Karyotypic Studies in South American *Lycium* (Solanaceae)

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The cosmopolitan genus *Lycium* L. contains around 75 woody species that grow mainly in arid or semiarid environments (Bernardello 1986). It is included in subfam. Solanoideae that is considered monophyletic and derived (Olmstead and Palmer 1992). As it is thought for the Solanaceae in general, South America is regarded as the centre of origin of the genus and the tribe *Lycieae* Hunz. (Bernardello 1987). *Lycium* is considered older and more complex than the other two genera of the tribe (*Grabowskia* Schlecht., *Phrodus* Miers). Most of the species (51) are confined to the American continent with two centres of diversification: Arizona (USA) and Argentina (Hitchcock 1932, Bernardello *l.c.*). The current infrageneric classification recognizes four sections: *Lycium*, *Mesocope*, *Schistocalyx*, and *Sclerocarpellum* (Chiang 1983a, Bernardello *l.c.*), although it is quite artificial and some species are not easily placed in them.

Most of the species are diploid with $n=12$, like most genera belonging to Solanoideae (Hunziker 1979). The basic chromosome number is considered $x=12$ (Bernardello 1982, Chiang 1982). Some American taxa have been already explored in their chromosome numbers (Bernardello 1982, Chiang 1982). However, there are no karyotypic studies of any species group of the genus, in spite of its importance in systematics as another morphological feature (Babcock 1947, Stebbins 1958, Jones 1970) and in the study of patterns and processes of evolution (Stebbins 1971, Levin and Wilson 1976, Bush *et al.* 1977). Cytological information is referred to mitotic and meiotic chromosome number reports, as it mostly happens in the family as a whole (Bernardello and Anderson 1990), except for the karyotypes of a hybrid and its parental species (Bernardello *et al.* 1995).

In this article, we qualitatively and quantitatively survey the karyotypes of most of the South American species of the section *Lycium* recognized by Bernardello (1986) with the objectives of: 1) clarifying the taxonomic relationships of the species, and 2) assessing the karyotype for possible insight into the evolution in the section.

Materials and methods

The seven species, two varieties, and 109 individuals studied with their specific collections (32) are listed in Table 1. Voucher specimens are deposited at CORD.

Mitosis in root tip cells was studied from squashes from primary roots of germinating seeds. Seeds were soaked for 1–2 days in running water, put in petri dishes on moist filter paper, and stored at room temperature in the dark. Fresh root tips were pretreated for 2 hr in a saturated solution of paradichloro-benzene in water at room temperature, rinsed in distilled water, and fixed in freshly made ethanol:acetic acid (3:1) at room temperature for 12–24 hr. Then they were placed in alcoholic hydrochloric acid-carmin (Snow 1963) for one week. Stained root tips were stored in 50% acetic acid until the squash was made. Meristem cells were isolated in a drop of 50% acetic acid on a slide, macerated, squashed, and heated gently. Slides were made permanent in Euparal by means of Bradley's method (1948).

Table 1. *Lycium* section *Lycium* taxa studied. Country, province, and place of origin of the collection, collector, and accession number are given. The number within parentheses indicates (the number of individuals studied for each accession, the number of studied cells). LB=L. Bernardello, LS=L. Stiefkens

<i>L. americanum</i> Jacq.	Arg. Córdoba, Miramar, LB 733 (5, 22) and 758 (5, 12).
<i>L. cestroides</i> Schlecht.	Arg. Córdoba, Carlos Paz, Perea w.n. (3, 10); Córdoba, Cosquín, LB w.n. (6, 21); Córdoba, Jesús María, LS 3 (3, 6); Córdoba, Córdoba, Cosa 107 (2, 16); Córdoba, Río Carnero, LB 824 (3, 13); Córdoba, Soto, LS 10 (1, 4); Jujuy, Río Perico, Barbosa 83 (4, 13) and 84 (6, 12).
<i>L. elongatum</i> Miers	Arg. Córdoba, Tulumba, LB 721 (11, 48); Córdoba, Chancani, Toledo w.n. (4, 39).
<i>L. infaustum</i> Miers	Arg. Córdoba, after Serrezuela, A. Hunziker <i>et al.</i> 25389 (6, 37) and 25389 bis (2, 6); Catamarca, Carranza, Carabús w.n. (3, 15); Córdoba, Salinas Grandes, LB 820 (1, 8).
<i>L. rachidocladum</i> Dun.	Chile, Coquimbo, La Serena, LB 866 (4, 10).
<i>L. tenuispinosum</i> Miers var. <i>tenuispinosum</i>	Arg. Córdoba, Tulumba, LB 722 (7, 17); Río Negro, Road 251 Km 104, Cocucci 442 (2, 18); Córdoba, Road 60 Km 896, LB 825 (2, 3); La Pampa, Parque Luro, LB 114 (2, 11); Chubut, Punta Pardela, LB 784 (2, 5); La Pampa, between La Reforma y Guía 25 de Marzo, Galetto 250 (2, 4); Córdoba, Salinas Grandes, LB 278 (2, 4); San Juan, before Matagusanos, LB 836 (3, 5).
<i>L. tenuispinosum</i> var. <i>calycinum</i> (Griseb.) Bernardello	Arg. La Rioja, Chilecito, A. Hunziker 25429 (4, 49); San Juan, before Matagusanos, A. Hunziker <i>et al.</i> 23204 (2, 5).
<i>L. tenuispinosum</i> var. <i>friesii</i> (Dammer) C. L. Hitchc.	Arg. Salta, El Gólgota, Barboza 80 (3, 3); San Juan, Caucete, Galetto 255 (2, 4), 256 (3, 2), and 257 (2, 3); San Juan, Vallecito, Galetto 258 (2, 3).

At least four cells per individual and 15 per species were examined. Ten to 14 metaphases of each species were photographed with phase contrast optics and Kodak PANATOMIC X film. The photographs were used to take measurements of short arm, long arm and total chromosome length for each chromosome pair. Centromeric index and arm ratio were calculated and used to classify the chromosomes after Levan *et al.* (1964). Satellites were classified according to Battaglia (1955). Total haploid chromosome length of the karyotype (tl), based on the mean chromosome lengths for each species, average chromosome length, and average arm ratio were calculated. In each cell, 12 pairs of homologous chromosomes were identified based on similarity in size and centromere position. Karyograms were constructed after Bernardello *et al.* (1994). Idiograms are based on the mean values for each taxon. Karyotype asymmetry was estimated using the indices of Romero Zarco (1986) and Stebbins' classification (1971). In addition, with data from 5 cells by species, the following indices were calculated for each chromosome: relative long arm length (RLL), relative short arm length (RSL), relative chromosome length (RL), and arm difference ratio (AD) after Watanabe *et al.* (1990). Based on these values, minimal chromosomal distance (MCD) and chromosomal identity (CI) were computed (Watanabe *et al.* 1990).

Phenograms were generated by UPGMA method using NTSYS-pc (Rohlf 1992), one based on CI values and another one based on twenty-nine variables for each species (mean length and mean arm ratio of each chromosome pair —24 var.—, both asymmetry indices, average chromosome length, average arm ratio, and genome length).

Results and discussion

The somatic chromosome number $2n=24$ was always found. According to previous data, the basic number in the tribe is $x=12$ (Ratera 1943, Khyos 1971, Di Fulvio 1977, Chiang 1982, 1983b, Bernardello 1982, 1985, Hunziker *et al.* 1985, Chiang *et al.* 1989), as it occurs in subfam. *Solanoideae* as well (Hunziker 1979). Like most of the Solanaceae, all species are diploid.

Karyotype formulae obtained, total haploid chromosome length, and mean chromosome length and arm ratios are summarized in Table 2. Fig. 1 shows examples of the range of chromosomes encountered. Idiograms calculated from means are given for each taxon in Fig. 2. Table 3 shows the data matrix of CI, whereas MCD was always 0 indicating there is an overall karyotypic similarity between the species.

The chromosomes are small (Table 2; Fig. 1). The average chromosome length for all the taxa as a whole is $2.01 \pm 0.18 \mu\text{m}$, while the range for individual species is quite short ($1.79\text{--}2.33 \mu\text{m}$). Consequently, the overall haploid genome is relatively homogeneous (range = $21.52\text{--}28.01 \mu\text{m}$, $\bar{x} = 24.18 \pm 2.25$; Table 2). The shortest measured chromosome pair was #11 in samples of *L. infaustum* ($1 \mu\text{m}$) and the longest one was pair #1 in a cell of *L. rachidocladum* ($3.7 \mu\text{m}$). Considering the total genome length, *L. rachidocladum* has the longest while *L. infaustum* and *L. tenuispinosum* var. *calycinum* the shortest ones.

All the species bear one pair of *m* chromosomes (#1) with terminal microstatellites located at the distal end of the short arms (Table 2; Fig. 1). Satellites were frequently seen in both members of the pair, although sometimes (around 25% of the studied cells) they were detected in only one of them.

The karyotypes in this section, like those in the family (Bernardello and Anderson 1990 and cites therein), consist of *m* and *sm* chromosomes (11 pairs and one pair respectively; Fig. 2; Table 2). Symmetry is estimated by the A_1 and A_2 indices (Table 2). Mean values obtained for the interchromosomal asymmetry index (A_2) are quite alike for the karyotypes studied (range = $0.08\text{--}0.16$). This indicates there is scarce variation between the size of the different chromosomes in each species. Data for the mean intrachromosomal asymmetry index (A_1) are also comparable evidencing no sharp differences between the chromosome arms in the different taxa (range = $0.15\text{--}0.20$). After Stebbins' (1971) asymmetry classification, all the species' karyotypes fall into category "1A" that includes highly symmetrical karyotypes. Our data indicate that *Lycium* is characterized by constant, symmetrical karyotypes, as is common in *Solanum* and other Solanaceae (Stebbins 1971, Moscone 1989, Bernardello and Anderson 1990, Bernardello *et al.* 1994). In contrast to annuals, karyotypes of perennials and woody plants are very stable and less diversified (Brandham 1983, Ehrendorfer 1983), a tendency that our data support.

This study shows a high degree of intraspecific uniformity for all variables measured in the different samples studied. This lack of interpopulational variation was also observed in other solanaceous plants (Goodspeed 1954, Takehisa 1964, Moscone 1989, Bernardello and Anderson 1990), although other taxa are cytologically quite variable (Pickersgill 1977, Wu and Li

Table 2. *Lycium* section *Lycium* taxa studied, codes, karyotype formulae, total haploid chromosome length (tl), mean chromosome length (c), mean arm ratio (r), and ranges of intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2)

Taxon	Codes	Karyotype formula ^a	tl ^b	c ^b	r	$\bar{x}$	A_1 range	$\bar{x}$	A_2 range
<i>L. americanum</i>	amer	11 m*+1 sm	22.74	1.89	1.22	0.16	0.12–0.21	0.12	0.10–0.15
<i>L. cestroides</i>	cest	11 m*+1 sm	25.78	2.14	1.22	0.17	0.10–0.26	0.12	0.08–0.16
<i>L. elongatum</i>	elon	11 m*+1 sm	25.01	2.08	1.22	0.16	0.12–0.21	0.12	0.07–0.15
<i>L. infaustum</i>	infa	11 m*+1 sm	21.52	1.79	1.25	0.18	0.13–0.23	0.13	0.10–0.19
<i>L. rachidocladum</i>	rach	11 m*+1 sm	28.01	2.33	1.28	0.20	0.14–0.24	0.16	0.12–0.20
<i>L. tenuispinosum</i>									
var. <i>tenuispinosum</i>	tent	11 m*+1 sm	23.63	1.96	1.21	0.16	0.14–0.18	0.10	0.08–0.16
var. <i>calycinum</i>	tenc	11 m*+1 sm	21.52	1.79	1.21	0.15	0.11–0.19	0.12	0.08–0.16
var. <i>friesii</i>	tenf	11 m*+1 sm	25.29	2.10	1.22	0.15	0.10–0.25	0.12	0.09–0.13

^a See text for nomenclature. ^b Lengths in μm . * One pair of chromosomes bears a satellite on the short arm.

Fig. 1. Photomicrographs of mitotic metaphases of *Lycium* sect. *Lycium* roman. (a) *L. infustum* (#820). (b) *L. cestroides* (#83). (c) *L. tenuispinosum* var. *tenuispinosum* (#422). (d) *L. americanum* (#733). (e) *L. elongatum* (#721). Bar = 5 μ m, all at the same scale. Arrow heads point satellites.

Fig. 2. Idiograms for each taxon. Bar=1 μm, all at the same scale. Species codes given in Table 2.

Table 3. Data matrix of chromosomal identities of the *Lycium* taxa investigated (species codes in Table 2)

	amer	cest	elon	infa	rach	tent	tenc	tenf
amer	1							
cest	0.9	1						
elon	1	0.9	1					
infa	0.7	0.7	0.5	1				
rach	0.6	0.5	0.7	0.6	1			
tent	0.7	0.9	0.8	0.6	0.6	1		
tenc	1	0.9	1	0.5	0.8	0.9	1	
tenf	1	0.8	0.9	0.7	0.6	1	1	1

Fig. 3. UPGMA phenograms. (a) derived from average taxonomic distance (29 variables, cf. material and methods). (b) derived from CI data (Table 3). Species codes given in Table 2.

1985, Trivedi and Sinha 1986, Moscone 1990).

Karyotype composition of all the taxa is comparable. Previous data on a population of *L. cestroides* agree with our results, as well as data on *L. ciliatum*, belonging to sect. *Schistocalyx*, and the hybrid *L. ciliatum* × *cestroides* (Bernardello *et al.* 1995).

Cluster analysis based on 29 cytological variables grouped the species mainly according to the chromosome length (Fig. 3): 1) *L. rachidocladum* isolated with the longest chromosomes ($tl=28.01\ \mu m$), 2) *L. infaustum*, *L. americanum*, and *L. tenuispinosum* var. *calycinum* with shorter chromosomes ($tl=21.52\text{--}22.74\ \mu m$), and 3) *L. cestroides*, *L. elongatum*, *L. tenuispinosum* var. *tenuispinosum* and var. *friesii* with comparatively longer chromosomes ($tl=23.63\text{--}25.78\ \mu m$). A different and more realistic clustering is observed in the phenogram based on CI: most of the taxa are very close or even identical, from the cytological point of view, being *L. rachidocladum* and *L. infaustum* comparatively somewhat different.

The studied species are morphologically diverse and differentiated, as previously shown by morphological, anatomical, and phenetic studies (Bernardello 1986, 1987, Bernardello and Leiva-González 1993, respectively). However, morphological differentiation of the species of the section was not accompanied by some kind of variation in the chromosomes, a situation that differs from data on two sections of *Solanum* (Bernardello and Anderson 1990, Bernardello *et al.* 1994) and seed plants as a whole (Levin and Wilson 1976). This perspective suggests that speciation in the group has not been followed either by changes in chromosome number or by large chromosomal rearrangements. Previous meiotic studies (cf. *supra*) mostly reported normal bivalent formation suggesting there were not large inversions or translocations.

Summary

Mitotic chromosomes of seven South American species and two varieties of *Lycium* sect.

Lycium were studied to clarify their taxonomic relationships and to assess the karyotype for possible insight into the evolution in the section. All species have $2n=2x=24$. The chromosome numbers of *L. infaustum* and *L. rachidocladum* are reported for the first time and data on karyotype composition are mostly new. The generalized karyotype of the section shows it is highly symmetrical. The chromosomes are metacentric or submetacentric with the formula: $11m+1sm$. Microsatellites are present in chromosome pair #1 and are attached to the short arms. The section is karyotypically homogeneous. Cluster analysis using several cytological data and chromosomal identity index (CI) shows that most of the taxa are very close or even identical. There are no indications of major chromosomal rearrangements within the section. Karyotypic features obtained suggest that morphological differentiation in the group was not followed by chromosomal divergence.

Key words: Karyotype composition, *Lycium* sect. *Lycium*, phenetics, South America.

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