ORIGINAL
ARTICLE

Chloroplast DNA variation in the *Turnera sidoides* L. complex (Turneraceae): biogeographical implications

P. R. Speranza[1], J. G. Seijo[2,3], I. A. Grela[1] and V. G. Solís Neffa[3,4]*

[1]*Facultad de Agronomía, Universidad de la República, Av. E. Garzón 780, Montevideo, 12900, Uruguay,* [2]*Facultad de Ciencias Exactas y Naturales y Agrimensura (UNNE), Av. Libertad 5500, 3400 Corrientes, Argentina,* [3]*Instituto de Botánica del Nordeste (UNNE-CONICET), Sargento Cabral 2131, 3400 Corrientes, Argentina and* [4]*Facultad de Ciencias Agrarias (UNNE), Sargento Cabral 2131, 3400 Corrientes, Argentina*

ABSTRACT

Aim To analyse the current geographical structure of chloroplast DNA variation in the *Turnera sidoides* L. complex in order to establish historical biogeographical hypotheses for the mid-latitude South American lowlands. During the Quaternary, the climate shifted from tropical humid to cold dry, and the vegetation cover has not been stable. The consequences of these processes on the current distribution of the vegetation of this area have received very little attention.

Location The mid-latitude South American lowlands extend between *c.* 20 and 40°S and include Uruguay, northern, central and eastern Argentina, southern Brazil, and parts of southern Paraguay and Bolivia. They are surrounded by higher-elevation systems.

Methods *Turnera sidoides* is a well-studied polyploid complex of perennial rhizomatous herbs occurring throughout the area of interest. We analysed 321 individuals from 79 populations of the five recognized subspecies. We also included progenies from artificial crosses in order to analyse chloroplast inheritance. After screening sequences for four non-coding chloroplast DNA regions, the *trnL–trnF* spacer was selected to characterize the collection.

Results Three haplotypes can be easily identified, with each differing from the others in two independent characters. A clear geographic structure is revealed when haplotypes are plotted for the complex as a whole regardless of subspecies and cytotype. Three distinct regions can be identified.

Main conclusions We propose three putative refugial areas for the *Turnera sidoides* complex, which are associated with the orographical systems of the region. Ravines and slopes in the Haedo Cuchilla system in northern Uruguay, the elevations of the western side of the area in Argentina, and the eastern Serranías system in south-eastern Uruguay may each have served as refugia in which the A, B and C haplotypes became fixed during the drier climatic phases. Biogeographical patterns in the area covered by *T. sidoides*, particularly east of the Uruguay River, have not previously been analysed from a historical perspective.

Keywords

Biogeography, chloroplast DNA, diversity, Quaternary climatic change, refugia, South American lowlands, *Turnera sidoides*.

*Correspondence: V. G. Solís Neffa, Instituto de Botánica del Nordeste (UNNE-CONICET), Sargento Cabral 2131, 3400 Corrientes, Argentina.
E-mail: viviana@agr.unne.edu.ar

INTRODUCTION

Geological processes coupled with Quaternary climatic episodes are considered by many to have played a major role in shaping current plant distributions and evolution. A great amount is known about the biogeographical effects and stimulus for speciation and intraspecific differentiation of Quaternary climatic changes in the temperate regions of the Northern Hemisphere (Pielou, 1991; Hewitt, 1996, 2000; Comes & Kadereit, 1998; Vargas, 2003). Climatic oscillations affected different parts of the globe differently, depending on distance from the equator, continental mass, and the presence

of mountain ranges. During the Last Glacial Maximum (LGM), ice sheets covered much of the Northern Hemisphere (Hewitt, 1996); the Southern Hemisphere, however, was not subjected to extensive glaciations, although its climate was considerably modified (Damuth & Fairbridge, 1970; Ab'Sáber, 1977, 1982; Markgraf & Bradbury, 1982; Clapperton, 1993; Iriondo, 1999).

The biotic consequences of climatic changes in South America have been discussed in relation to the contraction and expansion of the Andean flora (Simpson & Todzia, 1990; Premoli *et al.*, 2000; Pastorino & Gallo, 2002; Pastorino *et al.*, 2004; Bull-Hereñu *et al.*, 2005; Muellner *et al.*, 2005), the contraction of the Amazonian forest to islands or refugia (Ledru, 1993; van der Hammen & Absy, 1994; Colinvaux *et al.*, 1996a,b; Prance, 1996), the expansion of tropical and sub-tropical open formations (Markgraf & Bradbury, 1982; Markgraf, 1991; Soubies *et al.*, 1991; Behling, 1995, 1997, 2002), and the expansion of seasonally dry forests (Prado & Gibbs, 1993; Pennington *et al.*, 2000). Notwithstanding the above, not much attention has been paid to the subtropical and temperate lowlands of southern South America.

Mid-latitude South American lowlands

The area of interest (Fig. 1a) corresponds to the southern end of the South American Platform (Instituto Brasileiro de Geografia e Estatística, 1997). This mostly low-lying area includes the whole of Uruguay, northern, central and eastern Argentina, southern Brazil, the southern half of Paraguay, and southern Bolivia. This area is surrounded by higher-elevation ranges locally known as *sierras* and *cuchillas*. To the west, its limit is an arch formed, north to south, by the Andean Piedmont and Subandean Sierras, and the Pampean Sierras. To the south, the area reaches the Ventania and Tandilia sierras at *c.* 39°S, and to the east, it is limited by the southern Brazilian high plains (Planalto). The northern limit includes on the eastern side the east-west southern edge of the Planalto at *c.* 30°S, and on the western side the area extends further north to include the southern half of Paraguay and part of Bolivia, to *c.* 20°S. The mid-latitude South American plains on the western side are mostly of Quaternary sedimentary origin, while to the east of the Uruguay River sediments range from Devonian to Holocene and are deposited among and over the Brazilian Shield. The resulting landscape is known in Brazil and Uruguay as Campos. The southern end of the Brazilian Shield sinks to the west under the sedimentary plains of Argentina, which are known as Pampa and Chaco. We include in this area the ranges that surround the region and their inward extensions to the west and south. In the east, the transition in elevation and climate into the Brazilian Planalto is much sharper.

The region has a subtropical to temperate climate, which varies from humid in the east (annual precipitation > 1600 mm) to semi-arid and arid (< 400 mm) in the west and south-west. Rainfall increases again (> 2300 mm) on the eastern slopes of the Subandean Sierras. The decrease in
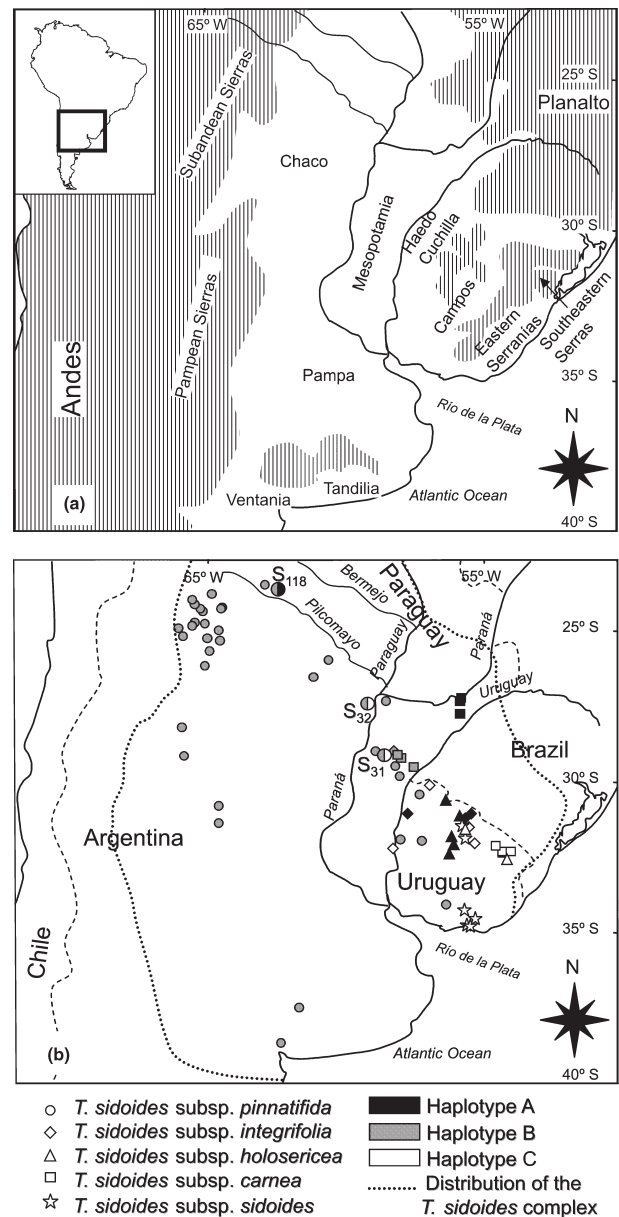
Figure 1 Major geographical features of the area under study and sample distribution. (a) Shaded areas represent major orographical systems. The inset shows the magnified area in relation to the South American continent. (b) Geographical distribution of the sampled populations of *Turnera sidoides* L. and their cpDNA haplotypes.

rainfall is accompanied by a lengthening of the dry season, and the temperature decreases gradually with latitude (Burgos, 1970; Cabrera & Willink, 1973; Iriondo & García, 1993).

The predominant vegetation corresponds to grasslands and woodland formations with important regional variations in floristic composition and life-cycle attributes (Cabrera, 1971; Soriano, 1991). The sierras (excluding Tandilia and Ventania) and river banks within this area are predominantly covered by woody vegetation. West of the Paraná River the vegetation is

mostly dominated by xerophytic forests and grasslands (Cabrera, 1971; Cabrera & Willink, 1973; Prado, 1993a,b). Biogeographically, Morrone (2000, 2006) assigned most of these areas to the Chacoan and Pampean provinces of the Chacoan subregion. From a phytogeographical point of view, Cabrera (1971) and Cabrera & Willink (1973) include most of these grasslands in the Pampean and Chacoan provinces, although they acknowledge that they extend into the Paranaense province (Campos district). The Chaco plain itself is characterized by saline soils and a highly seasonal climate resulting in xeromorphic forest formations. These conditions effectively limit the westward expansion of the Paranaense forest, although the immigration of non-Chacoan species is made possible by a net of gallery forests (Spichiger *et al.*, 2004). Alternatively, the generally xerophytic woody vegetation of the Pampean Sierras is included by Prado (1993a,b) in the Chaco province *sensu stricto*, while the semi-deciduous forests found along rivers and the Andean piedmont are classified as seasonally dry forest (as defined by Prado, 2000), which surrounds the typical Chaco flora, with abundant transitional formations. The woody vegetation of the hilly areas of Uruguay was considered by Grela (2004) as a southward extension of the Paranense forest formations.

In the mid-latitude South American lowlands, the numerous episodes of geomorphological and climatic changes of the Quaternary must have been critical in determining current species distribution patterns (Ab'Sáber, 1977; Burnham & Graham, 1999). However, the mechanisms of vegetation retraction and expansion in this area are not well understood. Pollen records are scarce for this area, and most information on Pleistocene and Holocene palaeoenvironmental changes has come from geomorphological and stratigraphical studies, including fossil wood, phytoliths and fossil vertebrate assemblages (Tonni & Fidalgo, 1978; Iriondo, 1981, 1991, 1992; Iriondo & García, 1993; Zárate & Blasi, 1993; Prieto, 1996; Zucol *et al.*, 2005; Iriarte, 2006). Most of these studies support the notion that the subtropical elements have repeatedly expanded and contracted in a SW–NE direction. Consequently, Quaternary events must have disrupted species ranges, caused the extinction of local populations, and changed selective pressures, producing a considerable genetic reorganization that may be reflected in the gene pools of the modern species. Ab'Sáber (1977) proposed that, during the drier phases, the elements of the current flora that are adapted to more humid conditions survived in refugial grassland formations associated with the slopes of the uplands. According to this hypothesis, the current geographical patterns of genetic variation within the appropriate biological systems will be associated with these higher-elevation ranges. Finding a biological model may not be straightforward. In this paper we will assess the existence of detectable chloroplast DNA (cpDNA) genetic variability in the *Turnera sidoides* L. (Turneraceae) complex as a whole, and whether such variability is geographically structured. The geographical distribution of genetic variability will be interpreted in relation to the geomorphological and climatic history of the area.

The biological system

Turnera sidoides is a well-studied polyploid complex of perennial rhizomatous herbs that ranges naturally over Uruguay and Argentina, reaching 39°S and including southern Bolivia, Brazil, and south-western Paraguay (Arbo, 1985; Solís Neffa, 2000). The complex occupies a wide range of habitats, growing throughout the area wherever sufficient moisture is available locally at least seasonally. *Turnera sidoides* is an obligate outbreeder owing to dystily (Arbo, 1985) and genetic self-incompatibility (Solís Neffa, 2000). Seeds are dispersed by gravity and ants, and tend to concentrate in localized areas. Discrete populations, ranging from less than ten to hundreds of plants, are frequently observed. Populations are usually separated from one another by a few to several kilometres (Solís Neffa, 2000). Five infraspecific taxa (subspecies) have been recognized based on the variability of leaf shape and indumentum (Arbo, 1985). *Turnera sidoides* subsp. *pinnatifida* (Juss. ex Poir.) Arbo is clearly distinguished by its pinnatifid to pinnatisect leaves, while the other four subspecies show elliptic to obovate leaves and are differentiated by the type of leaf indumentum. *Turnera sidoides* subsp. *carnea* (Cambess.) Arbo has a lax indumentum; subsp. *holosericea* (Urb.) Arbo has glaucus leaves with lanate-sericeus indumentum; *T. sidoides* subsp. *integrifolia* (Griseb.) Arbo is characterized by its hirsute indumentum; and subsp. *sidoides* has stellated hairs (Arbo, 1985). Except for subsp. *pinnatifida*, the distributions of the remaining subspecies are partially overlapping.

In addition to the morphological and geographical variation, *T. sidoides* shows different ploidy levels based on $x = 7$ (Fernández, 1987; Solís Neffa & Fernández, 2001). Diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) cytotypes occur in each of subspecies *carnea*, *holosericea*, *pinnatifida* and *integrifolia*. In subsp. *integrifolia*, octoploid cytotypes ($2n = 8x = 56$) have also been reported. *Turnera sidoides* subsp. *sidoides* is mostly tetraploid, although near-pentaploid aneuploids have also been reported (Fernández, 1987). In the complex as a whole, diploids have restricted and disjunct distributions; tetraploids are the most widespread; and populations with higher ploidy levels are rare and disjunct (Solís Neffa & Fernández, 2001; Solís Neffa & Seijo, 2003; Solís Neffa *et al.*, 2004).

MATERIALS AND METHODS

Sampling

Three hundred twenty-one plants sampled from 79 populations (Fig. 1b) covering the geographical range of the *T. sidoides* complex in Argentina and Uruguay were analysed for this study. Six of these samples were obtained from herbarium specimens, and the rest were collected in the field by the authors. An effort was made to provide a thorough representation of the entire region to avoid artefactual patterns caused by gaps in the distribution of the samples. Taxonomic determinations were based on the treatment of Arbo (1985). Natural populations

were randomly sampled, and plants were transported to Corrientes (Argentina), where they were grown in a greenhouse. Voucher specimens from each population have been deposited in the Herbarium of the Instituto de Botánica del Nordeste (CTES). Detailed information about collection sites, ploidy levels and the number of individuals for each population is provided as Supplementary Material (Table S1).

DNA extraction and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of cpDNA

Total DNA was extracted from silica-gel-dried leaves using a modified hexadecylmethylammonium bromide (CTAB) protocol taken from Doyle & Doyle (1987) and Cullings (1992). Up to eight individuals were analysed per population (Table S1). Ten-microlitre PCRs were set up using 1 μL of undiluted DNA using primers E and F (Taberlet *et al.*, 1991). The PCR mix contained 0.16 units of NEB Taq polymerase (New England Biolabs, Beverly, MA, USA) 1.5 mM $MgCl_2$, 0.4 μM of each primer, and 0.1 mM of each dNTP in the manufacturer's buffer. Amplification was carried out in a Biometra® T3 Thermoblock. The program consisted of an initial 5 min at 95°C, followed by one cycle of 1 min at 94°C, 1 min at 58°C, and 2 min 30 s at 72°C; the annealing temperature was then decreased by 1°C for 6 cycles, and then 32 additional cycles were carried out with an annealing temperature of 52°C, followed by a final elongation step of 5 min at 72°C. Amplification was confirmed by agarose gel electrophoresis, and product concentration was estimated visually by comparison with known standards. Aliquots containing *c.* 100 ng of the amplification product were digested with 2 units of *Mse*I for 3 h at 37°C. Restriction fragments were separated by electrophoresis in 2% agarose gels at 6 V cm^{-1} for 2 h. After electrophoresis, gels were stained with ethidium bromide, destained for 1 h in distilled water, and examined under UV light.

Sequencing

Four individuals that appeared clearly differentiated based on preliminary isozyme data (Solís Neffa, 2000; Solís Neffa & Faloci, 2003) were used for sequencing. Four non-coding chloroplast regions were amplified and sequenced: the *trn*T (UGU)–*trn*L (UAA) and *trn*L (UAA)–*trn*F (GAA) intergenic spacers, and the *trn*L (UAA) and *trn*G(UCC) introns. The universal primers of Taberlet *et al.* (1991) were used for the first three regions, and the

*trn*G intron was amplified with the external primers reported in Shaw *et al.* (2004). PCR amplification and sequencing were carried out as described in Vaio *et al.* (2005). The sequences were edited manually using SEQUENCHER™ (V4.1.4, Genecodes, Ann Arbor, MI, USA), and the same software was used to identify putative restriction sites.

cpDNA inheritance

Three diploid hybrid progenies whose parents showed different PCR-RFLP haplotypes were analysed ($S_{159}L \times S_{187}B$, $S_{215}B \times S_{159}L$ and $S_{159}L \times S_{116}B$). The number of individuals available for analysis in these progenies was four, one and three, respectively.

RESULTS

A total of five variable features were identified in the sequences: a 2-bp length variation in a polyG tract in the *trn*T–*trn*L spacer; a 6-bp inversion in the *trn*L intron (not shown); and a 4-bp indel, a variable length polyA/T, and a variable *Mse*I restriction site in the *trn*L–*trn*F spacer (Fig. 2). No variability was found in the *trn*G intron. All sequences were submitted to GenBank (accession numbers DQ110381–DQ110388 and DQ110390–DQ110402). The *trn*L–*trn*F spacer was selected to characterize all the samples because of the easily detectable variability in standard agarose gels. Three haplotypes can be recognized in this region: A (308 bp), B (304 = 189 + 115 bp) and C (313 to 315 = (200 to 198) + 115 bp). Considering only the *trn*L-*trn*F spacer, these three haplotypes differ from one another by two independent characters each. Haplotype C differs from A in both the presence of the restriction site and an indel; haplotype B differs from A in the presence of the *Mse*I restriction site and another indel; and the 11-bp length difference between B and C that we detected in the gels is caused by the simultaneous presence of two independent indels (Fig. 2).

The analysis of the hybrid progenies showed that all individuals exhibited the maternal haplotype. Results for $S_{159}L \times S_{187}$ B and $S_{215}B \times S_{159}L$ progenies are shown in Fig. 3. All of the populations screened were monomorphic, except for three variable populations of subsp. *pinnatifida* (S_{118}, S_{31} and S_{32}; Table S1, Fig. 1b).

Not all the haplotypes found in polyploids within a given subspecies were found in the corresponding diploids. The subspecies *carnea* and *integrifolia* contained the three haplotypes. In both subspecies, haplotype C was the most frequent

Subspecies	Population		Total length	Haplotype
sidoides	S_{101}	AAAAATTCTTTATCT (105bp) ATCCTTTTTTTTTTATTTTTAATT	315	C
integrifolia	S_{35}	AAAAATTCTTTATCT (105bp) ATCCTTTTTTTTT--TTTTTAATT	313	C
pinnatifida	S_{118}	AAAAATT----ATCT (105bp) ATCCTTTT-------TTTTTAATT	304	B
pinnatifida	S_{169}	AAAAATT----ATCT (105bp) ATCCTTTT-------TTTTTAATT	304	B
pinnatifida	S_{187}	AAAAATT----ATCT (105bp) ATCCTTTT-------TTTTTAATT	304	B
carnea	S_{215}	AAAAATT----ATCT (105bp) ATCCTTTT-------TTTTTAATT	304	B
integrifolia	S_{226}	AAAAATT----ATCT (105bp) ATCCTTTT-------TTTTTAATT	304	B
pinnatifida	S_{73}	AAAAATT----ATCT (105bp) ATCCTTTT-------TTTTTAATT	304	B
holosericea	S_{131}	AAAAATTCTTTATCT (105bp) ATCCTTTT-------TTTTGAATT	308	A
holosericea	S_{159}	AAAAATTCTTTATCT (105bp) ATCCTTTT-------TTTTGAATT	308	A

Figure 2 Differences among the sequences of the three haplotypes detected for the *trn*L–*trn*F spacer. Bases are counted from the first base of primer E (*trn*L), and total lengths (bp) are up to the last base of primer F (*trn*F). The target site of *Mse*I is underlined. Note that the C haplotype may vary in length.
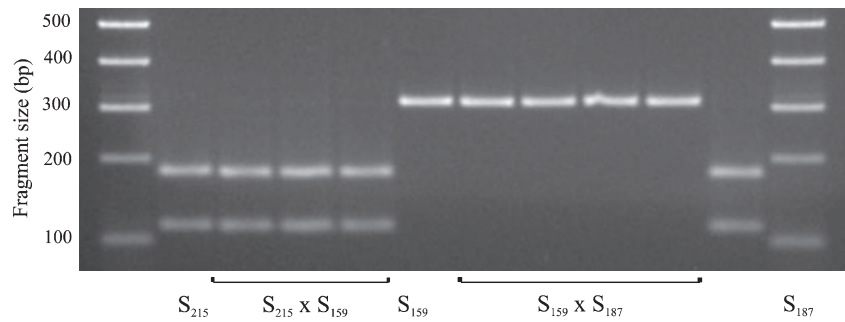
Figure 3 PCR-RFLP of the *trn*L–*trn*F spacer in two intersubspecific diploid hybrid progenies of *Turnera sidoides* L. The female progenitor is shown on the left of each progeny, and the pollen donor on the right.

(50%). In subsp. *carnea*, diploids exhibited haplotype B; tetraploids, haplotypes B and C; and hexaploids, haplotype A. In subsp. *integrifolia*, the only diploid population analysed showed haplotype A, while all three haplotypes were found among its tetraploids. In subsp. *holosericea*, the prevailing haplotype was A (71.4%), which was observed among diploid, tetraploid and hexaploid populations; haplotype C was observed among tetraploid populations (14.3%). *Turnera sidoides* subsp. *pinnatifida*, exhibited haplotype B exclusively, except for the mixed diploid population (S_{118}), which also contained haplotype A, and the tetraploids S_{31} and S_{32}, which contained haplotypes B and C. Finally, in subsp. *sidoides*, populations analysed contained haplotype C. The correspondence between cytotypes and haplotypes is shown in Table S1.

A clear geographic structure of haplotypes is revealed when haplotypes are plotted for the complex as a whole regardless of subspecies and cytotype (Fig. 1b). On the basis of haplotype distribution, three distinct regions may be identified within the *T. sidoides* area. The western part of the range is characterized by the presence of haplotype B and almost completely coincides with the present distribution area of subsp. *pinnatifida*, both in Argentina and Uruguay. This haplotype region occurs in the relatively more xeric locations within the species range, from mountain regions in the Andes (valleys and mountain grasslands) and sierras (Subandean, Pampean, Tandilia and Ventania), to the Chaco-Pampa and Mesopotamia plains. The second region, where populations harbouring haplotype A are found, includes north-eastern Argentina and northern Uruguay (Cuchilla de Haedo region). The eastern slope of the Haedo Cuchilla constitutes the western limit of the third region, occupied by populations with haplotype C. This region extends towards eastern Uruguay through the eastern Serranías region. The only exceptions are two populations harbouring haplotype C that occur near the Uruguay River; however, the sequence obtained from one of these populations (S_{35}) is slightly different from that found in subsp. *sidoides* (S_{101}; Fig. 2).

DISCUSSION

cpDNA inheritance in T. sidoides

Length polymorphisms in the *trn*L–*trn*F spacer and its *Mse*I restriction patterns have provided informative cpDNA variability in the *T. sidoides* complex. The biological and biogeographical interpretation of this variability is dependent on cpDNA pattern of inheritance. A strong bias towards paternal inheritance, with some evidence of biparental transmission, has been reported in the *Turnera ulmifolia* complex (Shore *et al.*, 1994; Shore & Triassi, 1998). In our study, however, we found strictly maternal inheritance in the eight available hybrids whose parents could be differentiated based on the current marker. Although the occurrence of occasional biparental or even paternal inheritance cannot be excluded completely, our results indicate that the cpDNA haplotypes described herein mostly represent the history of seed flow within *T. sidoides*.

Intrapopulational variability

In *T. sidoides*, propagation may occur vegetatively by rhizomes or sexually by seeds (Solís Neffa, 2000). Seeds do not show any adaptation for long-distance dispersal (Solís Neffa, 2000), so it is likely that most populations originate from a few founder individuals; this is consistent with the lack of intrapopulational variability. As a consequence, our sampling probably reflects quite accurately the actual distribution of the haplotypes in the field, in spite of the rather low number of individuals analysed per population.

Populations of subsp. *pinnatifida* only showed haplotype B, despite the wide range and morphological polymorphism of this subspecies. The only exceptions were the mixed populations S_{118}, S_{31} and S_{32} (Fig. 1b). Population S_{118}, which contains haplotypes B and A, is very distant from the nearest source of haplotype A. The presence of an *Mse*I restriction site is the only difference between haplotypes A and B that is detectable with our technical approach. This restriction site may have been lost independently; therefore, we cannot state unambiguously that population S_{118} represents a geographical outlier. A different situation is represented by populations S_{31} and S_{32}. Both exhibit haplotypes B and C. Population S_{31} is geographically close to a population of subsp. *integrifolia* with haplotype C. Even though intermediate forms between subspecies *integrifolia* and *pinnatifida* are not known from herbarium specimens (Solís Neffa, personal observation), artificial crosses between these two subspecies at the tetraploid level resulted in very fertile hybrids (pollen fertility over 90%) (Solís Neffa, 2000). Thus, ongoing intersubspecific hybridization could account for the introgression of haplotype C into population S_{31}. Population S_{32}

grows about 400 km away from the nearest sampled populations with haplotype C; thus variation within this population is not easily explained by recent hybridization, and is probably the result of long-range dispersal or long-term *in situ* survival of a polymorphic population. Other than this, no unequivocal evidence for recent long-range seed dispersal was found in our data set.

cpDNA variation among and within subspecies

The only two subspecies within which haplotype variability was not recorded were *sidoides* and *pinnatifida*. *Turnera sidoides* subsp. *sidoides* has a restricted range, while subsp. *pinnatifida* is widely distributed and has been well represented in this study. The lack of haplotypic variation recorded here may be the result either of a relatively fast expansion of the subspecies to its current range or of the inability of our marker to reveal it. At the other extreme, *carnea* and *integrifolia*, which are morphologically and geographically more homogeneous, had three cpDNA haplotypes. Introgression of cpDNA haplotypes across subspecific boundaries may provide one possible explanation for this observed variability. Reproductive barriers among the subspecies of the complex are incomplete (Solís Neffa, 2000), especially at the tetraploid level, and intermediate forms have been found in herbarium specimens and included in our data set. Given the hypothesized low mobility of seeds, and the presence of self-incompatibility, it is possible that the cytoplasm of a small population, or of a single individual at a given site, could be captured by the pollen supply from an advancing neighbouring population of a different subspecies (Potts & Reids, 1988). An equivalent mechanism has been proposed to explain a similar pattern of species-independent cpDNA variation in oaks (Dumolin-Lapègue *et al.*, 1999).

The mechanism explained above may also account for the morphological correspondence between diploid and polyploid cytotypes within each subspecies in spite of haplotypic diversity. Diploids of *T. sidoides* have been reported to produce unreduced gametes (Solís Neffa, 2000). The production of $2n$ gametes by diploids would offer a mechanism for cytoplasmic gene flow from diploid to polyploidy cytotypes. The reverse direction of gene flow from a higher to a lower ploidy level has been proposed for some polyploid complexes (Savidan & Pernès, 1981), but there is no evidence that this could occur in *T. sidoides*. Sexual polyploidization could account for several independent origins of polyploidy within different subspecies. In fact, there is abundant evidence for multiple origins of polyploids in the literature (Soltis & Soltis, 1999). As long as the entities involved are not completely isolated reproductively, there would not necessarily be a correspondence between their chloroplast haplotypes and that of the diploids that they are derived from, because chloroplast capture could have occurred before, during, or after polyploidization. With the current techniques, and given the proposed mechanisms, we do not expect to find detectable differential signatures for the occurrence of a limited number or multiple polyploidization events. Several and more sensitive markers would be required to analyse such a scenario.

Biogeographical implications

The analysis of cpDNA variation across the distributional range of *T. sidoides* has revealed that, in this species complex, the haplotype distribution tends to be geographically structured. Historical explanations have been suggested for cpDNA geographic patterns in various species of the Northern Hemisphere (Whittemore & Shaal, 1991; Ferris *et al.*, 1993; Petit *et al.*, 1993, 2002; Soltis *et al.*, 1997; Taberlet *et al.*, 1998). These studies inferred that present geographic structuring is a consequence of survival in peripheral refugia and subsequent re-immigration into vacant areas after the glaciers retreated, or alternatively of long-term *in situ* survival in glaciated regions in isolated ice-free areas above the ice shield (Brochmann *et al.*, 2003; Stehlik, 2003). Climatic changes during the Quaternary in southern South America did not involve the exclusion of vegetation by the formation of extensive ice sheets. The climatic changes in this area are mostly explained by minor shifts in the location of the Atlantic and Pacific anticyclones. While changes in temperature were modest, shifts in the circulation patterns produced major changes in the distribution of precipitation (Iriondo, 1992, 1999; Iriondo & García, 1993). A dry period occurred at the end of Pleistocene, during the LGM, and a briefer and less severe one in the Upper Holocene (Iriondo & García, 1993). During the drier periods, a pronounced aeolian activity deflated and redeposited large masses of silt and fine sand over most of the lowlands area (Popolizio, 1982, 2003; Iriondo & García, 1993; Panario & Gutiérrez, 1999). The vegetation cover of the region has not been stable since the LGM owing to oscillations in the balance between xerophitic and tropical and subtropical types of vegetation. The former have advanced repeatedly towards the north-east during the dry and colder periods and retreated towards the south-west during the humid and warmer periods (Popolizio, 1970, 2003; Iriondo, 1992). On the eastern side of the range, there is also independent evidence for the same drier or highly seasonal periods (Bracco *et al.*, 2005), although the results of Iriarte (2006) do not support the occurrence of dry conditions during the Upper Holocene. After the LGM, on the western side of the range, the thawing of ice sheets on the adjacent Andes gave rise to massive fresh-water lakes in depressions along the Paraguay and Paraná rivers, reaching southern Paraguay and the northern part of the province of Corrientes (Tapia, 1935). In the northern part of Buenos Aires province, another massive lake extended westwards to the Andes. During the cold-dry phases, the river valleys and ravines in the hilly areas of Argentina, Uruguay and Rio Grande do Sul, as well as some lowland areas, may have served as refugia for a flora adapted to more humid climates (Vuilleumier, 1971; Ab'Sáber, 1977; Haffer, 1982). The fragmentation of this flora may have led to the genetic differentiation of plant populations in isolation. In this

scenario, genetic drift would have fixed the haplotypes found in *T. sidoides* in three possible refugial centres, from which in turn individuals harbouring each haplotype may have recolonized the present area.

Some of the elevations at the western limit of the study area, which were proposed by Ab'Sáber (1977), Brown (1982), and Gentry (1986) as refugia, may have served as a centre for the fixation of the B haplotype. Within this area, the north-west may be of particular relevance, because today it is an important centre of morphological variation and is the main location of diploid populations of subsp. *pinnatifida* (Solís Neffa *et al.*, 2004). It has also been suggested that tetraploids, which are scattered over the entire Chaco-Pampean region, would have expanded their ranges up to the current boundaries from those refugia (Solís Neffa & Fernández, 2001). Our data are congruent with this hypothesis, in that all of the populations analysed in this region contain haplotype B. The alluvial plains along the palaeochannels of the main rivers of the Chaco-Pampean and Mesopotamian plains may have served as dispersion routes, as suggested by Popolizio (1970) and Spichiger *et al.* (2004).

A second refugial centre, suggested by the distribution of haplotype A, may have included the upper Uruguay river valley (as hypothesized by Ab'Sáber, 1977), or the ravines of the Haedo Cuchilla system in northern Uruguay, which still harbour a subtropical type of flora requiring moister conditions than those found in the surrounding areas (Grela, 2004). Today the region represents a peripheral area where remnants of subtropical rain forest are replaced by subtropical medium-tall grasslands. Alternatively, these ravine systems or the Uruguay river may have served as dispersion routes if refugial areas were located farther north.

Finally, haplotype C extends over an area where the most salient geomorphological feature is the southernmost outcrop of the Brazilian Shield, which includes the eastern Serranías in Uruguay and the south-eastern Serras in Rio Grande do Sul. Ab'Sáber (1977) suggests that the slopes of these hilly systems may have provided moister refugia for the local flora during the dry-cold phases of the climatic oscillations. The floristic distinctiveness of this region has previously been noted by Rambo (1954), who hypothesized that these elevated metamorphic formations may have been isolated in the past by marine ingressions.

A floristic connection among the higher-elevation ranges within this area is an old hypothesis (Rambo, 1954; De la Sota, 1967; Crisci *et al.*, 2001), and it has been suggested that these areas constitute present-day refugia for some species (Grela, 2004). Likewise, it has been suggested that these orographic features may have provided refugia for a flora adapted to more humid climates during the dry-cold phases that have occurred since the LGM (Ab'Sáber, 1977). The overall distribution of the *T. sidoides* complex is congruent with the proposed floristic connections; however, the clear geographic structure of cpDNA haplotypes suggests specific separate refugial areas in the past, and, therefore, provides novel hypotheses to be addressed by future research. The current analysis of the

T. sidoides complex indicates that it is an informative biogeographical model system and that further efforts to test the hypotheses provisionally proposed here would be worthwhile.

CONCLUSIONS

We propose three putative refugial areas for the *T. sidoides* complex associated with major orographical systems in the region. Ravines and slopes in the Haedo Cuchilla system in northern Uruguay, the elevations on the western side of the area in Argentina, and the eastern Serranías system in south-eastern Uruguay may have served as refugia in which the A, B and C haplotypes, respectively, became fixed during the drier climatic phases. Our results constitute a first attempt to interpret the influence of historical events in the current distribution and diversification of the mid-latitude South American lowlands flora using cpDNA analysis. Further studies, of other taxa, are required to determine if there are general phylogeographic patterns for this region.

REFERENCES

Ab'Sáber, A.N. (1977) Espaços ocupados pela expansão dos climas secos na America do Sul, por ocasião dos períodos glaciais quaternários. *Paleoclimas*, **3**, 1–19.

Ab'Sáber, A.N. (1982) The paleoclimate and paleoecology of Brazilian Amazonia. *Biological diversification in the Tropics* (ed. by G.T. Prance), pp. 41–59. Plenum Press, New York, NY.

Arbo, M.M. (1985) Notas taxonómicas sobre turneráceas Sudamericanas. *Candollea*, **40**, 175–191.

Behling, H. (1995) A high resolution Holocene pollen record from Lago do Pires, SE Brazil, vegetation, climate and fire history. *Journal of Paleolimnology*, **14**, 253–268.

Behling, H. (1997) Late Quaternary vegetation, climate and fire history in the *Araucaria* forest and campos region from Serra Campos Gerais (Paraná), S Brazil. *Review of Palaeobotany and Palynology*, **97**, 109–121.

Behling, H. (2002) Carbon storage increases by major forest ecosystems in tropical South America since the Last Glacial

Maximum and the early Holocene. *Global and Planetary Change*, **33**, 107–116.

Bracco, R., del Puerto, L., Inda, H. & Castiñeira, C. (2005) Mid–late Holocene cultural and environmental dynamics in Eastern Uruguay. *Quaternary International*, **132**, 37–45.

Brochmann, C., Gabrielsen, T.M., Nordal, I., Landvik, J.Y. & Elven, R. (2003) Glacial survival or tabula rasa? The history of North Atlantic biota revisited. *Taxon*, **52**, 417–450.

Brown, K.S. (1982) Paleoecology and regional pattern of evolution in neotropical forest butterflies. *Biological diversification in the Tropics* (ed. by G.T. Prance), pp. 255–306. Plenum Press, New York, NY.

Bull-Hereñu, K., Martínez, E.A. & Francisco, A. (2005) Structure and genetic diversity in *Colliguaja odorifera* Mol. (Euphorbiaceae), a shrub subjected to Pleisto-Holocenic natural perturbations in a mediterranean South American region. *Journal of Biogeography*, **32**, 1129–1138.

Burgos, J.J. (1970) El clima de la región noreste de la República Argentina en relación con la vegetación natural y el suelo. *Boletín de la Sociedad Argentina de Botánica*, **11**, 37–101.

Burnham, R.J. & Graham, A. (1999) The history of Neotropical vegetation: new developments and status. *Annals of the Missouri Botanical Garden*, **86**, 546–589.

Cabrera, A.L. (1971) Fitogeografía de la República Argentina. *Boletín de la Sociedad Argentina de Botánica*, **14**, 1–55.

Cabrera, A.L. & Willink, A. (1973) *Biogeografía de América Latina*. Secretaría General OEA, Washington, DC.

Clapperton, C.M. (1993) Nature of environmental changes in South America at the Last Glacial Maximum. *Palaeogeography, Palaeoclimatology, and Palaeoecology*, **101**, 189–208.

Colinvaux, P.A., De Oliveira, P.E., Moreno, J.E., Miller, M.C. & Bush, M.B. (1996a) A long pollen record from lowland Amazonia: forest and cooling in glacial times. *Science*, **274**, 85–88.

Colinvaux, P.A., Liu, K.-B., de Oliveira, P.E., Bush, M.B., Miller, M.C. & Steinitz Kannan, M. (1996b) Temperature depression in the lowland tropics in glacial times. *Climatic Change*, **32**, 19–33.

Comes, H.P. & Kadereit, J.W. (1998) The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science*, **3**, 432–438.

Crisci, J.V., Freire, S.E., Sancho, G. & Katinas, L. (2001) Historical biogeography of the Asteraceae from Tandilia and Ventania mountain ranges (Buenos Aries, Argentina). *Caldasia*, **23**, 21–41.

Cullings, K.W. (1992) Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology*, **1**, 233–240.

Damuth, E. & Fairbridge, R.W. (1970) Equatorial Atlantic deep-sea arkosic sands and Ice-Age aridity in tropical South America. *Bulletin of the Geological Society of America*, **81**, 189–206.

De la Sota, E.R. (1967) Composición, origen y vinculaciones de la flora pteridológica de las sierras de Buenos Aires (Argentina). *Boletín de la Sociedad Argentina de Botánica*, **2–3**, 105–128.

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, **19**, 11–15.

Dumolin-Lapègue, A., Kremer, A. & Petit, R.J. (1999) Are chloroplast and mitochondrial DNA variation species independent in oaks? *Evolution*, **53**, 1406–1413.

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

Ferris, C., Oliver, R.P., Davy, A.J. & Hewitt, G.M. (1993) Native oak chloroplasts reveal an ancient divide across Europe. *Molecular Ecology*, **2**, 337–344.

Gentry, A.H. (1986) Endemism in tropical vs. temperate plant communities. Conservation biology. *The science of scarcity and diversity* (ed. by M.E. Soulé), pp. 153–181. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Grela, I.A. (2004) Geografía florística de las especies arbóreas de Uruguay: propuesta para la delimitación de dendrofloras. Msc thesis, Programa de Desarrollo de las Ciencias Básicas, Ministerio de Educación y Cultura, Universidad de la República, Montevideo, Uruguay.

Haffer, J. (1982) General aspects of the refuge theory. Part 1: The refuge theory. *Biological diversification in the Tropics* (ed. by G.T. Prance), pp. 6–24. Columbia University, New York, NY.

van der Hammen, T. & Absy, M.L. (1994) Amazonia during the last glacial. *Palaeogeography, Palaeoclimatology, and Palaeoecology*, **109**, 247–261.

Hewitt, G.M. (1996) Some genetic consequence of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Instituto Brasileiro de Geografia e Estatística (1997) *Recursos naturais e meio ambiente: uma visão do Brasil*. Río de Janeiro, Brazil.

Iriarte, J. (2006) Vegetation and climate change since 14,810 ^{14}C yr B.P. in southeastern Uruguay and implications for the rise of early formative societies. *Quaternary Research*, **65**, 20–32.

Iriondo, M.H. (1981) Antigüedad del último cambio climático en el Litoral. *Ecología*, **6**, 5–8.

Iriondo, M.H. (1991) El Holoceno en el Litoral. *Comunicaciones del Museo Provincial de Ciencias Naturales 'Florentino Ameghino' (Nueva Serie)*, **3**, 1–39.

Iriondo, M.H. (1992) El Chaco. *Holoceno*, **1**, 50–63.

Iriondo, M.H. (1999) Climatic changes in the South American plains: records of a continent-scale oscillation. *Quaternary International*, **57–58**, 93–112.

Iriondo, M.H. & García, N.O. (1993) Climatic variations in the Argentine plains during the last 18,000 years. *Palaeogeography, Palaeoclimatology, and Palaeoecology*, **101**, 209–220.

Ledru, M.P. (1993) Late Quaternary environmental and climatic changes in Central Brazil. *Quaternary Research*, **39**, 90–98.

Markgraf, V. (1991) Younger Dryas in southern South America? *Boreas*, **20**, 63–69.

Markgraf, V. & Bradbury, J.P. (1982) Holocene climatic history of South America. *Striae*, **16**, 40–45.

Morrone, J.J. (2000) What is the Chacoan subregion? *Neotropica*, **46**, 51–68.

Morrone, J.J. (2006) Biogeographic areas and transition zones of Latin America and the Caribbean Islands, based on panbiogeographic and cladistic analyses of the entomofauna. *Annual Review of Entomology*, **51**, 467–494.

Muellner, N.A., Tremetsberger, K., Stuessy, T. & Baeza, C.M. (2005) Pleistocene refugia and recolonization routes in the southern Andes: insights from *Hypochaeris palustris* (Asteraceae, Lactuceae). *Molecular Ecology*, **14**, 203–212.

Panario, D. & Gutiérrez, O. (1999) The continental Uruguayan Cenozoic: an overview. *Quaternary International*, **62**, 75–84.

Pastorino, M.J. & Gallo, L.A. (2002) Quaternary evolutionary history of *Austrocedrus chilensis*, a cypress native to the Andean-Patagonian forest. *Journal of Biogeography*, **29**, 1167–1178.

Pastorino, M.J., Gallo, L.A. & Hattemer, H.H. (2004) Genetic variation in natural populations of *Austrocedrus chilensis*, a cypress of the Andean-Patagonian forest. *Biochemical Systematics and Ecology*, **32**, 993–1008.

Pennington, R.T., Prado, D.E. & Pendry, C.A. (2000) Neotropical seasonally dry forests and Quaternary vegetation changes. *Journal of Biogeography*, **27**, 261–273.

Petit, R.J., Kremer, A. & Wagner, D.B. (1993) Geographic structure of chloroplast DNA polymorphism in European oaks. *Theoretical and Applied Genetics*, **87**, 122–128.

Petit, R.J., Csaikl, U.M., Bordács, S., Burg, K., Coart, E., Cottrell, J., van Dam, B., Deans, J.D., Dumolin-Lapègue, S., Fineschi, S., Finkeldey, R., Gillies, A., Glaz, I., Goicoechea, P.G., Jensen, J.S., König, A.O., Lowe, A.J., Madsen, S.F., Mátyás, G., Munro, R.C., Olalde, M., Pemonge, M.H., Popescu, F., Slade, D., Tabbener, H., Taurchini, D., de Vries, S.G.M., Ziegenhagen, B. & Kremer, A. (2002) Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, **156**, 5–26.

Pielou, E.C. (1991) *After the Ice Age*. The University of Chicago Press, Chicago, IL.

Popolizio, E. (1970) Algunos rasgos de la geomorfología del Nordeste Argentino. *Boletín de la Sociedad Argentina de Botánica*, **11**(Suppl.), 17–35.

Popolizio, E. (1982) Geomorphology of the Argentine Northeast. *Water International*, **7**, 162–177.

Popolizio, E. (2003) El Paraná, un río y su historia geomorfológica. PhD thesis, Universidad del Salvador, Buenos Aires, Argentina.

Potts, B.M. & Reids, J.B. (1988) Hybridization as a dispersal mechanism. *Evolution*, **42**, 1245–1255.

Prado, D.E. (1993a) What is the Gran Chaco vegetation in South America? I. A review. Contribution to the study of the flora and vegetation of the Chaco. *Candollea*, **48**, 145–172.

Prado, D.E. (1993b) What is the Gran Chaco vegetation in South America? II. A redefinition. Contribution to the study of the flora and vegetation of the Chaco. *Candollea*, **48**, 615–629.

Prado, D.E. (2000) Seasonally dry forest of tropical South America: from forgotten ecosystems to a new phytogeographic unit. *Edinburgh Journal of Botany*, **57**, 437–461.

Prado, D.E. & Gibbs, P.E. (1993) Patterns of species distributions in the dry seasonal forest of South America. *Annals of the Missouri Botanical Garden*, **80**, 902–927.

Prance, G.T. (1996) Islands in Amazonia. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **351**, 823–833.

Premoli, A.C., Kitzberger, T. & Veblen, T.T. (2000) Isozyme variation and recent biogeographical history of the long-lived conifer *Fitzroya cupressoides*. *Journal of Biogeography*, **27**, 251–260.

Prieto, A.R. (1996) Late Quaternary vegetational and climatic changes in the Pampa grassland of Argentina. *Quaternary Research*, **45**, 73–88.

Rambo, B. (1954) Análise histórica da flora de Pôrto Alegre. *Sellowia*, **6**, 9–112.

Savidan, Y. & Pernès, J. (1981) Diploid-tetraploid-dihaploid cycles in the evolution of *Panicum maximum* Jacq. *Evolution*, **36**, 596–600.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.S., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E. & Small, R.L. (2004) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, **92**, 142–196.

Shore, J.S. & Triassi, M.T. (1998) Paternally biased cpDNA inheritance in *Turnera ulmifolia* (Turneraceae). *American Journal of Botany*, **85**, 328–332.

Shore, J.S., McQueen, K.L. & Little, S.H. (1994) Inheritance of plastid DNA in the *Turnera ulmifolia* complex (Turneraceae). *American Journal of Botany*, **81**, 1636–1639.

Simpson, B.B. & Todzia, C.A. (1990) Patterns and process in the development of the High Andean flora. *American Journal of Botany*, **77**, 1419–1432.

Solís Neffa, V.G. (2000) Estudios biosistemáticos en el complejo Turnera sidoides L. (Turneraceae, Leiocarpae). PhD thesis, Universidad Nacional de Córdoba, Argentina.

Solís Neffa, V.G. & Faloci, M. (2003) *Caracterización isoenzimática de poblaciones diploides de las subespecies del complejo* Turnera sidoides *L. (Turneraceae)*. XIV Comunicaciones Científicas y Técnicas. Facultad de Ciencias Agrarias, UNNE, Argentina.

Solís Neffa, V.G. & Fernández, A. (2001) Cytogeography of the *Turnera sidoides* L. complex (Turneraceae, Leiocarpae). *Botanical Journal of the Linnean Society*, **137**, 189–196.

Solís Neffa, V.G. & Seijo, J.G. (2003) Citogeografía del complejo autopoliploide *Turnera sidoides* L. (Turneraceae). *Boletín de la Sociedad Argentina de Botánica*, **38**(Suppl.), 247–248.

Solís Neffa, V.G., Panseri, A.F., Reynoso, W.L. & Seijo, J.G. (2004) Variación del color de flores y números cromosómicos en el noroeste del área de distribución de *Turnera sidoides* (Turneraceae). *Bonplandia*, **13**, 117–128.

Soltis, D.E. & Soltis, P.S. (1999) Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution*, **14**, 348–352.

Soltis, D.E., Gitzendanner, M.A., Strenge, D.D. & Soltis, P.S. (1997) Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematic and Evolution*, **206**, 353–373.

Soriano, A. (1991) Río de la Plata grasslands. *Natural grasslands. Introduction and Western Hemisphere*: (ed. by R.T. Coupland), pp. 367–407. Elsevier, Amsterdam.

Soubies, F., Suguion, K., Martin, L., Leprun, J., Servant, M., Turcq, B., Fournier, M., Delaune, M. & Sfeddine, A. (1991) The Quaternary deposits of the Serra dos Carajas (State of Pará, Brazil) – ages and other preliminary results. *Boletim Instituto de Geosciencias-Universidade de São Paulo, Publicaçao Especial*, **8**, 223–243.

Spichiger, R., Calenge, C. & Bise, B. (2004) Geographical zonation in the Neotropics of tree species characteristic of the Paraguay-Paraná Basin. *Journal of Biogeography*, **31**, 1489–1501.

Stehlik, I. (2003) Resistance or emigration? Response of alpine plants to the Ice Age. *Taxon*, **52**, 499–510.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Tapia, A. (1935) *Pilcomayo. Contribución al conocimiento de las llanuras argentinas*. Dirección de Minas y Geología, Boletín No. 40, Buenos Aires, Argentina.

Tonni, E.P. & Fidalgo, F. (1978) Consideraciones sobre los cambios climáticos durante el Pleistoceno tardío-reciente en la Provincia de Buenos Aires. Aspectos ecológicos y zoogeográficos relacionados. *Ameghiniana*, **15**, 235–253.

Vaio, M., Speranza, P., Valls, J.F., Guerra, M. & Mazzella, C. (2005) Localization of the 5S and 45S rDNA sites and cpDNA sequence analysis in species of the *Quadrifaria* group of *Paspalum* (Poaceae, Paniceae). *Annals of Botany*, **96**, 191–200.

Vargas, P. (2003) Molecular evidence for multiple diversification patterns of alpine plants in Mediterranean Europe. *Taxon*, **52**, 463–476.

Vuilleumier, B. (1971) Pleistocene changes in the fauna and flora of South America. *Science*, **173**, 529–554.

Whittemore, A.T. & Shaal, B.A. (1991) Interspecific gene flow in oaks. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 2540–2544.

Zárate, M. & Blasi, A. (1993) Late Pleistocene–Holocene eolian deposits of Southern Buenos Aires Province, Argentina: a preliminary model. *Quaternary International*, **17**, 15–20.

Zucol, A.F., Brea, M. & Scopel, A. (2005) First record of fossil wood and phytolith assemblages of the Late Pleistocene in El Palmar National Park (Argentina). *Journal of South American Earth Sciences*, **20**, 33–43.

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1. Detailed information about the populations of *Turnera sidoides* L. included in this study.

This material is available as part of the online article from: http://www.blackwell-synergy.com/http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2699.2006.01622.x (This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

BIOSKETCHES

Pablo R. Speranza has a faculty position at the Universidad de la República (Uruguay). He has been working on the evolutionary patterns of polyploid complexes since 1992 using cytogenetic and molecular approaches.

J. Guillermo Seijo is a researcher at the National Research Council of Argentina and a Professor at the Universidad Nacional del Nordeste. His research is focused on the cytogenetics and evolutionary biology of plant species native to temperate and subtropical areas of South America.

Iván Grela has a faculty position at the Universidad de la República (Uruguay). He is currently working on natural range patterns and the geographic distribution of species richness in Uruguay.

Viviana G. Solís Neffa is a researcher of the National Research Council of Argentina and is Assistant Professor at the Universidad Nacional del Nordeste. Her research interests lie in the evolutionary biology of South American polyploid plant species, mainly focused on the *Turnera sidoides* complex.

Editor: Chris Humphries