NRC Research Press

ARTICLE

Inferences on the origins of polyploid *Turnera* species (Passifloraceae) based on molecular data

Alicia López, Aveliano Fernández, and Joel S. Shore

Abstract: We explore the evolution of polyploids in subseries *Turnera*, testing hypotheses on their origins using DNA sequences (partial *ndhF* and *trnT-L*) from the plastid genome, as well as sequences of the nuclear ribosomal internal transcribed spacer (ITS). We construct phylogenies (with both Bayesian and maximum parsimony methods) using both the plastid and ITS sequences. We test hypotheses concerning the genome contributors to polyploids where previous cytogenetic studies had designated various diploid species as possessing A or C genomes and had proposed various genomic constitutions for the polyploids. Our analyses support the occurrence of a C genome clade of species and the origin of autooctoploid *T. fernandezii* Arbo from *T. grandiflora* (Urb.) Arbo (a C genome diploid). Nuclear ITS data support the hypothesis that *T. concinna* Arbo (an A genome species) contributed a genome to the segmental allotetraploid *T. grandidentata* (Urb.) Arbo, whereas analyses of *ndhF* and *trnT-L* sequences did not lead to identification of the plastid (or additional nuclear genome) donor. Our analyses support the origins of allooctoploids *T. aurelii* Arbo and *T. cuneiformis* Poir. from hexaploid *T. orientalis* (Urb.) Arbo. We found no evidence that hexaploid *T. velutina* Presl. possesses a C genome. We provide evidence, using Bayes factors, supporting the hypothesis that the segmental allohexaploids have had independent origins.

Key words: cpDNA, ITS, *Turnera*, Passifloraceae, phylogeny, polyploidy.

Résumé : Les auteurs ont exploré l'évolution des polyploïdes de la sous-série *Turnera*, en testant des hypothèses sur leurs origines et utilisant les séquences ADN (*ndfF* partiel et *trnT-L*) du génome plastidique ainsi que des séquences de l'espaceur interne transcrit de l'ADN ribosomal (ITS). Ils ont construit des polygénies (avec les méthodes Bayésienne et de la parsimonie maximale) en utilisant à la fois les séquences plastidiques et les ITS. Ils ont testé des hypothèses portant sur les génomes contribuant aux ploïdies où des études cytogénétiques antérieures avaient désigné diverses espèces diploïdes comme possédant les génomes A ou C et avaient proposé diverses constitutions génomiques pour les polyploïdes. Les analyses effectuées supportent l'occurrence d'un clade d'espèces à génome C et l'origine de l'autooctoploïde du *T. fernandezii* Arbo à partir du *T. grandiflora* (Urb.) Arbo (un génome diploïde C). Les données de l'ITS nucléique supportent l'hypothèse que le *T. concinna* Arbo (une espèce à génome A) a contribué au génome à l'allélotétraploïde *T. grandidentata* (Urb.) Arbo, alors que les analyses des séquences *ndhF* et *trnT-L* ne conduisent pas à l'identification du donneur plastidique (ou génome nucléique additionnel). Les analyses supportent les origines des espèces allooctoploïdes *T. aureli* Arbo et *T. cuneiformis* Poir. à partir de l'hexaploïde *T. orientalis* (Urb.) Arbo. Les auteurs ont trouvé aucune preuve que l'héxaploïde *T. velutina* Presl. possède un génome C. Ils présentent une preuve, en utilisant les facteurs Bayésien, supportant l'hypothèse que les allohéxaploïdes segmentaires auraient eu des origines indépendantes.

Mots-clés : ADNcp, ITS, *Turnera*, Passifloraceae, phylogénie, polyploïde.

Introduction

The origin of polyploid plants has been a subject of considerable interest in evolutionary biology and agriculture given the prevalence of polyploid species in the Angiosperms. The advent of molecular methods has provided new insights into the evolution of polyploids, allowing tests of hypotheses pertaining to progenitor–descendant relationships, and the demonstration of multiple origins of polyploids (e.g., Soltis and Soltis 1999; Doyle et al. 2003, 2004; Wendel and Cronn 2003; Soltis et al. 2004). More recently, the exploration of evolutionary changes subsequent to the evolution of polyploids has been undertaken. This has included investigations of genomic reorganization and changes in gene expression (Wendel et al. 1995; Adams and Wendel 2004; Levi and Feldman 2004; Hegarty and Hiscock 2008; Ilut et al. 2012). The time frame and causes of such changes have also been explored by comparing synthetic and natural polyploids (Song et al. 1995; Hegarty and Hiscock 2008; Chang et al. 2010). The application of next-generation sequencing to polyploid evolution has just begun and should allow considerable progress to be made in extending analyses to non-model plant species (Buggs et al. 2012; Ilut et al. 2012).

Polyploidy is a significant evolutionary process in the genus *Turnera*, with approximately 60% of chromosome counts indicating polyploidy (Solís Neffa and Fernández 2000; Fernández and Solís Neffa 2004; Shore et al. 2006). Here we focus on polyploidy in a monophyletic group, subseries *Turnera* ($x = 5$), where ploidy levels range from diploid to octoploid, including allopolyploids, segmental allopolyploids, and autopolyploids (Fernández 1987; Solís Neffa and Fernández 2000; Truyens et al. 2005; Shore et al. 2006; Arbo and Espert 2009). The nature and origins of polyploids in various species have been defined largely on the basis of chromosome pairing within species and in artificial interspecific hybrids (summarized in Fig. 1).

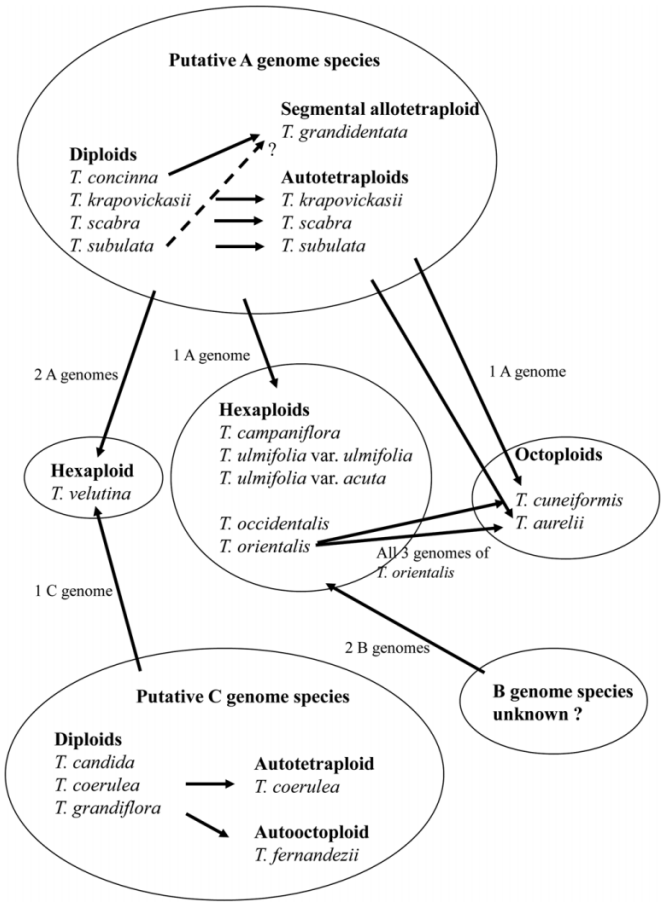
Species within subseries *Turnera* include the *T. ulmifolia* L. complex originally recognized by Urban (1883) and species investi-

Received 6 June 2012. Accepted 8 December 2012.

A. López. Instituto de Botánica Darwinion (CONICET – ANCEFN), Labardén 200, CC 22, B1642HYD, San Isidro, Buenos Aires, Argentina.
A. Fernández. Instituto de Botánica del Nordeste (UNNE – CONICET), Sargento Cabral 2131, CC 209, 3400, Corrientes, Argentina.
J.S. Shore. Department of Biology, York University, 4700 Keele Street, Toronto, ON M3J 1P3, Canada.
Corresponding author: Alicia López (e-mail: alopez@darwin.edu.ar).

Published at www.nrcresearchpress.com/cjb on 2 January 2013.

Fig. 1. Scheme summarizing the genomic constitutions of species and origins of polyploids based on cytogenetic analyses.



gated and described more recently (Arbo and Fernández 1983, 1987; Fernández 1987, 1997; Fernández and Arbo 1989, 1990, 1993*a*,1993*b*, 1996, 2000*a*, 2000*b*; Solís Neffa and Fernández 1993, 2000; Arbo 2005). Morphological, cytogenetic, and molecular phylogenetic analyses support monophyly of series *Turnera* (Arbo and Fernández 1983, 1987; Fernández 1987, 1997; Fernández and Arbo 1989, 1990, 1993*a*, 1993*b*,1996, 2000*a*, 2000*b*; Fernández and Solís Neffa 2004; Truyens et al. 2005; Solís Neffa 1996; Solís Neffa and Fernández 1993, 2000; Arbo and Espert 2009). Cytogenetic analyses have resulted in the assignment of different genome designations to various species (Table 1; Fig. 1). Diploid yellow-flowered species (*T. concinna* Arbo, *T. subulata* Smith, *T. scabra* Millspaugh, and *T. krapovickasii* Arbo) possess variants of an "A" genome. Diploid white- and blue-flowered species (*T. coerulea* DC., *T. grandiflora* (Urb.) Arbo, and *T. candida* Arbo) have the "C" genome designation. No viable diploid hybrids have been produced in crosses between the A and C genome species, and no diploid species have been assigned to the B genome (Fernández and Arbo 1989, 1990, 1993*a*, 1993*b*, 1996).

Species showing high frequencies of quadrivalents and tetrasomic inheritance include autotetraploid accessions of the A genome species, *T. krapovickasii*, *T. scabra*, and *T. subulata* (Fernández and Arbo 1989; Shore 1991*a*, 1991*b*). *Turnera fernandezii* Arbo appears to be an autooctoploid derived from a diploid C genome species, *T. grandiflora* (Fernández 1987; Fernández et al. 2010). *Turnera grandidentata* (Urb.) Arbo ($A^gA^gA^rA^r$; Fernández and Arbo 1990), a segmental allotetraploid, may have close affinities with diploid *T. concinna*, based on cytogenetic analyses (Fernández and Arbo 1993*a*, 1993*b*), geographic overlap, and phylogenetic analysis using nuclear ribosomal internal transcribed spacer (ITS) sequences (Truyens et al. 2005). Strong genomic in situ hybridization signals to one genome of *T. grandidentata* using a *T. subulata* probe suggest that it possesses two A genomes, yet weaker hybridization to the remaining chromosomes indicates considerable homeology among the genomes that it possesses (López et al. 2010*a*). Species exhibiting partly homologous chromosome sets are considered segmental allopolyploids (Solís Neffa and Fernández 2000).

The origins of the hexaploid species are uncertain. All appear to form only bivalents at first metaphase of meiosis, suggesting that they are allopolyploids. Meiotic studies of artificial interspecific hybrids and genomic in situ hybridization (GISH), however, support segmental allopolyploid origins for *T. occidentalis* Arbo & Shore, *T. orientalis* (Urb.) Arbo, *T. ulmifolia* L., and *T. velutina* Presl. (López et al. 2010*a*, 2010*b*). Hexaploids *T. orientalis* ($A^oA^oBBB^oB^o$) and *T. occidentalis* ($A^{oc}A^{oc}BBB^oB^o$) are karyotypically similar (López et al. 2010*b*) and may share a single origin. Two octoploid species, *T. cuneiformis* Poir. ($A^{cu}A^{cu}A^oA^oBBB^oB^o$) and *T. aurelii* Arbo ($A^aA^aA^oA^oBBB^oB^o$), are thought to share three genomes in common with hexaploid *T. orientalis* (Table 1; Fig. 1; Fernández and Arbo 2000*a*). *Turnera ulmifolia* likely had an independent hexaploid origin as it is hypothesized to carry different variants of the A and B genomes ($A^uA^uB^aB^aB^uB^u$) in contrast to those of *T. orientalis* and *T. occidentalis* (Table 1; Fig. 1; Fernández and Arbo 1993*b*; Fernández and Solís Neffa 2004; López et al. 2010*a*).

Although a number of possible A genome diploids have been described (Table 1; Fig. 1), the putative B genome donor(s) of the hexaploids and octoploids remain unknown. Hexaploid *T. velutina* may possess a C genome ($AAA^vA^vC^vC^v$; Fernández and Arbo 2000*b*; López et al. 2010*a*), donated from a *T. coerulea*-like progenitor (Arbo 2005). Artificial hybrids between *T. velutina* and *T. grandiflora* (C^gC^g; Fernández and Arbo 1993*b*) showed nine bivalents, consistent with some level of homology between the *T. grandiflora* genome and one of the genomes of *T. velutina* (Fernández and Arbo 2000*b*). The possession of a C genome is also supported by GISH (López et al. 2010*a*).

The purpose of this study is to gain further insight into the origins of polyploid species of subseries *Turnera* by testing hypotheses generated from previous biosystematic studies. We carry out phylogenetic analyses using two sequences from the chloroplast genome (portions of the *ndhF* gene and *trnT-L*) in an attempt to discover the plastid donors of the polyploids and gain insight into their origins. The *ndhF* gene has been successfully used in a molecular phylogenetic analysis of *Passiflora* (Muschner et al. 2003). Sequences of *trnL-F* have been informative in a biogeographical analysis of the *Turnera sidoides* complex in series *Leiocarpae* (Speranza et al. 2007) and for higher level phylogenetics of the Turneraceae clade of the Passifloraceae (Thulin et al. 2012). Note that the species of subseries *Turnera* that have been investigated, exhibit variable and paternally biased plastid inheritance such that 68% of plastid transmission is paternal, 17% is maternal, and 15% is biparental (Shore et al. 1994; Shore and Triassi 1998). Analyses of sequence data from the chloroplast genome will not necessarily reflect the maternal lineage. Finally, we compare our analyses of cpDNA sequence data with a re-analysis of nuclear ITS sequences of Truyens et al. (2005).

Materials and methods

We obtained *ndhF* and *trnT-L* sequences from 31 accessions of 19 taxa (18 species including two taxonomic varieties of *T. ulmifolia*) of *Turnera*, with 17 taxa belonging to *Turnera* subseries *Turnera* Arbo. *Turnera pumilea* L. (series *Leiocarpae* Urb.) and *T. joelii* Arbo (series *Turnera* subseries *Umbilicatae* Arbo) were chosen as more distantly related species, with the former used as an outgroup in analyses. Our sample includes all but three species of subseries *Turnera* (South American *T. lucida* Urb. and two African species, *T. thomasii* (Urb.) Story and *T. oculata* Story). Accessions were sampled as seed collected in the field and subsequently grown in the glass-

Table 1. Species, code to distinguish accessions of the same species, ploidy level, putative genomic constitution, genome size, collector and locality, and GenBank accession numbers for *trnT-L*, *ndhF*, and ITS.

Species	Code	Ploidy level	Genome constitution[a]	Genome size[b]	Collector and locality	Accession numbers		
						trnT-L	ndhF	ITS
A Genome								
T. concinna		2*x*	$A^{co}A^{co}$		Arbo et al. 8900, San Pedro, Paraguay	JQ771880	JQ771849	AY973353
T. grandidentata		4*x*	$A^{g}A^{g}A^{r}A^{r}$	0.99	Arbo 6109, Paraguarí, Paraguay	JQ771878	JQ771847	AY973354
T. krapovickasii	K2	2*x*	$A^{k}A^{k}$	0.91	Ahumada 4549, Tarija, Bolivia	JQ771887	JQ771856	
T. krapovickasii	K4	2*x*	$A^{k}A^{k}$		Krapovickas 39099, Puerto Margarita, Bolivia	JQ771885	JQ771854	
T. krapovickasii	46355	2*x*	$A^{k}A^{k}$		Krapovickas and Cristobal 46355, Salta, Argentina			AY973355
T. krapovickasii	K5	4*x*	$A^{k}A^{k}A^{k}A^{k}$	0.80	Schinini 19514, El Quebrachal, Anta, Salta, Argentina	JQ771888	JQ771857	
T. krapovickasii	K7	4*x*	$A^{k}A^{k}A^{k}A^{k}$	0.84	Krapovickas and Cristobal 40303, El Quebrachal, Anta, Salta, Argentina	JQ771886	JQ771855	
T. scabra	BR	2*x*	$A^{sc}A^{sc}$		Barrett 1128, Barreirinhas, Brazil	JQ771879	JQ771848	
T. scabra	CR	2*x*	$A^{sc}A^{sc}$		Cascante and Vargas 315, Santa Rosa, Costa Rica	JQ771882	JQ771851	
T. scabra	ES	2*x*	$A^{sc}A^{sc}$		Shore 150, El Salvador	JQ771881	JQ771850	
T. scabra	MI	2*x*	$A^{sc}A^{sc}$		Athanasiou 1, Isla Margarita, Venezuela	JQ771889	JQ771858	
T. scabra	NI	2*x*	$A^{sc}A^{sc}$		Shore 308, Managua, Nicaragua	JQ771883	JQ771852	AY973358
T. scabra	CO	4*x*	$A^{sc}A^{sc}A^{sc}A^{sc}$		Barrett 689, Dagua, Colombia	JQ771884	JQ771853	AY973357
T. scabra	DR	4*x*	$A^{sc}A^{sc}A^{sc}A^{sc}$		Shore 303, San Fco de Macoris, Domincan Republic	JQ771891	JQ771860	same as AY973356
T. subulata	2*x*	2*x*	$A^{su}A^{su}$	0.69	Arbo 2410, Sao Luis, Maranhao, Brazil	JQ771894	JQ771863	
T. subulata	1374	2*x*	$A^{su}A^{su}$		Barrett and Shore 1374, Arco Verde, Brazil			AY973356
T. subulata	1380	4*x*	$A^{su}A^{su}A^{su}A^{su}$		Barrett and Shore 1380, Recife, Brazil			same as AY973356
T. subulata	4*x*	4*x*	$A^{su}A^{su}A^{su}A^{su}$	0.67	Krapovickas and Cristóbal 37115, Pará, Santarem, Brazil	JQ771890	JQ771859	
C Genome								
T. candida		2*x*	$C^{c}C^{c}$		Jardim et al. 2588, Bahia, Brazil	JQ771866	JQ771835	AY973349
T. coerulea		4*x*	CCCC		Wood 7989, Tomina, Bolivia	JQ771867	JQ771836	AY973348
T. fernandezii		8*x*	$C^{g}C^{g}C^{g}C^{g}C^{g}C^{g}C^{g}C^{g}$	0.74	Dematteis et al. 2908, Amambay, Paraguay	JQ771868	JQ771837	AY973352
T. grandiflora	AY97	2*x*	$C^{g}C^{g}$		Orginal source unknown, cultivated at Univ. Toronto			AY973351
T. grandiflora	G11	2*x*	$C^{g}C^{g}$	0.81	Fernández 366, Corrientes, Argentina	JQ771870	JQ771839	
T. grandiflora	G12	2*x*	$C^{g}C^{g}$		Arbo 8884, Amambay, Paraguay	JQ771869	JQ771838	
T. grandiflora	Schin	2*x*	$C^{g}C^{g}$		Schinini et al. 19260, Corrientes, Argentina			AY973350
Allopolyploids								
T. aurelii		8*x*	$A^{a}A^{a}A^{o}A^{o}BBB^{o}B^{o}$	0.70	López and Vanini 111, Formosa, Argentina	JQ771877	JQ771846	same as AY973364
T. campaniflora		6*x*		0.46	Barrett 1337, Quaco Rock, Jamaica	JQ771872	JQ771841	AY973367
T. cuneiformis		8*x*	$A^{cu}A^{cu}A^{o}A^{o}BBB^{o}B^{o}$	0.44	Arbo 5449, Bahia, Brazil	JQ771875	JQ771844	same as AY973364
T. occidentalis		6*x*	$A^{oc}A^{oc}BBB^{o}B^{o}$	0.58	Sagástegui 14896, Cajamarca, Perú	JQ771876	JQ771845	AY973365
T. orientalis	O2	6*x*	$A^{o}A^{o}BBB^{o}B^{o}$	0.60	Cabral 358, Teyú Cuaré, Misiones, Argentina	JQ771873	JQ771842	
T. orientalis	O5	6*x*	$A^{o}A^{o}BBB^{o}B^{o}$		Schinini and Daviña 24717, San Ignacio, Misiones, Argentina	JQ771874	JQ771843	
T. orientalis	1538	6*x*	$A^{o}A^{o}BBB^{o}B^{o}$		Arbo 1538, Corrientes, Argentina			AY973361 AY973362 AY973363
T. orientalis	312	6*x*	$A^{o}A^{o}BBB^{o}B^{o}$		Shore 312, Paso de la Patria, Argentina			AY973364
T. ulmifolia var. acuta		6*x*	$A^{u}A^{u}B^{a}B^{a}B^{u}B^{u}$		Correll 40638, Pelican Lake, Bahamas	JQ771871	JQ771840	
T. ulmifolia var. ulmifolia		6*x*	$A^{u}A^{u}B^{a}B^{a}B^{u}B^{u}$	0.46	Arbo 2698, Florida, U.S.A	JQ771892	JQ771861	
T. ulmifolia var. ulmifolia	l07	6*x*	$A^{u}A^{u}B^{a}B^{a}B^{u}B^{u}$		Shore and Schappert 107, Falmouth, Jamaica			AY973366
T. velutina		6*x*	$AAA^{v}A^{v}C^{v}C^{v}$	0.72	Koch and Fryxell 78341, Oaxca, México	JQ771893	JQ771862	AY973360 AY973359
Outgroup								
T. joelii		2*x*			Barrett and Shore 1373, Brazil	JQ771895	JQ771864	AY973368
T. pumilea		2*x*			Arbo 8839, Corrientes, Argentina	JQ771896	JQ771865	AY973375

[a]Genomic formulae for various species were taken from Fernández and Arbo (1989·1990, 1993*b*, 1996, 2000*a*, 2000*b*), Fernández and Solís Neffa (2004), Fernández et al. (2010), and López et al. (2010).
[b]Genome size measurements (in pg) are from López et al. (2011) and are presented as a per 1*x* genome level.

house at York University, Toronto, Canada, or at the Instituto de Botánica del Nordeste, in Corrientes, Argentina. Vouchers are deposited in the herbarium (CTES) of the latter institution.

DNA extraction, amplification, and sequencing

Total DNA was extracted from fresh flower buds or leaf material using the CTAB method of Doyle and Doyle (1987). Primers used for *trnT(UGU)-trnL(UAA)* amplification and sequencing were *trnT a* (5′-CATTACAAATGCGATGCTCT) and *trnL d* (5′-GGGGATAGAGGGACTTGAAC), as described in Taberlet et al. (1991). Primers used for the *ndhF* gene were *ndhF-f4* (5′-CCGCAGTAATTTTGAAAATGC) and *ndhF-r7* (5′-GCTCGACTTCTTCCTCTTTTC), which correspond to sequence positions 850 and 1360 of the *Nicotiana tabacum ndhF* gene. PCR followed the methods of Truyens et al (2005) and was performed at 60 °C annealing temperature. Amplicons were gel-purified (using QIA Quick Gel Extraction Kit, QIAGEN) and directly sequenced in both directions using an ABI377 sequencer (Core Molecular Facility at York University) or at Agencourt Bioscience Corporation (Beverly, Massachusetts).

Both *trnT-L* and *ndhF* were sequenced in all accessions. To explore intraspecific variation, we included a number of accessions of some species. We obtained partial sequences (527 bp) of the *ndhF* gene that encodes a subunit of NADH dehydrogenase and 1069 bp of the *trnT-L* region that includes an intron and an intergenic spacer that flank the 3′ *trnL* gene. Both regions are located in the plastid genome.

ITS sequences

We obtained 26 sequences of ITS from GenBank (Table 1) that were sequenced by Truyens et al. (2005). ITS sequences of one accession of *T. orientalis*, *T. cuneiformis*, and *T. aurelii* were identical (Table 1), and we included only one in the phylogenetic analyses but placed all three taxa at the appropriate terminal of the phylogenetic tree after the analysis. Likewise, ITS sequences of two *T. subulata* accessions were identical to those of one *T. scabra* accession, and they were dealt with in a similar manner (Table 1). Of the 26 ITS sequences, 16 were obtained from the same accessions (and indeed the same DNA sample) as those used for *trnT-L* and *ndhF* sequencing (Table 1). Truyens et al. (2005) used single-strand conformation polymorphism (SSCP) analysis to identify and sequence two different copies of ITS from *T. velutina* and three different copies for one accession of *T. orientalis* (Table 1). We have included these sequences in our analyses and distinguish them by the codes c1, c2, and c3, following Truyens et al. (2005), to indicate they were derived from the same individual (Table 1).

Phylogenetic analyses

Sequences were aligned using CLUSTAL_X (Thompson 1997) and further aligned by eye using MacClade 4.05 (Maddison and Maddison 2000). Indels in *trnT-L* were coded as the simple gap characters of Simmons and Ochoterena (2000) using the program SeqStat version 1.4.1 (Müller 2005). Voucher information and GenBank accession numbers for sequences are provided (Table 1).

Phylogenetic analyses of the cpDNA (combined *trnT-L* and *ndhF*) and nuclear (ITS) sequences were performed separately and compared. To compare the results from different methods of phylogenetic inference, trees were reconstructed using both Bayesian inference as implemented in MRBAYES ver. 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012) and parsimony as implemented in TNT (Goloboff et al. 2008).

Bayesian analyses

Bayesian analysis was carried out for the combined *ndhF* and *trnT-L* sequences, including the corresponding *trnT-L* indels. We explored initially three different partitionings of the data. The most highly partitioned data set included five partitions (with their associated parameters to be estimated separately): one for the indels, one for *trnT-L*, and three for *ndhF*, the latter of which was partitioned into 1st, 2nd, and 3rd codon positions. The least extreme partitioning included two partitions: one for the *trnT-L* indels and one for all remaining sequence data. We decided to use three partitions: the *trnT-L* indels and one partition for *trnT-L* and another for *ndhF* (without specifying codon positions separately). We adopted this final partitioning because the shape parameters (α) from the gamma distribution could not be reliably estimated for the most extreme partitioning as the variances for the three shape parameters for the codon positions of *ndhF* were very large relative to their estimates. We felt that the least partitioned data set, treating all sequences as a single partition, might be inappropriate given that the two regions likely evolve at different rates given that much of the *trnT-L* sequence is non-coding.

The indel data partition was analysed using a binary model in MRBAYES 3.2.1 (Ronquist et al. 2012) specifying the ascertainment bias as variable. For the sequence substitution data, we used the "model-jumping" approach of Huelsenbeck et al. (2004) as implemented in MRBAYES 3.2.1 (Ronquist et al. 2012), which samples across all 203 general time reversible rate models using the posterior probabilities as weights in obtaining final parameter estimates.

Following initial explorations, we ran the Bayesian analysis for 3 000 000 generations using the default setting of two independent runs with four chains each (one of which was heated), the default priors, and sampling every 1000 generations. We assessed convergence of the runs by exploring a number of parameter estimates, including the average standard deviation of split frequencies, assuring that the potential scale reduction factor (PSRF) for each parameter estimate was close to 1.000, as well as assessing convergence of the posterior probabilities of the substitution models explored using the model-jumping routine. We used a burn-in of 50% (i.e., discarded the first 50% of samples). Commonly, the average standard deviation of split frequencies was in the range of 0.04 to 0.003 (average 0.009), the PSRFs ranged from 0.999 to 1.010 (average 1.000), and the standard deviation of posterior probabilities from the various substitution models ranged from 0.02 to 0.003.

We ran a similar Bayesian analysis for the ITS sequence data with a single partition of the data (there were few indels in the ingroup and we did not use them). We used the model-jumping and convergence criteria as above, using 2 000 000 generations, and a burn-in of 50%.

In a further analysis of the *trnT-L* and *ndhF* data, we constrained *T. scabra* and *T. subulata* to both be monophyletic and then used the approach above to obtain a phylogeny. We then compared this model with the unconstrained model above using Bayes factors to determine whether monophyly was an adequate fit. We subtracted the harmonic mean of the marginal likelihood of the constrained model from that of the unconstrained model to determine whether there was strong support for the lack of monophyly (Kass and Raftery 1995; Ronquist et al. 2012). We carried out a comparable analysis to test the hypothesis that the segmental allohexaploids and allooctoploids are monophyletic, using both the ITS and *trnT-L* and *ndhF* data.

Parsimony analysis

A parsimony search was performed for the combined *trnT-L* and *ndhF* data (including indels) using TNT (Tree Analysis Using New Technology, vers. 1.1; Goloboff et al. 2008). All characters (sequence and indel data) were equally weighted and considered unordered, running 1000 replicates of random addition sequences (RAS), with tree bisection and reconnection (TBR), saving a maximum of 10 trees in each RAS. Support was estimated in a JK analysis using heuristic searches, and frequencies were estimated from 5000 pseudoreplicates (Farris et al. 1996; Schuh 2000). We carried out a comparable analysis for the ITS sequence data. Trees were viewed and edited using Tree View (Page 1996) and Mesquite (Maddison and Maddison 2011).

In the following, support will be referred to as strong (JK support ≥ 88% or for a Bayesian posterior probability ≥ 0.91), moderate (77%–87% JK support, 0.85–0.90 posterior probability), weak (63%–75% JK support, 0.75–0.84 posterior probability), or ambiguous (<63% JK support, <0.75 posterior probability) following Zander (2004) and Antonelli (2008).

Results

Sequence data from *ndhF* gave a total of 527 characters (bp), yielding 36 parsimony informative characters. The *trnT-L* data were comprised of 1069 bp of sequence, yielding 90 parsimony informative characters, as well as indels coded as 41 simple binary characters (Simmons and Ochoterena 2000), providing an additional 18 parsimony informative characters. Therefore, a total of 144 characters were parsimony informative. Parsimony analysis of the *trnT-L ndhF* data yielded three equally parsimonious trees of length 422, a consistency index of 0.76, and a retention index of 0.85 (Supplementary Fig. S1*a*).[1] The ITS data included 22 sequences of 612 bp of which 55 characters were parsimony informative. Parsimony analysis of the ITS data yielded 15 equally parsimonious trees of length 166, a consistency index of 0.83, and a retention index of 0.84 (Supplementary Fig. S1*b*).

With a few exceptions, there were no major conflicts between the trees yielded by Bayesian and the maximum parsimony analyses in the sense that there were no clades that were strongly supported in one analysis but contradicted in the other. We provide phylogenetic trees including estimated branch lengths based on Bayesian analyses (Fig. 2). Below, we compare the results of the Bayesian analyses and associated posterior probabilities (PP) of various clades (Fig. 2) with the parsimony trees and associated JK support (Supplementary Fig. S1).

Turnera grandiflora clade

There was strong support for a white- and blue-flowered C genome clade (referred to as the *Turnera grandiflora* clade) in both the Bayesian (PP = 1.0) and parsimony (JK = 100) analyses of the *trnT-L ndhF* data (Fig. 2). The topology of this clade was identical in both analyses. The clade includes *T. candida*, *T. coerulea*, *T. fernandezii*, and *T. grandiflora*. Interestingly, autooctoploid *T. fernandezii* is sister to the diploid *T. grandiflora* accession (PP = 1.0, JK = 97) that was sampled nearby in Amambay, Paraguay (Table 1). These species do, however, differ in sequence by six substitutions and two indels. Analyses of ITS sequences revealed comparably strong support for the *T. grandiflora* clade (PP = 1.0, JK = 96) and also showed that *T. fernandezii* was sister to two *T. grandiflora* accessions, although this latter relationship was less well supported (PP = 0.9, JK = 63). The accessions of *T. grandiflora* used for ITS were different from those used in the *trnT-L ndhF* analysis (Table 1).

Turnera orientalis clade

All analyses provided strong support (*trnT-L ndhF* data, PP = 1.0, JK = 99; ITS data, PP = 1.0, JK = 86) for a clade (referred to here as the *T. orientalis* clade) comprised of segmental allohexaploids *T. orientalis* and *T. occidentalis* and allooctoploids *T. cuneiformis* and *T. aurelii*. For the *trnT-L ndhF* data, the five species–accessions are collectively distinguished by just three substitutions. *Turnera cuneiformis* (Bahia, Brazil) has a sequence identical to that of the *T. orientalis* accession from Misiones, Argentina. For the ITS data, the sequence of one accession of *T. orientalis* from Paso de la Patria, Argentina (Fig. 2; Table 1), was identical to that of *T. cuneiformis* and *T. aurelii* (Truyens et al. 2005). Included in the ITS analysis are three sequences obtained from a single plant of *T. orientalis* (*T. orientalis* Arbo 1538). Truyens et al (2005) used SSCP to identify and clone sequence variants of ITS under the hypothesis that these might represent contributions from different genome donors. These sequences of *T. orientalis* Arbo 1538 are distinguished with the codes c1, c2, or c3 (Fig. 2), and all fall within the *T. orientalis* clade.

Turnera ulmifolia clade

A clade of hexaploids from the Caribbean (referred to as the *T. ulmifolia* clade) is strongly supported in all analyses (*trnT-L ndhF* data, PP = 1.0, JK = 99; ITS data, PP = 1.0, JK = 96).

Turnera velutina

Turnera velutina is the remaining hexaploid to be considered. It does not fall into either of the polyploid clades (*T. ulmifolia* or *T. orientalis* clades) based on *trnT-L ndhF* or the ITS analyses. The two sequence variants of ITS cloned out of a single plant of *T. velutina* (c1 and c2) do not form a clade (Fig. 2*b*).

Assessing monophyly of the polyploid clades

We tested the hypothesis that the segmental allohexaploids and allooctoploids are monophyletic by using Bayes factors (Kass and Raftery 1995; Ronquist et al. 2012). For both the *trnT-L ndhF* and ITS data, we constrained *T. aurelii*, *T. cuneiformis*, *T. occidentalis*, *T. orientalis*, *T. ulmifolia*, *T. campaniflora*, *T. ulmifolia*, and *T. velutina* to be monophyletic and re-ran the Bayesian analysis. The difference in the harmonic mean of the marginal likelihoods of the unconstrained versus the constrained analysis was 13.82 for the *trnT-L ndhF* data and 6.63 for the ITS data, both providing very strong evidence that these polyploids do not form a monophyletic group.

A genome diploids and tetraploids

A clade of all accessions of four species (*T. concinna*, *T. krapovickasii*, *T. scabra* and *T. subulata*) was strongly supported in both the Bayesian (PP = 1.0) and parsimony (JK = 91) analyses of *trnT-L ndhF*. This clade also received strong support in the Bayesian analysis of ITS (PP = 0.99) but weak support in the parsimony analysis (JK = 68). The clade included *T. grandidentata* as the sister group of *T. concinna* in the ITS analysis (PP = 1.0, JK = 94) but not in the *trnT-L ndhF* analysis. Thus the placement of *T. grandidentata* is incongruent in analyses based on ITS versus *trnT-L ndhF*.

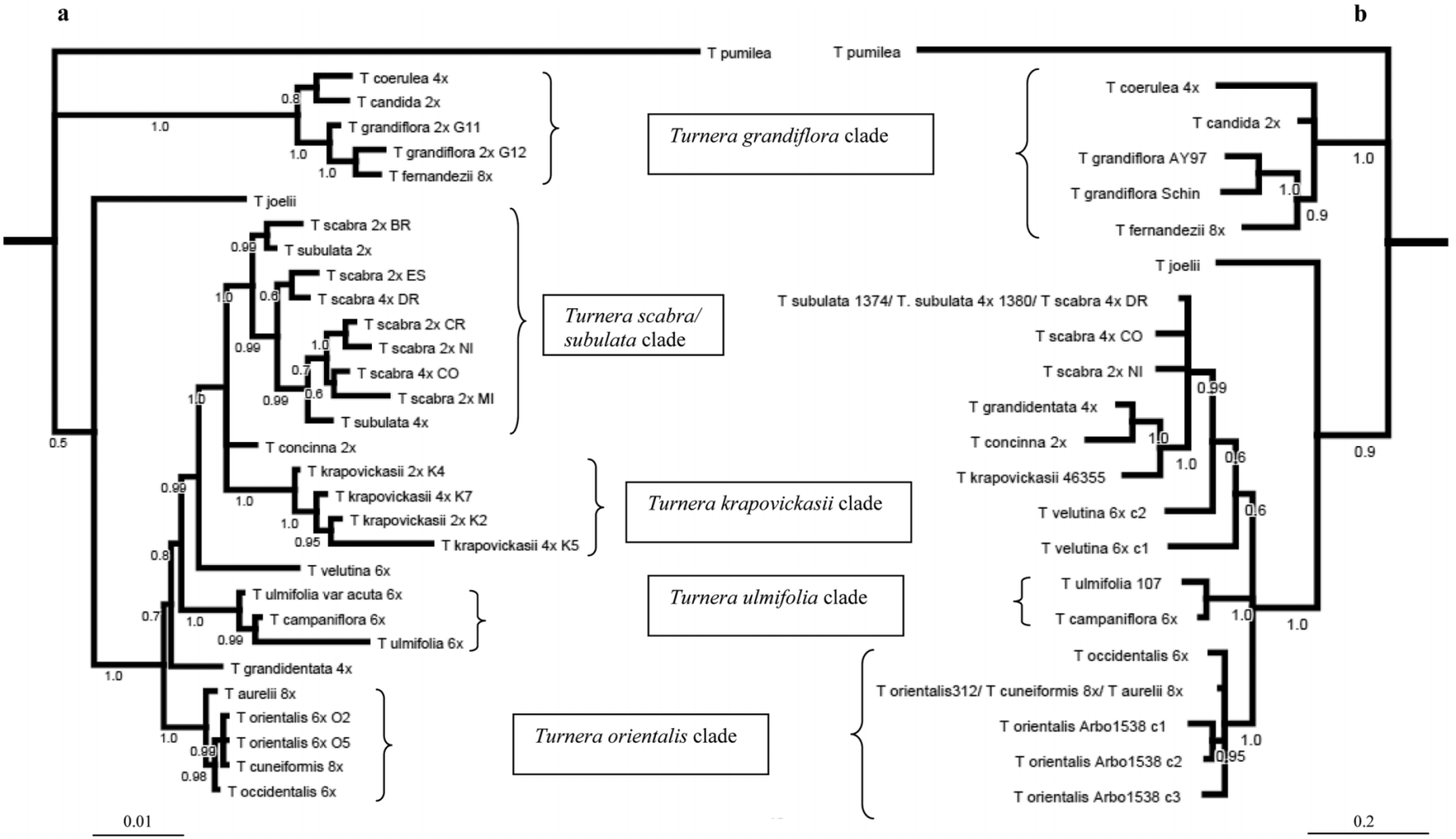
All four *T. krapovickasii* accessions (two diploid and two tetraploid) form a clade in the Bayesian analysis of *trnT-L ndhF* (PP = 1.0, JK = 86). Only a single accession of *T. krapovickasii* was available for the ITS sequence analysis. All accessions of *T. subulata* and *T. scabra* form a clade (*trnT-L ndhF*, PP = 1.0) in the Bayesian analysis, but there is no such clade apparent in the parsimony analysis as various species–accessions fall into a polytomy along with *T. concinna* and the *T. krapovickasii* clade.

We used Bayes factors to assess monophyly of *T. subulata* and *T. scabra* by re-running the Bayesian analysis while constraining both species to be monophyletic. The difference in the harmonic mean of the marginal likelihood of the unconstrained (–4637.77) versus the constrained (–4657.53) phylogeny gave a value of 19.56, indicating very strong support for a lack of monophyly (Kass and Raftery 1995; Ronquist et al. 2012). Although we did not carry out a comparable analysis for ITS, we note that two of the *T. subulata* accessions from northeastern Brazil had sequences identical to those of a *T. scabra* accession from the Dominican Republic (Fig. 2*b*).

Geographic proximity may account for some of the structure of the clade comprised of *T. subulata* and *T. scabra* accessions. For example, two diploid accessions from northeastern Brazil, *T. scabra* BR (Barreirinhas, Brazil) and *T. subulata* (Sao Luis, Brazil), are strongly supported sister taxa in the Bayesian analysis (PP = 0.99) and moderately supported in the parsimony analysis (JK = 76). Likewise, diploid *T. scabra* accessions from Costa Rica and Nicaragua (*T. scabra* NI and CR) received strong support in the Bayesian analysis (PP = 1.0), but support was ambiguous in the

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2012-0135.

Fig. 2. (*a*) Bayesian tree (phylogram) based on *trnT-L* and *ndhF* combined data. (*b*) Bayesian tree based on the analysis of ITS. Values at the nodes represent posterior probabilities. Scale bar represents the expected number of changes per site.



parsimony analysis (JK = 57) (Fig. 2*a*; Supplementary Fig. S1*a*). These two sequences differ by 5 bp substitutions but share a unique indel not present in the other accessions–species.

Discussion

We used cpDNA (*trnT-L ndhF*) and nuclear (ITS) sequence data to gain insights into the origins of polyploid species in subseries *Turnera* and test hypotheses generated previously. Our analyses provide strong support for the occurrence of a clade of C genome species (the *T. grandiflora* clade) and for the origin of autooctoploid *T. fernandezii* from *T. grandiflora*.

Studies of chromosome pairing in hybrids (Fernández and Arbo 2000*b*), as well as GISH (López et al 2010*a*), led to the proposition that allohexaploid *Turnera velutina* might have had a C genome progenitor (Table 1). This hypothesis is not supported by ITS or *trnT-L ndhF* sequence analyses as *T. velutina* does not fall in the C genome clade. We cannot, however, rule out this possibility with certainty as a C genome species might not have contributed its cpDNA to *T. velutina*. Likewise, ITS might have been homogenized to sequences contributed by donor that was not a C genome species through processes of concerted evolution (Wendel et al. 1995). Two ITS sequences were, however, identified and cloned out of a single plant of *T. velutina* (Truyens et al. 2005), possibly indicating the lack of complete homogenization of ITS from different genome donors. Neither of these sequences is, however, closely allied with any C genome ITS sequences. The progenitors of *T. velutina* remain unclear.

A clade comprised of hexaploids *T. orientalis* and *T. occidentalis* and octoploids *T. cuneiformis* and *T. aurelii* was strongly supported in our analyses. *Turnera orientalis* and *T. occidentalis* may have resulted from speciation at the hexaploid level following a single origin of hexaploidy in this clade. Although cytogenetic data support a close relationship between these species, they are, however, thought to possess different variants of the A genome (Table 1; López et al. 2010*b*). The origin of octoploid *T. cuneiformis* from hexaploid *T. orientalis* is well supported, with *T. cuneiformis* (Bahia, Brazil) having *trnT-L ndhF* sequences identical to that of the *T. orientalis* accession from Misiones, Argentina. Similarly, the origin of allooctoploid *T. aurelii* from *T. orientalis* is well supported, and ITS sequences of one accession of *T.* orientalis and that of *T. aurelii* are identical. Thus our results are consistent with the hypothesis of Fernández and Arbo (1993*b*, 2000*a*) that both octoploid *T. cuneiformis* and *T. aurelii* have three genomes in common with each other and with hexaploid *T. orientalis* (which also contributed its cpDNA). The putative additional genome contributors distinguishing these octoploids remain unknown. Likewise, there are no known diploid or tetraploid donors of the B genomes that these species are thought to possess (Table 1).

Interestingly, allooctoploid *T. aurelii* has a genome size considerably greater than that of hexaploids *T. orientalis* and *T. occidentalis* on a per 1*x* genome level (López et al. 2011; Table 1). In contrast, allooctoploid *T. cuneiformis* has a genome size smaller than that of the hexaploids, suggesting that there has been a marked reduction in its genome size since its origin (Table 1; López et al. 2011). We presume that the difference in genome size of *T. aurelii* versus *T. cuneiformis* may in some way be a function of the additional unknown genome contributors to these octoploids. Fernández and Arbo (2000*a*) observed that five large univalents occur at meiosis in artificial hybrids of *T. aurelii* × *T. orientalis* and that *T. aurelii* possesses larger chromosomes than *T. cuneiformis* (Solís Neffa and Fernández 1993).

A separate clade of hexaploids from the Caribbean (the *T. ulmifolia* clade), divergent from the *T. orientalis* clade, is well supported in all analyses. Our analysis using Bayes factors indicates that the three clades (*T. orientalis*, *T. ulmifolia*, and *T. velutina* clades) are not monophyletic, suggesting independent origins for two or more of these clades. *Turnera ulmifolia* var. *ulmifolia* and *T. campaniflora* Arbo Shore & Barrett have comparable genome sizes that are less than those of *T. orientalis* (Table 1), perhaps lending support for an independent origin of these hexaploid clades.

Diploid and tetraploid accessions of *T. krapovickasii* form a single clade in the analysis of *trnT-L ndhF* data, consistent with an autoploid origin of the tetraploid accessions. In contrast, *T. subulata* and *T. scabra* (including diploid and tetraploid accessions of both species) do not show monophyly based on the *trnT-L ndhF* analyses. These species were considered to be taxonomic varieties within the *T. ulmifolia* complex (Urban 1883). They exhibit reasonably high fertility as hybrids (Arbo and Fernández 1987). Additional accessions collected throughout the native range and sequences of additional genes will likely be required to explore the origins and relationships of these species more closely.

It is possible that lineage sorting of ancestral variation, hybridization between species, or perhaps misassignment of some accessions to the correct species explain the lack of monophyly of *T. scabra* and *T. subulata*. For example, *T. scabra* BR (Barreirinhas, Brazil) exhibits a polymorphism for presence versus absence of a purple petal spot in its flowers, and the purple spot is characteristic of *T. subulata*, leading to some uncertainty about the species to which this accession belongs. Tetraploid *T. scabra* CO (Dagua, Colombia) also exhibits this polymorphism. The petal spot is known to be determined by a single dominant gene (Shore and Barrett 1987). The polymorphism could indicate that hybridization has occurred among *T. scabra* and *T. subulata*, a possibility that had been suggested by Arbo and Fernández (1987).

Cytogenetic analyses and GISH indicate that there is considerable homology among the genomes of *T. krapovickasii*, *T. scabra*, and *T. subulata*, which along with *T. concinna* and *T. grandidentata*, share variants of an A genome (Fernández and Arbo 1989; López et al. 2010*a*). Analysis of ITS sequence data likewise placed these species within a clade.

Cytogenetic investigations had suggested that diploid *T. concinna* might have contributed to the origins of the segmental allotetraploid *T. grandidentata* (Fernández and Arbo 1993*a*), and indeed the two species represent sister taxa in an analysis based on ITS sequences (Truyens et al. 2005; Fig. 2*b*). The plastid genes *trnT-L ndhF* sequenced here provide no evidence for such a relationship (Fig. 2*a*). This incongruence between molecular data from the nucleus versus the plastid genome can be interpreted as a sign of hybridization (Doyle et al. 2003) consistent with a segmental alloploid origin of *T. grandidentata*. The analysis of *trnT-L ndhF* did not reveal any particularly close affinity to a plastid donor for *T. grandidentata*, leaving open the question of the source of the additional progenitor contributing to its origin. Studies using GISH supported the possibility that *T. subulata* might have contributed a genome to *T. grandidentata*, but this is not supported by our analysis. Collectively, these results suggest that *T. concinna* likely contributed a nuclear genome to tetraploid *T. grandidentata* (based on ITS data), but the plastid donor of *T. grandidentata* (and the presumed contributor of its additional nuclear genome) remains unknown.

CpDNA exhibits paternally biased but variable inheritance patterns (including maternal, paternal, and biparental inheritance) for intraspecific crosses of *T. ulmifolia* and for the reciprocal interspecific cross *T. velutina* × *T. ulmifolia* (Shore et al. 1994; Shore and Triassi 1998). Comparably variable patterns of inheritance have been observed for *Passiflora* species (Hansen et al. 2007) but not for crosses of the *T. sidoides* complex, where strict maternal inheritance was observed for the eight progeny analyzed (Speranza et al. 2007). An effect of paternal inheritance via pollen could be that it enhances gene flow of cpDNA, allowing greater opportunities for hybridization, in contrast to species exhibiting strict maternal inheritance. In *Turnera* species, the arillate seeds are ant-dispersed (Barrett 1978), presumably minimizing seed dispersal distances relative to those of pollen. From a phylogenetic perspective, Hansen et al. (2007) indicate that heteroplasmy resulting from

biparental inheritance could lead to misinterpretations of phylogenies based on cpDNA sequences. The proportion of progeny exhibiting biparental transmission of cpDNA is approximately 15% (Shore et al. 1994; Shore and Triassi 1998). Although this limits the possibility of heteroplasmy, we cannot rule out its occurrence.

Acknowledgments

The authors thank D. Salariato, L. Packer, and P. Chafe for advice and M.M. Arbo for advice and samples of a number of species. The work was supported by Natural Sciences and Engineering Research Council of Canada Discovery grant to J.S. Shore and a GSEP fellowship awarded to A. López.

References

Adams, K.L., and Wendel, J.F. 2004. Exploring the genomic mysteries of polyploidy in cotton. Biol. J. Linn. Soc. **82**: 573–581. doi:10.1111/j.1095-8312.2004.00342.x.

Antonelli, A. 2008. Higher level phylogeny and evolutionary trends in Campanulaceae subfam. Lobelioideae: molecular signal overshadows morphology. Mol. Phylogenet. Evol. **46**: 1–18. doi:10.1016/j.ympev.2007.06.015. PMID: 18042405.

Arbo, M.M. 2005. Estudios Sistemáticos en *Turnera* (Turneraceae). III. Series *Anomalae* y *Turnera*. Bonplandia, **14**: 115–318.

Arbo, M.M., and Espert, S.M. 2009. Morphology, phylogeny and biogeography of *Turnera* L. (Turneraceae). Taxon, **58**: 457–467.

Arbo, M.M., and Fernández, A. 1983. Posición taxonómica, citología y palinología de tres niveles de ploidía de *Turnera subulata* Smith. Bonplandia, **5**: 211–216.

Arbo, M.M., and Fernández, A. 1987. Cruzamientos intra e interespecíficos en *Turnera*, serie Canaligerae. Bonplandia, **6**: 23–38.

Barrett, S.C.H. 1978. Heterostyly in a tropical weed: the reproductive biology of the *Turnera ulmifolia* complex (Turneraceae). Can. J. Bot. **56**: 1713–1725. doi:10.1139/b78-203.

Buggs, R.J.A., Renny-Byfield, S., Chester, M., Jordon-Thaden, I.E., Viccini, L.F., Chamala, S., Leitch, A.R., Schnable, P.S., Barbazuk, W.B., Soltis, P.S., and Soltis, D.E. 2012. Next-generation sequencing and genome evolution in allopolyploids. Am. J. Bot. **99**: 372–382. doi:10.3732/ajb.1100395. PMID: 22268220.

Chang, P.L., Dilkes, B.P., McMahon, M., Comai, and Nuzhdin, S.V. 2010. Homoeolog-specific retention and use in allotetraploid *Arabidopsis suecica* depends on parent of origin and network partners. Genome Biol. 11: 1–17.

Doyle, J.J., and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**: 11–15.

Doyle, J.J., Doyle, J.L., Rauscher, J.T., and Brown, A.H.D. 2003. Diploid and polyploid reticulate evolution throughout the history of the perennial soybeans (*Glycine* subgenus *Glycine*). New Phytol. **161**: 121–132. doi:10.1046/j.1469-8137.2003.00949.x.

Doyle, J.J., Doyle, J.L., Rauscher, J.T., and Brown, A.H.D. 2004. Evolution of the perennial soybean polyploid complex (*Glycine* subgenus *Glycine*): a study of contrasts. Biol. J. Linn. Soc. **82**: 583–597. doi:10.1111/j.1095-8312.2004.00343.x.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., and Kluge, A.G. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics, **12**: 99–124. doi:10.1111/j.1096-0031.1996.tb00196.x.

Fernández, A. 1987. Estudios cromosómicos en *Turnera* y *Piriqueta* (Turneraceae). Bonplandia, **6**: 1–21.

Fernández, A. 1997. Estudio citogenéico en híbridos entre una especie octoploide, *Turnera aurelii* y dos diploides, *T. caerulea* y *T. joelii*. Bonplandia, **9**: 281–286.

Fernández, A., and Arbo, M.M. 1989. Relaciones genómicas entre cuatro especies diploides de *Turnera* con flores amarillas (serie *Canaligerae*). Bonplandia, **6**: 983–109.

Fernández, A., and Arbo, M.M. 1990. Gametas no reducidas y relaciones genómicas en tres especies de *Turnera* (Turneraceae). Darwiniana, **30**: 21–26.

Fernández, A., and Arbo, M.M. 1993*a*. Citogenética de híbridos entre *Turnera grandidentata* (4*x*) y *T. subulata* y *T. scabra* (2*x*) (Turneraceae). Bonplandia, **7**: 119–127.

Fernández, A., and Arbo, M.M. 1993*b*. Relaciones genómicas entre seis especies de *Turnera* (Serie *Canaligerae*) del Paraguay. Candollea, **48**: 305–318.

Fernández, A., and Arbo, M.M. 1996. Relaciones genómicas entre las especies diploides de flores blanco-azuladas de *Turnera* (Serie *Canaligerae*). Bonplandia, **9**: 95–102.

Fernández, A., and Arbo, M.M. 2000*a*. Cytogenetic relationships between *Turnera aurelii*, *T. cuneiformis* (2*n*=8*x*=40), and *T. orientalis* (2*n*=6*x*=30) (Turneraceae). Cytologia, **65**: 97–102.

Fernández, A., and Arbo, M.M. 2000*b*. Relaciones genómicas entre dos especies hexaploides de *Turnera*, *T. orientalis* y *T. velutina*, y una diploide, *T. grandiflora* (Turneraceae, serie *Turnera*). Bonplandia, **10**: 181– 187.

Fernández, A., and Solís Neffa, V.G. 2004. Genomic relationships between *Turnera krapovickasii* (2*x*, 4*x*) and *Turnera ulmifolia* (6*x*) (Turneraceae, *Turnera*). Caryologia, **57**: 45–51.

Fernández, A., Rey, H., and Solís Neffa, V.G. 2010. Evolutionary relationships between the diploid *Turnera grandiflora* and the octoploid *T. fernandezii* (Turneraceae). Ann. Bot. Fenn. **47**: 321–329.

Goloboff, P.A., Farris, J.S., and Nixon, K.C. 2008. TNT, a free program for phylogenetic analysis. Cladistics, **24**: 774–786. doi:10.1111/j.1096-0031.2008.00217.x.

Hansen, A.K., Escobar, L.K., Gilbert, L.E., and Jansen, R.K. 2007. Paternal, maternal, and biparental inheritance of the chloroplast genome in *Passiflora* (Passifloraceae): implications for phylogenetic studies. Am. J. Bot. **94**: 42–46. doi:10.3732/ajb.94.1.42. PMID:21642206.

Hegarty, M.J., and Hiscock, S.J. 2008. Genomic clues to the evolutionary success of polyploid plants. Curr. Biol. **18**: R435–R444. doi:10.1016/j.cub.2008.03.043. PMID:18492478.

Huelsenbeck, J.P., and Ronquist, F. 2001. MRBAYES: bayesian inference of phylogenetic trees. Bioinformatics, **17**: 754–755. doi:10.1093/bioinformatics/17.8.754. PMID:11524383.

Huelsenbeck, J.P., Larget, B., and Alfaro, M.E. 2004. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Mol. Biol. Evol. **21**: 1123–1133. doi:10.1093/molbev/msh123. PMID:15034130.

Ilut, D.C., Coate, J.E., Luciano, A.K., Owens, T.G., May, G.D., Farmer, A., and Doyle, J.J. 2012. A comparative transcriptomic study of an allotetraploid and its diploid progenitors illustrates the unique advantages and challenges of RNA-seq in plant species. Am. J. Bot. **99**: 383–396. doi:10.3732/ajb.1100312. PMID:22301896.

Kass, R.E., and Raftery, A.E. 1995. Bayes factors. J. Am. Stat. Assoc. **90**: 773–795. doi:10.1080/01621459.1995.10476572.

Levi, A.A., and Feldman, M. 2004. Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. Biol. J. Linn. Soc. **82**: 607–613. doi:10.1111/j.1095-8312.2004.00346.x.

López, A., Fernández, A., and Poggio, L. 2010*a*. Genomic affinities in *Turnera* (subseries *Turnera*, Turneraceae) inferred by in situ hybridization techniques. Genome, **53**(8): 594–598. doi:10.1139/G10-037. PMID:20725146.

López, A., Poggio, L., and Fernández, A. 2010*b*. Genomic relationships between *Turnera orientalis* (Urb.) Arbo (6*x*) and *T. occidentalis* Arbo and Shore (6*x*) (Turneraceae, subseries *Turnera*). Annal. Bot. Fenn. **47**: 471–476.

López, A., Panseri, A., Fernández, A., and Poggio, L. 2011. Nuclear DNA content in the polyploid complex *Turnera ulmifolia* (*Turnera* L., Passifloraceae). Plant Syst. Evol. **296**: 225–230. doi:10.1007/s00606-011-0489-0.

Maddison, D.R., and Maddison, W.P. 2000. MacClade 4: analysis of phylogeny and character evolution. Version 4.05. Sinauer, Sunderland, Mass.

Maddison, D.R., and Maddison, W.P. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75. Available at http://mesquiteproject.org.

Müller, K. 2005. SeqState — primer design and sequence statistics for phylogenetic DNA data sets. Appl. Bioinform. **4**: 65–69. doi:10.2165/00822942-200504010-00008.

Muschner, V.C., Lorenz, A.P., Cervi, A.C., Bonatto, S.L., Souza-Chies, T.T., Salzano, F.M., and Freitas, L.B. 2003. A first molecular phylogenetic analysis of *Passiflora* (Passifloraceae). Am. J. Bot. **90**: 1229–1238. doi:10.3732/ajb.90.8.1229. PMID:21659223.

Page, R.D.M. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comp. Appl. Biol. Sci. **12**: 357–358.

Ronquist, F., Teslenko, M., van der, Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., and Huelsenbeck, J.P. 2012. MRBAYES 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61**: 539–542. doi:10.1093/sysbio/sys029.

Schuh, R.T. 2000. Biological systematics. Principles and applications. Cornell University Press, Ithaca, N.Y.

Shore, J.S. 1991*a*. Chromosomal evidence for autotetraploidy in *Turnera ulmifolia* complex (Turneraceae). Can. J. Bot. **69**(6): 1302–1308. doi:10.1139/b91-169.

Shore, J.S. 1991*b*. Tetrasomic inheritance and isozyme variation in *Turnera ulmifolia* vars. *elegans* Urb. and *intermedia* Urb. (Turneraceae). Heredity, **66**: 305–312.

Shore, J.S., and Barrett, S.C.H. 1987. Inheritance of floral and isozyme polymorphisms in *Turnera ulmifolia* L. J. Hered. **78**: 44–48.

Shore, J.S., and Triassi, M. 1998. Paternally biased cpDNA inheritance in *Turnera ulmifolia* (Turneraceae). Am. J. Bot. **85**(3): 328–332. doi:10.2307/2446324. PMID: 21684915.

Shore, J.S., McQueen, K.L., and Little, S.H. 1994. Inheritance of plastid DNA in the *Turnera ulmifolia* complex (Turneraceae). Am. J. Bot. **81**: 1636–1639. doi:10.2307/2445342.

Shore, J.S., Arbo, M.M., and Fernández, A. 2006. Breeding system variation, genetics and evolution in the Turneraceae. New Phytol. **171**: 539–551. 16866957.

Simmons, M.P., and Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. **49**: 369–381. doi:10.1093/sysbio/49.2.369.

Solís Neffa, V.G. 1996. Cariotipos de especies de *Turnera* (Turneraceae). Bonplandia, **9**: 121–127.

Solís Neffa, V.G., and Fernández, A. 1993. Estudios cromosómicos en especies de *Turnera* (Turneraceae). Bonplandia, **7**: 101–118.

Solís Neffa, V.G., and Fernández, A. 2000. Chromosome studies in *Turnera* (Turneraceae). Genet. Mol. Biol. **23**: 925–930. doi:10.1590/S1415-47572000000400037.

Soltis, D.E., and Soltis, P.S. 1999. Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. **14**: 348–352. doi:10.1016/S0169-5347(99)01638-9. PMID:10441308.

Soltis, D.E., Soltis, P.S., Pire, J.C., Kovarik, A., Tate, J.A., and Mavrodiev., E. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic,

genomic and genetic comparisons. Biol. J. Linn. Soc. **82**: 485–501. doi:10.1111/j.1095-8312.2004.00335.x.

Song, K., Lu, P., Tang, K., and Osborn, T.C. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc. Natl. Acad. Sci. U.S.A. **92**: 7719–7723. doi:10.1073/pnas.92.17.7719. PMID: 7644483.

Speranza, P.R., Seijo, J.G., Grela, I.A., and Solís Neffa, V.G. 2007. Chloroplast DNA variation in the *Turnera sidoides* L. complex (Turneraceae): biogeographical implications. J. Biogeogr. **34**: 427–436. doi:10.1111/j.1365-2699.2006.01622.x.

Taberlet, P., Gielly, L., Pautou, G., and Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. **17**: 1105–1109. doi:10.1007/BF00037152. PMID:1932684.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X Windows interface: flexible strategies for multiple sequences alignment aided by quality analysis tools. Nucleic Acids Res. **25**: 4876–4882. doi:10.1093/nar/25.24.4876. PMID:9396791.

Thulin, M., Razafimandimbison, S.G., Chafe, P., Heidari, N., Kool, A., and Shore, J.S. 2012. Phylogeny of the Turneraceae clade (Passifloraceae s.l.): Trans-Atlantic disjunctions and two new genera in Africa. Taxon, **61**: 308–323.

Truyens, S., Arbo, M.M, and Shore, J.S. 2005. Phylogenetic relationships, chromosome and breeding system evolution in *Turnera* (Turneraceae): inferences from ITS sequence data. Am. J. Bot. **92**: 1749–1758. doi:10.3732/ajb.92.10.1749. PMID:21646092.

Urban, I. 1883. Monographie der familia der Turneraceen. Jahrb. Königl. Bot. Gart. **2**: 1–152.

Wendel, J.F., and Cronn, R.C. 2003. Polypoidy and the evolutionary history of cotton. Adv. Agron. **78**: 139–186. doi:10.1016/S0065-2113(02)78004-8.

Wendel, J.F., Schnabel, A., and Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. U.S.A. **92**: 280–284. doi:10.1073/pnas.92.1.280. PMID: 7816833.

Zander, R.H. 2004. Minimal values for reliability of bootstrap and jackknife proportions, decay index, and bayesian posterior probability. Phyloinformatics, **2**: 1–13.