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REGULAR PAPER

Patterns of cytotype variation of *Turnera sidoides* subsp. *pinnatifida* (Turneraceae) in mountain ranges of central Argentina

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Abstract Cytogeographical variability among 564 plants from 26 populations of *Turnera sidoides* subsp. *pinnatifida* in mountain ranges of central Argentina was analysed with meiotic chromosome counts and flow cytometry and is described at regional and local scales. Populations were primarily tetraploids ($2n = 4x = 28$), although diploid ($2n = 2x = 14$), hexaploid ($2n = 2x = 42$), and mixed populations of diploids and triploids ($2n = 3x = 21$) were also found. Diploids, triploids, and hexaploids were fewer in number and restricted to narrow areas, while tetraploids were the most common and geographically widespread cytotype. Diploids grew at higher altitudes and in colder and wet locations; tetraploids had the broadest ecological spectrum, while hexaploids occurred at the lowest altitudes and in drier conditions. The cytotypes were also spatially segregated at a microgeographical scale. Diploids grew in the piedmont, tetraploids were in the adjacent valley, and in the contact zone of both cytotypes, patches of diploids and triploids were found. At a regional scale, the distribution of the cytotypes may be governed by a combination of ecological and historical variables, while segregation in the contact zone may be independent of the selective environment because the cytotypes are unable to coexist as a result of reproductive exclusion. The role of triploids is also discussed.

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Introduction

Polyploidy plays a significant role in the evolution of vascular plants; as many as 70–80% of angiosperm species and up to 95% of pteridophytes have a polyploid origin (Masterson 1994; Soltis and Soltis 1999; Bennett 2004). Polyploidy can potentially contribute to the acquisition of new morphological, genetic and/or physiological features, which may enhance the competitive ability, fitness or ecological tolerance of polyploids compared to the diploid parents. These events, which have occurred at temporal scales from ancient to contemporary, are thought to have a fundamental role in plant adaptation and range expansion (Levin 1983; Udall and Wendel 2006). As a consequence, polyploids may have a distinct or peripheral distribution compared to the parental diploids along climatic or environmental gradients (Levin 2002). Such differences in geographical ranges among cytotypes may reflect historical patterns of colonization or genetic differentiation that has occurred in association with or subsequent to polyploid formation (Levin 1983). Alternatively, the spatial patterns of cytotypes may be explained non-adaptatively, through frequency-dependent production of hybrids with low frequency (Levin 1975).

Polyploidy is also proposed to be a major force in the evolution of *Turnera sidoides* L. (Fernández 1987; Solís Neffa and Fernández 2001). This complex of outcrossing, perennial, rhizomatous herbs is distributed from the southern regions of Bolivia and Brazil, through Paraguay to Uruguay and Argentina, reaching 39°S (Arbo 1987; Solís

Neffa 2000). Five subspecies are recognized on the basis of the geographical distribution and the great variability of some morphological features (Arbo 1985). From chromosome studies, subspecies are known to exhibit different ploidy levels based on $x = 7$, ranging from diploid ($2n = 2x = 14$) to octoploid ($2n = 8x = 56$) (Fernández 1987; Solís Neffa and Fernández 2001; Solís Neffa et al. 2004). Both meiotic chromosome associations and karyotype analysis suggest that polyploid cytotypes are of autopolyploid origin (Fernández 1987; Solís Neffa 2000; Solís Neffa and Fernández 2002).

Because the extent of geographical differentiation of cytotypes provides useful insights into the evolutionary dynamics and ecological differentiation of polyploids, we have taken a cytogeographical approach for *T. sidoides*. The results so far have revealed that, at a geographical scale and considering the complex as a whole, diploids have restricted and disjunct distributions, tetraploids are the most widespread, while populations with higher ploidy levels are rare and disjunct. Proximal and historical non-exclusive hypotheses have been proposed to explain the spatial patterns of the cytotypes in this species (Solís Neffa 2000; Solís Neffa and Fernández 2001; Solís Neffa et al. 2004). Because the widespread latitudinal and longitudinal range of *T. sidoides* encompasses a great diversity of climates and ecological regions with contrasting precipitation regimes, the geographical separation of the cytotypes was first hypothesized to result from differences in habitat requirements. However, taking into consideration the relative frequency and the geographical distribution of cytotypes, it was also hypothesized that the distribution of extant cytotypes may reflect the geomorphologic and climatic changes that affected most of the present-day geographical area of *T. sidoides* before and after the Large Glacial Maximum. Consequently, the restricted diploids may be relictual populations that have survived these periods of change, while such historical events could provide favorable conditions for the establishment of polyploids, with tetraploids having the widest distribution as a result of their range expansion (Solís Neffa and Fernández 2001; Solís Neffa et al. 2004). However, considering that the distribution of the cytotypes in relation to current climatic conditions varies among the subspecies (Solís Neffa and Fernández 2001) and that different morphogenetic and climatic historical processes gave rise to a myriad of landscapes within the range of *T. sidoides*, the available data are not enough to discriminate how extant environmental conditions and/or historical events that affected each landscape have contributed to the present cytotype distribution.

Moreover, a preliminary survey in the mountain ranges of central Argentina (Sierras de Córdoba) reveals the existence of diploid and tetraploid cytotypes of *T. sidoides* subsp. *pinnatifida* (Juss. ex Poir.) Arbo (Solís Neffa and Fernández 2001; Speranza et al. 2007). This mountain region is of particular interest because altitude and climate are important factors influencing plant distribution, and thus, we can examine the cytotype distribution along an environmental gradient. In addition, the fact that diploid plants were detected in only one locality, near (<2 km) tetraploids (V. Solís Neffa and G. Seijo personal communication), offers the opportunity to examine the interaction between cytotypes and to infer the evolutionary forces influencing the establishment of polyploids and coexistence with diploid individuals in natural populations. Nevertheless, more information is needed to determine the exact distribution of cytotypes at a microgeographical scale, identify any mixed populations, and understand the dynamics of the diploid–tetraploid contact zones for *T. sidoides*.

Thus, to better understand the evolution of polyploidy in *T. sidoides* complex and to infer the factors influencing cytotype frequency and distribution, our objective in this paper was to analyse the distribution of the different cytotypes of *T. sidoides* subsp. *pinnatifida* in Sierras de Córdoba at both the regional and local scales. To do this, we first report new ploidy level determinations and assess the frequency of cytotypes. Moreover, we analyse the spatial distribution of ploidy levels at a regional scale and relate the distributional patterns to climatic and historical factors to evaluate evidence of range expansion and habitat differentiation through polyploidy. Finally, we examine the cytotypes structure in a contact zone of diploids and tetraploids as a first step in understanding the possible evolutionary forces governing the cytotype frequency and distribution at a microgeographical scale.

Materials and methods

Plant habitats

Plant material for the present study was obtained during field collections during 2005–2008 in Sierras de Córdoba, a mountain system located in central Argentina (Fig. 1a). These ranges extend along 200 km with an N-NE to S-SW direction, from 29° to 33°30′S, rising from 500 to 3,000 m a.s.l., and comprising four major orographic units: Sierras del Norte, Sierras Orientales (or Chicas), Sierras Centrales (or Grandes), and Sierra Occidental (or de Pocho), which are separated by low valleys (Fig. 1a).

This group of mountains comprises crystalline basement uplifts that became active in the late Miocene. All these ranges present a western abrupt flank and an oriental-extended slope where the old erosion surfaces are preserved, sometimes covered by Tertiary and Quaternary sediments.

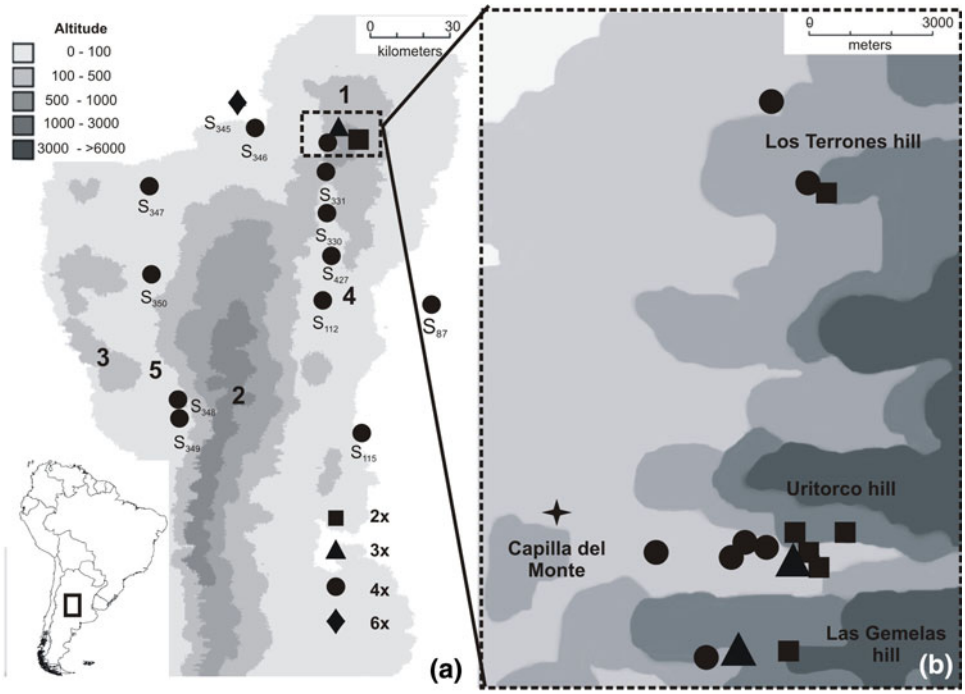

Fig. 1 Study area. **a** Map showing the distribution of the population and cytotypes of *Turnera sidoides* subsp. *pinnatifida* in Sierras of Córdoba, central Argentina (indicated by a *rectangle*). For location details see Table 1. *1* Sierras Chicas. *2* Sierras Grandes. *3* Sierras Occidentales. *4* Punilla valley. *5* Traslasierra valley. **b** Blown-up area shows the distribution of cytotypes in the contact zone of diploid and tetraploid in Capilla del Monte. *Shading* represents altitudinal gradient (m a.s.l.)

The intermountain depressions are relatively flat and filled with sediments. Soils are derived from the weathering of the granitic substrate and fine-textured eolian deposits.

The mountain system is within a temperate and semi-arid climate zone. The annual temperature ranges are lower than in the plain and fluctuate between 10 and 16°C. Above 2,000 m a.s.l., the lower temperatures in winter cause isolated snowfalls. Precipitation increases according to elevation from 500 to 900 mm/year, with most rainfall concentrated in the warmer season.

At a local scale, fieldwork was conducted in Capilla del Monte, located in the Punilla valley at the western slope of the Sierras Chicas (Fig. 1).

Plant sample collections

Populations were located using records from the herbaria of the Instituto de Botánica del Nordeste (CTES) and the Instituto Multidisciplinario de Biología Vegetal (CORD). Voucher specimens have been deposited in the Herbarium of the Instituto de Botánica del Nordeste. Details of the geographical position and altitude of the material studied are given in Table 1.

In each locality, leaf samples and floral buds of at least 15 plants were randomly collected. Sampled individuals were approximately 1 m apart. Some individual plants of each site were also transported to Corrientes (Argentina) where they were grown under homogeneous greenhouse conditions.

To examine the spatial segregation at local scale, in Capilla del Monte, we randomly collected plants along a transect (4 km), from the piedmont of the Uritorco hill towards the valley. Moreover, to test whether the pattern observed in this hill extended into this region, we also sampled sites from the piedmont of Los Terrones and Las Gemelas hills toward the valley (Fig. 1b).

Chromosome counts and ploidy level estimation

Ploidy level of sampled plants was inferred from counts of chromosome in meiosis and from estimates of relative DNA content using flow cytometry.

Chromosomes in meiotic metaphases were counted for some plants of populations S235, S235b, S234, S236, S321, S322 and S345. Meiotic chromosomes were examined in pollen mother cells of young buds collected in the field or from plants grown in the greenhouse after fixation in 5:1 absolute ethanol:lactic acid (Fernández 1973) for 12 h at 4°C and storage in 70% ethanol at 4°C. Pollen mother cells were extracted in a drop of 2% aceto-orcein after the tip of pollen sacs was cut. Then, a slight squash was done. Slides were made permanent in Euparal using Bowen's (1956) method. Chromosomes were viewed and photographed with a Leica DMRX microscope equipped with a computer-assisted Leica DC 350 digital camera system, at a magnification of ×1000.

Flow cytometry analyses were used to determine the ploidy level for most plants using leaf tissue and using the recommendations with the Partec kit CySatin UV Precise P (05-5002), which was used to prepare the samples. Briefly, 0.5 cm^2 of leaf material was placed in a Petri dish with a comparable amount of tissue from an internal standard (the diploid S235). After adding 0.5 ml of extraction buffer

Table 1 Geographical location and results of ploidy level estimations of populations of *Turnera sidoides* subsp. *pinnatifida* of Sierras de Córdoba (central Argentina) analyzed in this study

Population	Locality (voucher references)	Latitude (S)	Longitude (W)	Altitude (m)	Ploidy level
S326a	Capilla del Monte, C° Las Gemelas (Elías 6)	30°53′12.30″	64°29′52.10″	1249	$2x^{a}$
S344	Capilla del Monte, C° Los Terrones (Elías s/n)	30°46′46.90″	64°29′38.80″	1118	$2x^{a}$
S235	Capilla del Monte, Uritorco hill (Solís Neffa and Seijo 967)	30°51′50.83″	64°29′36.24″	1186	$2x^{a}$
S235b	Capilla del Monte, Uritorco hill, La Toma (Elías s/n)	30°51′55.37″	64°29′29.64″	1207	$2x^{a}$
S235c	Capilla del Monte, Uritorco hill, Mirador (Elías s/n)	30°51′54.04″	64°29′35.04″	1191	$2x^{a}$
S319	Capilla del Monte, Uritorco hill, base (Elías s/n)	30°51′45.10″	64°29′42.71″	1168	$2x^{a}$
S235d	Capilla del Monte, Uritorco hill (Elías s/n)	30°50′00.00″	64°29′02.00″	1615	$2x^{a}$
S321	Capilla del Monte (Elías 13)	30°51′47.30″	64°29′52.20″	1017	$2x–3x^{a}$
S346	San Marcos Sierra (Elías 1)	30°47′00.35″	64°38′34.20″	635	$4x^{a}$
S347	La Higuera (Elías 2)	31°00′44.80″	65°06′20.50″	612	$4x^{a}$
S350	Salsacate (Elías 3)	31°18′46.70″	65°05′40.20″	928	$4x^{a}$
S349	Mina Clavero (Elías 4)	31°44′00.40″	65°00′26.10″	890	$4x^{a}$
S348	Nono (Elías 5)	31°47′49.00″	65°00′23.00″	861	$4x^{a}$
S326b	Capilla del Monte, C° Las Gemelas (Elías 7)	30°53′08.70″	64°30′37.40″	974	$2x–3x^{a}$
S344c	Capilla del Monte, C° Los Terrones (Elías 8)	30°46′55.30″	64°30′17.30″	938	$4x^{a}$
S344a	Capilla del Monte, C° Los Terrones (Elías 10)	30°46′43.40″	64°29′44.40″	1008	$4x^{a}$
S328	La Cumbre (Elías 11)	30°59′10.60″	64°30′22.00″	1133	$4x^{a}$
S330	Villa Giardino (Elías 12)	31°02′19.10″	64°29′59.60″	1043	$4x^{a}$
S331	La Falda (Elías 14)	31°04′60.00″	64°30′00.00″	952	$4x^{a}$
S427	Cosquín (Elías s/n)	31°16′00.00″	64°29′00.00″	952	$4x^{a}$
S326c	Capilla del Monte, C° Las Gemelas (Elías 7)	30°53′08.70″	64°30′37.40″	974	$4x^{a}$
S112	Villa Carlos Paz (Solís Neffa 486)	31°24′01.93″	64°31′01.14″	661	$4x^{b}$
S234	Capilla del Monte, road to Uritorco hill (Solís Neffa and Seijo 966)	30°51′44.96″	64°30′40.66″	1007	$4x^{a}$
S236	Capilla del Monte, road to Uritorco hill (Solís Neffa and Seijo 968)	30°51′41.55″	64°30′21.43″	1027	$4x^{a}$
S237	Capilla del Monte, route 38 and road to El Zapato hill (Solís Neffa and Seijo 969)	30°51′44.96″	64°31′49.76″	977	$4x^{c}$
S332	Capilla del Monte, road to Uritorco hill (Elías s/n)	30°51′42.49″	64°30′18.78″	1129	$4x^{a}$
S322	Capilla del Monte, road to Uritorco hill (Elías s/n)	30°51′40.14″	64°30′29.00″	1116	$4x^{a}$
S318	Capilla del Monte, road to Uritorco hill (Elías s/n)	30°51′38.50″	64°30′33.88″	1110	$4x^{a}$
S115	Dique Los Molinos (Solís Neffa s/n)	31°50′45.52″	64°32′28.09″	816	$4x^{b}$
S87	Córdoba (Solís Neffa & Seijo 985)	31°24′00.00″	64°11′00.00″	361	$4x^{a}$
S345	Cruz del Eje (Elías 15)	30°43′60.00″	64°47′60.00″	449	$6x^{a}$

[a] Ploidy level estimation of new populations

[b] Solís Neffa and Fernández 2001

[c] Speranza et al. 2007

(Partec P kit CyStain UV Precise P 05-5002), the tissue was chopped with a razor blade. Following a 2-min incubation, samples were filtered through a 50-µm nylon mesh into the sample tube with 1.5 ml of DAPI (4′,6-diamidino-2-phenylindole) staining solution (Partec P kit CyStain UV Precise P 05-5002). The mixture was incubated for 2 min at room temperature and analysed. The fluorescence intensity of DAPI-stained nuclei was determined using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) with the detector operating at 355 nm. About 3,000 nuclei were measured per sample. Ploidy levels were estimated by comparing the DNA peak of the samples to the internal standard. Data analysis was performed using PA II's Partec FloMax software.

Geographical distribution of cytotypes

The distribution of the ploidy levels of the populations analysed and of populations reported previously (Solís Neffa and Fernández 2001; Speranza et al. 2007) was plotted on maps using the shareware Diva-Gis (Hijmans et al. 2004).

To assess the potential influence of altitude and climate in shaping cytotype distributions, altitudes for the collecting locations of the plants were obtained with a GPS, while the following climatic variables were extracted from the WorldClim data base (Hijmans et al. 2005): annual mean temperature, mean monthly temperature range, isothermality, temperature seasonality, temperature annual range, annual precipitation, precipitation of driest month and precipitation seasonality. We chose these variables because they represent general trends (means), variation (seasonality), and limiting variables (i.e., minimum and maximum temperatures).

The mean, standard deviation, and range of variation of each variable were also calculated for each cytotype. To evaluate the existence of significant differences for each trait among diploids, triploids and tetraploids, we analysed the data with a one-way ANOVA at a significance level of 5% ($\alpha = 0.05$) after a Bartlett's test of homogeneity. Also, Tukey's honestly significant difference (HSD) test (at 5% level) was used to test differences between each pair of means.

A principal components analysis (PCA) from a data matrix of 26 populations × 8 climatic variables was also performed. The calculation of the PCA was done on the correlation matrix estimated from the resulting data set. Standardized data were used for the multivariate analyses.

All statistical analyses were performed using the program Infostat version 2008.

The cytotype distribution in the contact zone was mapped using the shareware Diva-Gis (Hijmans et al. 2004). This software was also employed to calculate the distances among cytotype patches in this zone.

Results

Ploidy level estimation

New counts for 564 plants from 26 populations of *T. sidoides* subsp. *pinnatifida* are reported. Ploidy levels obtained from both meiotic divisions and flow cytometry estimations (Fig. 2) of each population analysed are presented in Table 1. Populations studied were primarily tetraploids ($2n = 4x = 28$; 68.97%), although diploid ($2n = 2x = 14$; 20.69%), hexaploid ($2n = 2x = 42$; 3.44%) and mixed populations of diploid and triploid ($2n = 3x = 21$; 6.90%) were also found.

Cytotype distribution at a regional scale

The different cytotypes mostly occurred in single areas. Diploids are largely restricted to Capilla del Monte (Punilla valley). Tetraploids are widespread over the entire studied area, although they are lacking in Cruz del Eje where hexaploids were found (Fig. 1a).

The environmental traits that characterize the geographic area of each cytotype are summarized in Table 2. Each cytotype was collected from a wide altitudinal range (Table 2). Diploids were found at higher altitudes (1,118–1,615 m a.s.l.), while hexaploids were located at lower altitudes (449 m a.s.l.); nevertheless, tetraploids occurred at both lower and higher altitudes, from 361 in Córdoba city (population S87) to 1,133 m a.s.l. in La Cumbre (population S328).

One-way analysis of variance showed that only the annual mean temperature differed significantly among cytotypes (Table 2). Multivariate analysis (PCA) based on climatic data showed that the first two components accounted for 86% of the total variance. Most of the climatic data variability (60%) was explained by the first ordination axis (Fig. 3), that represented a major gradient in temperatures (isothermality, temperature seasonality and temperature annual range) and precipitation seasonality. The highest values on the first axis correspond to diploids, triploids and tetraploids of Punilla valley; tetraploids of Traslasierra valley, Córdoba (S87) and Los Molinos (S115) as well as hexaploids of Cruz del Eje are scattered in the middle of the plot, followed by tetraploids of La Higuera (S347), that are in the left of the ordination axis. The second component, accounting for the remaining 26% of the variance is closely related to precipitation variables (annual precipitation and precipitation of the driest month) and mean monthly temperature range, and separated the tetraploids of Córdoba (S87), Los Molinos (S115) and Cosquín (S427) from the remainder of the populations in the Punilla and Traslasierra valleys, that had an intermediate position, followed by tetraploids of La Higuera (S347), that are in the bottom part of the ordination axis. The cophenetic correlation was high ($r = 0.99$), indicating a very good fit between the distance among populations in the two dimensional plot and the distance in the original multidimensional space.

Cytotypes distribution at a microgeographical scale

Spatial segregation of cytotypes was also seen at a microgeographical scale in Capilla del Monte. The census of cytotypes in this locality indicated a predominance of tetraploids (54.43%) over diploids (44.98%), with few triploids (0.68%). In this locality, diploids individuals grew in the piedmont of the Uritorco, Los Terrones and Las Gemelas hills, while tetraploids were in the adjacent valley (Fig. 1b). In the contact zone of both cytotypes, located in the Uritorco and Las Gemelas hills, patches of diploids and triploids plants were found. Distances from these mixed sites of diploids and triploids to the nearest diploid and

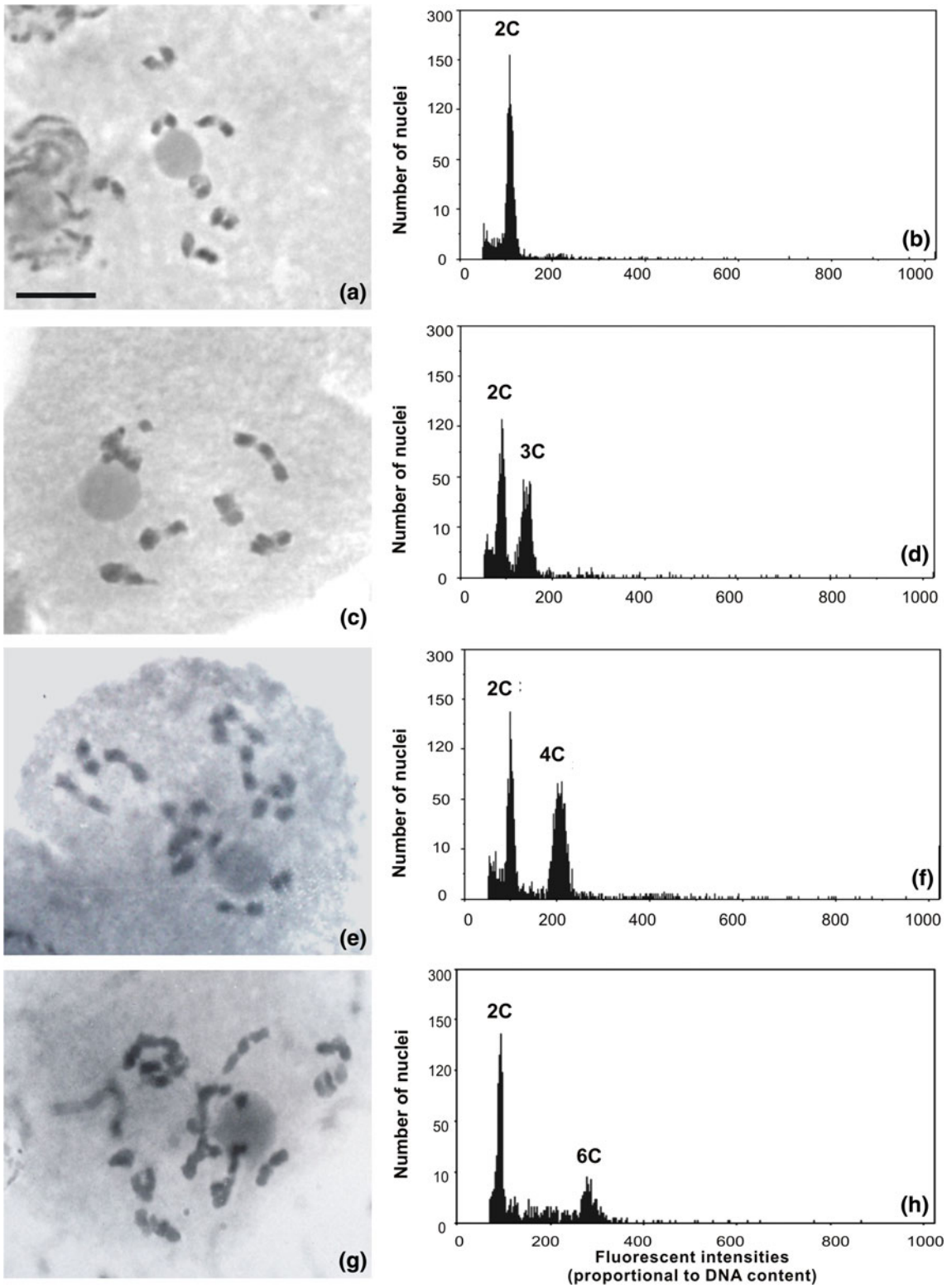

Fig. 2 Meiotic chromosomes at diacinesis and flow cytometric profiles of diploid and polyploid plants of *Turnera sidoides* subsp. *pinnatifida*. **a** and **b** Diploid ($2n = 2x = 14$). **a** 7 II. **b** Flow cytometry histogram of a diploid plant and the internal standard (S235) with a high peak at 2C. **c** and **d** Triploid ($2n = 3x = 21$). **c** 7 III. **d** Flow cytometry histogram showing a peak at 3C and the standard S235 (2C). **e** and **f** Tetraploid ($2n = 4x = 28$). **e** 14 II. **f** Flow cytometry histogram of a tetraploid plant with a high peak at 4C and the diploid standard S235 (2C). **g** and **h** Hexaploid ($2n = 6x = 42$). **g** 6 II + 2 III + 6 IV. **h** Flow cytometry histogram of a hexaploid plant with a peak at 6C and the diploid standard S235 (2C). *Bar* 5 μm

Table 2 Altitude and climatic variables analysed in the geographical area of each cytotype of *Turnera sidoides* subsp. *pinnatifida* in Sierras de Córdoba, central Argentina

Variable	Ploidy level				*F*
	2*x*	3*x*	4*x*	6*x*	
Altitude	1206.60 ± 81.00	995.50 ± 30.40	818.10 ± 235.50	462.00	3.48
	1118–1615	974–1017	361–1108		
Annual mean temperature (°C)	13.80 ± 0.30[a]	14.70[a] ± 0.00	16.00[b] ± 1.30	18.40	4.84**
	13–14	14.70–14.70	14.10–18.00		
Mean monthly temperature range (°C)	14.10 ± 0.00	14.10 ± 0.00	14.00 ± 0.10	13.90	0.03
	14.10–14.10	14.10–14.10	13.80–14.20		
Isothermality	53.80 ± 0.10	53.70 ± 0.20	51.90 ± 1.50	50.80	2.90
	53.70–53.90	53.50–53.80	49.80–53.60		
Temperature seasonality (°C)	436.20 ± 1.30	437.00 ± 6.40	468.60 ± 25.70	478.00	3.06
	433.80–437.20	432.50–441.56	437.50–501.60		
Temperature annual range (°C)	26.20 ± 0.10	26.20 ± 0.10	27.10 ± 0.60	27.40	3.32
	26.20–26.20	26.20–26.30	26.30–27.80		
Annual precipitation (mm^3)	662.80 ± 8.70	637.50 ± 2.10	637.50 ± 53.60	541.00	0.93
	648.00–668.00	636.00–639.0	536.00–749.00		
Precipitation of driest month (mm^3)	8.00 ± 0.00	8.00 ± 0.00	9.20 ± 1.50	7.00	1.39
	8.00–8. 00	8.00–8.00	7.00–12.00		
Precipitation seasonality	69.10 ± 0.50	70.00 ± 0.06	70.40 ± 2.70	74.30	1.01
	68.80–70.10	70.00–70.10	67.40–76.10		

Results of the ANOVA test ($P < 0.05$) for each variable are shown in the last file. *Within a column*, means with *different superscripts* are significantly different (Tukey post hoc test for multiple comparisons of means, $P < 0.05$)

Basic statistic: average, standard deviation and range of variation of each trait

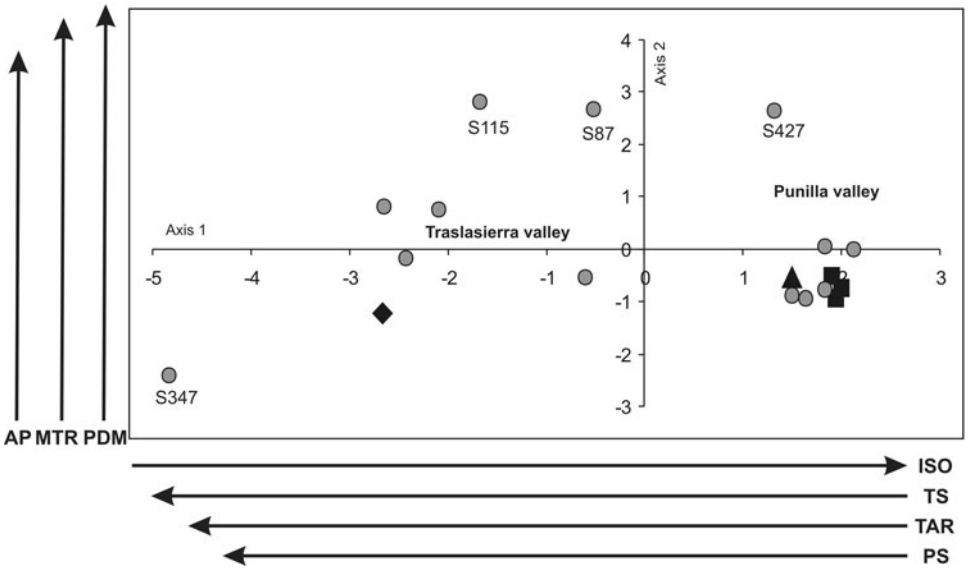

Fig. 3 Ordination plot of populations analyzed in the plane of the first two principal components (PCA) based on climatic data. The climatic variables associated to axis I and II is shown by *arrows*. The length and the direction of the *arrows* represent the relative contribution of the climatic variables to axis I and II. *ISO* isothermality, *TS* temperature seasonality. *TAR* temperature annual range, *PS* precipitation seasonality, *PDM* precipitation of driest month, *MTR* mean monthly temperature range and *AP* annual precipitation. *Black squares*, *black triangle*, *grey circles* and *black diamond* represent diploids, triploids; tetraploids and hexaploids, respectively

tetraploid patches ranged between 500–1200 m and 400–1700 m, respectively (Fig. 1b).

Discussion

Ploidy level estimations

Our cytological survey of *T. sidoides* subsp. *pinnatifida* has confirmed the occurrence of diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) individuals. Although near pentaploid aneuploids were reported for *T. sidoides* subsp. *sidoides* (Fernández 1987), our finding of the triploid cytotype ($2n = 3x = 21$) constitutes the first odd polyploid reported for natural populations of *T. sidoides* complex and for the genus *Turnera*.

Cytotype distribution at a regional scale

As in many other polyploid species (van Dijk and Bakx-Schotman 1997; Husband and Schemske 1998; McArthur and Sanderson 1999), diploids and polyploids of *T. sidoides* subsp. *pinnatifida* are geographically separated in the Sierras de Córdoba. In this region, diploids, triploids and hexaploids are fewer in number and are more restricted, while tetraploids are the most common and widespread cytotype.

Such distribution patterns were proposed to be the result of differences among cytotypes in their adaptability to the underlying heterogeneity of environmental factors (Lewis 1980). This hypothesis has been supported by several investigations which demonstrate that polyploids are better adapted than their diploid relatives (e.g. *Parnassia* L.: Wentworth and Gornall 1996; *Artemisia* L.: McArthur and Sanderson 1999; *Solanum* L.: Hijmans et al. 2007) to more extreme ecological environments. Nevertheless, this hypothesis has been disputed by other authors who proposed that the widespread cytotypes may have been more effective colonizers of areas that became available after the amelioration of the climate at the end of the Pleistocene (Stebbins 1971; van Dijk and Bakx-Schotman 1997).

Comparisons of altitude and climatic variables among the localities revealed differences in habitat among cytotypes of *T. sidoides* subsp. *pinnatifida*. Diploids grow at higher altitudes and in colder and more humid locations; tetraploids are widespread and have a broader ecological spectrum than the other cytotypes; while the hexaploid population occurs at the lowest altitude and in warmer and drier conditions than diploids. Such an environmental relationship is suggestive of a differentiation of environmental preferences among the cytotypes and likely explains the geographical distribution of cytotypes in the Sierras de Córdoba. However, considering the relative abundance of tetraploids in comparison with diploids as well as the distribution of cytotypes in this mountain system, the cytotype structure may also reflect historical vegetation and climatic changes.

Tropical or subtropical vegetation covered the Sierras de Córdoba during the Tertiary; however, the climatic changes induced by the Andes uplift gradually yielded arid environments and more xeric vegetation (Luti et al. 1979). During such events, the valleys and ravines along the hilly systems would have had more stable environments (Ab'Sáber 1977), while in the surrounding areas, pulses of expansions and contractions of the vegetation caused by the cycles of cold–warm and arid–wet climates could have allowed the establishment of plants that were adapted to either cool or warm as well as dry or moist conditions (Iriondo and García 1993). Taking into account these historical environmental changes, the occurrence of extant diploids in more temperate and humid locations of the study area suggests that diploid populations may have had a wider distribution along this mountain system before the aridification events. Hence, the diploids in Capilla del Monte may be remnant from this wider distribution; conversely, the widespread distribution of extant tetraploids suggests that this cytotype may have been a more effective colonizer of areas that became available after the climatic change throughout the Sierras de Córdoba. Moreover, the finding of one hexaploid population in the northwestern limit of the study area with more stressful ecological conditions (higher temperature and less available water) than diploids is coincident with the occurrence of higher ploidy levels (hexaploids and octoploid) of *T. sidoides* in the most extreme ecological conditions of the complex area (Solís Neffa and Fernández 2001; V. Solís Neffa and G. Seijo personal communication). This fact and the wider distribution of tetraploids support the hypothesis that the range expansion associated with polyploidy may have played an important role in the evolutionary history of *T. sidoides*.

Consequently, the cytotype structure of *T. sidoides* subsp. *pinnatifida* at Sierras de Córdoba may be governed by a combination of ecological and historical variables. The geomorphologic and climatic changes that occurred in this region from the Miocene onward (Ab'Sáber 1977; Luti et al. 1979; Iriondo and García 1993) would explain the frequency and distribution of the cytotypes, while the current climate and ecological factors may contribute to the maintenance of the spatial patterns detected.

Cytotype distribution at a microgeographical scale

In the contact zone in the Capilla del Monte, diploids and tetraploids of *T. sidoides* subsp. *pinnatifida* are also spatially segregated, although both cytotypes can occur in

close proximity. The frequency and distribution of cytotypes in the contact zones of diploid and polyploid was explained by ecological sorting in heterogeneous environments (Fowler and Levin 1984); however, whether the two cytotypes are ecologically similar, segregation may also result from competitive exclusion whereby the rarer cytotype mates mainly with incompatible partners and is replaced spatially by a superior cytotype (Levin 1975; Husband 2000). As a result, in the contact zones of diploid and tetraploid, populations having multiple cytotypes might reflect relatively minor negative interactions between them (Husband and Schemske 1998), while the lack of mixed cytotypes population despite geographic proximity may be due to exclusion (van Dijk and Bakx-Schotman 1997).

In Capilla del Monte, diploids and tetraploids occur under similar climatic conditions; thus, it is unlikely that climate influences cytotype distribution in the contact zone. The similar ecological preferences of both cytotypes and the lack of mixed populations of diploid and tetraploid suggest that the separate distribution of cytotypes in the contact zone may be independent of the selective environment; diploids and tetraploids are unable to coexist as a result of reproductive exclusion. However, because the relationships of cytotype distribution with either different microclimatical or edaphical conditions were not considered in our study, the influence of such environmental variables in the spatial segregation of cytotypes cannot yet be discarded.

One interesting finding of this study is the occurrence of triploids in the contact zone of the diploid and tetraploid. In similar contact zones of other autopolyploid complexes, triploids may never or rarely occur (Lumaret et al. 1987; van Dijk et al. 1992), while in others they may often be present (Husband and Schemske 1998). When triploids are present, they may arise through the union of reduced (n) and unreduced ($2n$) gametes of diploids or by crossing among diploids and tetraploids (Ramsey and Schemske 1998).

In *T. sidoides* subsp. *pinnatifida*, the finding of triploids may be explained by both of the aforementioned mechanisms. Because the production of unreduced gametes, although at low rates, was detected in diploids of *T. sidoides* complex (Panseri et al. 2008), the presence of triploids in mixed patches of diploids and triploids may reflect triploid formation in natural diploid populations through the union of n and $2n$ gametes. Alternatively, considering that in Capilla del Monte diploid and tetraploid plants grow close to each other (within 400 m) and that the major pollinators of *T. sidoides* are small bees (Solís Neffa 2000; Benítez-Vieyra et al. 2007) that fly distances over 500 m, triploids could also arise in the contact zone from pollinations between both cytotypes.

If triploids arise by the union of n and $2n$ gametes within diploid populations, their distribution at the lower altitudinal limit of the diploid range may be related to both the tendency of the $2n$ producers to be concentrated as a result of low seed dispersal and to environmental factors favouring a higher production of $2n$ gametes. Alternatively, triploids may originate along the altitudinal range of diploids; however, they would only be able to survive and establish in the marginal area of diploids.

The finding of triploids in the contact zone raises a new hypothesis about the origin of polyploids in *T. sidoides*. Because all polyploids so far analysed have an even ploidy level (Fernández 1987; Solís Neffa and Fernández 2001; Solís Neffa et al. 2004), bilateral polyploidization was expected to be the most important mechanism of polyploid origin in the complex (Panseri et al. 2008). However, our finding of triploid plants suggests that unilateral polyploidization by a triploid bridge may be an alternative mechanism of polyploid formation.

Triploids may also play an important role in the dynamics of the contact zone of diploid and tetraploid in Capilla del Monte. The production of sterile triploids as a result of hybridizations between diploid and tetraploid can favour the evolution of reproductive isolation among the cytotypes and hence may play a critical role in the local distribution of related cytotypes. On the contrary, if triploids are partially fertile, then they may contribute to the gene flow between diploid and tetraploids, preventing local adaptation of cytotypes to different environments (Lenormand 2002; Pannell et al. 2004). Preliminary cytogenetic studies revealed that triploids in the contact zone are partially fertile (Elías and Solís Neffa 2008). This fact, with the lower frequency of triploids, indicates that the role of triploid plants in the spatial segregation of diploids and tetraploids in the contact zone may be limited. Nevertheless, because triploids produce viable, though scarce, reduced (n) and unreduced ($2n$) gametes (Elías and Solís Neffa 2008), such triploid plants may contribute to the gene flow among cytotypes in the contact zone.

Current studies on the relative fitness of diploids, triploids and tetraploids, as well as the patterns of mating and reproductive isolation of cytotype will improve our understanding of the relative importance of triploids in the dynamics of the diploid and tetraploid contact zones in *T. sidoides*.

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