# Cytogeographic analysis of southern South American species of Stemodia (Scrophulariaceae) 

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Chromosome numbers for 52 populations representing eight South American species of Stemodia (Scrophulariaceae) were determined. The numbers $2 \mathrm{n}=22$ found in S. hassleriana and S. palustris, and 2n = 44 in S. lobelioides are the first records for the species, while those found in S. hyptoides $(2 \mathrm{n}=22,44)$ and $S$. stricta $(2 \mathrm{n}=22)$ constitute new cytotypes for those species. The basic chromosome number $x=11$ was confirmed for the New World species. Chromosome numbers indicate the existence of a polyploid series in $S$. hyptoides with $2 \mathrm{n}=22,44,66$. Moreover, the existence of at least three different ploidy levels, both within and among species, indicates that polyploidy has been one of the mechanisms involved in the evolution of the genus. The geographical distribution of different species and cytotypes are analysed and discussed in the light of their extant morphological variation and taxonomic implications.

Key words: chromosome numbers, cytotypes, geographical distribution, polyploidy, Stemodia

## Introduction

The genus Stemodia (Scrophulariaceae) comprises 49 perennial or annual herbaceous species that occur in temperate and warm climates around the world. The species may have erect or prostrate stems and axillary flowers, or the flowers are arranged in terminal spikes. Based on the combination of morphological characters and geographical distribution, Minod (1918) recognized 31 species in his revision for the Americas. According to his criteria all species with flowers in spikes live in South America, except S. bartsioides, which is restricted to Mexico. Species
with axillary flowers were segregated by that author in three monotypic genera.

In a more recent revision, Turner and Cowan (1993a) recognized 29 species for the Americas, including species with both types of flower arrangement, 16 exclusive for South America, nine for North America and four distributed in both. Considering the species distribution cited by those authors, three centers of species concentration may be distinguished in South America: the first in the NW (Peru, Ecuador, Colombia and Venezuela) with five species, the second in NE of Brazil with 10 species; and the third in North Argentina, Paraguay, SW of Brazil and

Uruguay, with eight taxa. Our study is focused on this latter center with seven endemic species and $S$. verticillata that ranges to North America (Turner \& Cowan 1993b). According to Cabrera's (1971) and Cabrera and Willink's (1973) phytogeographical treatments of South America, four of these species are mostly restricted to the phytogeographic Chaco Domain, one to the Cerrado Province of the Amazonian Domain, whereas the remaining are distributed in both domains.

Some taxa of this southern center of species concentration exhibit much morphological variation at intra or inter-population level, which hampers their precise taxonomic identification (Turner \& Cowan 1993a, 1993b; M. M. Sosa pers. obs.). Examples include S. stricta and $S$. hyptoides that live in partial sympatry in the Argentinean Mesopotamia. Minod (1918) pointed out the presence of a "Typus Intermedius" within the $S$. stricta complex, which was considered by Turner and Cowan (1993b) to be a hybrid between $S$. stricta and S. hyptoides. More drastically, Dawson (1979) treated S. stricta as a variety of S. hyptoides. Therefore, the boundaries between some species particularly in the southern region of South America are blurred and the identity of some taxa is often confused in herbarium specimens.

Several genetic mechanisms may contribute to shadow the morphological differentiation between phylogenetically related species. Among them, the most commonly cited are: interspecific gene flow in hybrid zones (Rieseberg et al. 1999, Arnold 2000); morphological variation due to chromosome doubling and generation of polyploid series (Stebbins 1971, Lewis 1980); or combination of both mechanisms giving rise to polyploid and hybrid complexes (Grant 1989). Since reproductive biology in Stemodia is poorly understood, estimations of gene flow between interspecific populations will have to wait until more basic knowledge on this genus became available. However, chromosome number determinations may provide valuable data to determine the causes of morphological continuity between species.

Chromosome numbers for species of Stemodia were usually provided as part of larger studies in Scrophulariaceae or included in general
chromosome number lists. These reports cited $2 \mathrm{n}=28$ and $2 \mathrm{n}=42$ for the Old World species $S$. viscosa, and $2 \mathrm{n}=22$ and $2 \mathrm{n}=44$ for S. verticillata and S. multifida, respectively, of the New World (Fedorov 1974, Subramanian \& Pondmudi 1987, Turner \& Cowan 1993a). A more recent contribution on South American species reported $2 n=22$ for S. ericifolia, S. lanceolata and S. verticillata, $2 \mathrm{n}=44$ for S. stricta and 2 n $=66$ for S. hyptoides (Sosa \& Seijo 2002). Based on these preliminary results, additional studies on chromosome numbers of southern South American species were conducted to shed light on (1) the mechanisms contributing to species diversification, and (2) the causes of morphological continuity among species.

## Material and methods

The species analysed, chromosome numbers, localities and collectors are listed in the Appendix. Voucher specimens have been deposited at the herbarium of the Instituto de Botánica del Nordeste (CTES), and plants from several populations are cultivated under greenhouse conditions.

For mitotic studies, root tips were pre-treated with 8-hydroxyquinoline 0.002 M for about 3 hrs at room temperature, then fixed in 5:1 ethanol:lactic acid (Fernández 1973) and kept in $70 \%$ aqueous ethanol at $4^{\circ} \mathrm{C}$ until use. Roots were stained following the Feulgen's technique and meristems were macerated and squashed in a drop of 3\% acetic orcein. Permanent slides were prepared using Euparal as mounting media.

The map showing chromosome number distribution includes the results obtained in this report and those cited by Sosa and Seijo (2002).

## Results

Chromosome numbers of 52 populations belonging to eight species of Stemodia from Argentina, Paraguay and Uruguay were determined and are listed in the Appendix. Stemodia ericifolia (Fig. 1A), S. hassleriana, S. lanceolata, S. palustris, S. stricta and $S$. verticillata were diploids with $2 \mathrm{n}=2 \mathrm{x}=22$; S. lobelioides was tetraploid with $2 \mathrm{n}=4 \mathrm{x}=44$; whereas $S$. hyptoides showed three

Fig. 1. Mitotic chromosomes in species of Stemodia. - A: S. ericifolia, $2 n=2 x=22 .-B: S$. hyptoides, $2 \mathrm{n}=2 \mathrm{x}=22$. $-\mathbf{C}$ : S. hyptoides, $2 \mathrm{n}=4 \mathrm{x}=$ 44. - D: S. hyptoides, 2 n $=6 x=66$. Scale bar = $5 \mu \mathrm{~m}$.

cytotypes $2 n=2 x=22$ (Fig. 1B), $2 n=4 x=44$ (Fig. 1C) and $2 \mathrm{n}=6 \mathrm{x}=66$ (Fig. 1D). Among the tetraploids of $S$. hyptoides is included one population that was formerly misidentified as $S$. stricta (Sosa \& Seijo 2002).

Diploids are the most common and distributed throughout the study area. Tetraploids are more restricted and hexaploids are found rarely (Fig. 2). Among the 19 populations of S. hyptoides, seven are diploids, nine tetraploids and three hexaploids. Diploid populations of this species complex are concentrated along the Paraná River in the northwest of the Misiones Province (Argentina); tetraploid populations are more dispersed, ranging from north Uruguay to the Misiones Province (Argentina), whereas hexaploids are scarce, growing only in the Misiones and Corrientes Provinces (Argentina) close to the Paraná River (Fig. 2). Only one contact zone between diploid and tetraploid cytotypes was detected in the western limit of this species range. In this area, individuals showed a great range of morphological variation, mainly in the size of the leaf blades (length and width) and length of corolla. Most diploid individuals analysed in this
area resemble those belonging to $S$. stricta. The latter species has disjunct areas, one related to the Paranaense forest in eastern Argentina and NW Uruguay, and the other related to the Tucumanooranense forest in western Argentina.

## Discussion

The chromosome numbers found in the species here analysed revealed a basic chromosome number of $x=11$, in agreement with the data previously reported for species of the New World (Fedorov 1974, Turner \& Cowan 1993a, Sosa \& Seijo 2002). These results suggest that American species have exclusively $\mathrm{x}=11$, while the Old World species have $x=11$ and $x=7$. Species within the Scrophulariaceae show a wide range of haploid numbers, from $n=6$ to $n=84$ (Darlington \& Wylie 1956, Moore 1973, Fedorov 1974, Goldblatt 1981, 1984, 1985, 1988; Goldblatt \& Johnson 1990, 1991, 1994, 1996, 1998, $2000,2003,2006)$. Since the commonest number in the family is $n=8$, it has been proposed that this may be the original number of the family,


Fig. 2. Distribution of cytotypes of Stemodia ericifolia $(+)$, S. hassleriana ( $\square$ ), S. hyptoides ( $\triangle$ ), S. lanceolata $(\bigcirc)$, S. palustris ( $\square$ ), S. stricta $(\diamond)$, S. verticillata $(\times)$, all the species with $2 \mathrm{n}=2 \mathrm{x}=22$. S. hyptoides $(\mathbf{\Delta})$ and $S$. Iobelioides $(\boldsymbol{\bullet})$ with $2 \mathrm{n}=4 \mathrm{x}=44$. S. hyptoides $(\boldsymbol{)})$ with $2 n=6 x=66$. The arrow indicates the contact zone.
while $\mathrm{n}=10,11,12,13,14$, etc. were derived by aneuploid series followed by polyploidy (Subramanian \& Pondmudi 1987). However, an inverse occurrence of this mechanism (i.e., polyploid series followed by aneuploidy) would have been more parsimonious, because chromosome losses are better tolerated after chromosome duplication. According to this scenario, the American species of Stemodia can be considered as derived since they are all based on $x=11$.

Chromosome numbers for $S$. hassleriana, $S$. lobelioides, S. palustris and S. stricta are the first reports, while those found in S. ericifolia, S. lanceolata and S. verticillata are in agreement with previous records (Fedorov 1974, Sosa \& Seijo 2002). The diploid and tetraploid levels detected in S. hyptoides are new cytotypes for the species, while the hexaploid cytotype was reported previously (Sosa \& Seijo 2002). The chromosome numbers found in the populations of this species demonstrate that they constitute a polyploid series. The fact that $25 \%$ of the analysed species and $57 \%$ of the populations of S. hyptoides were polyploid suggests that polyploidy has been one of the major mechanisms involved in the evolution of Stemodia.

Among those South American species that have only diploid cytotype, there is a remarkable morphological differentiation, mainly in the growth habit of plants, leaf shape and floral characters. This variation probably led Minod (1918) to segregate S. ericifolia, S. hassleriana
and $S$. verticillata in different monotypic genera. However, three of the diploid species are distantly distributed (S. ericifolia, S. hassleriana and $S$. palustris), while S. lanceolata, S. stricta and $S$. verticillata are widespread, with overlapping ranges. In spite of the partially sympatric distribution, no putative hybrids have so far been found, either in herbarium or in natural populations, suggesting that a high degree of reproductive isolation is operating among these species.

In contrast, species having polyploid cytotypes exhibit much morphological inter- and intra- populational variation; some plants with intermediate characters between $S$. hyptoides and $S$. stricta were noted by Minod (1918) and Turner and Cowan (1993a, 1993b) and putative hybrids were repeatedly cited, based on characters such as plant height and length of the corolla. Although the genetics of these traits are not known for Stemodia, they are usually determined as quantitative traits and are often cited as having been affected by the gigas effect due to polyploidy (Stebbins 1971, Lewis 1980, Grant 1989, Solís Neffa 2000, Almada et al. 2006). Diploid specimens of $S$. hyptoides are usually shorter than polyploids and have smaller leaf blades, but the length of corolla has an inverse relationship with ploidy level (Sosa 2005, 2007). In this respect, diploids of S. hyptoides resemble S. stricta in plant height and in size of the leaf blade, but they are individuals that better fit the description of S. hyptoides (based on original description of Chamisso \& Schlechtendal 1828) when the corolla size is considered. Therefore, the variation observed in the intermediate individuals and putative hybrids may be explained, to some extent, by the gigas effect. Clearly, a wide and detailed analysis of the variability of these two species in combination with chromosome number counts has to be done in order to precisely delimit these taxa.

Although some species have partially overlapping ranges, habitat segregation is observed among the diploid ones (Fig. 2). Stemodia ericifolia is restricted to the dryer part of the western Chaco Province where it is found in the herbaceous stratum of xerophytic deciduous forests on sedimentary soils of fluvio-lacustrine origin that are flooded frequently. Stemodia palustris is distributed in a narrow band in association with
the Uruguay River basin in the NE extreme of the Pampeana Province and the eastern Espinal Province. Stemodia hassleriana is endemic to the Cerrado Province and occurs in riparian forest. Stemodia lanceolata, S. stricta and S. verticillata are more widespread, but although they are adapted to a wider range of soils, they only occur in places with a pluviometric range over 1000 mm per year. Habitat segregation, mainly for species with restricted areas, suggests that morphological differentiation and speciation of diploids occurred in allopatry. This hypothesis is supported because the areas of the present day distribution of the allopatric diploids are considered as refugia for the flora during the Quaternary climate changes, i.e. lowlands in the western Dry Chaco region, Amambay ranges in the Cerrado Province and southern ranges of the Brazilian Plateau (Ab'Sáber 1977, Solís Neffa \& Fernández 2001, Speranza et al. 2007).

Among the polyploid taxa, S. lobelioides is restricted to the Pampeana Province in riversides of the Uruguay River basin. Its morphological similarity and geographical distribution downstream of $S$. palustris suggest that the species are closely related, and it can be hypothesized that the tetraploid condition of $S$. lobelioides allowed its expansion southwards during the more benign periods of the Quaternary. Stemodia hyptoides has a wide distribution throughout the east of the Espinal and Chaco Provinces, NE of the Pampeana Province and in the SW of the Paranaense Province of the Amazonian Domain. Diploid populations were collected only toward the NE of the species range (in the Paranaense Province), tetraploids are distributed throughout the area, whereas hexaploids are restricted to SW of Misiones and Corrientes (Argentina). This pattern of cytotype distribution is characteristic of mature polyploid complexes (Stebbins 1971) and agrees with the direction of flora retreats and expansions in a NE-SW direction during the Quaternary (Iriondo 1999). The current distribution of diploids in the NE extreme of the analysed area comprises a region, in which remnants of the subtropical flora persisted during the glaciations, while tetraploids and hexaploids are currently found in the areas that became more temperate and humid only in interglacial periods (Iriondo 1999).

Our results provide evidence that diploid species may have originated in allopatry and that the lack of a clear delimitation of the taxa in Stemodia is, in part, due to the existence of species with polyploid series as well as closely related diploid and polyploid species. Moreover, the geographical distribution of cytotypes suggest that, at least for S. hyptoides, increases in ploidy levels may have favored the range expansion of the species.

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Appendix. Chromosome numbers, locality and voucher specimens of species of Stemodia studied. *Chromosome numbers reported previously.

| Species | 2 n | Locality and vouchers | Lat. S | Long. W |
| :---: | :---: | :---: | :---: | :---: |
| S. ericifolia | 11* | Argentina, Formosa, Dep. Matacos, Ing. Juarez. Schinini et al. 35286 | $23^{\circ} 54^{\prime}$ | $61^{\circ} 51{ }^{\prime}$ |
|  | 22 | Argentina, Formosa, Dep. Matacos, Ing. Juarez. Seijo et al. 2717 | $23^{\circ} 54^{\prime}$ | $61^{\circ} 51^{\prime}$ |
|  | 22 | Argentina, Formosa, Dep. Bermejo, Laguna Yema. Sosa et al. 220 | $24^{\circ} 13^{\prime}$ | $61^{\circ} 17^{\prime}$ |
|  | 22 | Argentina, Salta, Dep. Rivadavia, J. Pagé. Sosa et al. 223 | $23^{\circ} 41^{\prime}$ | $62^{\circ} 28^{\prime}$ |
|  | 22 | Argentina, Salta, Dep. Rivadavia, Pluma de Patos. Sosa et al. 224 | $23^{\circ} 22^{\prime}$ | $63^{\circ} 06^{\prime}$ |
| S. hassleriana | 22 | Paraguay, Dep. Amambay, Bella Vista. Sosa et al. 211 | $22^{\circ} 07^{\prime}$ | $56^{\circ} 31^{\prime}$ |
|  | 22 | Paraguay, Dep. Amambay, near to Bella Vista. Sosa et al. 213 | $22^{\circ} 07^{\prime}$ | $56^{\circ} 31^{\prime}$ |
| S. hyptoides | 22 | Argentina, Misiones, Dep. Eldorado, Eldorado. Sosa \& Keller 121 | $26^{\circ} 22^{\prime}$ | $54^{\circ} 40^{\prime}$ |
|  | 22 | Argentina, Misiones, Dep. Eldorado, Eldorado. Sosa et al. 247 | $26^{\circ} 22^{\prime}$ | $54^{\circ} 40^{\prime}$ |
|  | 22 | Argentina, Misiones, Dep. Iguazú, P. N. Iguazú. Sosa et al. 131 | $25^{\circ} 40^{\prime}$ | $54^{\circ} 27^{\prime}$ |
|  | 22 | Argentina, Misiones, Dep. Iguazú, Isla San Martín. Sosa et al. 133 | $25^{\circ} 40^{\prime}$ | $54^{\circ} 27^{\prime}$ |
|  | 22 | Argentina, Misiones, Dep. San Martín, Puerto Rico. Sosa \& Rodríguez 251 | $26^{\circ} 48^{\prime}$ | $55^{\circ} 01^{\prime}$ |
|  | 22 | Argentina, Misiones, Dep. Montecarlo, Puerto Piray. Sosa \& Rodríguez 249 | $26^{\circ} 28^{\prime}$ | $54^{\circ} 42^{\prime}$ |
|  | 22 | Argentina, Misiones, Dep. Montecarlo, Puerto Montecarlo. Sosa \& Rodríguez 250 | $26^{\circ} 34^{\prime}$ | $54^{\circ} 46^{\prime}$ |
|  | 44 | Argentina, Corrientes, Dep. Gral. Paz, Caá Catí. Sosa 93 | $27^{\circ} 45^{\prime}$ | $57^{\circ} 37^{\prime}$ |
|  | 44 | Argentina, Corrientes, Dep. Mercedes, El Socorro. Sosa et al. 245 | $28^{\circ} 31^{\prime}$ | $57^{\circ} 10^{\prime}$ |
|  | 44 | Argentina, Corrientes, Dep. Saladas, San Lorenzo. Sosa 45 | $28^{\circ} 07^{\prime}$ | $58^{\circ} 46^{\prime}$ |
|  | 44 | Argentina, Corrientes, Dep. Saladas, Paso Naranjo. Sosa 128 | $28^{\circ} 28^{\prime}$ | $58^{\circ} 32^{\prime}$ |
|  | 44 | Argentina, Corrientes, Dep. San Miguel,San Miguel. Sosa 134 | $28^{\circ} 00^{\prime}$ | $57^{\circ} 36^{\prime}$ |
|  | 44 | Argentina, Corrientes, Dep. San Roque, near to Santa Lucía river. Sosa 241 | $28^{\circ} 34^{\prime}$ | $58^{\circ} 43^{\prime}$ |
|  | 44 | Argentina, Misiones, Dep. Apóstoles, Chimiray stream. Sosa \& Rodriguez 244 | $27^{\circ} 55^{\prime}$ | $55^{\circ} 40^{\prime}$ |
|  | 44 | Argentina, Misiones, Dep. Capital, Pindapoy Chico stream. Sosa \& Rodriguez 243 | $27^{\circ} 22^{\prime}$ | $55^{\circ} 52^{\prime}$ |
|  | 44 | Uruguay, Dep. Tacuarembó, route 5. Sosa et al. 76 | $32^{\circ} 09^{\prime}$ | $56^{\circ} 06^{\prime}$ |
|  | 66* | Argentina, Corrientes, Dep. Mburucuyá, Paso Aguirre. Sosa 42 | $28^{\circ} 03^{\prime}$ | $58^{\circ} 13^{\prime}$ |
|  | 66 | Argentina, Misiones, Dep. Capital, Posadas. Sosa \& Rodríguez 138 | $27^{\circ} 22^{\prime}$ | $55^{\circ} 52^{\prime}$ |
|  | 66 | Argentina, Misiones, Dep. San Ignacio, Santo Pipó stream. Sosa \& Rodríguez 252 | $27^{\circ} 07^{\prime}$ | $55^{\circ} 25^{\prime}$ |
| S. Ianceolata | 22* | Argentina, Corrientes, Dep. Capital. Riachuelo. Sosa \& Seijo 37 | $27^{\circ} 34^{\prime}$ | $58^{\circ} 45^{\prime}$ |
|  | 22 | Argentina, Formosa, Dep. Patiño, Ibarreta. Sosa et al. 218 | $25^{\circ} 12^{\prime}$ | $59^{\circ} 50^{\prime}$ |
|  | 22 | Argentina, Formosa, Dep. Patiño, route 81. Sosa et al. 219 | $24^{\circ} 34^{\prime}$ | $60^{\circ} 47^{\prime}$ |
|  | 22 | Argentina, Salta, Dep. Rivadavia, J. Pagé. Sosa et al. 222 | $23^{\circ} 41^{\prime}$ | $62^{\circ} 28^{\prime}$ |
|  | 22 | Argentina, Salta, Dep. Orán, Tabacal. Sosa et al. 228 | $23^{\circ} 15^{\prime}$ | $64^{\circ} 13^{\prime}$ |
| S. lobelioides | 44 | Argentina, Corrientes, Dep. Monte Caseros, Uruguay river. Sosa \& Schinini 210 | $30^{\circ} 15^{\prime}$ | $57^{\circ} 38^{\prime}$ |
|  | 44 | Uruguay, Dep. Artigas, Bella Unión. Sosa et al. 70 | $30^{\circ} 15^{\prime}$ | $57^{\circ} 36^{\prime}$ |
| S. palustris | 22 | Argentina, Corrientes, Dep. San Roque, route 12. Sosa 94 | $28^{\circ} 34^{\prime}$ | $58^{\circ} 43^{\prime}$ |
|  | 22 | Argentina, Corrientes, Dep. Saladas, Estancia Fortín. Sosa 95 | $28^{\circ} 34^{\prime}$ | $58^{\circ} 43^{\prime}$ |
|  | 22 | Uruguay, Dep. Artigas, Guabiyú stream. Sosa \& Schinini 209 | $28^{\circ} 07^{\prime}$ | $58^{\circ} 46^{\prime}$ |

Appendix. Continued.


