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Research Article

Data reassessment in a phylogenetic context gives insight into chromosome evolution in the giant genus *Solanum* (Solanaceae)

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Chromosome data are fundamental in evolution. However, there has been no attempt to synthesize and evaluate the significance of such information from a phylogenetic perspective in the giant genus *Solanum*, which was the aim of this work. New and published information of the main cytotaxonomic features (chromosome number, polyploidy, total length of the haploid complement, mean chromosome length, mean arm ratio, karyotype formula, nuclear DNA amount, number/position of rDNA sites) was compiled and mapped onto an embracing Solanaceae phylogeny, performing Ancestral States Reconstruction. There were 506 *Solanum* species with chromosome counts (49.7% from an estimated total of 1,018 spp.), with x = 12 being the most frequent number (97%). Species with karyotypes represent 18.8%, while 8% have been studied with any molecular cytogenetic technique. Chromosome characters showed transitions associated with supported nodes, some of which have undergone fewer transitions than others. The common ancestor of all *Solanum* was a diploid with 2n = 24, a karyotype with st and/or t chromosomes, 2C DNA content of 1–1.2 pg, one locus of 18–5.8–26S rDNA and one of 5S, both loci being asyntenic. The chromosomal variables behave as homoplastic, with reversions in all branches. The analysed characters were sorted from more to less conserved: asynteny of rDNA loci; number of sites of 18–5.8–26S; chromosome number; karyotype formula; number of 5S loci. This pattern of chromosomal evolution distinguishes *Solanum* from closely related genera and from genera from other families with a similar number of species.

Key words: ancestral state reconstruction, chromosome evolution, chromosome number, DNA content, karyotype, rDNA loci, *Solanum*, synteny

Introduction

Solanaceae is an outstanding Angiosperm family, valued by botanists and by society. It includes major crops (e.g., potato: *Solanum tuberosum*; tomato: *S. lycopersicum*; peppers: *Capsicum* spp.), ornamentals, weeds, and several species used as biological models. Within Solanaceae, there is a major lineage termed the 'x = 12 clade', whose members share such cytological synapomorphy (Olmstead et al., 2008). According to Olmstead and Bohs (2006), ~2300 species belong to this clade, including the giant genus *Solanum* L. Such a number of species presumably sharing the same basic chromosome number suggests questions on evolutionary pathways at different levels, despite apparent chromosome uniformity.

Solanum is the largest and most complex genus in the family. Together with *Astragalus* (Fabaceae), *Euphorbia* (Euphorbiaceae), *Carex* (Cyperaceae), *Piper* (Piperaceae), and *Bulbophyllum* and *Epidendrum* (Orchidaceae), *Solanum* is among the 10 largest genera of Angiosperms (Stevens, 2001 onwards). It comprises herbs, shrubs, trees, and woody vines; its centre of diversification is in South America and its estimated age is 17 mya (Bohs, 2005; Särkinen, Bohs, Olmstead, & Knapp, 2013). The number of species varies from 1,000 to 1,500 according to the author (Bohs, 2005; Olmstead et al., 2008; Särkinen et al., 2013). Recent molecular data have clarified phylogenetic relationships at all taxonomic levels in Solanaceae (Olmstead et al., 2008; Olmstead, Sweere, Spangler, Bohs, & Palmer, 1999) and in *Solanum* (Bohs, 2005; Bohs & Olmstead, 1997, 1999, 2001; Olmstead & Palmer, 1997; Särkinen et al., 2013; Spooner, Anderson, & Jansen, 1993; Weese & Bohs, 2007). These studies recognized 13 major clades within *Solanum*. The Thelopodium clade is resolved as the first branching group. The remaining species are divided into two strongly supported clades: Clade

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I (with all non-spiny species, herbaceous without stellate hairs, woody climbers, or shrubs) and Clade II (Leptostemonum, shrubs or small trees, most with prickles and/or stellate hairs, with 13 major clades, Särkinen et al., 2013; Stern, Agra, & Bohs, 2011). Within Clade I, two clades are resolved: the Potato clade, with Regmandra clade as the first branching group, and Clade M, including Morelloid, Dulcamaroid, Archaesolanum, Normania, and the African non-spiny clades.

Angiosperm genomes are plastic in their ability to tolerate epigenetic, genetic, and karyotypic changes (Lim et al., 2007; Weiss-Schneeweiss & Schneeweiss, 2013). Chromosomes within a family, a genus or even a species may vary in number, size, morphology, and staining properties (e.g., Sharma & Sen, 2002). The knowledge of the structural and quantitative characteristics of the karyotype has proven to be important in evolutionary and taxonomic studies in several Angiosperm groups (e.g., Guerra, 2000, 2012; Stebbins, 1971, 1985). Chromosomes provide valuable information for inferring phylogenetic relationships, since they are hereditary elements of the whole nuclear genome and discrete hereditary units of mutation. Variation in chromosomal features used in cytotaxonomy may be continuous (e.g., total length of the chromosome set, asymmetry indices, nuclear DNA amount) or discontinuous (e.g., chromosome number, heterochromatic bands, number of rDNA sites). Chromosome number and morphology have been the most common karyotype characters examined (Guerra, 2008). Since the beginning of cytotaxonomy, chromosome number has been a common character employed. It is the most easily obtained information and the only one that is known for most plant groups, although data are still missing for most plant species. Karyotype features represent an important aspect in plant speciation, since chromosomal differences establish immediate post-zygotic crossing barriers (e.g., Rieseberg, 1997). Evolution of karyotypes is expected to be congruent with clade differentiation. Therefore, karyological data provide important characters for plant systematics and evolution (Stace, 2000), especially when combined with molecular phylogenies (e.g., Baltisberger & Horandl, 2016; Weiss-Schneeweiss, Tremetsberger, Schneeweiss, Parker, & Stuessy, 2008).

Different staining techniques can identify other levels of karyotype variation, allowing species discrimination (Moraes, dos Santos Soares Filho, & Guerra, 2007). A widely applied banding technique uses fluorescent dyes: chromomycin A3 (CMA), which labels CG-rich heterochromatin, and 4′,6-diamidino-2-phenylindole (DAPI), which labels AT-rich heterochromatin (Schweizer, 1976). Heterochromatin is the chromosome fraction that remains condensed throughout the cellular cycle, and is characterized by low gene density and high repetitive clusters of satellite DNA and transposable elements (Grewal & Jia, 2007). Different techniques may indicate different heterochromatin amounts for the same species, but only fluorescent banding is considered a specific technique.

In the same vein, fluorescent *in situ* hybridization (FISH) is a molecular technique effective in detecting chromosomal rearrangements involved in chromosome speciation (e.g., Melo & Guerra, 2003; Srebniak, Rasmussen, & Małuszyńska, 2002). Homologous chromosomes in a complement can be identified using FISH; thus, related species can be compared and evolutionary questions can be answered (e.g., Chacón, Sousa, Baeza, & Renner, 2012; Chiarini, Santiñaque, Urdampilleta, & Las Peñas, 2014). The most common markers are ribosomal DNA genes (5S and 18–5.8–26S rDNA), which are abundant and highly conserved in higher plants (Heslop-Harrison & Schwarzacher, 2011).

DNA content of organisms (C-value for unreplicated haploid nuclei) is another source of information (Bennett & Leitch, 2005). Comparative C-values have helped explain genome size evolution (Bennett & Leitch, 2005), which is correlated with some characteristics, such as life history, phenology, and minimum generation time (Ohri, 1998). In addition, nuclear DNA amounts are useful in the study of phylogenetic relationships between related groups (e.g., Ohri, 1998). The data on DNA content in plant species and populations have grown in the last 15 years and form one of the most widely used cytotaxonomic parameters (Bennett & Leitch, 2012).

Numerous scattered cytogenetic analyses dealing with *Solanum* species (particularly those of economic importance) have been performed using classical and molecular techniques (e.g., Dong et al., 2000; Tanskley et al., 1992). Karyotypes have been reported for several American species of *Solanum* and have proved to be useful in differentiating taxa and evolutionary trends in several clades, such as Basarthrum (Bernardello & Anderson, 1990), Lasiocarpa (Bernardello, Heiser, & Piazzano, 1994), Leptostemonum (Chiarini & Bernardello, 2006), and *Solanum* as a whole (e.g., Acosta, Bernardello, Guerra, & Moscone, 2005; Acosta, Guerra, & Moscone, 2012; Chiarini, Moreno, Barboza, & Bernardello, 2010; Melo, Martins, Oliveira, Benko-Iseppon, & Carvalho, 2011; Rego, da Silva, Torezan, Gaeta, & Vanzela, 2009). However, the karyology of less than half of *Solanum* species has been studied (Goldblatt & Johnson, 1979 onwards; Rice et al., 2015).

Despite the accessibility of this information, to date there has been no attempt to synthesize and evaluate the significance of available data from a phylogenetic perspective, which is the objective of this work. Several articles have analysed karyological data using phylogenetic trees in genera from several families (e.g., Baltisberger & Hörandl, 2016; Lim et al., 2007; Lim, Matyášek, Lichtenstein, & Leitch, 2000; Peruzzi, Leitch, & Caparelli, 2009; Watanabe, Yahara, Denda, & Kosuge, 1999). Wu, Pannetta, Xu, and Tanksley (2009), combined data from multiple sets of single-

copy conserved orthologous marker (COSII) studies, and other comparative mapping studies performed in tomato, potato, eggplant, pepper, and diploid *Nicotiana* species, and deduced the features and outcomes of chromosomal evolution in the family over the past 30 million years. These results provide a broad overview of chromosomal evolution in the Solanaceae, estimating the rates and timing of chromosomal changes.

We will focus on the published information of the main features used in cytotaxonomy: chromosome number, presence of polyploidy, total length of the haploid chromosome set, mean chromosome length, mean arm ratio, karyotype formula, nuclear DNA amount, and number, position, and chromosome type with 5S and 18-5.8-26S rDNA sites. In addition, new data on FISH from 24 species are included. This review will provide information and insights into the genome evolution of *Solanum*. In order to discuss the dynamics of these changes, data were mapped onto the most embracing phylogeny for the Solanaceae available (Särkinen et al., 2013), using Character mapping and Ancestral States Reconstruction (ASR).

Materials and methods

Revisited data

A perusal of cytogenetic literature published until August 2017 was conducted on the main databases available online: IPCN (Goldblatt & Johnson, 1979 onwards), US National Library of Medicine, National Institutes of Health Search database (PubMed ®, 2017, https://www.ncbi.nlm.nih.gov/pubmed), Chromosome counts Database (CCDB, http://ccdb.tau.ac.il/; Rice et al., 2015), Scopus©, https://www.scopus.com/ (2017), Web of Science©, http://ipscience.thomson reuters.com/ (Thomson Reuters, 2016). The keywords used for the search were: *Solanum*, chromosome, karyotype, rDNA, and heterochromatin.

Since chromosome counts and karyotypes came from a wide range of different sources, the available data were standardized as follows:

1) For counts, gametophytic numbers were transformed into sporophytic. Odd single numbers were considered incorrect counts (e.g., $2n = 6$) and were disregarded.
2) The following data were gathered: mean chromosome length (C), total haploid chromosome length (TL), mean arm ratio (r), 2C DNA content, and haploid karyotype formula. When needed, chromosome categories were modified to fit the widely used classification of Levan, Fredga, and Sandberg (1964). When articles did not report measurements, but did present photographs or camera lucida drawings with scales, measurements were calculated from them. Species that had more than one datum for a variable were averaged according to the requirements of the different analyses.
3) Articles using banding and FISH exhibit a wide disparity in the nomenclature of the different band types. Because of this, and given that the reduced size of *Solanum* chromosomes makes it difficult to establish the precise band location, band types were converted into two main categories: interstitial (including centromeric, pericentromeric, and proximal) and terminal (including subterminal, telomeric, and distal).

The validity of species names was checked using the Tropicos© (2017) database. The infrageneric classification system follows Bohs (2005) and Särkinen et al. (2013). To calculate the total number of species of the genus and of each infrageneric group, the number of species in the latest revision for each group was added: Thelopodium (Knapp, 2000), Archaesolanum (Symon, 1981), Normania (Bohs, 2005; Bohs & Olmstead, 2001), African non-spiny (Knapp & Vorontsova, 2016), Morelloid (Särkinen, Barboza, & Knapp, 2015), Dulcamaroid (Knapp, 2013), Nemorense (Child, 1983; Whalen, 1984), Cyphomandra (Bohs, 2007), Geminata (Knapp, 2002), Leptostemonum (Levin, Myers, & Bohs, 2006; Stern et al., 2011; Vorontsova & Knapp, 2016), Androceras (Stern, Weese, & Bohs, 2010; Whalen, 1979, 1984), Carolinense (Wahlert, Chiarini, & Bohs, 2015), Acanthophora (Nee, 1999), Erythrotrichum (Agra, 2008; Nee, 1999), Elaeagnifolium (Levin et al., 2006; Stern et al., 2011), Torva (Nee, 1999; Whalen, 1984), Micracantha (Nee, 1999; Stern et al., 2011; Stern & Bohs, 2016; Whalen, 1984), Asterophorum (Nee, 1999; Stern et al., 2011; Whalen, 1984), Lasiocarpa (Bohs, 2004), Bahamense (Stern et al., 2011; Strickland-Constable, Schneider, Ansell, Russell, & Knapp, 2010; Whalen, 1984), Sisymbriifolium (Stern et al., 2011), Thomasiifolium (Stern et al., 2011; Whalen, 1984), Gardnerii (Stern et al., 2011), Old World clade (Aubriot, Singh, & Knapp, 2016; Vorontsova, Stern, Bohs, & Knapp, 2013), Allophyllum/Wendlandii or Aculeigerum (Bohs, 2005; Clark, Nee, Bohs, & Knapp, 2015), Potato (Tepe, Anderson, Spooner, & Bohs, 2016), Pteroidea/Herpystichum (Knapp & Helgason, 1997; Tepe, Farruggia, & Bohs, 2011), Regmandra (Bennett, 2008), Articulatum (Tepe et al., 2016), Anarrhichomenum and Basarthrum (Correll, 1962; Tepe et al., 2016), Etuberosum (Contreras & Spooner, 1999), Juglandifolia, Lycopersicoides, and Lycopersicon (Peralta, Spooner, & Knapp, 2008), and Petota (Spooner, Clausen, Peralta, & Alvarez, 2016).

Character mapping and ancestral states reconstruction

The chromosomal features were mapped on extant species and then the history of chromosomal evolution was estimated on a maximum credibility tree of Solanaceae, using comparative methods. The tree was obtained by re-running the analyses with the matrix and the parameter settings provided by Särkinen et al. (2013). Species in the resulting tree were pruned according to their availability for chromosomal data. The following features were coded as discrete to infer character history: chromosome number; karyotype formula; number of 18S–5.8S–26S loci/nucleolar organizer regions (NORs); synteny (i.e., co-localization) of the 18S–5.8S–26S and 5S rDNA genes, and number and position of 5S loci (Appendix S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at https://doi.org/10.1080/14772000.2018.1431320). DNA content (2C value) and the arm ratio (r) were coded as continuous characters (Appendix S1, see supplemental material online). The character history was traced either under a model where all transition rates were different ('ARD' model) or equal ('ER' model) among chromosome characters. The models of character evolution were selected using *ace* function from *APE* packages in R software and compared using a Chi square test of Log likelihood value from each model evaluated (Paradis, Claude, & Strimmer, 2004). To estimate the history of discrete characters, a Bayesian stochastic character mapping approach was used (Huelsenbeck, Nielsen, & Bollback, 2003; Nielsen, 2001), with the *make.simmap* function from the phytools R package (Revell, 2012). One thousand ancestral-state histories on the maximum credibility tree of Solanaceae were simulated. Because data accessibility varied according to species, a stochastic character mapping was performed using different species number. In order to compare the degree of variation among chromosomal characters, the ratio between the numbers of changes estimated (C) and the numbers of nodes (N) in the trees were used. Thus, it was possible to compare the variation among chromosomal traits in *Solanum* and to estimate which is more conservative in the clade history.

In order to determine changes of continuous characters during the phylogenetic history, the extant species scores for arm ratio (r) and 2C DNA content were mapped and plotted onto the estimated phylogenetic tree, calculating ancestral character states through a Maximum likelihood (ML) based procedure, which assumes that characters evolve under a Brownian motion model. The mapping of r scores on the phylogenetic tree was carried out using *ContMap* function in the phytools (Revell, 2012) package for R version 3.3.2 (R Core Team, 2016).

New FISH data

Species studied and voucher specimens are included in Appendix S2 (see supplemental material online). Mitotic chromosomes were examined in root tips obtained from germinating seeds following the protocol explained in Chiarini (2014). The location and number of rDNA sites were determined by FISH using two probes: the p*Ta*71 containing the 18–5.8–26S gene of wheat (Gerlach & Bedbrook, 1979) and a 5S rDNA fragment obtained by PCR from *Solanum stuckertii* using the primers 5S rDNA-3 (5′-GTG CTT GGG CGA GAG TAG TA-3′) and 5SrDNA-4 (5′-GGT GCG TTA GTG CTG GTATG-3′) (Fulneček, Matyášek, Kovařík, & Bezděk, 1998). The FISH protocol followed Schwarzacher and Heslop-Harrison (2000), with minor modifications (Chiarini, 2014). At least 10 metaphases of each species and from at least three different individuals were photographed with a Zeiss Axiophot microscope equipped with epifluorescence and a digital image capture system. The free software ImageJ (http://rsbweb.nih.gov/ij/) was used for the merging of images.

Results

Chromosome counts

The total number of *Solanum* species with counts, both in meiosis and mitosis, was 506 species, i.e., 49.7% from an estimated total of 1,018 spp. (Table 1). The absolute number of counts amounted to 842, with 398 being in meiosis (47.25%) and 444 in mitosis (52.75%). Comparatively, there were scarce data available on the clades: African non-spiny (1 sp. out of 14), Dulcamaroid (15/45), Regmandra (2/11), Pteroidea/Herpystichum (1/20), Brevantherum (11/60), Thomasiifolium (1/11), and Gardneri (1/7); there were no data on Thelopodium and Asterophorum (Table 1).

The most frequent number was x = 12, recorded in 97% of the examined species as diploid (2n = 24, 77%), tetraploid (2n = 48, 14%), hexaploid (2n = 72, 4%), triploid (2n = 36, 2%), or octoploid (2n = 96, 0.2%); the latter is the highest chromosome number recorded for the genus. Other numbers recorded were 2n = 46 (1.5%), which was found exclusively in Archaesolanum, 2n = 22 (only in *S. mammosum* and *S. platense*), and 42, 60, and 64 (1%) in several species from different clades.

Polyploidy was common in the clades Potato (mainly in Petota), Morelloid, Leptostemonum (Old World, Carolinense, Elaeagnifolium), and Archaesolanum. Overall, tetraploidy, with 2n = 48, was the most common level. On the other hand, polyploidy was rare in the Brevantherum and Geminata clades.

Karyotypes

The percentage of species subjected to karyotypic studies was 18.8%. The total number of karyotypes was slightly

Table 1. Summary of known chromosome counts and karyotypes per species in *Solanum*.

Major clades	Sub-clades	Total of species	Species with counts	Species with karyotypes (percentage)
Thelopodium		3	–	–
Archaesolanum		9	7	1
Normania		3	1	–
African non-spiny		14	1	–
Morelloid		76	65	47 (61%)
Dulcamaroid		45	15	11 (24%)
Cyphomandra		50	20	20 (40%)
Geminata		140	51	12 (8,5%)
Brevantherum		60	11	6 (10%)
Nemorense		6	1	–
Leptostemonum				
	Acanthophora	20	14	14 (70%)
	Erythrotrichum	22	6	5 (22%)
	Androceras/Crinitum	20	17	4 (20%)
	Carolinense	11	7	4 (36%)
	Elaeagnifolium	4	4	2 (50%)
	Torva	45	17	13 (29%)
	Micracantha	11	1	–
	Asterophorum	2	–	–
	Lasiocarpa	12	12	9 (75%)
	Bahamense	3	1	–
	Sisymbriifolium	2	1	1 (50%)
	Thomasiifolium	11	1	1 (9%)
	Gardnerii	7	1	–
	Old World Clade	ca. 240	106	9 (4%)
Allophyllum/Wendlandii		8	2	1
Potato				
	Lycopersicon	13	12	8 (62%)
	Lycopersicoides	2	1	1
	Juglandifolia	2	1	–
	Petota	112	105	7 (6%)
	Basarthrum	14	14	11 (79%)
	Regmandra	11	2	1
	Pteroidea/Herpystichum	20	1	–
	Etuberosa	3	3	1
	Anarrhichomenum	ca. 15	4	4 (27%)
	Articulatum	2	1	–
Total		1018	506	192 (18.8%)

higher (24%), considering that several species have been the subject of many studies (e.g., *S. tuberosum*, *S. melongena*, *S. surattense*, *S. nigrum*, etc.). A few groups were better studied, with more than 60% of their members being analysed: Basarthrum, Lasiocarpa, Acanthophora, and Morelloid (Table 1). On the other hand, no data are available on Thelopodium, African non-spiny, Normania, Pteroidea/Herpystichum, Articulatum, Juglandifolia, Nemorense, Gardneri, Bahamense, and Micrantha (Table 1).

Karyotypes were typically symmetrical. All accessions had m (metacentric) chromosomes (from 1 to 12 pairs in diploids, with 10 pairs being the most frequent), whereas 94% of them had sm (submetacentric) chromosomes (1 to 11 pairs in diploids, with 2 pairs being the most frequent). St (subtelocentric) and t (telocentric) chromosomes were not common: 22% of the species showed between 1 and 6 st chromosomes in diploids (with 1 pair being the most frequent) and 5% of the taxa had between 1 and 2 pairs of t chromosomes in diploids. Table 2 synthesizes the frequency of chromosome types recorded in the studied clades. The most asymmetrical karyotypes were found in Potato (*S. pinnatisectum*: 2m+7sm+2st+1t, Li, Chen,

Table 2. Chromosome types and their frequencies in *Solanum*.

Clade	Chromosome type	Number of species	Range	Mode
Brevantherum	m	6	5–11	6
	sm	6	1–7	6
Cyphomandra	m	20	5–10	9
	sm	20	1–6	3
	st	8	1–2	1
Dulcamaroid	m	18	2–12	8
	sm	16	1–9	2
	st	7	1–2	1
Geminata	m	13	4–11	9
	sm	13	1–6	2
	st	9	1–2	1
Leptostemonum	m	93	1–12	10
	sm	87	1–11	2
	st	13	1–6	1
	t	5	1–2	1
Morelloid	m	47	2–12	10
	sm	43	1–11	2
	st	5	1–4	1
Potato	m	47	2–12	6
	sm	43	1–12	6
	st	13	1–3	1
	t	8	1–2	1
Total	m	246	1–12	10
	sm	230	1–11	2
	st	55	1–6	1
	t	13	1–2	1

Note: m = metacentric; sm = submetacentric; st = subtelocentric; t = telocentric.

Beasley, Lynch, & Goettel, 2006), Dulcamaroid (*S. crispum*: 2m+9sm+1st, Moyetta, Stiefkens, & Bernardello, 2013), and Leptostemonum clades (*S. indicum*: 1m+9sm+2st, Krishnappa & Chennaveeraiah, 1975; *S. palinacanthum*: 4m+2sm+6st, Acosta et al., 2005).

The overall mean chromosome length was 2.31±1.40 (n = 258; range 0.30–8.98; Appendix S3, see supplemental material online). The Cyphomandra clade was outstanding because it had the longest chromosomes, which were statistically significant from the remaining clades; in addition, the average chromosome size of the Leptostemonum clade was significantly higher than that of the Potato clade (Appendix S4). The mean smallest chromosomes were found in Archaesolanum, whereas the absolute small chromosomes in Morelloid and Leptostemonum.

The overall mean arm ratio (r) was 1.67±0.34 (n = 210; range = 1.19–3.71; Appendix S3, see supplemental material online). No statistical differences were detected among most clades, except for Leptostemonum, which differed from Potato and Morelloid.

2C values ranged from 1.25 in *S. chacoense* (Potato) to 49.6 in *S. circinatum* (Cyphomandra, sub nom. *C. hartwegii*), with a mean of 5.75±8.14 (Appendix S5). Results of a linear regression determined that the total length of the haploid karyotype (TL) predicted the 2C value ($r^2 = 0.75$, t = 16.97, $P<0.0001$, N = 98; 2C value = (−3.15±0.54) + (0.23±0.01 * TL), Appendix S6, see supplemental material online).

In general, no correlations were found, except for mean chromosome length with total karyotype length, and for these two variables with 2C value (Appendix S7, see supplemental material online).

Chromosome banding and FISH

A total of 86 species, including 24 studied here for the first time (Appendices S2, S8–10, see supplemental material online), have been examined with fluorescent banding and/or FISH, representing a small percentage of the genus (~8%). Of these, 29 species were studied with CMA and 40 with the double staining CMA/DAPI. Only species of four major clades were targeted, with Leptostemonum and Potato being the best represented.

Heterochromatin percentage, reported in 37 species, was not statistically correlated to TL; it varied from 1.86 in *S. aculeatissimum* to 35.43% in *S. villosum*, with a mean value of 13.42 (Appendix S3). Results of chromosome banding reported three different heterochromatin types: (1) a strong pair of CMA^+ signals (corresponding to GC-rich heterochromatin regions) associated with the secondary constrictions (i.e., NORs) in terminal position, which were observed in all species, (2) additional CMA^+/$DAPI^-$ heterochromatin blocks not associated with NORs and located in interstitial regions were detected in 22 species (46%); the number of these bands varied from one pair (in five species) to 26 pairs (only in *S. stuckertii*), (3) additional CMA^+/$DAPI^-$ heterochromatin blocks not associated with NORs and located in terminal or subterminal regions were observed in 24 species (51%). The number of these bands varied from one pair (in two species) to 22 pairs (i.e., all chromosome pairs having terminal bands) in *S. sandwicense*.

Concerning FISH assays, 82 species have been studied with probes for any of the two rDNA loci; 72 of them have been studied simultaneously with probes for both loci and 10 only with probe for the 18–5.8–25S (Table 3). Diploid species studied with a 5S probe amounted to 70, and 76 were studied with probe for the 18–5.8–25S. Thus, only ~8% species have been studied in their rDNA and none of the 13 major clades has been studied in more than 20% of its species. Geminata received the least attention, with only 4 of 140 species analysed (Table 3).

For the 5S rDNA loci, probes of four different origins were recorded (Brasileiro-Vidal, Melo-Oliveira, Carvalheira, & Guerra, 2009; Chiarini et al., 2014; Rego et al., 2009; Xiang-Hui, Young-Hua, Liu, & Chao-Wen, 2011).

Table 3. Summary of the *Solanum* species in which number and position of rDNA signals for the 5S and 18–5.8–25S genes were reported. The data comprise the location of signals in terminal (t) or interstitial (i) position and in metacentric (m), submetacentric (sm), or subtelocentric (st) chromosomes.

Solanum clades (species studied with any of the rDNA probes/total species in the clade)	Diploid species studied with FISH									
	5S					18–5.8–25S				
	i m	i sm	t m	t sm	2 or more pairs	i	t m	t sm	t st	2 or more pairs
Archaesolanum (1/9)										
Brevantherum (4/60)		2		1	1		1	3		
Cyphomandra (5/50)	3	1			1			1		4
Dulcamaroid (7/45)	3	1	1		2		7			
Geminata (4/140)	1	1	1		1		1	2		1
Leptostemonum (25/410)	12	4	1	1	3		13	9	1	
Morelloid (14/76)	4	2	4	1	1		3	7		3
Potato* (21/194)	8	2		-		2	10	1		
Allophyllum/Wendlandii (1/8)	1						1			
TOTAL (82/1018)	32	13	7	3	9	2	36	23	1	8

Note:* = In six species the morphology of the chromosomes with signals was not informed.

With these probes, 72 species have been studied, of which 61 were diploids with one pair of signals per genome. Of these, bands were interstitial and located in an m chromosome in 32 species and interstitial in a sm chromosome in 13 species, whereas in the remaining 10 species they were terminal in either an m or sm chromosome (chromosome morphology was not indicated in 6 species). Seven species presented two pairs of bands in different combinations of position and chromosome type. Interestingly, two diploid species, *S. pallidum* (Appendix S8, see supplemental material online) and *S. stuckertii* (Appendix S9, see supplemental material online), showed more than two pairs of signals; *S. stuckertii* is remarkable for presenting 11 pairs of signals in both terminal and interstitial positions (i.e., dispersion of the 5S site). Only two species (*S. hjertingii*, *S. stoloniferum*) were polyploids with two or more pairs of signals, while in *S. elaeagnifolium*, both diploid and polyploids accessions were studied, with 5S signals being proportional to the ploidy level.

For the 18–5.8–25S rDNA loci, probes of three different origins were used (Brasileiro-Vidal et al., 2009; Gerlach & Bedrock, 1979; Unfried & Gruendeler, 1990). A total of 82 species have been studied using these probes, 76 of which were found to be diploid and six polyploid. Within the diploids, 68 species presented only one pair of signals and eight species, two pairs or more. Most species had terminal signals: 36 species in an m chromosome, 23 in a sm chromosome, and in one species in a st chromosome (in six species the morphology of the chromosomes with signals were not informed) (Table 3). Only two species (*S. habrochaites* and *S. pennellii*) showed signals for the 18–5.8–25S in interstitial positions, and three species (*S. habrochaites*, *S. crispum*, and *S. pallidum*) presented signals for the 5S and the 18–5.8–25S located in the same chromosome (i.e., synteny, according to Tang et al., 2008).

Dispersion of the 18–5.8–26S loci was found in *S. trichoneuron* (Appendix S9, see supplemental material online) and *S. pallidum* (Appendix S8, see supplemental material online), and consisted in small terminal fluorescent bands in several or even in all chromosomes of the complement.

Another type of heterochromatin was revealed when DAPI was applied after the denaturation/renaturation of DNA in the FISH procedure, detecting predominant AT specific bands (Bogunic, Siljak-Yakovlev, Muratovic, & Ballian, 2011). In the species here recorded, $DAPI^+$ bands after-FISH do not seem to coincide well with any bands visualized with the CMA/DAPI procedure. The presence of DAPI bands after-FISH is constant among the cells of a single individual examined and is useful to individualize species (Appendices S8–9, see supplemental material online).

In eight species, some members of a chromosomal pair were heteromorphic, either because of their length (*S. lycopersicum*), the position of the centromere (*S. vespertilio*), their pattern of heterochromatic bands (*S. pennellii*), the size of the NORs (*S. reductum*, *S. palinacanthum*, *S. sandwicense*), or the number and position of rDNA sites (*S. pallidum*, *S. elaeagnifolium*).

DNA content

A summary of the 2C DNA content data is given in Appendix S5 (see supplemental material online). Of the 13 major clades, only eight had available data on DNA content, with Cyphomandra being the best known (24%).

No data are available for Nemorense, Normania, Allophyllum/Wendlandii, African non-spiny, and Thelopodium. The average 2C values per clade ranged from 1.74 pg in Dulcamaroid to 20.80 pg in Cyphomandra (i.e., a 12-fold variation), although the variation is much lower (~2-fold) when Cyphomandra is not considered. The lowest individual value (1.26 pg) corresponded to *S. chacoense* (Potato), which is ~6-fold lower than that of *S. macranthum* (Leptostemonum), and more than 30-fold lower than the highest value, which corresponds to *S. splendens* (Cyphomandra).

Character mapping and ASR

The main results of the ASR for discrete characters are summarized in Table 4. All characters showed transitions associated with supported nodes; for instance, transitions from symmetrical karyotype formulae to asymmetrical and vice versa occurred several times (Fig. 1, Table 4). Some characters underwent comparatively few transitions (e.g., chromosome number from diploid 2n = 24 to dysploid 2n = 22, Fig. 2; synteny and number of NORs, Appendices S11–12, see supplemental material online) compared with others that were more variable (e.g. number of 5S loci, Appendix S13). Results for character mapping of continuous characters are represented with heatmaps (Appendices S14–15, see supplemental material online).

Discussion

Chromosome numbers

Solanaceae exhibit a dysploid series from x = 7 to x = 14, although other numbers, such as x = 17, 19, and 23, were recorded (cf. Goldblatt & Johnson, 1979 onwards; Rice et al., 2015). A hypothesis on paths of chromosome number changes is still lacking, and even its original base number is a matter to be clarified. Raven (1975) postulated x = 12 as plesiomorphic. However, based on recent phylogenetic studies, Olmstead et al. (2008) suggested that this number is apomorphic. The most common base number is x = 12, characterizing an entire clade (including Solanoideae where it is almost universal) and was found in more than 50% of the species studied. Within the x = 12 clade, evidence for dysploid changes to x = 13 via Robertsonian translocations has been reported in *Capsicum* (Moscone et al., 2006) and *Solanum* (sub *Lycopersicon*, Banks, 1984). However, such type of chromosomal change is rare; most species with a chromosome number different from x = 12 belong to *Capsicum*, whereas in *Solanum* dysploidy (with x = 23) seems to be a synapomorphy of the Archaesolanum clade with a few species

Table 4. Summary of the Ancestral State Reconstruction from stochastic character mapping for discrete chromosomal characters. The data comprise traits, number of species, model of character evolution used, character states, mean total time spent in each state in percentage (MT), number of changes (C), number of changes per nodes (C/N, where N corresponds to #species −1), more frequent changes, rate of change and state of character estimated at the *Solanum* root.

Trait	# species	Model	Character states	MT	C	C/N	More frequent changes	rate	State at the *Solanum* root
Synteny of rDNA loci	60	ARD	0 = non syntenic	94.67	6.573	0.111	0 to 1	0.105 ± 0.066	0
			1 = syntenic	5.31					
NORs	65	ARD	0 = one pair	90.52	12.407	0.194	0 to 1	0.022 ± 0.007	0
			1= two pairs	9.48					
Chromosome number	310	ER	0 = diploid	72.45	75.304	0.244	0 to 1	1. 176 ± 0.129	0
			1= polyploid	17.46					
			2= dysploid	10.09					
Karyotype formulae	119	ARD	0 = one or two sm chromosomes and the rest m	34.33	87.871	0.744	1 to 0	0.069 ± 0.017	2
			1 = more than two pairs sm and the rest m	33.74					
			2 = one or more chromosomes st or t, and the rest m	31.93					
5S loci	60	ARD	0 = one pair	76.82	97.027	1.644	1 to 0	0.374 ± 0.055	0
			1= two pairs	16.65					
			2= more than two pairs	6.53					

Note: ARD = All rates different; ER = equal rates.

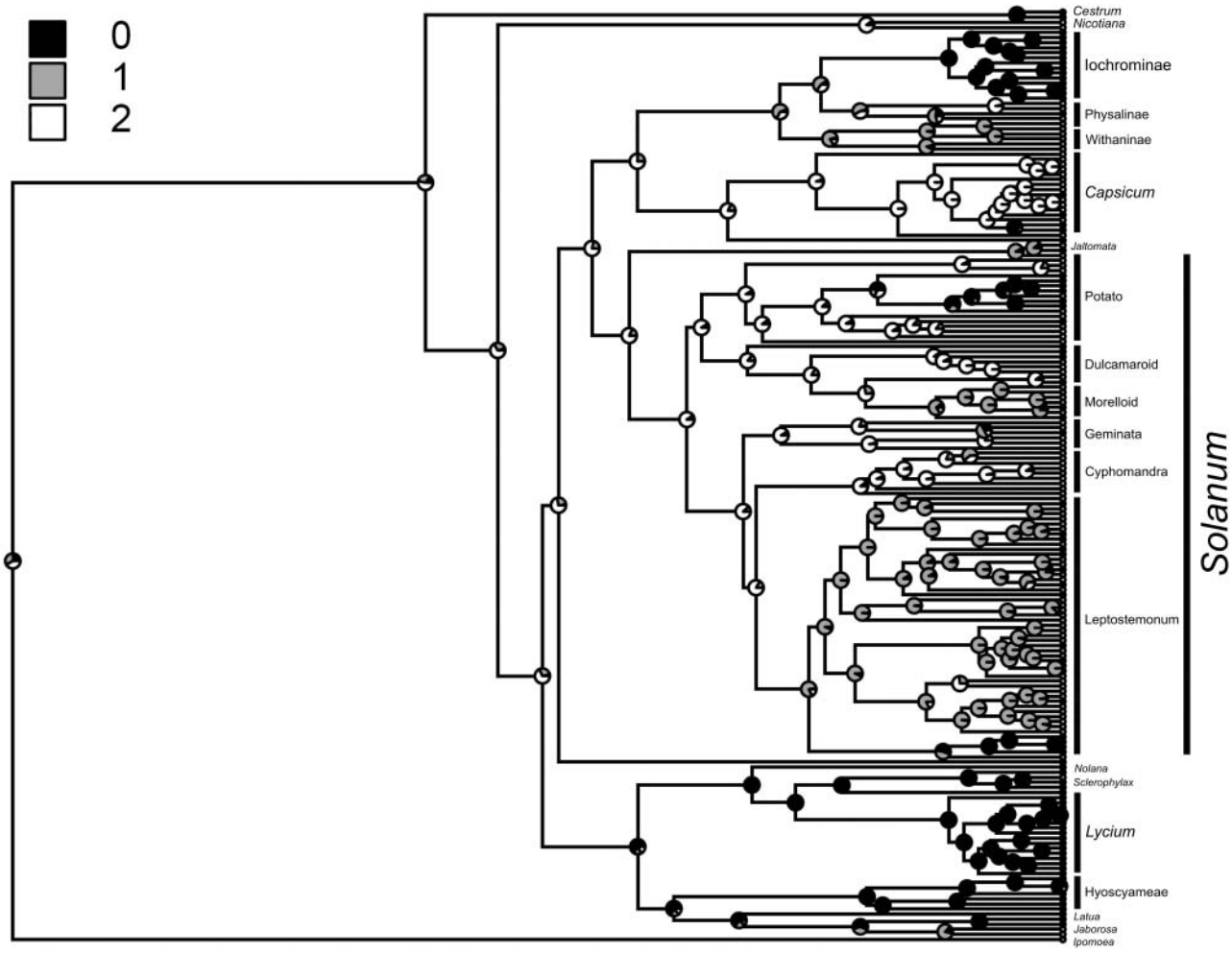

Fig. 1. Ancestral reconstruction of karyotype formula in *Solanum* using stochastic character mapping. Pies indicate the proportion of each character state (black, 0 = one or two sm chromosomes and the rest m; grey, 1 = more than two pairs sm and the rest m; white, 2 = one or more chromosomes st or t, and the rest m) estimated for each node from 1,000 simulations on the maximum credibility tree.

from different clades presenting odd numbers, as 2n = 22, 42, or 60.

Polyploidy has occurred in at least 22 genera from subfamilies Cestroideae (tribe Browallieae), Nicotianoideae (*Nicotiana* and tribe Anthocercideae), Solanoideae (*Mandragora*, *Lycium*, and tribes Solaneae, Hyoscyameae, and Physalideae). The highest chromosome number was found in *Scopolia japonica* (2n = 14x = 168, Lee & Oh, 1971). Polyploids were frequent in *Nicotiana*, where ~40% of the species are tetraploids often by amphidiploidy (Kenton, Parokonny, Gleba, & Bennett, 1993). Polyploid series are conspicuous in some genera of the x = 12 clade, i.e., *Mandragora* (2x, 7x, 8x), *Lycium* (2x, 3x, 4x, 8x, 10x), *Physalis* (2x, 4x, 6x), *Chamaesaracha* (2x, 3x, 4x, 6x), *Withania* (2x, 3x, 4x, 8x), and *Scopolia* (4x, 7x, 14x). In *Solanum*, polyploidy arose independently several times, characterizing some clades: Morelloid (with 2x, 4x, 6x, 8x), Petota (2x, 3x, 4x, 5x), and Carolinense (2x, 4x, 6x). Thus, in *Solanum* polyploidy represents the main number alteration. Within flowering plants, polyploidy has been related to several biological aspects, as habit, life form, mating system, geographic range, and invasion of new habitats (Sonnleitner et al., 2015; Stebbins, 1985) as there would be advantages conferred by genome duplication (Soltis, Soltis, Bennett, & Leitch, 2003; Soltis, Visger, & Soltis, 2014; Stebbins, 1985; te Beest et al., 2011; Wendel, 2000). Polyploidy is the most important source of number variation in the evolution of flowering plants, being a highly recurrent state. Even within a single species, polyploid individuals or populations may originate independently. Intraspecific polyploidy is underestimated because most species are cytologically known from a few samples, and phenotypic differences between cytotypes are often not evident (Levin, 2002). Within *Solanum*, polyploidy seems to be related to clonal propagation in the Carolinense and Elaeagnifolium clades, and to

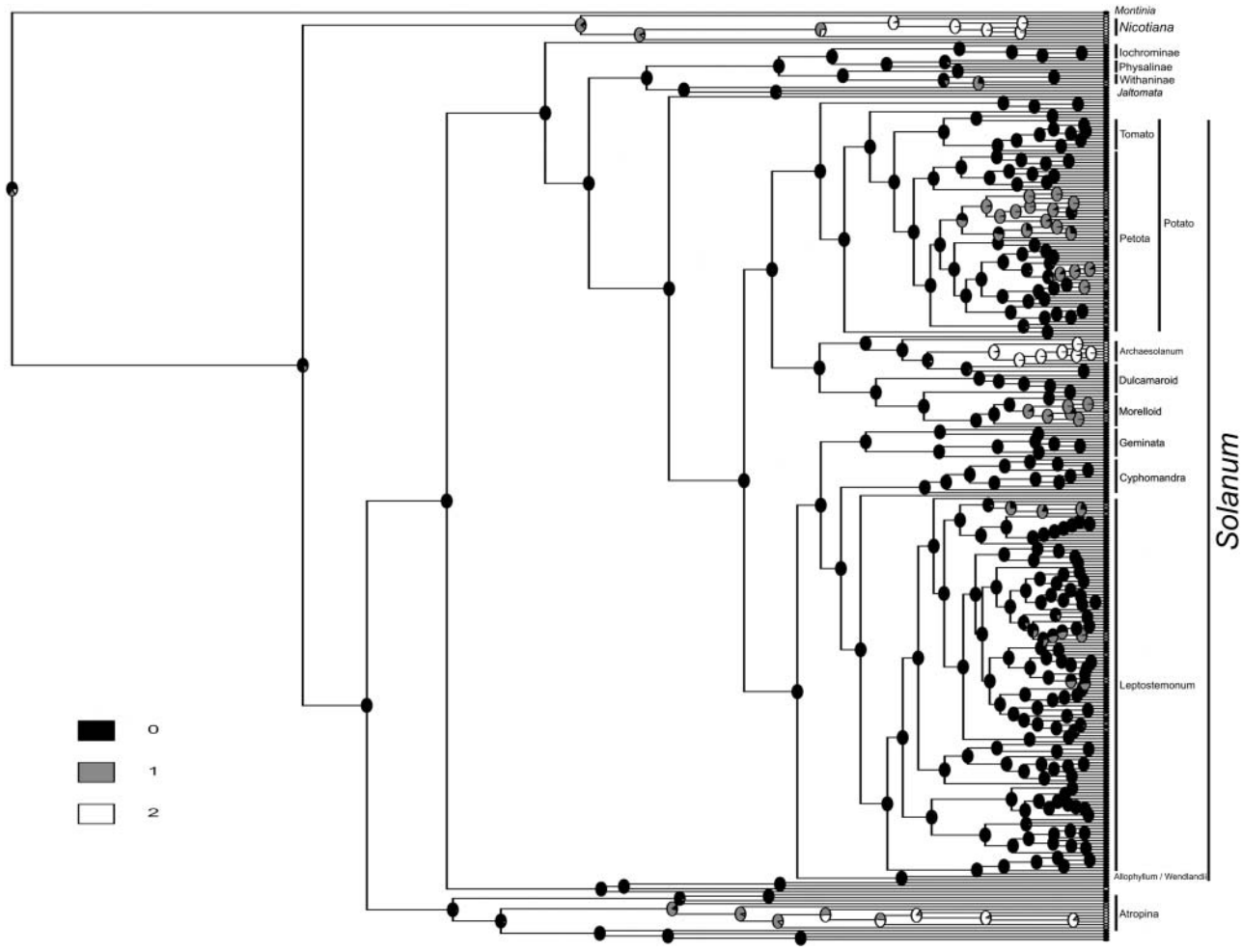

Fig. 2. Ancestral reconstruction of chromosome number in *Solanum*, using stochastic character mapping. Pies indicate the proportion of each character state (black, 0 = diploid; grey, 1 = polyploidy; white, 2 = dysploid) estimated for each node from 1,000 simulations on the maximum credibility tree.

perennial habit with tubers in the Petota clade. However, a cause-effect relationship is difficult to draw, and more data are needed to be conclusive.

Considering the number of counts, *Solanum* is relatively better known than other large Angiosperm genera (*Astragalus*, *Euphorbia*, *Bulbophyllum*, *Carex*, *Piper*, *Epidendrum*; Appendix S16, see supplemental material online). The number x = 12 is a stable character and species diversification within *Solanum* would not have included changes in basic number. Since *Solanum* is a genus of recent speciation (Särkinen et al., 2013; Stebbins, 1971), and genetic divergence is a function of time (Frary, Doganlar, & Frary, 2016), uniformity of chromosome number in the genus could be attributed to these facts. While *Piper* and *Euphorbia* are older and present many numbers, other genera of similar age and size (*Bulbophyllum*, *Epidendrum*) are not as chromosomally explored as *Solanum*. Only in *Astragalus*, the available data suggest that its number is not as conserved as in *Solanum*. Other reasons beyond the time of speciation should be investigated. For instance, chromosome uniformity within *Aloe* (Asphodelaceae) has been attributed to climatic stability and similarity of habitats (Vosa, 2005), although species of *Solanum* live in all kinds of habitats. In *Carex*, whose age and number of species are comparable to *Solanum*, it is clear its many numbers are due to its holocentric chromosomes (Escudero, Hipp, Waterway, & Valente, 2012). Considering that the number x = 12 characterizes a whole clade with about 2300 spp. in Solanaceae, the case would be comparable to Cactaceae, a family of ±30 mya (Tank et al., 2015) with about 1800 species and where most of the ~870 counts are x = 11. Cactaceae would represent an analogous case to the clade x = 12, where a number is conserved despite an enormous morphological diversification; however, most cacti are succulents adapted to dry environments and Solanaceae show a wide range of habitats and life forms.

Karyotypes

Chromosome length is important because it is useful to single out individuals, samples, populations or species, besides being an indirect indicator of the total DNA amount. However, it has the disadvantage of being affected by the degree of chromatin condensation and by the pretreatment method. Thus, comparisons in different assays are not completely reliable. Despite this downside, Solanaceae data are in the same order of magnitude, allowing general comparisons. Solanaceae show notable variation in chromosome size, with a range of 1.0–1.5 μm (in *Metternichia*, Basarthrum clade of *Solanum* and *Atropa*) to 6.5–11.51 μm (in *Cestrum* and the Cyphomandra clade of *Solanum*). Most species have small or medium-sized chromosomes with average lengths of 1.5–5.0 μm. *Solanum* is characterized by small- to medium-sized chromosomes with respect to other Solanaceae and Angiosperms (e.g., Badr, Khalifa, Aboel-Atta, & Abou-El-Enain, 1997; Guerra, 2000), with most species being within the 1–3 μm range. Chromosomes of ~6 μm are an exceptional synapomorphy of Cyphomandra clade (Bohs, 1994, 2001).

Stebbins (1971) pointed out an association between habit and chromosome size, with perennial species having small chromosomes. However, within woody Solanaceae, chromosomes were either small (e.g., *Lycium*, Stiefkens & Bernardello, 2000; *Lycianthes*, Acosta et al., 2005), or medium-sized (e.g., *Saracha punctata*, *Latua pubiflora*, Chiarini et al., 2010); within *Solanum*, these chromosomes were found among annuals, perennials, herbs, trees and vines, whereas Cyphomandra clade species are shrubs or trees. Stebbins' ideas may seem irrational in the current genetic knowledge; however, other phenomena may be related to chromosome length. As DNA content is directly related to chromosome size, the alleged factors affecting the former may indirectly affect the latter, as the speed of DNA replication and the duration of the life cycle. Another idea is that long chromosomes would undergo a higher number of chiasmata than small ones (Turney, de los Santos, & Hollingsworth, 2004). For ferns, it has been proposed that low recombination due to small chromosomes is compensated by high ploidy levels, which increases the number of chiasmata (Nakazato, Jung, Housworth, Rieseberg, & Gastony, 2006). Further studies are needed to establish if there are differences in recombination rates among *Solanum* with small chromosomes.

In Solanaceae, there are groups showing a high constancy in chromosome number, with symmetrical karyotypes and a majority of m chromosomes of rather similar size, e.g., *Lycium* (Stiefkens & Bernardello, 2000), but there are also groups in which complements include st and t chromosomes, e.g., *Nicotiana*, *Capsicum*, *Jaborosa*, *Physalis*, and the Acanthophora clade of *Solanum* (Acosta et al., 2005; Chiarini & Barboza, 2008; Menzel, 1951). Some *Nicotiana* species are unique in having karyotypes mostly with st chromosomes (Goodspeed, 1954) and karyotypes with at least one st pair were found in *Hyoscyamus*, *Capsicum*, and *Solanum* (Acosta et al., 2005; Bernardello et al., 1994; Moscone et al., 2006; Sheidai, Mosallanejad, & Khatamsaz, 1999). Karyotypes that are highly symmetrical have been considered primitive (Stebbins, 1971), but, at the same time, karyotype orthoselection was proposed for the maintenance of complements with m and sm chromosomes of approximately the same length (Brandham & Doherty, 1998; Moscone et al., 2003). It is difficult to determine the direction of such karyotype evolution, as many reversals might have occurred (Stace, 2000). For instance, in Brassicaceae, karyotype asymmetry might be a transitory state rather than a derived evolutionary end point (Mandáková & Lysák, 2008). Also in Solanaceae, when data of karyotype symmetry are interpreted in relation to the latter phylogenetic hypotheses, the resulting picture is complex, with values of symmetry changing back and forth. The genera within the x = 12 clade would have followed different pathways, with examples of uniform, symmetrical formulae (*Lycium*, Stiefkens & Bernardello, 2000), uniform and asymmetrical formulae (*Capsicum*, *Physalis*, Menzel, 1951), or heterogenous asymmetrical formulae (*Jaborosa*, Chiarini, Moreno, Moré, & Barboza, 2016). Within *Solanum*, 10m + 2sm is the most frequent formula, but species with st or t chromosomes are interspersed in the phylogenetic tree, whereas formulae with more than two sm chromosomes predominate in clade Leptostemonum. In a general survey of Solanaceae, Badr et al. (1997) reported values of r ranging from 1.17 to 2.78, whereas in *Solanum* from 1.19 to 3.71. Thus, karyotype asymmetry of *Solanum* does not represent a uniform situation with respect to the family (Appendix S14, see supplemental material online).

Relationships among karyotype formulae and different macroscopic features (as habit and life form) were not corroborated. Transitions between karyotype formulae can be interpreted as evidence of rearrangements. Particularly, formulae with st or t chromosomes are probably the result of a deletion or translocation of the entire or part of one arm. Evidence suggests that the direction of evolutionary changes due to chromosome rearrangements implies karyotype re-patterning.

DNA content

Most genera of Angiosperms do not show a variation of DNA content higher than 2-fold (Bennett & Leitch, 2012). In *Solanum*, measurements of the nuclear content are available for few species and show that genome size (1C-value) of diploid species varies from 0.63 pg (588 Mbp) in *S. chacoense* and *S. tripartitum* to 24.8 pg (24304 Mbp) in *S. splendens* (sub *Cyphomandra hartwegii* var.

ramosa, Pringle & Murray, 1991). Nevertheless, an extreme discontinuity is observed in the Cyphomandra clade, whereas most *Solanum* species share an average 1C value of ~1.35 pg. Thus, *Solanum* is an example of great variation, although the extreme values are restricted to a single clade. The increase in DNA content in Cyphomandra clade is not attributable to any particular factor, since its species share most features with other *Solanum* clades. For instance, *S. corymbiflorum* (Cyphomandra, 1C = 6.75 pg) and *S. robustum* (Leptostemonum, 1C = 3.13 pg) are shrubs or treelets that co-occur in the forests of SE Brazil and NE Argentina.

Different patterns of addition of DNA amount were reviewed by Peruzzi et al. (2009). Plants increase their content in three ways: genome duplications or polyploidy, simultaneous homogeneous increase in all chromosomes (i.e., proportional increase or 'concerted evolution'), and increase restricted to a subset of chromosomes via amplification/deletion of blocks of certain families of tandem repeats ('unequal increase'). The third case is evident when one of the chromosomes of the complement is markedly larger (e.g., de la Herrán et al., 2001), which is confirmed by molecular techniques. In the proportional increase pattern, the amount added to each chromosome arm is proportional to its length and does not result in a change in asymmetry when genome size changes, as observed in several genera (Brandham & Doherty, 1998). In many Liliaceae, Peruzzi et al. (2009) found an unequal increase pattern, i.e., the amount added varies between longer and shorter chromosome arms unequally, resulting in an increase in the intrachromosomal asymmetry. In *Solanum*, the mechanism which led to large chromosomes in the Cyphomadra clade was a concerted evolution or changes of the proportional type, since its species are diploid and have formulae with a majority of m chromosomes and their asymmetry indexes are low. Neither bimodal karyotypes nor complements with some pairs distinctively larger have been noticed in the genus.

Data of DNA content are available for only 11 of the 98 genera of Solanaceae, and total measurements per species represent ~7% of its species. *Petunia* is the best known genus (almost all of its 25 species were examined), followed by *Nicotiana* (82% of its 67 species), whereas *Datura* and *Calibrachoa* have been studied in half of their species. *Solanum* is poorly known, having only 70 measurements (± 7%). However, *Solanum* is more variable in DNA content than *Nicotiana*, *Petunia*, or *Calibrachoa* (see Appendix S5, see supplemental material online). Average 2C content values of *Solanum*, not including the Cyphomandra clade, are ~2.69 pg, which is similar to the values of *Petunia* or *Calibrachoa*, and lower than *Nicotiana* or *Capsicum.* More measurements in closely related genera (such as *Jaltomata*, *Physalis*, *Lycianthes*) are necessary to understand the evolutionary trends in the x = 12 clade.

Compared with the largest genera of Angiosperms, *Solanum* is the best known genus. The available data suggest that, not including the chromosome number, *Euphorbia* is also extremely variable, with a 40-fold variation. In Cactaceae, variation in DNA content is also moderately high (~8-fold), much lower than in *Solanum* (Bennett & Leitch, 2012).

Chromosome banding and FISH

Solanum did not show extreme or discontinuous changes in the heterochromatin patterns as occur in genera from other families (Guerra, 2000): percentages vary gradually from 1.8 to 35.4, with a mean value of 14. The reported 75% (Peterson, Price, & Johnston, 1996) or 64.9% (Chang et al., 2008) in *S. lycopersicum* are exceptional values and result from differences in heterochromatin concept between these authors. Peterson et al. (1996) used Feulgen staining and considered heterochromatin as the entire proximal heteropycnotic condensed region of the chromosome; this technique, however, is not specific for heterochromatin. Chang et al. (2008) also suggested a high percentage, based on the distribution of the Cot-1, Cot-10, and Cot-100 fractions, which hybridized over all chromosomes, covering large regions recognized by these authors as heterochromatin.

The occurrence of GC-rich heterochromatin sequences adjacent to or co-localized with NORs has been frequently described for many plant species (Guerra, 2000); nevertheless, not all CMA^+ bands are associated with NOR in *Solanum*, which implies that GC-rich sequences are independent of rDNA genes (Jo et al., 2009). Heterochromatin patterns appear more variable within *Solanum* than within other genera of the x = 12 clade (*Lycium* and *Sclerophylax*), where heterochromatin is restricted to the NOR-associated regions and heterochromatin percentages are low (Blanco, Las Peñas, Bernardello, & Stiefkens, 2012; Lujea & Chiarini, 2017; Stiefkens, Las Peñas, & Bernardello, 2009). On the other hand, *Jaborosa* presented a notable variability in heterochromatin (Chiarini et al., 2016). These variable patterns have been interpreted as evidence of intense chromosomal rearrangements (e.g., Chiarini et al., 2016; Evtushenko et al., 2016; Grewal & Jia, 2007) associated with species' diversification and colonization of new habitats, since these patterns function as rapidly evolving species barriers (Hughes & Hawley, 2009).

The heterochromatic bands not associated with NORs were located in terminal positions in several species, corresponding to an equilocal pattern distribution, which agrees with the heterochromatin dispersion model proposed by Schweizer and Loidl (1987). Some species presented small terminal bands in both arms of all chromosomes. Possibly, this telomeric heterochromatin is present in all species, but may sometimes go undetected because of the resolution of the technique and the reduced

size of the bands. Other *Solanum* species presented interstitial $CMA^+/DAPI^-$ bands, which could be evidence of inversions or translocations.

In Solanaceae, species with symmetrical karyotypes were found to present simple heterochromatin patterns, whereas species with many heterochromatin bands had more asymmetrical karyotypes (Blanco et al., 2012; Chiarini et al., 2016; Stiefkens et al., 2009). Within *Solanum*, this relationship between asymmetry and heterochromatin has been corroborated in species of Acanthophora clade (Chiarini et al., 2014), but not in species of the Dulcamara and Morelloid clades (Moyetta, Urdampilleta, Chiarini, & Bernardello, 2017) or Lycopersicum clade (Brasileiro-Vidal et al., 2009), and in general, arm ratio is not statistically correlated to heterochromatin percentage. Thus, heterochromatin does not present a homogenous pattern within *Solanum* and no clear tendencies related to clade diversification have been found.

Concerning the $DAPI^+$ bands after FISH, in some *Solanum* species they coincide with the bands visualized with the CMA/DAPI procedure (Chiarini et al., 2014), whereas in others, they do not (Chiarini & Gauthier, 2016). However, the only way to prove co-localization of both band types is to perform all the techniques sequentially on the same slide, which has never been attempted in *Solanum*. The presence of these $DAPI^+$ bands post-FISH was constant in all cells of a single individual and is therefore useful as a species-specific character.

In different plant families, species with large chromosomes have more heterochromatic bands than those with small chromosomes (Chiarini et al., 2014; Guerra, 2000; Moscone et al., 2006) and a correlation between karyotype length (and consequently, chromosome size) and the number or length of heterochromatic bands has already been reported (e.g., Chiarini et al., 2014, 2016; Moscone et al., 2006; Pringle & Murray, 1993). However, while there are examples of positive (Las Peñas, Bernardello, & Kiesling, 2008) and negative (Stebbins, 1971) correlations between heterochromatin amount and chromosome asymmetry, those trends are difficult to infer from the available data on *Solanum*.

In the last 20 years, the number and position of 18–5.8–26S and 5S rDNA sites have been described using FISH for more than 1000 plant species (Garcia, Garnatje, & Kovařík, 2012). These sites have been used as a tool to estimate the karyotypic similarity among species or to understand the karyotype evolution. Several factors make 5S and 18–5.8–26S rDNA loci suitable markers for karyotype characterization (Weiss-Schneeweiss & Schneeweiss, 2013). However, there are some restrictions to the use of rDNA sites to identify chromosome homologies, such as intraspecific variability. A survey of 18–5.8–26S rDNA loci number and distribution published for 749 species belonging to 175 genera indicated that the most frequent numbers of sites per diploid karyotype were two and four, and that they most often occur at terminal positions (45%), usually within the short arms, and frequently on telocentric chromosomes where they usually occupy the whole arm (Roa & Guerra, 2012). According to the available data, *Solanum* follows this general trend, with a few exceptions (*S. habrochaites*, *S. pennellii*, *S. pallidum* and *S. trichoneuron*). At the same time, the number and position of the rDNA loci seem to be homoplastic; some populations would have multiplied these loci after species divergence. In some taxa, the gain of rDNA genes has been associated with polyploidy events and/or increase of the genome length (Hasterok et al., 2006; Pellicer, Garcia, Vallès, Kondo, & Garnatje, 2013). Nevertheless, the increase of rDNA genes is not related to such phenomena in *Solanum*.

The two rDNA genes may evolve differentially (Roa & Guerra, 2012). In some plant groups, the 18–5.8–26S is more variable than the 5S (Chacón et al., 2012; Fulneček et al., 1998; Książczyk, Taciak, & Zwierzykowski, 2010). Contrarily, in other genera the number of 5S rDNA sites is more flexible (Fukushima, Imamura, Nagano, & Hoshi, 2011; Morales, Aguiar-Perecin, & Mondin, 2012). Accordingly, our data on *Solanum* suggest a differential evolution of the rDNA genes, with the 18–5.8–26S site more being stable than the 5S. In the span of 24 mya (the age of $x = 12$ clade), there were few losses of an 18–5.8–26S site and dispersion events, whereas the number and position of the 5S sites underwent several changes. A similar situation was observed in other Solanaceae, like *Jaborosa* (Chiarini et al., 2016), whereas in *Lycium* both rDNA sites seem to be stable (Blanco et al., 2012).

Several mechanisms have been hypothesized to be responsible for the mobility of rDNA sites. These include, among others, unequal recombination, transposition, conversion/homogenization of repeats among loci (Raskina et al., 2008; Volkov, Medina, Zentgraf, & Hemleben, 2004). Different types of transposable elements have been postulated as being responsible for the rapid change of the copy number and chromosomal location of rDNA in plants (Datson & Murray, 2006; Evtushenko et al., 2016; Raskina et al., 2004). The copy number of migrated rDNA repeats would be amplified by unequal crossing over, to the extent that these new sites can be detected by FISH (Fukushima et al., 2011).

In most *Solanum* species, there are two main 18–5.8–26S sites per basic genome, although several species presented minor sites along the complement. Such dispersion of the 18–5.8–26S was also observed in other Solanaceae as *Cestrum* (Urdampilleta, Chiarini, Stiefkens, & Bernardello, 2015). The presence of minor sites could represent the final stage of DNA loss for these loci (Chiarini et al., 2014; Kotseruba et al., 2010).

In angiosperms, both the number and localization of 18–5.8–26S and 5S rDNA loci are largely independent from one another (Małuszynska, Hasterok, & Weiss,

1998). An exception is some clades of Asteraceae, where these loci are physically linked (Garcia, Panero, Siroky, & Kovařík, 2010). The phylogenetic distribution of such linked arrangements suggests its recurrent origin and/or reversal (Garcia et al., 2010). The degree of synteny is a function of the time since their divergence, with translocation, inversion, and transposition being the main mechanisms of chromosome rearrangement. Knowledge of genome synteny and collinearity makes it easier to leverage resources from one species to another (Frary et al., 2016). As synteny is the result of descent from a common ancestor, disruption in conserved syntenic segments can be used to deduce the mechanisms of chromosome rearrangements that accompanied species divergence (Frary et al., 2016). In *Solanum*, it has been suggested that inversions can occur independently among different lineages, with some regions of the genome being subject to rearrangements more frequently than others (Szinay et al., 2012). For instance, synteny between eggplant and tomato was first investigated by Doganlar, Frary, Daunay, Lester, and Tanksley (2002). Further work with the same population performed by Doganlar et al. (2014) and Wu et al. (2009) showed that two chromosomes, 1 and 8, were found to be completely syntenic. Moreover, *S. lycopersicum* and *S. lycopersicoides* present complete collinearity (Chetelat & Meglic, 2000); two wild potatoes, *S. ochranthum* and *S. juglandifolium*, showed overall synteny with respect to tomato, with a shared arrangement of chromosome 10 (Szinay et al., 2012). According to Wu and Tanskley (2010), chromosome 1 of tomato is totally collinear with respect to potato and differs from that of eggplant by one inversion. Chromosome 11 of potato differs from that of tomato by one inversion, and chromosome 11 of eggplant is involved in one translocation with chromosome 4 and two inversions. D'Agostino et al. (2013) demonstrated that in *S. dulcamara*, five chromosomes (1, 3, 6, 8, and 9) were completely collinear with the respective tomato counterparts; chromosomes 2, 5, 7, and 10 contain inversions relative to their tomato homeologues, and also detected translocations on chromosomes 4, 11, and 12, as observed in other Solanaceae that have different combination of chromosome arms. All this background suggests that certain chromosomes are unstable and have been rearranged more than once over the evolutionary time. One of the chromosomes that seems to be more stable is chromosome 2 of tomato, which carries the genes of the large ribosomal subunit, whereas chromosome 11, which bears the genes for the small unit, is apparently more unstable. This is consistent with the summary of locations for the 5S and 18 5.8 26S recorded here: the 5s site seems to be more variable in number and position than the 18 5.8 26S. A homeology of chromosome 2 can be established for all *Solanum* species studied with the 18 5.8 26S probe. This chromosome is usually the largest of the complement and rarely also carries the 5S gene in synteny; thus, it can be deduced that translocation between chromosomes 2 and 11 has been infrequent during the *Solanum* evolutionary story.

Rates of chromosomal evolution

Doganlar et al. (2002, 2014) and Wu et al. (2009) estimated for Solanaceae a rate of 0.19 rearrangements per chromosome per million years. This is a moderate rate of chromosome evolution, in which paracentric inversions of conserved syntenic segments would be the primary mechanism involved. According to these authors, translocations would have been of secondary importance in the divergence of eggplant and tomato/potato. In the comparison of the maps of tomato, potato, eggplant (all in *Solanum*) and pepper (*Capsicum*) genomes, Wu and Tanskley (2010) also estimated rates of chromosomal evolution in the Solanaceae, with the calibration point for the tomato-coffee split being 86 mya. They calculated 0.1∼1 inversions per million years and 0.2∼0.4 translocations per million years across different species (i.e., 0.03∼0.12 rearrangements per chromosome per million years). These rates are hard to compare to those from other families due to differences in mapping techniques, criteria to identify rearrangements, and methods for estimating divergence time. Given the mostly constant chromosome number in Solanaceae and similar rates of chromosomal evolution across species, Wu and Tanskley (2010) considered that the family has a modest rate of chromosomal evolution. However, these studies (Doganlar et al., 2002, 2014; Wu et al., 2009; Wu & Tanskley, 2010) were performed by means of parsimony and using at most five species, which does not represent the whole family. They calculated these rates by dividing number of rearrangements between ancestor and living plants by mya since branch split. Parsimony does not take into account the branch length; therefore, it may not be the best model for representing chromosomal evolution, since it is proven that state character reversals often occur and chromosomal features seem to be quite homoplastic (Guerra, 2012; Stace, 2000). Namely, a rate can be obtained with our data, by dividing 30 mya (the age of the crown Solanaceae; Särkinen et al., 2013) by the number of changes in karyotype formula stochastically estimated (88), and one rearrangement (visible by means of the classical technique) per complement every 2.93 mya (or 0.34 rearrangement per million years) is obtained.

Conclusions

(1) According to our results on ASR, the common ancestor to all *Solanum* species was probably a diploid with 2n = 24, with a karyotype with st and/or t

chromosomes, an arm ratio ∼1–1.5, a 2C DNA content of ∼1–1.2 pg, one locus (a pair of signals) of 18–5.8–26S or NORs, one locus of 5S, and both rDNA loci being asyntenic (non-collinear). (2) All chromosome variables behave as homoplastic, in different degrees, with reversals of character states in all branches, and the same character states arising independently several times at different places of the phylogenetic tree. (3) The main evolutionary derivations with respect to the ancestor were: an increase in chromosome size and DNA content in the lineage that originated the Cyphomandra clade; a decrease or loss of st and/or t chromosomes in the Leptostemonum clade; dysploidy in Archaesolanum clade; an increase of polyploidy in the Petota, Carolinense, Elaeagnifolium, and Morelloid clades. (4) The analysed characters can be ordered from more to less conservative, as follows:

Asynteny of rDNA loci (rearrangement involving chromosome 1) → number of 18–5.8–26S sites or NORs → chromosome number → karyotype formula → number of 5S loci.

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