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ORIGINAL ARTICLE

Genome size and karyotype diversity in *Solanum* sect. *Acanthophora* (Solanaceae)

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Abstract In order to study the evolution of different cytogenetic characters in species of *Solanum* sect. *Acanthophora* in relationship to the known phylogeny for this group, the following techniques were used: CMA/DAPI chromosome banding; fluorescent in situ hybridization with probes for the 18-5.8-26S and the 5S rDNA genes in mitotic chromosomes; nuclear DNA quantification by flow cytometry. Depending on the species, 2–6 of the 12 basic chromosome pairs were identified. The heterochromatic banding patterns were shown to be species-specific. All species presented one chromosome pair bearing a 18-5.8-26S signal and one pair (rarely two) with a 5S signal, the two rDNA sites being non-syntenic. The techniques employed allowed us to establish two species groups within sect. *Acanthophora*: one with small, symmetric chromosomes, little heterochromatin and lower DNA content, and the other one with larger and more asymmetric chromosomes, more heterochromatin $CMA^{+}/DAPI^{-}$ (associated with NOR or not) and a higher DNA content. An elevated karyotype asymmetry would be associated with a high amount of heterochromatin and a high DNA content. The trend within sect. *Acanthophora* would be towards a loss of heterochromatin, a reduction of chromosome size, and an increase in symmetry.

Keywords CMA/DAPI chromosome banding · FISH · Flow cytometry · Heterochromatin · Phylogeny · *Solanum* sect. *Acanthophora*

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Introduction

Solanum subgenus *Leptostemonum* (Dunal) Bitter, with ca. 450 species, is the largest subgenus within *Solanum*, comprising almost one-third of the genus (Bohs 2005; Levin et al. 2006; Weese and Bohs 2007). Species of this subgenus have a worldwide distribution, with the highest species richness in Central and South America, Africa, and Australia. The members of subgen. *Leptostemonum* are commonly known as the "spiny solanums" because one of the main characteristics defining the subgenus is the presence of epidermal prickles on stems and leaves. Other features that characterize this group are the stellate hairs and the long, attenuate anthers with small terminal pores. The group includes economically important plants: *S. melongena* L. (aubergine or eggplant), *S. macrocarpon* L. (gboma eggplant), *S. aethiopicum* L. (scarlet eggplant), *S. sessiliflorum* Dunal (cubiu or cocona), and *S. quitoense* Lam. (lulo or naranjilla).

A distinguishable group within *Leptostemonum* is section *Acanthophora* Dunal, which includes about 20 mainly Neotropical species of herbs and small shrubs. Its center of diversity is eastern Brazil, where all but a few species are native (Nee 1991; Chiarini and Auler Mentz 2012). The distinctive trait of *Acanthophora* is the presence of unbranched hairs on the stems and upper leaf surfaces, whereas the undersurfaces may have mixed stellate and simple hairs (Nee 1991; Levin et al. 2005). Most species are adapted to disturbed habitats and secondary forest in open, sunny places. A number of species have been introduced and naturalized in other parts of the world. For example, *S. viarum* Dunal has become a noxious weed in USA and South Africa (Bryson and Byrd 1994; Welman 2003) and *S. palinacanthum* Dunal invades roadsides and crop fields in Argentina and Brazil (Nee 1991; Auler Mentz

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and Oliveira 2004). Other species are cultivated for their ornamental fruits (e.g., *S. mammosum* L., *S. capsicoides* All.) or for their alkaloid content (*S. viarum*, *S. mammosum*).

Acanthophora is especially diverse concerning the fruit, which can be juicy (as in *S. atropurpureum* Schrank) or spongy (as in *S. viarum*), small (1.35 cm diameter in *S. incarceratum* Ruiz et Pav.) or relatively large (4 cm diameter in *S. mammosum*), orange–red (*S. capsicoides*, *S. atropurpureum*), yellow (most species), or greenish yellow (*S. incarceratum* and *S. platense* Dieck.). Seeds can be winged (*S. platense*, *S. tenuispinum* Rusby), or bulky (*S. viarum*, *S. mammosum*) (Levin et al. 2005; Chiarini and Barboza 2009). Anatomical studies also revealed a variability of microscopic structures, tissues, and cell types (Chiarini and Barboza 2009).

The phylogeny of *Solanum* sect. *Acanthophora* was studied by Levin et al. (2005). The authors analyzed DNA sequence data from two nuclear regions (the granule-bound starch synthase gene [GBSSI or *waxy*] and ITS) and two plastid regions (*trnS–trnG* and *trnT–trnF*). They found the traditional circumscription of sect. *Acanthophora* to be non-monophyletic and proposed a narrower circumscription of the section. The so-called "Acanthophora clade" was also recovered in the phylogeny of Levin et al. (2006).

In *Acanthophora*, karyotype data from classical staining are available for 11 out of its ca. 20 species (Acosta et. al 2005; Chiarini and Bernardello 2006), but only one species was studied up to now by means of CMA/DAPI banding and/or the FISH technique (Melo et al. 2011). This is unfortunate because chromosomes can be an informative character in systematic studies. They are decisive in establishing linkage groups and natural classifications (Stebbins 1971). Cytogenetic techniques have shown to be useful detecting chromosome rearrangements involved in speciation (e.g., Fuchs et al. 1998; Srebniak et al. 2002; Melo and Guerra 2003). The FISH procedure allows homologous chromosomes in a complement to be differentiated and permits the comparison among related species, while the CMA/DAPI staining makes visible heterochromatin blocks, and both techniques, when combined with other markers, allow the detection of chromosome rearrangements and to establish phylogenetic hypotheses. These procedures have been successful in several genera of Solanaceae, as *Capsicum* (Moscone et al. 2006), *Nicotiana* (Lim et al. 2000), and *Nierembergia* (Tate et al. 2009), and they can be used to test the apparent chromosomal homogeneity within *Solanum*. In fact, *Solanum* is remarkable because the conservation of a single chromosome number and the supposed constancy in karyotype features, such as chromosome size and karyotype asymmetry (e.g., Bernardello and Anderson 1990; Acosta et al. 2005; Chiarini and Bernardello 2006).

Another feature of genomes that is a valuable source of information is the DNA content, which has been the subject of intense research since the last decades (Bennett and Leitch 2005). Comparative studies in angiosperms have shown that DNA contents or 'C-values' are correlated with features, such as plant phenology, minimum generation time, life history, frost resistance, biomass production, and ecological adaptations (Ohri 1998). Nuclear DNA amount has even shown to be a useful tool to study phylogenetic relationships between taxonomically related groups (Ohri 1998; Zonneveld 2001; Bennett and Leitch 2005). However, data of DNA content are available only for three species of *Acanthophora* (Bennett and Smith 1991).

Considering the phylogeny of Levin et al. (2005), it is possible to hypothesize about the evolution of other traits in *Acanthophora*. In order to assess the distribution of chromosome markers in the section, the framework molecular phylogeny of Levin et al. (2005) has been used here to map the chromosome characters onto the tree. The aims of this work were: (1) to describe the classical karyotype variables and heterochromatin patterns in the Acanthophora clade, (2) to characterize the distribution of the rDNA genes, (3) to determine the DNA content of species in sect. *Acanthophora*, and (4) to find relationships among karyotype, heterochromatin, DNA content, rDNA sites and phylogeny.

Materials and methods

Material examined

A total of nine species of *Solanum* sect. *Acanthophora* were studied. Details of voucher specimens are included in Table 1.

Chromosome preparations

Mitotic chromosomes were examined in root tips obtained from germinating seeds. Roots were pretreated in saturated *p*-dichlorobenzene in water for 2 h at room temperature, fixed in 3:1 ethanol: acetic acid, washed in distilled water, digested 45 min at 37 °C with Pectinex SP ULTRA® (Novozymes), and squashed in a drop of 45 % acetic acid. After coverslip removal in liquid nitrogen, the slides were stored at −20 °C.

Karyotypes and CMA/DAPI banding

Slides were stained with a drop of 0.5-mg/ml chromomycin A3 (CMA) in McIlvaine buffer, pH 7.0 and distilled water (1:1) containing 2.5-mM $MgCl_2$ for 90 min and subsequently stained with 2 µg/ml 4′-6-diamidino-2-phenylindole

Table 1 Provenance, collectors, and accessions numbers of the *Solanum* sect. *Acanthophora* species studied

Species	Source	Figures
Solanum acerifolium Dunal	Colombia. Cundinamarca, mun. Pacho, vereda Patasía, cerro Traga-Arepas, Feb. 11/2010, *Beltrán 62* (COL). Quindío, mun. Circasia, vereda Membrillal, 1,700 m.a.s.l., Feb. 13/2010, *Beltrán 82* (COL). Valle del Cauca, vía Cali-Buenaventura Km. 18, 1,900–2,000 m.a.s.l., Feb. 14/2010, *Beltrán 90* (COL).	1i, 2a
Solanum aculeatissimum Jacq.	Cultivated at the Botanical and Experimental Garden, Radboud University Nijmegen. Accession number: NLD020 (904750114), country of origin South Africa, Cape prov., near Komaga (original sample: 19750522, B.G. Birmingham University, UK, Donor number: BIRM/S.1696).	1h, 2f
Solanum atropurpureum Schrank	Argentina. Corrientes prov., Santo Tomé dep., Garruchos, Estancia San Juan Bautista, 128 m.a.s.l., 28°10′08.9″S, 55°38′47.7″W, Dec. 8/2008, *Barboza* et al. *2125* (CORD).	1d, 2e
Solanum capsicoides All.	Brazil. Río de Janeiro state, from Ubatuba to Parati, Km 591/592, Apr. 17/2008, *Barboza* et al. *2038* (CORD)—São Paulo state, from Salesópolis to Paraibuna, SP 077, km 108.5, Feb. 25/2006, *Barboza* et al. *1641* (CORD).	1c, 2d
Solanum mammosum L.	Cultivated at the Botanical and Experimental Garden, Radboud University Nijmegen. Accession numbers NLD020 (904750170), country of origin Ecuador, and NLD020 (944750071), country of origin Costa Rica, Collection site Chiapa de Corso.	1e, 2h
Solanum palinacanthum Dunal	Argentina. Córdoba prov., Calamuchita dep., Villa Amancay, 32°12′25″S, 64°35′55″W, Nov. 26/2011, *Chiarini 773* (CORD).	1g, 2c
Solanum platense Dieckmann	Argentina. Misiones prov., San Ignacio dep., ayo. Macaco, Dec. 7/2002, *Barboza* et al. *441* (CORD).	1f, 2g
Solanum tenuispinum Rusby	ARGENTINA. Tucumán prov., Tafí dep., Reserva Los Sosas, Km 36/37, on the road to Tafí del Valle, 27°00′49.7″S, 65°39′16.8″W, 1450 m.a.s.l., Feb. 24/2009, *Barboza* et al. *2151* (CORD).	1b, 2i
Solanum viarum Dunal	COLOMBIA. Quindío, mun. Circasia, vereda Membrillal, 1,700 m.a.s.l., Feb 13/2010, *Beltrán 80* (CORD)—Argentina. Corrientes prov., Ituzaingó dep., RN 12 on the road to San Carlos, May 14/2004, *Barboza* et al. *1006* (CORD).	1a, 2b

(DAPI) for 30 min, and finally mounted in McIlvaine's buffer–glycerol v/v 1:1 (Schweizer 1976; Schweizer and Ambros 1994). The amount of heterochromatin was expressed as a percentage of the total length of the haploid karyotype.

In order to compare the data obtained with the techniques here described, morphometric information for mitotic chromosomes was taken from previous works (Acosta et al. 2005; Chiarini and Bernardello 2006).

Fluorescent in situ hybridization (FISH)

The location and number of rDNA sites were determined by FISH using the following probes: the p*Ta*71 containing the 18-5.8-26S gene of wheat (Gerlach and Bedbrook 1979) labeled with biotin-14-dATP (BioNick, Invitrogen Carlsbad); a 5S rDNA fragment obtained by PCR (Kitamura et al. 2001) with DNA of *S. stuckertii*, labeled with digoxigenin-11-dUTP (DigNick, Roche). The FISH protocol was according to Schwarzacher and Heslop-Harrison (2000), with minor modifications. The preparations were incubated in 100-µg/ml RNAase, post-fixed in 4 % (w/v) paraformaldehyde, dehydrated in a 70–100 % graded ethanol series, and air-dried. On each slide 15 µl of hybridization mixture was added (4–6 ng/µl of probe, 50 % formamide, 10 % dextran sulfate, 2× SSC and 0.3 % SDS), previously denatured at 70 °C for 10 min. Chromosome denaturation/hybridization was done at 90 °C for 10 min, 48 °C for 10 min, and 38 °C for 5 min using a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany), and slides were placed in a humid chamber at 37 °C overnight. The 18-5.8-26S probe was detected with

avidin-FITC conjugate (Sigma-Aldrich), the 5S probe was detected with antidigoxigenin-rhodamine (Roche), and then counterstained and mounted with 25-µl antifade Vectashield® (Vector Lab.), containing 1.5 µg/ml of DAPI. Photomicrography was done with a Zeiss Axiophot microscope equipped with epifluorescence and a digital image capture system. For the mergence of images, the free software ImageJ was employed (http://rsbweb.nih.gov/ij/).

Flow cytometry

Plants to be used for C-value estimate were grown from seeds of the same sources used for chromosome preparations (Table 1). For every accession, 1–3 individuals were measured, three runs each. Nuclear suspensions were prepared according to Doležel et al. (2007): approximately 1 cm^2 of leaf tissue of *Solanum* species and the appropriate piece of internal standard leaves were chopped with a razor blade in a glass Petri dish containing 1 ml of ice-cold Otto I solution (0.1 M citric acid and 0.5 % v/v Tween 20). *Solanum lycopersicum* 'Stupické' ($2C = 1.96$ pg, Doležel et al. 1992) and *Zea mays* 'CE-777' ($2C = 5.43$ pg, Lysák and Doležel 1998) were used as internal reference standards, depending on the specimen. Nuclear suspension was then filtered through a 45-µm nylon mesh into a 5-mL cytometry tube. Subsequently, 1 mL of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$; Otto 1990), RNAse (50 µg/mL), and propidium iodide (50 µg/mL) were added to stain the DNA and to avoid staining of double stranded RNA. Samples were kept on ice and analyzed within a 10-min period in a FACSVantage flow cytometer (Becton–Dickinson, San José, California) using an Innova 300 laser at 488 nm and CELLQuest software (Becton–Dickinson, San José, California). Chicken red blood cells were used to calibrate the flow cytometer to optimize forward and side scatter and fluorescence parameters. Three DNA estimations were carried out for each plant (5,000–10,000 nuclei per analysis) in three different days to avoid errors due to instrumental drift. The 2*C*-value (amount of nuclear DNA contained within a diploid somatic cell of a eukaryotic organism) was calculated as: (sample peak mean/standard peak mean) × 2*C* DNA content of the standard (in pg). For comparison purposes, data of DNA content in the genus *Solanum* were taken from the Plant DNA *C*-values database (Bennett and Leitch 2010).

Results

Karyotypes and CMA/DAPI banding

The chromosome traits analyzed are summarized in Table 2. The chromosome banding showed three different heterochromatin types: (1) all species presented a strong pair of CMA signals, corresponding to GC-rich heterochromatin regions, associated with the secondary constrictions in terminal position (Fig. 1a–i); (2) in four species (*Solanum mammosum*, *S. platense*, *S. palinacanthum*, and *S. aculeatissimum*, Fig. 1e–h, respectively), additional $CMA^+/DAPI^-$ heterochromatin blocks not associated with NOR were located in subterminal or interstitial regions. In *S. platense*, most of $CMA^+/DAPI^-$ blocks occupied terminal positions (Fig. 1f); (3) *S. palinacanthum* is the only species that has conspicuous $DAPI^+/CMA^0$ bands in interstitial position (Fig. 1g).

The total amount of the CMA^+ heterochromatin ranged from 1.86 to 22.42 % of the total karyotype length. Number and positions of bands and percentage of heterochromatin for each species are summarized in Table 2.

Chromosomes bearing one secondary constriction are obvious in all species and are strongly marked with FISH using 18-5.8-26S rDNA probes, and all species showed two terminal sites (Fig. 2; Table 2), which coincide with a $CMA^+/DAPI^-$ block. The morphology of NOR-bearing chromosomes and the localization of the 18-5.8-26S rDNA loci was variable: the signal was located either in a metacentric or a submetacentric chromosome, and the size of this chromosome also varied among species. One species, *Solanum palinacanthum*, showed a heteromorphic satellite pair, with one of the 18-5.8-26S blocks smaller than the other (Fig. 3).

Fluorescent in situ hybridization (FISH)

The hybridization signals obtained with the 5S rDNA probe were two for each species, except for *Solanum atropurpureum* (Fig. 2e) and *S. acerifolium* (Fig. 2a), which presented two pairs of signals. The position of these signals was subterminal and/or interstitial (terminal only in *S. platense*), and placed either in the short or in the long arm, in a metacentric or submetacentric chromosome (Figs. 2, 3). In all cases, the 5S sites are non-syntenic with respect to the 18-5.8-26S.

After the FISH procedure, $DAPI^+$ bands were visualized in *S. palinacanthumm* (Fig. 2c) in coincidence with the $DAPI^+/CMA^0$ bands earlier evidenced with the CMA/DAPI technique.

Flow cytometry

The 2*C*-values obtained are within the range known for *Solanum* species. The lowest value correspond to *S. acerifolium* (2.18 pg) while *S. mammosum* showed the highest (5.35 pg). Results are summarized in Table 2 and displayed schematically in Fig. 3.

Table 2 Cytogenetic features in *Solanum* sect. *Acanthophora* species: chromosome numbers, number of FISH signals and heterochromatic bands, percentage of heterochromatin and DNA content (Mean 2*C*-values)

Species	2*n*	Pairs of FISH signals		Pairs of $CMA^+/DAPI^-$ bands			Pairs of $DAPI^+/CMA^0$ bands (Int)	Total of bands pairs	Total of chromosome pairs with bands	TL (μm)	% Heterochromatin	Mean 2*C*-values (pg)
		18-5.8-26S	5S	SC	Int	T						
S. acerifolium	24	1	1 Int + 1P	1				1	1	23.33	2.96	2.18
S. aculeatissimum	24	1	1 Int	1	3			4	4	22.28	1.86	2.68
S. atropurpureum	24	1	2 Int	1				1	1	19.12	2.92	2.25*
S. capsicoides	24	1	1T	1				1	1	23.72	2.55	2.38
S. mammosum	22	1	1 Int	1	13	1		15	11	28.04	20.45	5.35*
S. palinacanthum	24	1	1 Int	1	14	4	4	19	12	29.72	20.79	5.31
S. platense	22	1	1T	1	1	13		14	11	19.44	22.42	3.61
S. tenuispinum	24	1	1 Int	1				1	1	20.81	2.08	2.125
S. viarum	24	1	1 Int	1				1	1	29.20	3.17	2.65*

SC secondary constriction, *Int* intercalary band, *P* pericentromeric band, *T* terminal band, *TL* total haploid genome length, taken from literature (see text)

* Data obtained from http://data.kew.org/cvalues

Discussion

Karyotypes and CMA/DAPI banding:

Chromosome number

Our data confirm previous chromosome number reports (Acosta et al. 2005). Although the number $2n = 24$ is very conservative in *Solanum*, the *Acanthophora* clade is peculiar because it contains two species with $2n = 22$ (Chiarini and Bernardello 2006). To elucidate the emergence of such number, the more parsimonious explanation that fits with the phylogenetic tree of Levin et al. (2005), should be that the reduction from $2n = 24$ to $2n = 22$ took place independently twice: once in the split of *S. mammosum* from *S. palinacanthum*, and another time during the separation of *S. platense* from the ancestor of all the remaining species (Fig. 3). Considering that the closest relative with $2n = 24$ is *S. palinacanthum*, which has subtelocentric chromosomes, an alternative hypothesis could be that a Robertsonian fusion of two acrocentrics took place in an ancestor with a karyotype similar to that of *S. palinacanthum*, resulting in the $2n = 22$ of *S. mammosum* and *S. platense*. Banding techniques seem to reinforce the idea of a separate origin for *S. platense* and *S. mammosum* because the banding pattern between the two species is very different.

Heterochromatin patterns

Among angiosperms, there is a notable variability in heterochromatin distribution patterns, but extreme or discontinuous changes within a related group of species are rare (Guerra 2000). In most genera in which at least five species were studied (e.g., *Vigna*, Galasso et al. 1996; *Citrus*, Miranda et al. 1997; *Cestrum*, Fregonezi et al. 2006), the number of bands and the heterochromatin amount varied, but the general patterns were relatively constant. This is not the case of *Acanthophora* species, where a remarkable differentiation exists among the species placed at the base of the phylogenetic tree (*S. palinacanthum*, *S. mammosum*, *S. platense*) and the remaining species.

The occurrence of GC-rich heterochromatin sequences adjacent to or interspersed with NORs is extensively described for many plant species (Guerra 2000). In *Acanthophora*, not all CMA^+ bands are rDNA-associated, which implies that GC-rich sequences are independent of rDNA genes (Jo et al. 2009).

4′-6-Diamidino-2-phenylindole applied after denaturation/renaturation of DNA detects heterochromatin with predominant AT-content and can reveal specific bands, as shown by Bogunić et al. (2011) in species of *Pinus*. Furthermore, studying three species of different families,

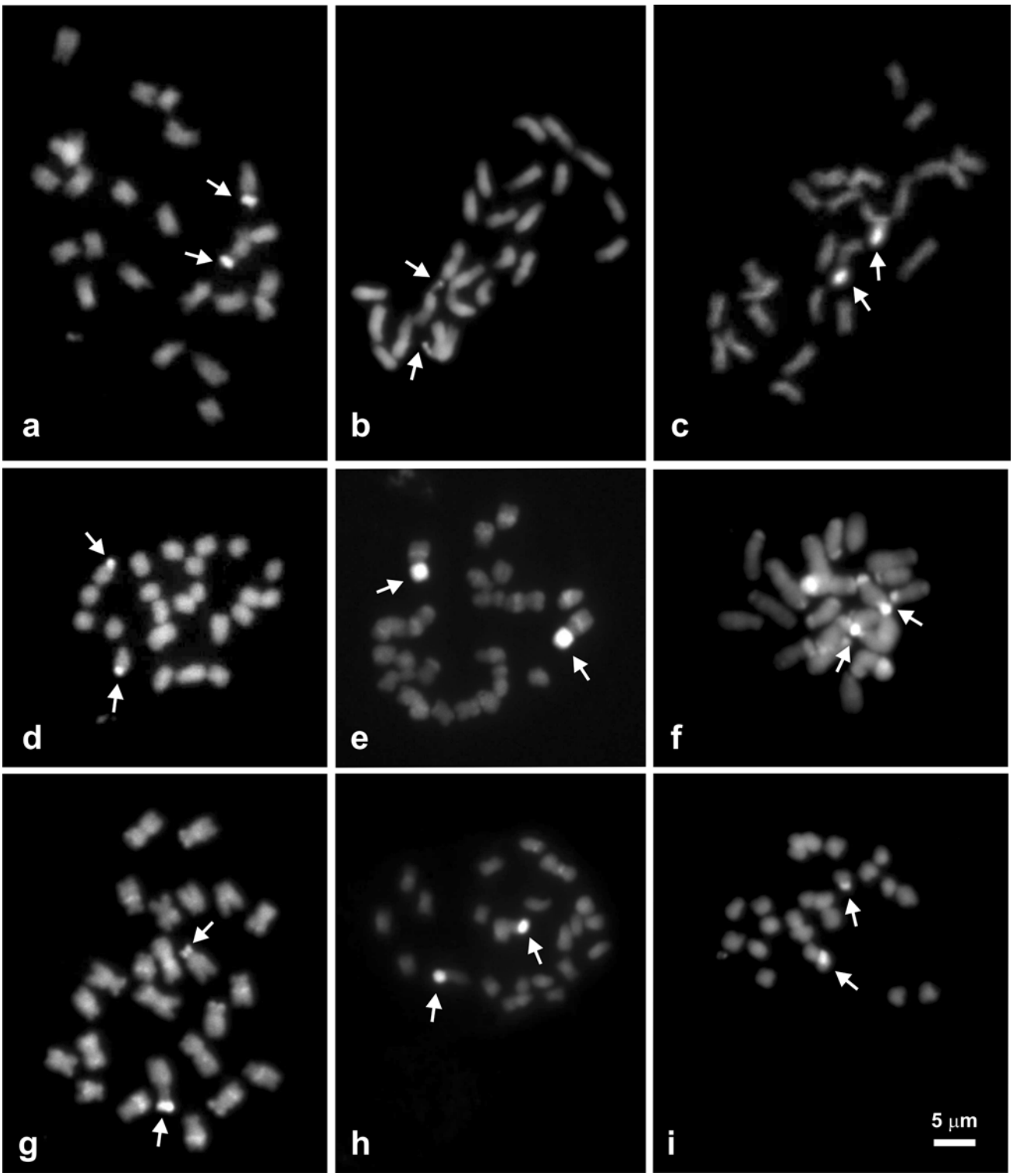

Fig. 1 Fluorochrome chromosome banding (CMA fluorescence) in *Acanthophora* species. **a** *S. viarum*. **b** *S. tenuispinum*. **c** *S. capsicoides*. **d** *S. atropurpureum*. **e** *S. mammosum*. **f** *S. platense*. **g** *S. palinacanthum*. **h** *S. aculeatissimum*. **i** *S. acerifolium*. *Arrows* indicate $CMA^+/DAPI^-$ NOR-associated heterochromatin

Barros e Silva and Guerra (2010) found that the number of $DAPI^+$ bands after FISH can be either the same as that detected with the CMA technique or a higher number. In the species studied here, after-FISH $DAPI^+$ bands seem to coincide very well with $DAPI^+$ bands visualized with the CMA/DAPI procedure. Such banding pattern is exclusive

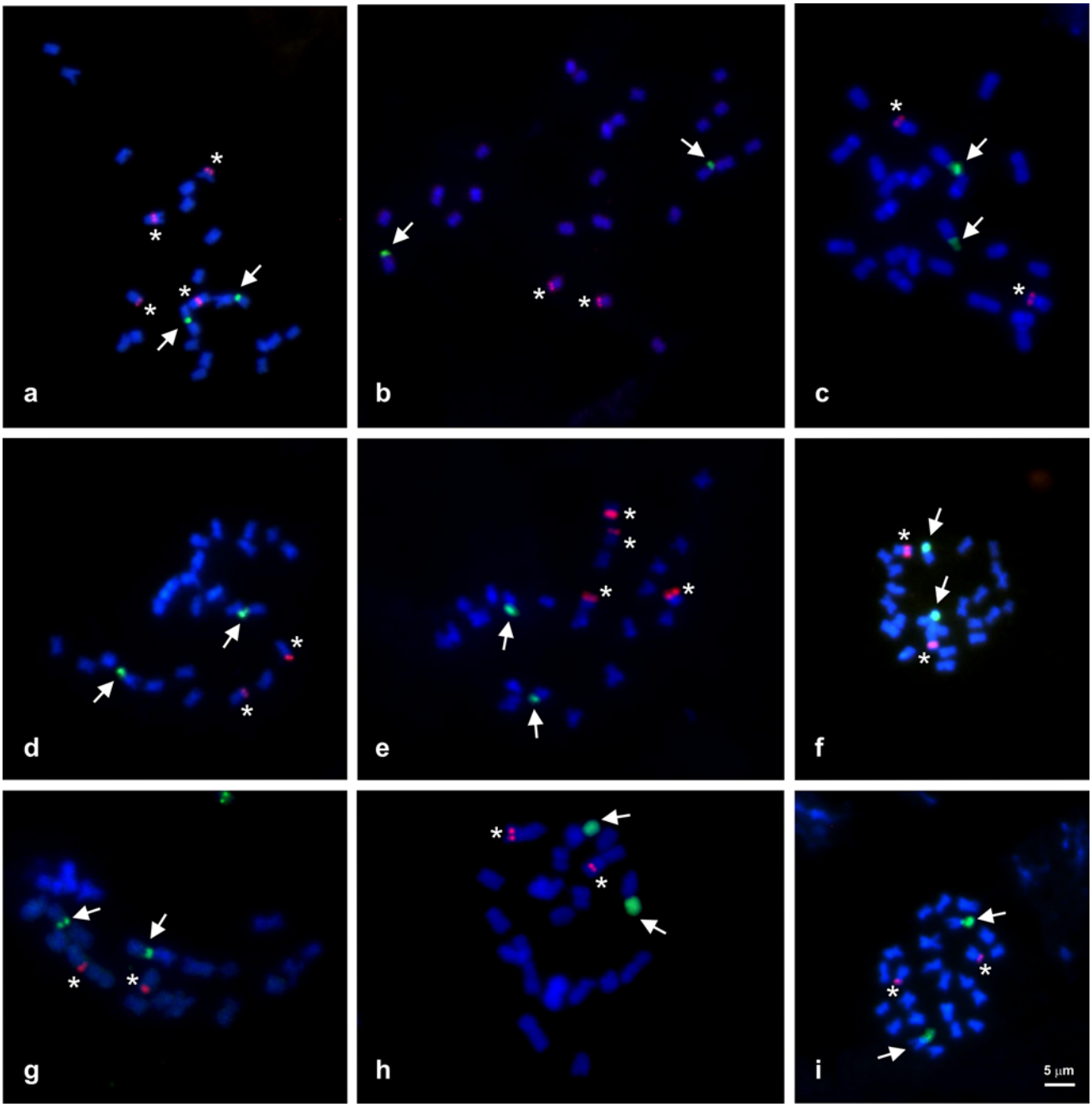

Fig. 2 Fluorescence in situ hybridization with 5S and 18-5.8-26S rDNA probes, in *Acanthophora* species. *Arrows* point at the chromosomes with the 18-5.8-26S signal, and *asterisks* indicate the chromosomes with a 5S signal. **a** *S. acerifolium*. **b** *S. viarum*. **c** *S. palinacanthu*m. **d** *S. capsicoides*. **e** *S. atropurpureum*. **f** *S. aculeatissimum*. **g** *S. platense*. **h** *S. mammosum*. **i** *S. tenuispinum*. Note the after-FISH DAPI bands in *S. palinacanthum*

of the large, asymmetrical karyotype of the basal *S. palinacanthum*.

The physical positioning of heterochromatic bands appeared in *S. mammosum* and *S. palinacanthum* in interstitial regions, corresponding to an equilocal pattern distribution. This situation agrees with the heterochromatin dispersion model proposed by Schweizer and Loidl (1987), which describes the transfer of heterochromatin between non-homologous members of a chromosome set at equilocal positions and between chromosome arms of similar lengths, as favored by chromosome proximity in the polarized mitotic interphase nucleus. This hypothesis also

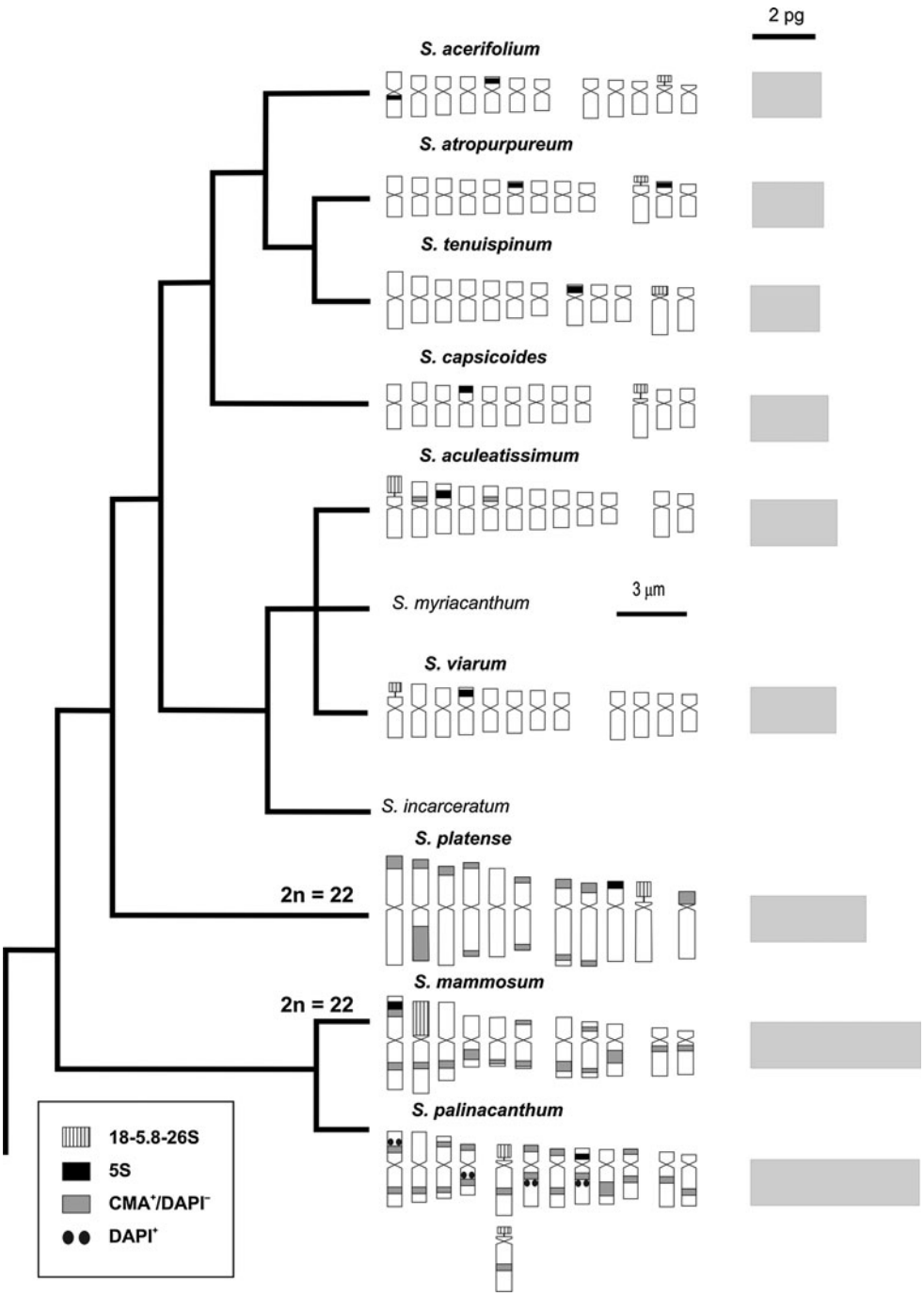

Fig. 3 Idiograms of *Acanthophora* species based on mean chromosome values, placed onto the phylogenetic tree of Levin et al. (2005), all at the same scale. Chromosomes are ordered from *longest* to *shortest* within each category, from *m* to *st*. *Gray blocks* indicate $CMA^{+}/DAPI^{-}$ bands, *circles* indicate positive pyknosis by DAPI staining after FISH, *black blocks* are 5S signals, and *hatched blocks* are 18-5.8-26S signals plus $CMA^{+}/DAPI^{-}$ band in the same position. *Gray bars* at the *right* represent the corresponding 2*C*-values in pg for each species. In *S. palinacanthum* both chromosomes of the heteromorphic pair are showed

fits in the case of *S. platense*, in which most heterochromatic bands occupy terminal positions.

Relationship among heterochromatin and others chromosome variables

A correlation between karyotype length (and consequently, chromosome size) and number or length of heterochromatic bands has been already noticed in some families (e.g., Calyceraceae and Dipsacaceae; Benko-Iseppon and Morawetz 2000), in some genera of Solanaceae (*Capsicum*, Moscone et al. 1996) and even within *Solanum* (Pringle and Murray 1993, sub nom. *Cyphomandra*), while examples of the contrary are few (Las Peñas et al. 2008). Apparently, heterochromatin could be an extra component of the genome because the species with large chromosomes have more heterochromatic bands than the ones with small chromosomes (Pringle and Murray 1993; Moscone et al. 1996). We corroborated this trend also in the *Acanthophora* species studied.

In the species here considered, those with the more asymmetric karyotypes present the highest number of heterochromatic bands, but no trends have been clearly described in this sense. There are examples of a positive correlation between heterochromatin amount and chromosome asymmetry (Las Peñas et al. 2008) and examples of the opposite situation (Stebbins 1971).

Fluorescent in situ hybridization (FISH):

The FISH experiments allowed us to study the evolution of the rDNA within *Acanthophora*. Considering the immensity of the genus *Solanum*, the 18-5.8-26S sites have been studied just in a few species (McGrath and Helgeson 1998; Li et al. 2006). The most remarkable contributions are those of Rego et al. (2009) and Melo et al. (2011), which studied a total of 11 diploid species of the Leptostemonum clade. They obtained the number of two signals per complement in all cases, always associated with satellites. Our results corroborated this trend. Thus, the location of 18-5.8-26S ribosomal genes follows the usual pattern found in other Solanaceae and in most plant species, always occurring in the terminal chromosome regions (Lim et al. 2000; Fregonezi et al. 2006; Kwon and Kim 2009). Outside of the Leptostemonum clade, the number of 18-5.8-26S sites seems to be variable. For example, more than one pair of sites occur in *S. corymbiflorum* from the Cyphomadropsis clade, and in other genera from the "$x = 12$ clade", such as *Vassobia breviflora* (Rego et al. 2009).

There are few data of FISH experiments with 5S rDNA probes in *Solanum*. Rego et al. (2009) provide the most important contribution, studying eight species of *Leptostemonum*. Those data, in addition to ours, support the observation that signals in the short arm of the smallest chromosomes of the complement are the commonest situation. Exceptions are *S. scuticum* and *S. acerifolium*, where signals in the large arm were found. *Solanum acerifolium* and *S. atropurpureum* are rare in having two pairs of 5S signals. Rego et al. (2009) found just one pair of signals in a sample of *S. atropurpureum*, which could be attributed to intraspecific variation. More than two 5S signals are also found in *Solanum trachytrichium* (from the Geminata clade) and in *S. gemellum* (Brevantherum clade) (Rego et al. 2009). Paracentromeric 5S rDNA sites have been detected in *S. lycopersicum* (Lapitan et al. 1991) and in the longest chromosome of the set in potato (Dong et al. 2000). These data suggest that the 5S rDNA could be a marker of species and/or species groups because its location and number of sites among species of *Solanum* is not conserved. A similar situation was observed in other Solanaceae genera, such as as *Nicotiana* (Lim et al. 2000; Kitamura et al. 2001) and *Cestrum* (Fregonezi et al. 2006). Variation in number and/or location of 5S sites seems to be common to other plant groups (Frello and Heslop-Harrison 2000; Urdampilleta et al. 2006; Datson and Murray 2006), suggesting their mobility. Chromosome rearrangements, transposition events, and unequal crossing-over have been proposed to account for variations in rDNA site number. Minor sites could have been added/deleted through non-homologous unequal crossing-over (Krishnan et al. 2001). It was also suggested that telomeric or subtelomeric positions of rDNA loci would possibly allow significant chromosome rearrangements/duplications to occur without deleterious effects to the cells.

The phylogenetic implications of variation in number and location of rDNA loci in *Acanthophora* species can be examined by mapping the number and relative positions of rDNA loci on the phylogenetic tree of these species obtained from GBSSI, ITS, *trnS–trnG*, and *trnT–trnF* (Fig. 3). The similarity in the positions of rDNA arrays reinforces the close phylogenetic relationships between *S. acerifolium*, *S. atropurpureum*, *S. tenuispinum*, and *S. capsicoides*, which share a 18-5.8-26S signal in a large *sm* or *st* chromosome and a terminal 5S signal in a *m* or *sm* chromosome; *S. viarum* and *S. aculeatissimum* share the 18-5.8-26S signal in the largest *m* pair and one intercalar 5S signal in a *m* pair; in *S. mammosum*, an intercalar 5S signal is located at a *m* pair, while in the allied *S. platense* and *S. palinacanthum* the signal is in a *sm* pair: these differences can be interpreted as an evidence for the chromosome rearrangement which yielded the $2n = 22$ from $2n = 24$.

Two more equally parsimonious hypotheses might explain the emergence of a second 5S rDNA loci within the clade formed by *S. acerifolium*, *S. atropurpureum*, and *S. tenuispinum*: either a duplication of the site in the ancestor of *S. tenuispinum*, *S. acerifolium*, and *S. atropurpureum*, with a later loss in the branch that originates *S. tenuispinum*, or two independent duplications, one in the branch of *S. acerifolium* and another in the branch of *S. atropurpureum.* The first hypothesis implies a later inversion or translocation, because in *S. acerifolium* one of the 5S sites is placed near the centromere in a large *m* chromosome, while in *S. atropurpureum* the two 5S sites are almost telomeric.

Flow cytometry:

Genome size

Regarding the DNA content of weeds, it is worth mentioning the study of Bennett et al. (1998). The authors compared 116 weeds to 2,473 non-weeds, finding a range of mean DNA amount per genome between 0.35 and 19.10 pg (with an average of 3.79 pg) for the first group. According to these authors, the weedy behavior is not a direct

consequence of the low DNA content, but is a result of other phenotypic and phenological characters (e.g., minimum generation time or rapid production of small seeds), closely correlated with the DNA amount. In the same vein, Kubešová et al. (2010) proposed that small genome size confer on alien plants an advantage at the stage of naturalization and need not be necessarily associated with the subsequent invasion. Our values for species in sect. *Acanthophora* are all within the weed category of Bennett et al. (1998). However, two species (*S. viarum* and *S. palinacanthum*) are recognized as aggressive weeds (Nee 1991; Auler Mentz and Oliveira 2004), yet they have the largest genomes. Thus, the general trend for angiosperm weeds is not reflected among species of *Acanthophora*.

There are several examples of the relationship between DNA content and chromosome size (Nagl and Ehrendorfer 1974; Dimitrova and Greilhuber 2000; Levin 2002; Garnatje et al. 2004). In the species here studied, although falling within the known range of *Solanum* subgenus. *Leptostemonum* (1.95–6.25 pg; Bennett and Leitch 2010), the 2*C*-values vary more than double. This is consistent with the available karyotype information: chromosomes range from symmetric and short (1.57 µm in *S. viaru*m) to asymmetric and large (2.93 µm in *S. platense*) (Acosta et al. 2005; Chiarini and Bernardello 2006). In fact, the two species with the highest *C*-values (*S. palinacanthum* and *S. mammosum*) have the larger chromosomes (and the highest values of *C* and TL, Table 2), while species with smaller chromosomes (*S. tenuispinum*, *S. acerifolium*, *S. atropurpureum*) showed the lowest *C*-values (Table 2). There is a discrepancy regarding the *S. viarum* data, which can be attributed to population differences and the use of different techniques. However, a clear separation in two groups can be made according to DNA content and chromosome size.

The relationship between genome size and heterochromatin is well established (e.g., Levin 2002). Tandem repeats (including satellite DNA, telomeric sequences and the genes coding for the 5S and 18-5.8-26S ribosomal RNA), organized as heterochromatin, are the main causes for the increasing in genome size. In sect. *Acanthophora*, we corroborate the relationship, although we cannot know the nature of the sequences involved in the heterochromatin increase. Although these sequences do not seem to be associated with NORs, in the Solanaceae, satellite DNA sequences can be originated from rDNA sequences (Hemleben et al. 2007). Curiously, the 5S genes are repeated in *S. acerifolium* and *S. atropurpureum*, both species with short chromosomes, little heterochromatin, and low DNA content.

Nucleotypic effects of DNA content

Among closely related plant species, DNA content tends to increase as species evolve from longer lived annuals to facultative or obligate perennials (Bennett 1972). In some genera, DNA content is related to life form, with annuals having lower DNA amounts than perennials (e.g., Albach and Greilhuber 2004; Price et al. 2005). Among the species of sect. *Acanthophora* studied, the highest *C*-value corresponds to *S. palinacanthum*, a perennial with clonal reproduction, while the lowest values are found among annual species (e.g., *S. viarum*, *S. atropurpureum*, *S. acerifolium*). However, additional measurements are needed to confirm a relationship of DNA content and life form in the *Acanthophora* as observed in the genera of other plant groups.

Understanding the relationships between nuclear DNA content and environmental conditions is complex, because several studies have produced conflicting results (e.g., Sims and Price 1985; Palomino and Sousa 2000). Genome size variations have been associated with annual precipitation (Wakamiya et al. 1993; Knight and Ackerly 2002) geography, habit, and altitude (e.g., Bureš et al. 2004; Šmarda and Bureš 2006). In the case of the species studied here, no clear environmental influence can be elucidated because species with different DNA content are sympatric (for instance, *S. atropurpureum*, *S. platense*, *S. viarum*, and *S. palinacanthum*).

Species of the same genus may vary enormously in their DNA content. Notable examples are found within *Crepis* (Jones and Brown 1976), *Bulnesia* (Poggio and Hunziker 1986), and *Allium* (Ohri 1998). In the Solanaceae, the highest variation is found in *Solanum*: from *S. chacoënse* to *S. sibundoyense* there is a ca. 24-fold increase (Bennett 1976; Pringle and Murray 1991). These two species belong to distantly related clades (Bohs 2005), but the differences found within a single clade, which is the case of *Acanthophora*, are also considerable: the 2*C*-value of *S. mammosum* is more than double the value of *S. tenuispinum*. Considering that the species with the lowest DNA content are more recently diverged, the tendency in *Acanthophora* would be to a reduce genome size. Unfortunately, there is little knowledge of the direction of genome size evolution during the speciation process (Levin 2002). There are examples of reduction and increase of genome size within a single monophyletic group, for instance, genus *Brachyscome* of the Asteraceae (Watanabe et al. 1999).

Conclusions

In this study, analyzing both rDNA as heterochromatin patterns, 2–6 of the 12 basic chromosome pairs were identified, depending on the species considered. All species can be differentiated by their heterochromatin and rDNA distribution patterns. For example, the closely related

S. tenuispinum, *S. acerifolium*, and *S. atropurpureum* can be distinguished by the chromosome markers used in this study, especially by the number and position of the 5S.

Morphological traits, such as fruit seem to respond quickly to selection constraints on the dispersal syndromes. For instance, *S. capsicoides* and *S. viarum*, although phylogenetically closely related, differ notably in fruit traits (Chiarini and Barboza 2009). Likewise at the cytogenetic level, these two species have undergone extensive chromosome rearrangements. The cytogenetic markers here employed have proved to be useful tools in detecting such rearrangements.

In short, there are two species groups within *Solanum* sect. *Acanthophora*: one group containing small, symmetric chromosomes, with less heterochromatin and a lower DNA content, and a second group, with larger and more asymmetric chromosomes, more heterochromatin CMA^+/$DAPI^-$ (associated with NOR or not) and a higher DNA content. Thus, elevated karyotype asymmetry seems to be associated with a higher amount of heterochromatin and a higher DNA content. The trend within *Acanthophora* appears to be a loss of heterochromatin, a reduction of chromosome size, and an increase in symmetry. The species with the shortest and more symmetric chromosomes are those placed at the top of the tree, while the species that occupy a basal position present the largest and asymmetric complements.

In the second group, we found some evidence of chromosomal rearrangements, but in the first, both rDNA loss and transposition could have occurred during genome evolution without major structural chromosome modifications. Genomic differentiation would be more likely due to genetic divergence than to large structural genomic divergence, associated with an increase/decrease of repeated sequences with possibly transposable elements and rDNA gene families. Further studies (such as genome mapping of repetitive DNA sequences) could support our conclusions.

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References

Acosta MC, Bernardello G, Guerra M, Moscone EA (2005) Karyotype analysis in several South American species of *Solanum* and *Lycianthes rantonnei* (Solanaceae). Taxon 54:713–723

Albach DC, Greilhuber J (2004) Genome size variation and evolution in *Veronica*. Ann Bot 94:897–911

Auler Mentz L, Oliveira PL (2004) *Solanum* (Solanaceae) na região sul do Brasil. Pesqui Bot 54:1–327

Barros e Silva AE, Guerra M (2010) The meaning of DAPI bands observed after C-banding and FISH procedures. Biotech Histochem 85(2):115–125

Benko-Iseppon AM, Morawetz W (2000) Cytological comparison of Calyceraceae and Dipsacaceae with special reference to their taxonomic relationships. Cytologia 65:123–128

Bennett MD (1972) Nuclear DNA content and minimum generation time. Proc R Soc Lond Biol Sci 181:109–135

Bennett MD (1976) DNA amount, latitude, and crop plant distribution. Environ Exp Bot 16:93–98

Bennett MD, Leitch IJ (2005) Plant genome size research: a field in focus. Ann Bot 95:1–6

Bennett MD, Leitch IJ (2010) Plant DNA C–values Database (release 5.0, December 2010). Available online: http://data.kew.org/cvalues/. Accessed 15 August 2011

Bennett MD, Smith JB (1991) Nuclear DNA amounts in angiosperms. Philos Trans R Soc Lond B Biol Sci 334:309–345

Bennett MD, Leitch IJ, Hanson L (1998) DNA amounts in two samples of Angiosperm weeds. Ann Bot 82(Suppl A):121–134

Bernardello LM, Anderson GJ (1990) Karyotypic studies in *Solanum* section basarthrum (Solanaceae). Amer J Bot 77:420–431

Bogunić F, Siljak-Yakovlev S, Muratović E, Ballian D (2011) Different karyotype patterns among allopatric *Pinus nigra* (Pinaceae) populations revealed by molecular cytogenetics. Plant Biol (Stuttg) 13(1):194–200

Bohs L (2005) Major clades in *Solanum* based on ndhF sequence data. In: Hollowell V, Keating R, Lewis W, Croat T (eds) A Festschrift for William D'Arcy. Monogr Syst Bot Missouri Bot Gard 104. Missouri Botanical Garden Press, St. Louis, pp 27–50

Bryson CT, Byrd JD (1994) *Solanum viarum* (Solanaceae), new to Mississippi. Sida 16:382–385

Bureš P, Wang YF, Horová L, Suda J (2004) Genome size variation in central European species of *Cirsium* (Compositae) and their natural hybrids. Ann Bot 94:353–363

Chiarini FE, Auler Mentz L (2012) A new species of *Solanum* sect. Acanthophora (Solanaceae) from Argentina and Brazil. Phytokeys 18:1–10. doi:10.3897/phytokeys.18.3903

Chiarini FE, Barboza GE (2009) Fruit anatomy of species of *Solanum* sect. Acanthophora (Solanaceae). Flora 204:146–156

Chiarini FE, Bernardello G (2006) Karyotypic studies in South American species of *Solanum* subgenus. Leptostemonum (Solanaceae). Plant Biol (Stuttg) 8:486–493

Datson P, Murray B (2006) Ribosomal DNA locus evolution in *Nemesia*: transposition rather than structural rearrangement as the key mechanism? Chromosom Res 14:845–857

Dimitrova D, Greilhuber J (2000) Karyotype and DNA-content evolution in ten species of *Crepis* (Asteraceae) distributed in Bulgaria. Bot J Linn Soc 132:281–297

Doležel J, Sgorbati S, Lucretti S (1992) Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. Physiol Plant 85:625–631

Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc 2:2233–2244

Dong F, Song J, Naess SK, Helgeson JP, Gebhardt C, Jiang J (2000) Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. Theor Appl Genet 101:1001–1007

Fregonezi JN, Fernandes T, Domingues Torezan JM, Vieira O, Vanzela ALL (2006) Karyotype differentiation of four *Cestrum* species (Solanaceae) based on the physical mapping of repetitive DNA. Genet Mol Biol 29:97–104

Frello S, Heslop-Harrison JS (2000) Chromosomal Variation in *Crocus vernus* Hill (Iridaceae) investigated by in situ hybridization of rDNA and a tandemly repeated sequence. Ann Bot 86:317–322

Fuchs J, Stehl S, Brandes A, Schweizer D, Schubert I (1998) Molecular cytogenetic characterization of the *Vicia faba* genome–heterochromatin differentiation, replication patterns and sequence localization. Chromosom Res 6:219–230

Galasso I, Saponetti LS, Pignone D (1996) Cytotaxonomic studies in Vigna III. Chromosomal distribution and reacting properties of the heterochromatin in five wild species of the section Vigna. Caryologia 49:311–319

Garnatje T, Vallès J, García S, Hidalgo O, Sanz M, Canela MA, Siljak-Yakovlev S (2004) Genome size in *Echinops* L. and related genera (Asteraceae, Cardueae): karyological, ecological and phylogenetic implications. Biol Cell 96:117–124

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucl Acids Res 7:1869–1885

Guerra M (2000) Patterns of heterochromatin distribution in plant chromosome. Genet Mol Biol 23:1029–1041

Hemleben V, Kovarik A, Torres-Ruiz RA, Volkov RA, Beridze T (2007) Plant highly repeated satellite DNA: molecular evolution, distribution and use for identification of hybrids. Syst Biodivers 5:277–289

Jo SH, Koo DH, Kim JF, Hur CG, Lee S, Yang TJ, Kwon SY, Choi D (2009) Evolution of ribosomal DNA-derived satellite repeat in tomato genome. BMC Plant Biol 9:42

Jones RN, Brown LM (1976) Chromosome evolution and DNA variation in *Crepis*. Heredity 36:91–104

Kitamura S, Inoue M, Shikazono N, Tanaka A (2001) Relationships among *Nicotiana* species revealed by the 5S rDNA spacer sequence and fluorescence in situ hybridization. Theor Appl Genet 103:678–686

Knight CA, Ackerly DD (2002) Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. Ecol Lett 5:66–76

Krishnan P, Sapra VT, Soliman KM, Zipf A (2001) FISH mapping of the 5S and 18S–28rDNA loci in different species of Glycine. J Hered 92:282–287

Kubešová M, Moravcová L, Suda J, Jarošík V, Pyšek P (2010) Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. Preslia 82:81–96

Kwon JK, Kim BD (2009) Localization of 5S and 25S rRNA genes on somatic and meiotic chromosomes in *Capsicum* species of chili pepper. Mol Cells 27:205–209

Lapitan NLV, Ganal MW, Tanksley SD (1991) Organization of the 5S ribosomal RNA genes in the genome of tomato. Genome 34:509–514

Las Peñas ML, Bernardello G, Kiesling R (2008) Karyotypes and fluorescent chromosome banding in *Pyrrhocactus* (Cactaceae). Pl Syst Evol 272:211–222

Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press, New York

Levin RA, Watson K, Bohs L (2005) A four-gene study of evolutionary relationships in *Solanum* section Acanthophora. Am J Bot 92:603–612

Levin RA, Myers NR, Bohs L (2006) Phylogenetics relationships among the "Spiny Solanums" (*Solanum* subgenus Leptostemonum, Solanaceae). Am J Bot 93:157–169

Li HY, Chen Q, Beasley D, Lynch DR, Goettel M (2006) Karyotypic evolution and molecular cytogenetic analysis of *Solanum pinnatisectum*, a new source of resistance to late blight and Colorado potato beetle in potato. Cytologia 71:25–33

Lim KY, Matyasek R, Lichtenstein CP, Leitch AR (2000) Molecular cytogenetic analyses and phylogeny of the *Nicotiana* section Tomentosa. Chromosoma 109:245–258

Lysák MA, Doležel J (1998) Estimation of nuclear DNA content in *Sesleria* (Poaceae). Caryologia 52:123–132

McGrath JM, Helgeson JP (1998) Differential behavior of *Solanum brevidens* ribosomal DNA loci in a somatic hybrid and its progeny with potato. Genome 41:435–439

Melo NF, Guerra M (2003) Variability of the 5S and 45S rDNA sites in *Passiflora* L. species with distinct base chromosome numbers. Ann Bot 92:309–316

Melo CAF, Martins MIG, Oliveira MBM, Benko-Iseppon AM, Carvalho R (2011) Karyotype analysis for diploid and polyploid species of the *Solanum* L. Plant Syst Evol 293:227–235

Miranda M, Ikeda F, Endo T, Morigucki T, Omura M (1997) Comparative analysis on the distribution of heterochromatin in *Citrus*, *Poncirus* and *Fortunella* chromosomes. Chromosom Res 5:86–92

Moscone EA, Lambrou M, Ehrendorfer F (1996) Fluorescent chromosome banding in the cultivated species of *Capsicum* (Solanaceae). Plant Syst Evol 202:37–63

Moscone EA, Scaldaferro MA, Grabiele M, Cecchini NM, Sanchez García Y, Daviña JR, Ducasse DA, Barboza GE, Ehrendorfer F (2006) The evolution of chili peppers (*Capsicum*-Solanaceae): a cytogenetic perspective. Acta Hort 745:137–170

Nagl W, Ehrendorfer F (1974) DNA content, heterochromatin, mitotic index, and growth in perennial and annual Anthemideae (Asteraceae). Plant Syst Evol 123:35–54

Nee M (1991) Synopsis of Solanum Section Acanthophora: A group of interest for glycoalkaloids. In: Hawkes JG, Lester R, Nee M, Estrada N (eds) Solanaceae III: Taxonomy, Chemistry, Evolution. Kew, Royal Botanic Gardens, pp 257–266

Ohri D (1998) Genome size variation and plant systematics. Ann Bot 82:75–83

Otto F (1990) DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. In: Crissman HA, Darzynkiewicz Z (eds) Methods in cell biology, vol 33. Academic Press, New York, pp 105–110

Palomino G, Sousa SM (2000) Variation of nuclear DNA content in the biflorus species of *Lonchocarpus* (Leguminosae). Ann Bot 85:69–76

Poggio L, Hunziker JH (1986) Nuclear DNA content variation in *Bulnesia*. J Hered 77:43–48

Price HJ, Dillon SL, Hodnett G, Rooney WL, Ross L, Johnston SJ (2005) Genome evolution in the genus Sorghum (Poaceae). Ann Bot 95:219–227

Pringle GJ, Murray BG (1991) Karyotype diversity and nuclear DNA variation in Cyphomandra. In: Hawkes G, Lester RN, Nee M, Estrada N (eds) Solanaceae III: taxonomy, chemistry, evolution. Kew, Royal Botanic Gardens, pp 247–252

Pringle GJ, Murray BG (1993) Karyotypes and C banding patterns in species of *Cyphomandra* Mart. ex Sendtn. (Solanaceae). Bot J Linn Soc 111:331–342

Rego LNAA, da Silva CRM, Torezan JMD, Gaeta ML, Vanzela ALL (2009) Cytotaxonomical study in Brazilian species of *Solanum*, *Lycianthes* and *Vassobia* (Solanaceae). Plant Syst Evol 279:93–102

Schwarzacher T, Heslop-Harrison P (2000) Practical in situ hybridization. Bios Scientific Publishers Limited, Oxford

Schweizer D (1976) Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma (Berlin) 58:307–324

Schweizer D, Ambros P (1994) Chromosome banding. In: Gosden JR (ed) Methods in molecular biology, chromosome analysis protocols. Humana Press, Totowa

Schweizer D, Loidl J (1987) A model for heterochromatin dispersion and the evolution of C-band patterns. Chromosom Today 9:61–74

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

Šmarda P, Bureš P (2006) Intraspecific DNA content variability in *Festuca pallens* on different geographical scales and ploidy levels. Ann Bot 98:665–678

Srebniak M, Rasmussen O, Małuszyńska J (2002) Cytogenetic analysis of an asymmetric potato hybrid. J Appl Genet 43:19–31

Stebbins GL (1971) Chromosomal evolution in higher plants. E. Arnold, London

Tate J, Acosta MC, McDill J, Moscone EA, Simpson BB, Cocucci AA (2009) Phylogeny and character evolution in *Nierembergia* (Solanaceae): molecular, morphological, and cytogenetic evidence. Syst Bot 34:198–206

Urdampilleta JD, Ferrucci MS, Torezan JM, Vanzela ALL (2006) Karyotype relationships among four South American species of *Urvillea* (Sapindaceae: paullinieae). Plant Syst Evol 258:85–95

Wakamiya I, Newton RJ, Johnston SJ, Price JH (1993) Genome size and environmental factors in the genus *Pinus*. Am J Bot 80:1235–1241

Watanabe K, Yahara T, Denda T, Kosuge K (1999) Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae); statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. J Plant Res 112:145–161

Weese T, Bohs L (2007) A three-gene phylogeny of the genus Solanum (Solanaceae). Syst Bot 32:445–463

Welman WG (2003) The genus *Solanum* (Solanaceae) in Southern Africa: subgenus Leptostemonum, the introduced sections Acanthophora and Torva. Bothalia 33:1–8

Zonneveld BJM (2001) Nuclear DNA contents of all species of *Helleborus* (Ranunculaceae) discriminate between species and sectional divisions. Plant Syst Evol 229:125–130