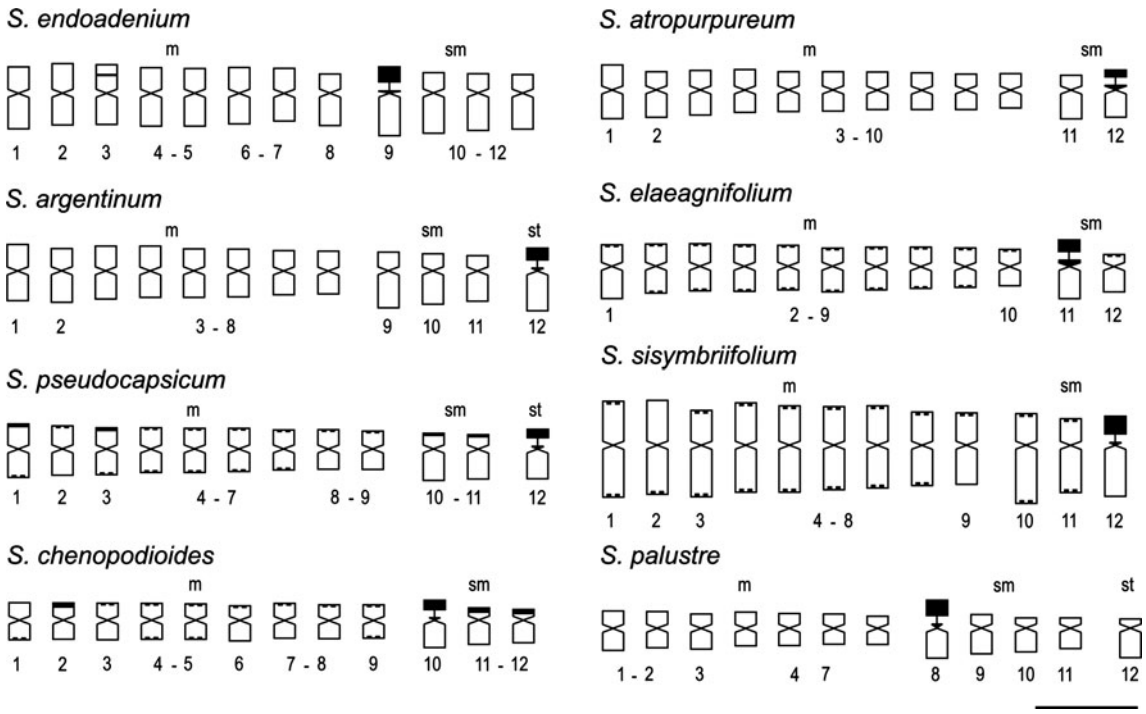

Fig. 4 Idiograms of *Solanum* species showing heterochromatic fluorochrome banding patterns. *Solid blocks* indicate $CMA^{+}/DAPI^{-}$ bands. *Scale bar* corresponds to 4 μm

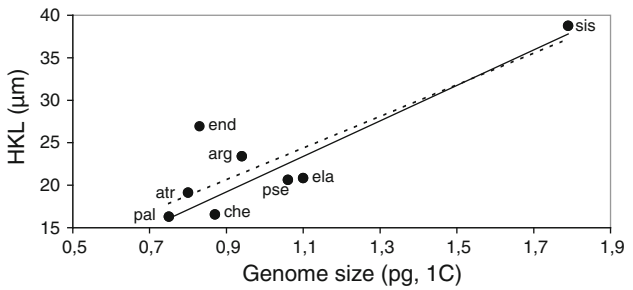

Fig. 5 Relationship between genome size (1C-value in pg) and haploid karyotype length (HKL) in *Solanum*. *Solid line* ($r = 0.86$; $P = 0.04$) and *dashed line* ($r = 0.64$; $P = 0.09$) represent the correlation without and with the *S. endoadenium* datum, respectively. *Species* codes as in Table 1

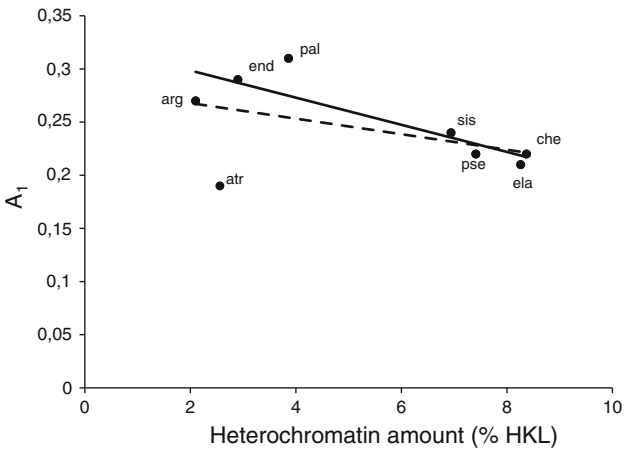

Fig. 6 Relationship between heterochromatin amount, expressed as percentage of haploid karyotype length (HKL), and intrachromosomal asymmetry index (A_1) in *Solanum* species. *Solid line* ($r = -0.78$; $P = 0.06$) and *dashed line* ($r = -0.30$; $P = 0.42$) represent the correlation without and with the *S. atropurpureum* datum, respectively. *Species* codes as in Table 1

S. pseudocapsicum has small terminal bands in whole chromosomes. The D'Arcy system (1972) proposed putting them in different subgenera (*S. argentinum*: subgenus *Brevantherum* and *S. pseudocapsicum*: subgenus *Solanum*).

Solanum endoadenium and *S. chenopodioides* belong to different clades (Dulcamaroid and Morelloid clades, respectively) but have a strong relationship (PBI *Solanum* project 2012). They have similar karyotype formulas and 1C-values but differ in karyotype length, A_1 asymmetry index, heterochromatic amount, and fluorescent banding patterns (more complex in *S. chenopodiodes*). D'Arcy's system put them in different subgenera (*S. endoadenium*: subgenus *Potato* and *S. chenopodioides*: subgenus *Solanum*). Notably, *S. chenopodioides* is karyologically more similar to *S. pseudocapsicum*, both with the same A_1 karyotype asymmetry index, similar heterochromatin amount, and heterochromatic banding pattern, and with slight differences in karyotype formula and length, and 1C-value; these taxa belong to subgenus *Solanum* in D'Arcy's system. *Solanum endoadenium* and *S. palustre* (Potato

clade) belong to subgenus *Potatoe* in D'Arcy's classification, and curiously they have very similar karyological characteristics, showing a simple fluorescence banding pattern, with only one chromosome pair with satellites and heterochromatic band on the short arms, and the most asymmetrical index estimates (A_1). They have similar karyotype formulas, NOR positions, and low 1C-values of DNA content, and they differed only in the mean chromosome length and in the presence of intercalary heterochromatin bands in *S. endoadenium*.

The three species of the Leptostemonum clade (subgenus *Leptostemonum* after D'Arcy) exhibited rather similar karyotype formulas and A_1 asymmetry index; nevertheless, *S. sisymbriifolium* and *S. elaeagnifolium* showed a banding pattern with minute terminal bands in the majority of chromosomes. The heterochromatin amount in both species was higher than in *S. atropurpureum* which is limited to the satellites. Finally, *S. sisymbriifolium* displayed a 1C-value and average chromosome length nearly twice as long as *S. atropurpureum* and *S. elaeagnifolium*, probably due to addition of repetitive dispersed DNA as transposable elements or perhaps segmental duplications of the genome (Heslop-Harrison and Schwarzacher 2011).

Considering the complicated infrageneric classification of *Solanum*, the present data suggest that detailed karyotype analysis and DNA content measurements may contribute to better understand of the phylogeny of the group. In several other genera with similar chromosome number and size, for example, *Spondias* L. (Almeida et al. 2007) and *Citrus* L. (Guerra 2009), detailed karyotype analysis has also demonstrated to be a useful tool to understand the relationships between species.

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