CSIRO PUBLISHING

Australian Journal of Botany
https://doi.org/10.1071/BT17245

Cryptic genetic diversity in *Solanum elaeagnifolium* (Solanaceae) from South America

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Abstract. *Solanum elaeagnifolium* is a global invasive weed that is polyploid in the South American range. Our aim was to establish the origin and distribution of these polyploid lineages, together with their relationship with the invasion of new habitats. Ploidy level was determined in 26 natural populations using chromosomal counts, and two non-coding plastid regions *trnL-trnF* and *rpl32F-trnL* were sequenced. Relationships among haplotypes were examined by a median-joining network, and groups of populations were identified by employing a spatial analysis of molecular variance. Phylogenetic relationships among haplotypes were reconstructed using Bayesian inference, and divergence times were estimated using BEAST. We found 21 haplotypes structured into three lineages: one with diploids in North America and invaded areas, and two in South America that developed independent polyploidisation events. The separation of lineages took place during the Pliocene with more recent divergences in the Early Pleistocene. The existence of three lineages can be attributed to the uplift of the Andes and the emergence of the Isthmus of Panama. Diversification within each lineage may be related to the recent cyclical glacial variation throughout the Pleistocene creating haplotype diversity and recurrent polyploidy. Thus, the South American populations of *S. elaeagnifolium* did not originate from a recent colonisation but are established ancient lineages.

Additional keywords: geological events, haplotypes, invasion, polyploidy, silverleaf nightshade, *Solanum elaeagnifolium*, weed.

Received 12 December 2017, accepted 19 October 2018, published online 21 November 2018

Introduction

Polyploidy is a frequent phenomenon in plants (te Beest *et al*. 2012; Soltis *et al*. 2014), serving as a source of genetic variability. It may lead to direct alterations in the genomic structure of organisms or through interactions with other factors. The effects of polyploidy can be seen through modifications to the cell cycle as well as changes in biomass production and stress resistance (Stebbins 1985; Deng *et al*. 2012). Polyploid species might be successful for many reasons such as positive changes in gene expression (Matzke and Matzke 1998), intergenomic cross talk (Wendel 2000), sexual dimorphism (Miller and Venable 2000) or alterations in plant–animal interactions (Thompson *et al*. 2004). Within a single species, polyploids are mostly formed from different progenitors in separated populations; phylogeographic approaches based on the analysis of DNA sequences have shown that recurrent polyploidy is the rule rather the exception (Soltis and Soltis 1999; Peirson *et al*. 2013). Fragments of the chloroplast genome, which is transmitted primarily via maternal inheritance, are suitable sequences for these type of studies due to a straightforward mode of genetic transmission without recombination (Avise 2000).

The role of genome duplications and changes in genome size during the invasion of new habitats has been the subject of intense debate (Stebbins 1985; Soltis *et al*. 2004). Polyploids frequently have a wider geographical range than their diploid parents (e.g. Schönswetter *et al*. 2007; Whittemore and Olsen 2011) possibly due to being preadapted to habitats and resources inaccessible to their parents (Levin 2004). Polyploids also have a potentially greater diversity of alleles that can confer a larger ecological niche than that of diploid progenitors (Brown 1984; Pound *et al*. 2004). The contemporary distribution of polyploids has been explained in relation to glaciations events, where diploid lineages persisted in refugia whereas tetraploids expanded to previously glaciated area (Burnier *et al*. 2009; Peirson *et al*. 2013). Similar studies undertaken in regions not directly affected by glaciations such as arid habitats that became cooler and drier in Pleistocene times (Rebernig *et al*. 2010), have mostly considered the northern hemisphere, whereas few have addressed occurrences of polyploid populations in relation to glaciations or evolution of arid or semiarid ecogeographical regions in South America (Speranza *et al*. 2007).

Solanum elaeagnifolium Cav., commonly known as 'silverleaf nightshade', is a global invasive weed that causes significant economic damage to human activities (Feuerherdt 2009). The probable native range of *S. elaeagnifolium* is the area around the Texas–Mexico border (Boyd *et al*. 1984), but it has spread into many regions in the world such as South America (Argentina, Chile, Colombia, Perú, Ecuador, Paraguay and Uruguay; Knapp *et al*. 2017), Australia (Gopurenko *et al*. 2014), Israel, Morocco, South Africa, Syria, and Tunisia (Boukhris-Bouhachem *et al*. 2007; Mekki 2007), Europe (Christodoulakis *et al*. 2009) and Turkey (Ilçim and Behçet 2007). In Argentina and Chile, populations with different ploidy levels have been documented and the geographical distribution of these cytotypes is apparently not random (Scaldaferro *et al*. 2012). Diploids are the most widespread cytotype and have been observed in a range of very different habitats whereas tetraploids occur in arid or semiarid regions with a mean annual rainfall of less than 500 mm and hexaploids are successful in colonising wetter areas (Scaldaferro *et al*. 2012).

Considering this background, we employed chromosomal counts and sequenced the intergenic spacer of the chloroplast genome in order to (1) gain an insight into genetic variability of *Solanum elaeagnifolium*, (2) determine if polyploid formation was a recurrent event in the evolutionary history of the species in South America, and (3) establish whether the distribution of genetic variation and cytotypes correspond to geographical patterns. In addition, we aimed to establish the times of haplotype divergence in order to test whether South America is part of the native range of the species or a more recent colonisation event.

We hypothesised that polyploidy has had different independent origins (i.e. recurrent polyploidy). Therefore, we expected that each cytotype would not form a clade or haplotype group but rather, diploid and polyploid individuals would be intermixed in the phylogenetic tree and the haplotype network. We also hypothesised that South America is an invaded range for *S. elaeagnifolium* and hence, we expected that divergence times between North American and South American haplotypes would be short, as low genetic variability is to be expected in South America if populations have recently expanded to this region.

Materials and methods

Plant material

Leaf material and seeds of *Solanum elaeagnifolium* Cav. were obtained from 1–2 individuals in 26 populations across its current native distribution range in Argentina, Chile, México, the United States and invaded places in Australia and Israel. Leaf tissue was dried in silica gel and seeds were preserved at 4°C until use. The provenance, geographical coordinates, and altitude of the studied populations are presented in Table 1. Voucher specimens were deposited in the herbarium of the Museo Botánico de Córdoba, Argentina (CORD).

Ploidy level determination

Mitotic chromosomes were examined in squashes of root tips obtained from germinated seeds. Root tips were pretreated in saturated p-dichlorobenzene at room temperature for 2 h, fixed in 3 : 1 ethanol : acetic acid, and stained with basic fucsin or Giemsa (Jong 1997; Guerra 1983). Slides were made permanent by freezing with liquid CO_2 (Bowen 1956) to remove the coverslip and then mounted with Euparal. The number of individuals per sample and the ploidy level are indicated in Table 1.

Molecular analyses

Total DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Wilkie 1997). Two non-coding regions of the chloroplast genome, *trnL-trnF* and *rpl32F-trnL*, were amplified using primer pairs described by Shaw *et al*. (2007). The PCR mix contained 2 µL of template DNA (10 ng), 0.625U GoTaq DNA polymerase (Promega, Madison, WI, USA), 5 µL of Green GoTaq 5X reaction buffer (Promega), 0.25 mM of each dNTP, and 0.3 µM of each primer in a total volume of 25 µL. The PCR cycling conditions were DNA denaturation at 80°C for 5 min followed by 30 cycles of denaturation at 95°C for 1 min, primer annealing at 50°C for 1 min, followed by a ramp of 0.3°C s^{-1} to 65°C, and primer extension at 65°C for 4 min followed by a final extension step of 5 min at 65°C. All reactions were carried out on an Eppendorf Mastercycler thermocycler (Eppendorf, Hamburg, Germany). Amplification products were separated by electrophoresis on a 1% agarose gel, stained with Syber Safe (Invitrogen, Eugene, OR, USA), and visualised with a UV transilluminator. The PCR-amplified products were sequenced by Macrogen Inc. (Seoul, South Korea). Sequences were deposited in GenBank (Accession numbers: *trnL-trnF*: MH030022-MH030048; *rpl32F-trnL*: MH039236-MH039262). For all subsequent analyses, the two chloroplast regions were concatenated for each individual. Sequences were aligned using MEGA 6.0 (Tamura *et al*. 2013), with manual adjustments made as needed. Gaps were coded following the 'simple indel coding' method (Simmons and Ochoterena 2000).

To examine genetic relationships among haplotypes, a median-joining network was constructed using NETWORK ver. 5.0.1.0 (Bandelt *et al*. 1999). Two ambiguous connections (loops) in the network were resolved using predictions from coalescent theory, principally the geographical criterion (Crandall and Templeton 1993). To identify groups of populations, a spatial analysis of molecular variance SAMOVA ver. 12.02 (Dupanloup *et al*. 2002) was performed based on 100 simulated annealing steps. This program defines geographically homogeneous groups of populations by maximising the differences between them, using the proportion of total genetic variance (FCT). Several runs were performed to evaluate different numbers of groups (K) from K = 2 to K = 20, to find the number of K at which FCT reaches a plateau and the obtained groups of populations have different haplotypes. Molecular diversity indices (Table 2) for *S. elaeagnifolium* species and groups of populations defined by SAMOVA analysis were calculated in ARLEQUIN ver. 3 (Schneider *et al*. 2000).

To estimate the haplotype divergences, we obtained sequences of *trnL-trnF* and *rpl32F-trnL* intergenic spacer regions from GenBank of *Schizanthus grahami* Gillies ex Hook., *Lycium cestroides* Schltdl., *Lycium ciliatum* Schltl.,

Table 1. Collection sites, vouchers, ploidy, haplotypes and SAMOVA groups of *Solanum elaeagnifolium* localities studied

Collector's names: *CA*, MC Acosta, *GB*, GE Barboza, *LB*, L Bohs, *JC*, J Chiapella, *FC*, F Chiarini, *AC*, AA Cocucci, *LG*, L Galetto, *AH*, AT Hunziker, *RJ*, R Juliani, *K*, Kursar, *EM*, EA Moscone, *MS*, MA Scaldaferro. Abbreviations: *NI* , introduction number of IADIZA (Mendoza); *w/n*, without number; *w/v*, without voucher

Locality and voucher specimen	Code	Geographic coordinates	Altitude (m above sea level)	Ploidy level (individuals counted)	Haplotype	SAMOVA group
ISRAEL. *NI 1466*	IS2	32.91°N, 35.10°E	2	2x (9)	H12	Red
UNITED STATES. Utah State, Washington County, near Zion National Park. *LB w/n*	UT2	37.45°N, 113.23°W	1543	2x (3)	H11	Red
UNITED STATES. Texas State, Big Bend National Park. *w/v*	TE2	29.81°N, 103.25°W	927	2x (3)	H12	Red
MEXICO. Durango State, Mapimí Locality. *NI 1503*	DU2	25.84°N, 103.84°W	1288	2x (10)[A]	H17 (2)	Red
MEXICO. Guanajuato State, San Pedro de los Pozos Locality. *FC 1272*	GU2	21.22°N, 100.49°W	2220	2x (3)	H13	Red
AUSTRALIA. New South Wales State, Narrandera Locality. *w/v*	AU2	34.4°S, 146.37°E	133	2x (3)	H18	Red
ARGENTINA. Chaco Province, 1° de Mayo Department. *FC 879*	CC2	27.37°S, 58.99°W	51	2x (3)	H8, H9 (2)	Blue
ARGENTINA. San Luis Province, Pueyrredon Department, El Chorrillo Locality. *NI 1276*	SL4	33.3°S, 66.27°W	785	4x (3)[A]	H7	Blue
ARGENTINA. Río Negro Province, Pichi Mahuida Department, RN 22. *NI 1544*	RN4	39.10°S, 64.67°W	147	4x (9)[A]	H14	Blue
ARGENTINA. Salta Province, General Güemes Department, Rosario de la Frontera Locality. *LG 214*	SA6	25.8°S, 64.97°W	768	6x (3)[A]	H15	Blue
ARGENTINA. Santa Fe Province, 9 de Julio Department, RN 95. *FC 917*	SF6	28.01°S, 61.18°W	56	6x (3)	H19	Blue
ARGENTINA. Santiago del Estero Province, Ojo de Agua Department, Villa Ojo de Agua Locality. *AH 25089*	SG6	29.50°S, 63.69°W	510	6x (9)[A]	H8	Blue
ARGENTINA. Córdoba Province, Capital Department. *FC 1028*	CP6	31.44°S, 64.20°W	423	6x (3)	H8	Blue
ARGENTINA. Córdoba Province, Calamuchita Department, RP 5. *EM 180*	CM6	31.93°S, 64.54°W	830	6x (13)[A]	H8	Blue
ARGENTINA. San Luis Province, Junín Department, Merlo Locality. *RJ 39*	SL6	32.35°S, 65.03°W	808	6x (2)[A]	H20	Blue
ARGENTINA. Formosa Province, Patiño Department, RN 81. *FC 892*	FO2	25.27°S, 59.76°W	87	2x (3)	H4	Yellow
CHILE. Atacama Region. Copiapó Province, Paipote Locality. *AC 369*	CH2	27.40°S, 70.68°W	436	2x (19)[A]	H5	Yellow
ARGENTINA. Santiago del Estero Province, La Banda Department, INTA. *NI 1444*	SG2	27.78°S, 64.25°W	177	2x (10)[A]	H21	Yellow
ARGENTINA. Córdoba Province, Cruz del Eje Department, RN 38. *MS 3*	CE2	30.62°S, 65.51°W	220	2x (8)[A]	H2	Yellow
ARGENTINA. Mendoza Province, Lavalle Department, Telteca Provincial Reserve. *NI 1453*	MZ2	32.39°S, 68.03°W	483	2x (9)[A]	H6	Yellow
ARGENTINA. Neuquen Province, Confluencia Department. *GB 76*	NE2	38.93°S, 69.23°W	612	2x (10)[A]	H3	Yellow

(*continued next page*)

Table 1. (*continued*)

Locality and voucher specimen	Code	Geographic coordinates	Altitude (m above sea level)	Ploidy level (individuals counted)	Haplotype	SAMOVA group
ARGENTINA. Córdoba Province, Minas Department. *GB 2010*	MI4	30.89°S, 65.01°W	564	4x (3)[A]	H2	Yellow
ARGENTINA. La Pampa Province, Lihuel Calel Department. *w/v*	LI4	38.00°S, 65.59°W	338	4x (3)	H3	Yellow
ARGENTINA. Buenos Aires Province, Tornquist Department, Sierra de La Ventana Locality. *GB 2308*	TO4	38.12°S, 61.80°W	246	4x (3)	H1	Yellow
ARGENTINA. Neuquen Province, Zapala Department, San Antonio Locality. *JC 1804*	ZA4	38.91°S, 69.79°W	785	4x (3)	H16	Yellow
ARGENTINA. Buenos Aires Province, Villarino Department, *GB 2321*	VI4	40.14°S, 62.66°W	36	4x (3)	H10	Yellow

[A]Samples for which the ploidy level were extracted from Scaldaferro *et al.* (2012).

Table 2. Molecular diversity indices calculated for *Solanum elaeagnifolium* and each SAMOVA group identified

Haplotype (h) and nucleotide (π) diversity are shown. Abbreviations: *P*, number of analysed populations; *N*, number of analysed individuals; *H*, number of haplotypes; %CG, CG content; Ps, number of polymorphic sites; ti, transitions; tv, transversions; *I*, indels. Alignment size without outgroups and range are indicated in base pairs (bp). Standard errors are indicated in parentheses

Grouping	*P*	*N*	Size	Range	*H*	CG%	Ps	ti	tv	*I*	h	π
S. elaeagnifolium	26	28	1885	1875–1833	21	30.39	39	13	18	8	0.9735 (0.0184)	0.007566 (0.003892)
Blue	9	10	1868	1868–1862	7	30.28	7	3	2	2	0.8667 (0.1072)	0.001405 (0.000926)
Yellow	11	11	1862	1861–1857	9	30.51	6	2	1	3	0.9636 (0.0510)	0.001406 (0.000917)
Red	6	7	1877	1875–1833	5	30.35	16	4	6	6	0.9048 (0.1033)	0.008321 (0.004857)

Capsicum baccatum L., *Capsicum chacoense* Hunz., *Solanum comptum* C.V. Morton, *Solanum hieronymi* Kuntze, *Solanum melongena* L., *Solanum houstonii* Martyn, *Solanum tridynamum* Dunal, *Solanum tuberosum* L., and *Solanum lycopersicum* L. The split of the genus *Schizanthus* was considered the root of the tree and was set to 30 million years ago (Mya). Additional time constraints were set for the split of *Lycium* from *Solanum* and *Capsicum* at 21 Mya, the split between *Solanum* and *Capsicum* genera at 19.1 Mya, the split of *S. melongena* from *S. tuberosum* and *S. lycopersicum* at 14.3 Mya, and between *S. tuberosum* and *S. lycopersicum* at 8 Mya; all dates were taken from Särkinen *et al*. (2013). Divergence times were estimated using BEAST version 1.6.2 (Drummond and Rambaut 2007). The input file was prepared in BEAUti ver. 1.6.2 (provided in the BEAST package). The substitution model was GTR with a Gamma site heterogeneity model with four categories, following the results of MrModelltest ver. 2.2 (Nylander 2004); the clock was set as an uncorrelated lognormal relaxed model, and the Yule process was selected as a prior for the distribution of divergence dates. The Monte Carlo Markov Chain was set to run for 20×10^6 generations, sampling every 1000 cycles.

Results

Three ploidy levels based on x = 12 were identified: diploid, tetraploid and hexaploid (Fig. 1; Table 1). All the analysed populations were monocytotypic. The geographical distribution of each ploidy level is presented in Fig. 2 and Table 1. The sequence analyses of 28 individuals sampled from 26 populations yielded 21 distinct cpDNA haplotypes (Table 1, Fig. 2). The length of *trn*L-*trn*F varied from 892 in haplotype H18 to 917 bp in haplotype H21, whereas the intergenic spacer *rpl*32F-*trn*L was more variable and ranged in size between 941 (H1, H2, H4, H18) and 959 bp (H11). Alignment length of the final matrix was 1884 bp and required the introduction of 7 gaps, ranging from 1 to 24 bp in length. Haplotype H18 had one deletion of 24 bp in *trnL-trnF* and haplotype H11 had one insertion of 16 bp in the *rpl*32F-*trn*L spacer. In addition to minor insertion of 1 or 2 bp, single-base deletions were found in two poly-A regions at *rpl*32F-*trn*L.

SAMOVA identified three groups of populations in the *S. elaeagnifolium* samples. The FCT value in K = 3 was 0.834 ($P < 0.0001$). One group consisted of populations from North America (NA) and the invasive range, here defined as the red lineage, and the other two groups were found in South America (SA) and are designated as the blue and yellow lineages. The configuration of the haplotype network (Fig. 2) was consistent with the groups defined by SAMOVA. We noted that the yellow group found in South America is more related to the red group from North America than to the blue lineage. The red group includes localities with only diploid individuals. Samples outside the natural range have the same haplotypes as or haplotypes derived from those found in the North American localities, suggesting that North America has been the source of these invasions (Fig. 2). According to the haplotype network,

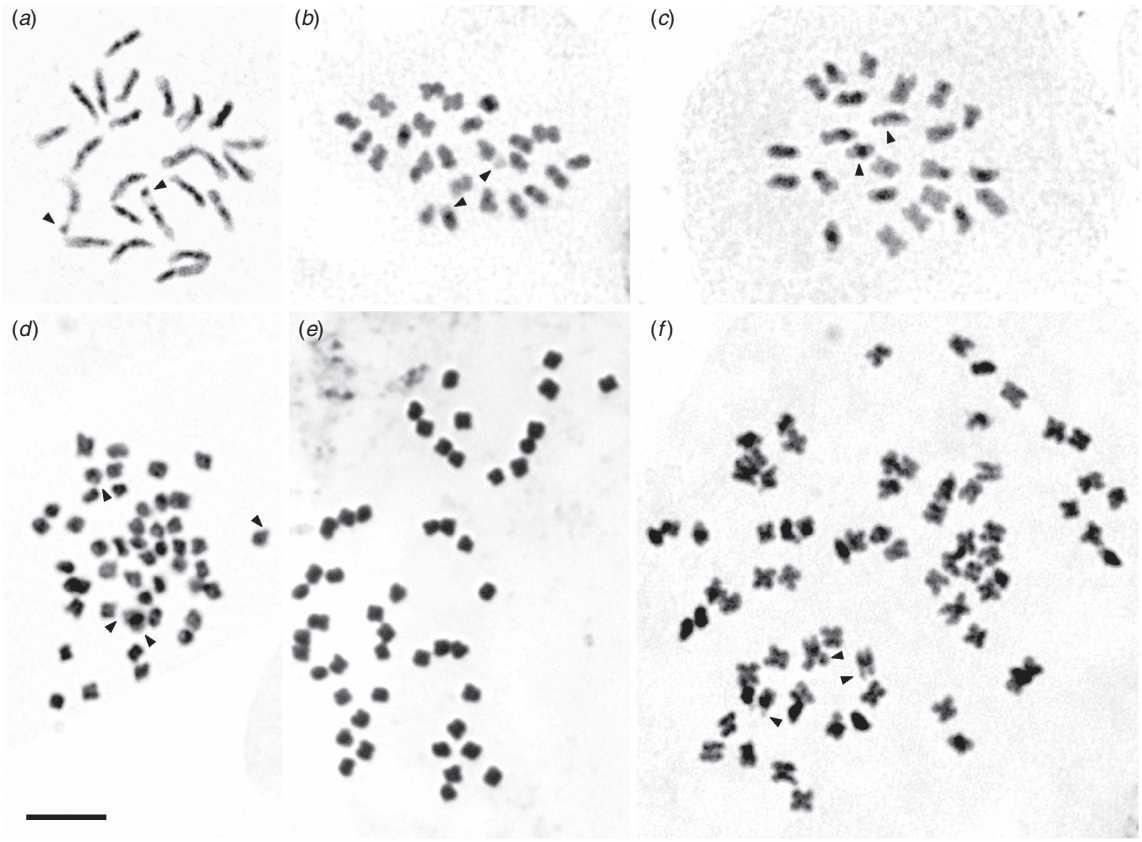

Fig. 1. Photomicrographs of somatic metaphases of *Solanum elaeagnifolium* accessions with different ploidy levels from the different chloroplast lineages. (*a*) Diploid cytotype ($2n = 24$), red lineage (NI 1466). (*b*) Diploid cytotype ($2n = 24$), blue lineage (FC 879). (*c*) Diploid cytotype ($2n = 24$), yellow lineage (NI 1453). (*d*) Tetraploid cytotype ($2n = 48$), blue lineage (NI 1544). (*e*) Tetraploid cytotype ($2n = 48$), yellow lineage (GB 2308). (*f*) Hexaploid cytotype ($2n = 72$), blue lineage (FC 917). Arrows indicate nucleolar organising regions (NORs). Scale = 5 µm.

polyploidy in South America has appeared independently several times. The yellow and blue groups have diploid and tetraploid cytotypes, whereas hexaploid samples were found only in the blue group (Fig. 2). Within the blue lineage, H8 was the most frequent and widespread haplotype and is central in the network. Within the yellow group, H2 and H3 were found in two localities each. Diploid localities from the blue lineage were only found in the Eastern Chaco district, whereas diploid localities of the yellow group were distributed in the Monte Province and Western Chaco. Polyploids from both lineages were found all throughout the *S. elaeagnifolium* distribution range in South America.

The phylogenetic tree (Fig. 3) yielded three major divergent clades in *S. elaeagnifolium* with high support values: a blue group (H7-H9, H14-H15, H19-H20; BPP (Bayesian posterior probability) = 1.00), a red group (H11-H13, H17-H18), and a yellow group (H1-H6, H10, H16, H21; BPP = 1.00). This topology is consistent with the groups previously described by SAMOVA and network. The blue lineage diverged at ~4.5 Mya (95% confidence and highest posterior density intervals = 2.20–7.76). The yellow group is more related to the red group. The divergence between the yellow and red clades was dated in 3.24 Mya (1.39–5.91). Most of the haplotype divergences, which are also the latest ones, took place between 1.85 Mya (0.58–3.84) and 1.57 Mya (0.42–3.56).

Discussion

We found recurrent polyploidy in *S. elaeagnifolium*. None of the cytotypes formed a clade or a haplotype group, and polyploid individuals appeared intermixed in the phylogenetic tree and in the haplotype network. Previous cytogenetic studies (Scaldaferro *et al*. 2012; Chiarini 2014) also provided evidence of recurrent polyploidy in *S. elaeagnifolium*. Polyploids increased their FISH marks proportionally to the ploidy level, which suggests that the polyploid series was recently formed and drastic rearrangements have not yet occurred (Chiarini 2014). However, the differences in the intensity of these marks in the 4x and 6x individuals could indicate that some loss of gene copies might be taking place at these loci, as demonstrated in *Nicotiana* L. (Renny-Byfield *et al*. 2012). Polyploidy independently arising several times in different populations of a single species has been observed in other plant families (e.g. Soltis and Soltis 1999). In fact, molecular data have revealed that most polyploid plant species are of multiple origin, with over 45 examples of recurrent polyploidy (Soltis and Soltis 1999, 2000), and with only a few examples of polyploid plants for which a single origin is probable (e.g. Kochert *et al*. 1996; Ainouche *et al*. 2004).

Solanum elaeagnifolium is characterised by notable phenotypic plasticity. Individual plants may vary in height,

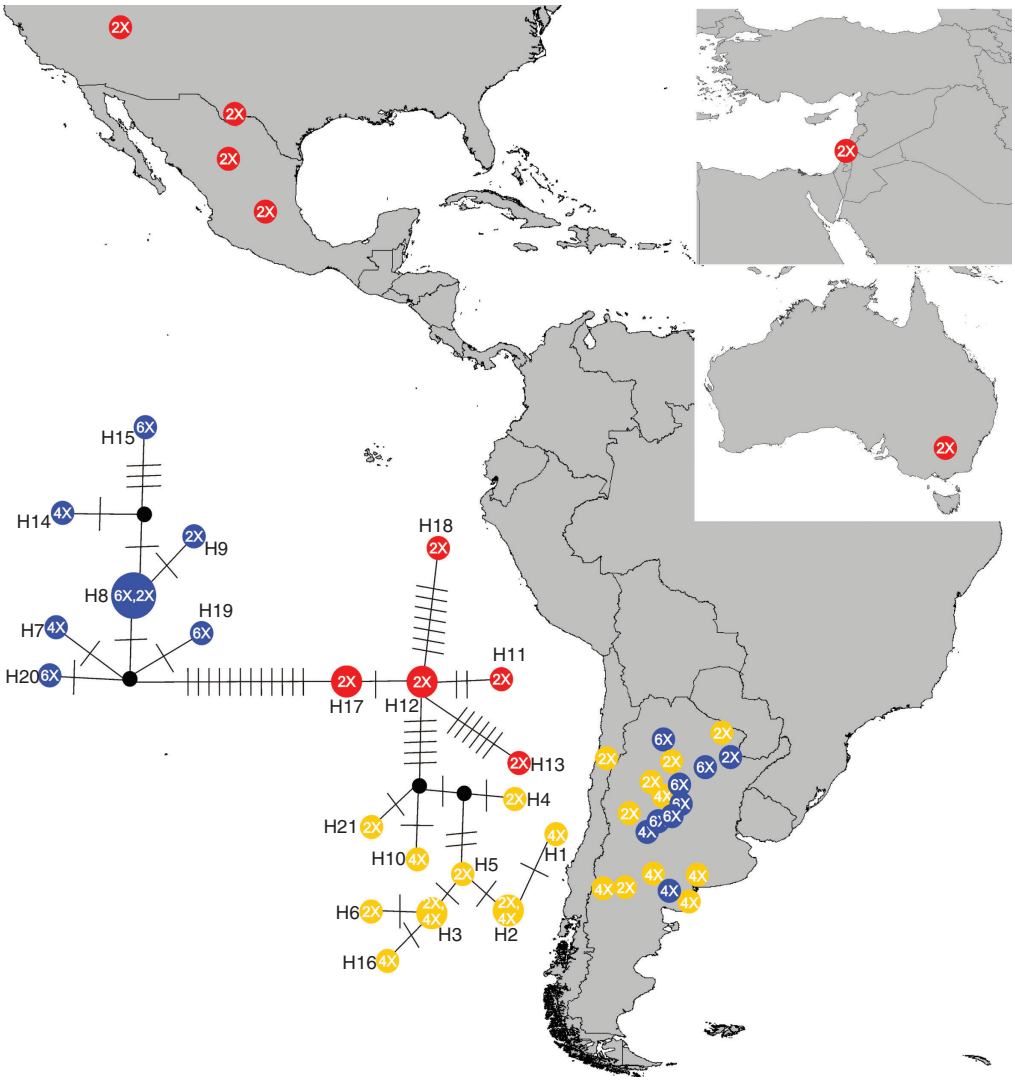

Fig. 2. Geographical distribution and genealogical relationships of the chloroplast DNA haplotypes found in *Solanum elaeagnifolium*. Lineages of chloroplast DNA haplotypes following SAMOVA results are indicated in color. Ploidy level is shown in the haplotype circle.

leaf size, leaf shape and proportions, number of prickles, fruit size, seed size and colour, number of seeds per fruit, flower dimensions and corolla colour (Chiarini and Barboza 2007; Mekki 2007; Chiarini 2014; Knapp *et al*. 2017). Not surprisingly, variability of *S. elaeagnifolium* also involves plastid DNA sequences. Here, a great haplotype diversity was found with 28 individuals having 21 different haplotypes ($h = 0.974$). This genetic study allowed us to classify the samples into three lineages, one restricted to North America (and the introduced range) whereas the two other lineages are growing in South America. The existence of markedly different lineages within *S. elaeagnifolium* was also observed by Gopurenko *et al*. (2014). Using the trnS–trnG intergenic spacer region, these authors inferred a genealogy among identified haplotypes of *S. elaeagnifolium* and compared the array of haplotypes detected in Australia to those found elsewhere. These authors concluded that all haplotypes observed in Argentina were unique to this country, indicating some degree of isolation of the *S. elaeagnifolium* populations in Argentina from the North American populations. The Argentine samples used in that study probably belonged to the 'blue lineage' observed here, given the degree of separation with respect to samples from Mexico, USA, South Africa and Australia.

According to our chronogram (Fig. 3), there is a notable time separation between the yellow and the blue lineages with divergence time calculated for the split of the two lineages being longer than the time estimated for the separation of two different species, such as *S. comptum*/*S. hieronymi* or *S. houstonii*/*S. tridynamum*. Indeed, although the two lineages are morphologically indistinguishable (for the time being), the difference in their chloroplast DNA sequences is similar to that of recognised species. This situation suggests two questions. First, are South American *S. elaeagnifolium* populations really a complex of cryptic species with different ploidy levels? And second, whether introgression from an unknown

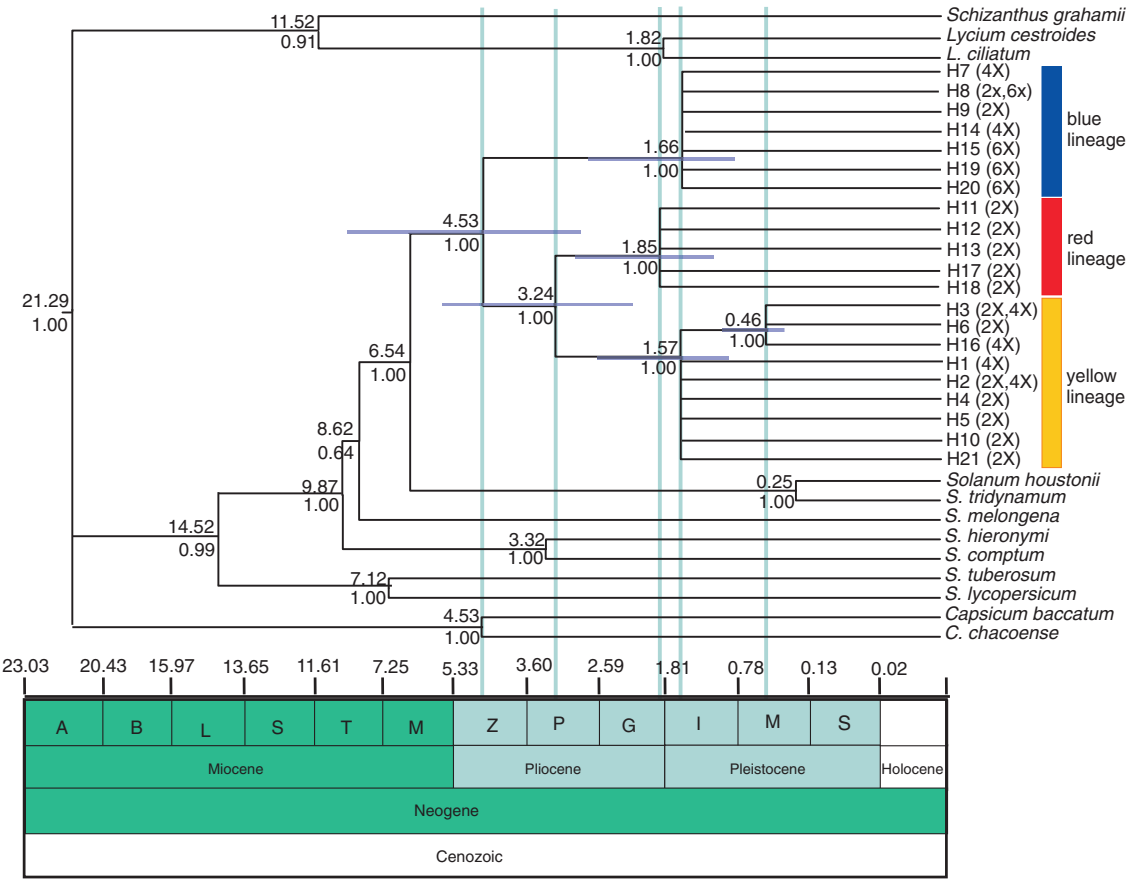

Fig. 3. Chronogram showing relationships among chloroplast haplotypes and lineages found in *S. elaeagnifolium*. Numbers above branches indicate the ages of nodes (millions of years ago) from the molecular dating analysis. Numbers below branches indicate posterior probabilities from Bayesian inference analysis (BPP).

or extinct species occurred in the past, resulting in the observed genetic variability and whether the hexaploids are old allopolyploids?

To answer the first question, morpho-anatomical and crossing studies are needed. We consider the term 'cryptic genetic diversity' describes this unexpected high variability detected by our molecular analyses since morphologically *S. elaeagnifolium* is considered to be a single species. Cryptic genetic diversity was also observed in other taxa where several populations were originally considered to be part of a single morphologically variable species and were later demonstrated to form a complex of several species (Flatscher *et al*. 2015). Further studies are needed to determine if there are other features (e.g. nuclear DNA data, morphology, ecological niche preferences) that distinguish these three lineages of *S. elaeagnifolium* to allow us to propose it as a complex of cryptic species.

In regards to the second question, a similar situation was postulated for *Cherleria sedoides* L., Caryophyllaceae (Moore *et al*. 2017). Further studies may answer this latter question including haplotype nets with nuclear markers using a broader taxon sampling as well as genomic *in-situ* hybridisation assays. Considering the pattern of phylogenetic relationships and variable ploidy levels of *S. hieronymi*, Wahlert *et al.* (2014) suggested a speciation process of hybridisation or introgression involving a co-occurring member of the Elaeagnifolium clade as the male parent, and a South American species from the Carolinense clade as the female parent.

The precise geographical origin of *S. elaeagnifolium* is a matter of controversy (Goeden 1971; Boyd *et al*. 1984). According to Levin *et al*. (2006), the strongly supported sister relationship with the Mexican species *S. tridynamum* and *S. hindsianum* indicates that the native range of *S. elaeagnifolium* could be in North America, with subsequent introduction to South America. At the same time, however, these authors suggested that plants of the disjunct areas should be examined in more detail using morphological and molecular approaches to confirm that these were conspecific. Later, a molecular phylogenetic study of the *Solanum* Carolinense clade (Wahlert *et al*. 2014) showed that *S. elaeagnifolium* was also related to *S. mortonii* and *S. homalospermum*, both endemic to Argentina. Thus, distribution information of related species does not clarify the issue of the origin of *S. elaeagnifolium*. According to our molecular dating, the presence of *S. elaeagnifolium* in South America is not due to an anthropogenic introduction, but rather it is because South American populations are native, especially considering that divergence of the lineages was within the Pliocene.

In addition, the haplotype variation found in South America (blue lineage h = 0.867; yellow, h = 0.964) is comparatively higher than the variation in an invaded range like Australia (h = 0.358; Gopurenko *et al*. 2014) further suggesting that South American populations are native rather than invaders. Further studies, with a higher number of samples from North America, are needed to establish the hemisphere where *S. elaeagnifolium* originated.

Complex geological scenarios such as orogeny and ice ages have been extensively used to explain the diversification observed in the biota (Hewitt 2001; Hoorn *et al*. 2010). The uplift of the mountains in the American continent ended in Pliocene times (Ortiz-Jaureguizar and Cladera 2006; Catalano *et al*. 2008), which is concomitant with the beginning of diversification into three lineages found in *S. elaeagnifolium* (dated at 4.5 Mya). This uplift established the rain shadow effect and the subsequent evolution of arid and semiarid ecogeographical regions. The distribution of the blue lineage only in Eastern Chaco indicates that some populations took refuge in humid biomes and diverged from the two other lineages. Scaldaferro *et al*. (2012) also noticed that hexaploids (included in the blue lineage) are generally distributed in humid areas. The completion of the Panamian Land Bridge linking North and South American semideserts ~3 Mya (Ortiz-Jaureguizar and Cladera 2006) would coincide with the existence of a common ancestor of the red and yellow lineages – estimated at 3.23 Mya in this work. The presence of this corridor would have allowed the movement of the hypothetical ancestor between North and South America. Finally, the remarkable haplotype diversification observed within each of the lineages may be related to more recent geological events. A major glaciation complex was recorded in Patagonia at ~2.3 Mya and continued until the Great Patagonian Glaciation ~1.5 Mya (Rabassa and Coronato 2009). These great glaciation processes increased the xeric conditions and have probably fragmented some areas of species distribution facilitating the occurrence of the high haplotype diversity observed in *S. elaeagnifolium* lineages (1.85–1.57 Mya).

Most of the explanations of the short-term success of polyploids are focussed on the effects of genomic changes and increased genetic variation, which can potentially affect the morphology, physiology and ecology of newly formed polyploids (Van de Peer *et al*. 2017). Thus, the increased genetic variation in *S. elaeagnifolium* polyploids might also lead to increased tolerance to a broader range and environmental conditions than that of diploid congeners. There is evidence that polyploidy affects the expression of genes involved in stress and hormone responses and that the production of unreduced gametes can be increased by changes in the temperature (Van de Peer *et al*. 2017). Polyploidisation was suggested as an important mechanism for generating new evolutionary lineages in areas that were glaciated during the Pleistocene in Brassicaceae (Jordon-Thaden and Koch 2008), Plantaginaceae (Albach 2007) and in Ranunculaceae (Hörandl *et al*. 2005). Several studies also address the dominant role played by polyploids in the Quaternary evolutionary history of the Arctic flora (e.g. Brochmann and Brysting 2008). In the same way, polyploidy would have facilitated the colonisation of *S. elaeagnifolium* to environments previously affected by glaciations, and there has probably promoted the secondary contact between the lineages. Further studies are necessary to establish the times and directions of these re-colonisations.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 112-201001-00153, PIP 112-201501-00690), Ministerio de Ciencia y Tecnología de la Provincia de Córdoba, and Secretaría de Ciencia y Tecnología (SECYT-UNC) (PID no. 203/14, 30720150100073CB). FCH, MAS, GB, and MCA are members of Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina and Universidad Nacional de Córdoba, Argentina. The authors thank Dr H. Wu (Charles Sturt University, Australia) who kindly provided some leaf material, L. Caeiro who provided valuable help in the laboratory, and J. Brasca who help in the edition of the text. Finally, thanks to two anonymous reviewers for suggestions in improving the paper.

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Handling Editor: XXX