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Karyotype analysis in Argentinean species of *Caesalpinia* (Leguminosae)

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Abstract — Somatic chromosomes of three *Caesalpinia* L. species (subfam. Caesalpinioideae, tribe Caesalpinieae: *C. gilliesii*, *C. mimosifolia* — both endemic to Argentina —, and *C. paraguariensis*, endemic to the Chaco region) were studied, being this the first chromosomic report for all of them. These species were diploid (2n = 24) and showed a variable percentage of tetraploid cells. Chromosomes were small: the average chromosome length was 1.90 ± 0.17 μm. The haploid karyotype length was relatively homogeneous (range: 20.67–24.74 μm, mean: 22.89 ± 2.06). Although *Caesalpinia* species were morphologically different, their differentiation was not followed by chromosomal variations. Effectively, all showed the same chromosome number and symmetrical haploid karyotype formula: 8 m + 4 sm. Microsatellites were present in chromosome pair no. 2 and were attached to the short arms. A cluster analysis based on karyotype features showed that *C. gilliesii* and *C. paraguariensis* were closer and that *C. mimosifolia* is more different because it has the shortest mean chromosome length and the highest mean arm ratio and A_2 values. Karyotypic features obtained suggest that no major visible chromosomal rearrangements have occurred during the differentiation in the group, although cryptic structural changes, as paracentric inversions or reciprocal translocations of segments of similar length, may have taken place.

Kew words: *Caesalpinia*, classical chromosome staining, karyotype evolution, karyosystematics, Leguminosae.

INTRODUCTION

The genus *Caesalpinia* L. belongs to subfam. Caesalpinioideae, tribu Caesalpinieae Benth. (Polhill and Vidal 1981). According to recent phylogenetic data, Caesalpinioideae is paraphyletic and some of its members are basal for the whole legume family (Doyle *et al.* 2000; Bruneau *et al.* 2001; Wojciechowski *et al.* 2004). *Caesalpinia* includes around 150 Pantropical woody species inhabiting Africa, Asia, and America that have great morphological variation among them (Ulibarri 1996; 1997). Many species are important as ornamental, medicinal, or timber producing (Burkart 1952). In South America, around 40 spp. are recognized, whereas in Argentina the genus is represented by 14 spp. (Ulibarri 1999).

Even though it is widely known that karyotypic features can be useful for studying patterns and processes of evolution (Stebbins 1971; Levin and Wilson 1976; Stuessy 1990), there are few available chromosomic data for *Caesalpinia*. The chromosome numbers of as few as 15 species were reported (cf. Fedorov 1969; Goldblatt 1981) and only six were studied in their karyotypes (Kumari and Bir 1989). Thus, many species remain karyologically unknown and the scarce published studies lack qualitative or quantitative analysis of the karyotypes.

In the present contribution, a morphometric karyotype analysis has been performed in three Argentinean species of *Caesalpinia* with the aims of: 1) reporting for the first time their chromosome numbers and karyotype data, 2) trying to cast light on the taxonomic relationships of species, and 3) exploring patterns of chromosome differentiation and karyoevolutionary trends.

The following are the studied species: 1) *Caesalpinia gilliesii* is a shrub up to 3 m high that bears showy yellow flowers of 15-25 mm long that have red, largely exserted stamens. In Argentina, where it is considered endemic, is common from Salta to Río Negro provinces (Ulibarri 1997; 1999). It is locally identified as "lagaña de perro" (Burkart 1936) and is pollinated by hawk moths (Cocucci

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et al. 1992). It grows subspontaneously in Chile and Uruguay (ULIBARRI 1997), being cultivated as ornamental in many countries all over the world and having antitumour activity in seeds (MABBERLEY 1997). 2) Known as "pishcalilla" (BURKART 1936), *C. mimosifolia* is endemic to Argentina and is typical of the Chaco and Monte phytogeographical regions (from Jujuy to San Luis provinces; ULIBARRI 1997; 1999). It is a shrub up to 2 m high with yellow, with 10-13 mm long flowers. 3) *Caesalpinia paraguariensis* is a tree that can reach up to 15 m high with comparatively small orange-yellowish, 7-9 mm long flowers. Known as "guayacán" or "ibirá-berá" (BURKART 1936), it has a widespread distribution in the Chaco region: Brazil, Bolivia, Paraguay, and Northern and Central Argentina — from Jujuy to San Luis provinces (ULIBARRI 1997; 1999). It is recommended for reforestation because of its valuable wood; in addition, it is used as source for tannin, and as forage with fruits available all year round (ARONSON and SARAVIA TOLEDO 1992). Its seed dispersal is through endozoochory (ABRAHAM DE NOIR *et al.* 2002).

MATERIALS AND METHODS

Table 1 includes collection data of the studied taxa, the number of individuals studied for each accession and the number of studied cells.

Seeds were manually scarified with sandpaper. Then, they were put in petri dishes, regularly watered, and conserved at 20°C in darkness. After 72 hours of this procedure, almost all seeds germinated. Primary roots of 1-2 cm long were used to study somatic chromosomes. Fresh root tips were pretreated for 2 hours in a saturated solution of paradichloro-benzene in water at room temperature (MEYER 1945), rinsed in distilled water, and fixed in freshly made ethanol:acetic acid (3:1) for 24 hours. Then, they were placed in alcoholic hydrochloric acid-carmine (SNOW 1963) for 7 days. Stained root tips were stored in 50% acetic acid until the squash was made. Meristem cells were isolated, macerated, and squashed in a drop of 50% acetic acid and heated gently. Slides were made permanent in Euparal without removing the coverslip by means of BRADLEY's method (1948).

A total of 60 individuals and 180 cells were analysed (20 individuals and 50-70 cells per species) under a Zeiss Axiophot microscope (Table 1). Ten metaphase plates from each species were photographed using Kodak T-Max film. The photomicrographs were used to take measurements of

Table 1 — Populations studied of *Caesalpinia* species, all from Argentina, province of San Luis. The numbers within brackets indicates: (the number of individuals studied for each accession, the number of studied cells). Vouchers were deposited at the Herbarium from the Universidad Nacional de San Luis (UNSL) and were determined by Ing. L. DEL VITTO.

Species	Collection data
C. gilliesii (WALL. ex HOOK.) D. DIETR.	Dpto. La Capital, Potrero de los Funes, A. CANGIANO *et al.* 1, 15 February 1998 (10, 30)
	Dpto. La Capital, Ruta 3 y Aguada de Pueyrredón, A. CANGIANO *et al.* 2, 24 February 1998 (10, 40)
C. mimosifolia GRISEB.	Dpto. Belgrano, Parque Nacional Sierras de las Quijadas, A. CANGIANO & M. SOMBRA 3, 15 February 2003 (20, 60)
C. paraguariensis (D. PARODI) BURKART	Dpto. Junín, Bajo de Véliz, L. DEL VITTO *et al.* 9249, 15 March 2003 (20, 50)

the following features for each chromosome pair: s (short arm length), l (long arm length), and c (total chromosome length). The arm ratio (r = l/s) was calculated and utilized to classify the chromosomes as recognized by LEVAN *et al.* (1964) as: m – metacentric (r = 1.00–1.69) or sm – submetacentric (r = 1.70–2.99). BATTAGLIA's (1955) terminology for satellites was used. The satellite lengths were added to the lengths of the corresponding arms. In addition, haploid karyotype length (kl) based on the mean chromosome lengths for each species, average chromosome length (C), average arm ratio (r), and ratio between the longest and the shortest chromosome of the complement (R) were calculated. Idiograms were based on the mean values for each species. The chromosomes were arranged first into groups according to their increasing arm ratio and then according to the decreasing length within each group. Karyotype asymmetry was estimated using the following parameters: A_1 = intrachromosomal asymmetry index, which indicates the length difference among the chromosome arms, and A_2 = interchromosomal asymmetry index, which indicates the size variation among the chromosomes (ROMERO ZARCO 1986). STEBBINS' (1971) karyotype asymmetry categories were also considered.

Six variables (kl, C, r, R, A_1, A_2) were statistically compared with ANOVA and TUKEY's tests using the program SPSS (release 6.0 for Windows, SPSS Inc., 1993). In addition, a phenogram was generated as follows: the studied taxa were the OTUs and the data matrix included the variables previously mentioned and the percentage of poly-

ploid cells; these variables were standardised and the taxonomic distance coefficient and the UPGMA (unweighted pair-group method using arithmetic averages) method were used with the program SYSTAT (release 7.0 for Windows, SPSS Inc., 1997).

RESULTS

The somatic chromosome number 2n = 24 was found in all species examined (Fig. 1). They were diploid, but showed some polyploid cells (Fig. 2). The percentage of tetraploid cells (2n = 4x = 48) varied among each species: in *C. gilliesii* 30% of the studied cells, in *C. mimosifolia* 12%, and in *C. paraguariensis* 50%.

The chromosomes were small (Table 2; Figs. 1-3). The average chromosome length as a whole was 1.90 ± 0.17 μm, ranging from 1.72 to 2.06 μm. The haploid karyotype length was relatively homogeneous (range: 20.67-24.74 μm, mean: 22.89 ± 2.06; Table 2). The shortest measured chromosome was pair no. 8 of *C. mimosifolia* (1.12 μm) and the longest was pair no. 1 of *C. gilliesii* (2.70 μm) (Table 2).

All species had one metacentric chromosome pair (no. 2) bearing satellites in the short arms (Figs. 1, 3). The frequency of appearance of the satellites was 70% of the examined metaphases in *C. gilliesii*, 40% in *C. paraguariensis*, and 30% in *C. mimosifolia*. Usually, satellites were seen in both members of the respective chromosome pair, although they may appear in just one homologue. They were always terminal microsatellites and had ca. 0.3 μm long.

Karyotypes were symmetrical considering both centromere position and chromosome size variation (Table 2, Fig. 3). The haploid karyotype formula was identical for the three species: 8 m + 4 sm chromosome pairs. In addition, arm ratios were quite homogeneous among the species studied.

Asymmetry has been estimated by the A_1 and A_2 indices. Most values were comparable among the three species, but *C. mimosifolia* showed a higher A_2 value (Table 2). Following Stebbins' (1971) karyotype asymmetry classification, *C. gilliesii* and *C. paraguariensis* fell into category 2A and *C. mimosifolia* into category 2B (Table 2).

A statistical analysis was performed among six variables related to the genome, using ANOVA. Results showed that there were significant differences among all of them (Table 3). Results of Tukey's test (Table 4) demostrated that all the variables analysed differentiate *C. mimosifolia* and *C. paraguariensis*, whereas kl, C, R, and A_2 separate *C. mimosifolia* from *C. gilliesii*, and only A_1 is useful to distinguish *C. gilliesii* from *C. paraguariensis* (see Table 4).

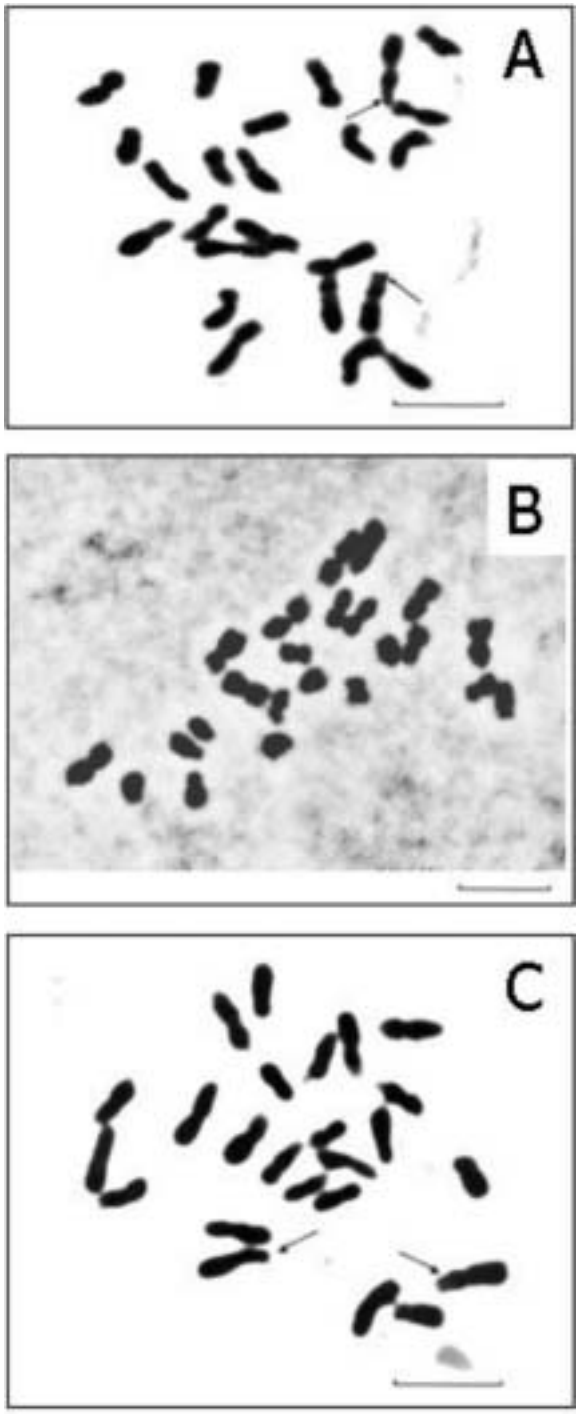

Fig.1 — Photomicrographs of mitotic metaphases of *Caesalpinia* species. A) *C. gilliesii*. B) *C. mimosifolia*. C) *C. paraguariensis*. Bar 5 μm, all at the same scale. Arrows point satellites.

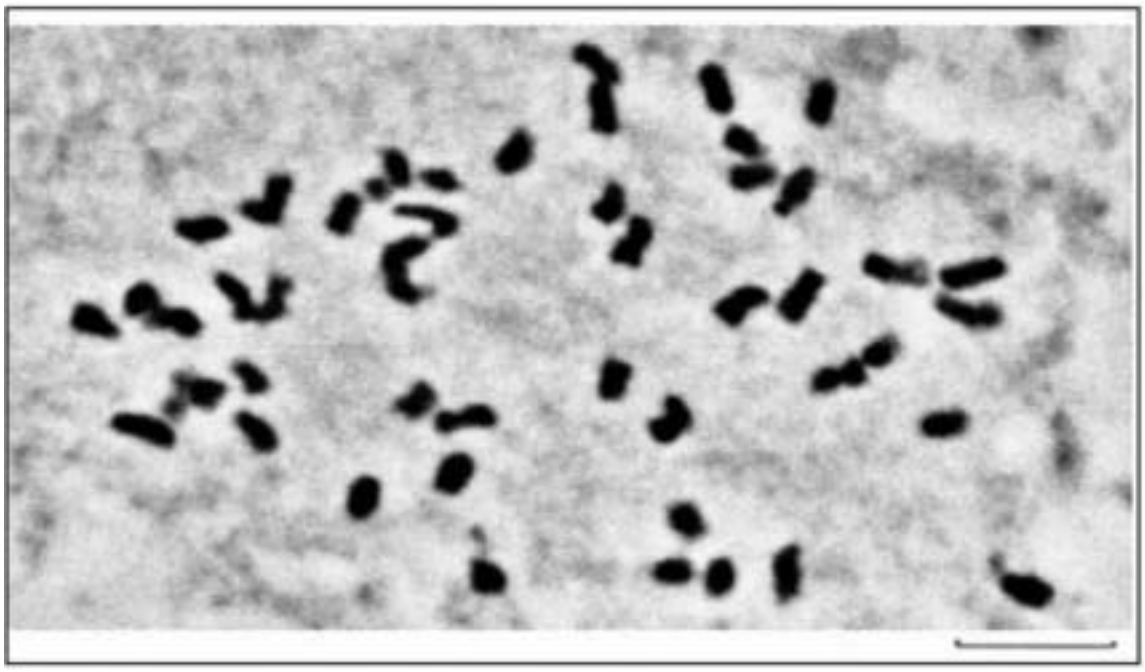

Fig. 2 — Photomicrograph of a polyploid mitotic metaphase of *Caesalpinia paraguariensis* (2n = 48). Bar 5 μm.

Table 2 — Karyotype data from *Caesalpinia* species studied. kl: haploid karyotype length, C: average chromosome length, r: average arm ratio, R, ratio between the longest and the shortest chromosome of the complement, A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index, St: Stebbins' (1971) asymmetry category. Lengths are given in μm. The asterisk indicates that the second chromosome pair bears satellites on the short arm.

Taxa	Haploid karyotype formulae	kl	C	r	R	A_1	A_2	St
C. gilliesii	8 m* + 4 sm	24.74	2.06	1.50	1.75	0.28	0.18	2A
C. mimosifolia	8 m* + 4 sm	20.67	1.72	1.55	2.18	0.29	0.24	2B
C. paraguariensis	8 m* + 4 sm	23.26	1.93	1.45	1.70	0.24	0.17	2A

The cluster analysis, based on the same six variables and percentage of polyploid cells, showed that dissimilarity values between species range from 0.08 to 0.14 (Fig. 4). There was a cluster formed by *C. gilliesii* and *C. paraguariensis*, indicating that they were closer, and *C. mimosifolia* separated, because it had with the shortest mean chromosome length and the highest R and A_2 values (Table 2).

DISCUSSION

Leguminosae is regarded as having a base number of x = 7, with x = 14 established early in their evolution (Goldblatt 1981). Within it, subfam. Caesalpinoideae is considered polyploid, with a predominance of tetraploids with the base number x = 7, followed by a dysploid series, with 2n = 28 as the most common chromosome number (Bandel 1974; Goldblatt 1981; Bairiganjan and Patnaik 1989). In tribe Caesalpinieae, the basic number x = 14 is also present in several cytologically explored genera, including some of the least specialized, e.g. *Gymnocladus* Lam. and *Gleditsia* L., as well as several specialized generic groups (Goldblatt 1981). However, the majority of *Caesalpinia* species show x = 12 (e.g., Goldblatt 1981; Kumari and Bir 1989; Souza and Benko-Iseppon 2004). There are some exceptional counts on *C. decapetala* (Roth) Alston with 2n = 22 together with 2n = 24 (cf. Goldblatt 1981; Goldblatt and Johnson 1990, 1991), but they should be rechecked before considering x = 11 as a secondary basic number. The

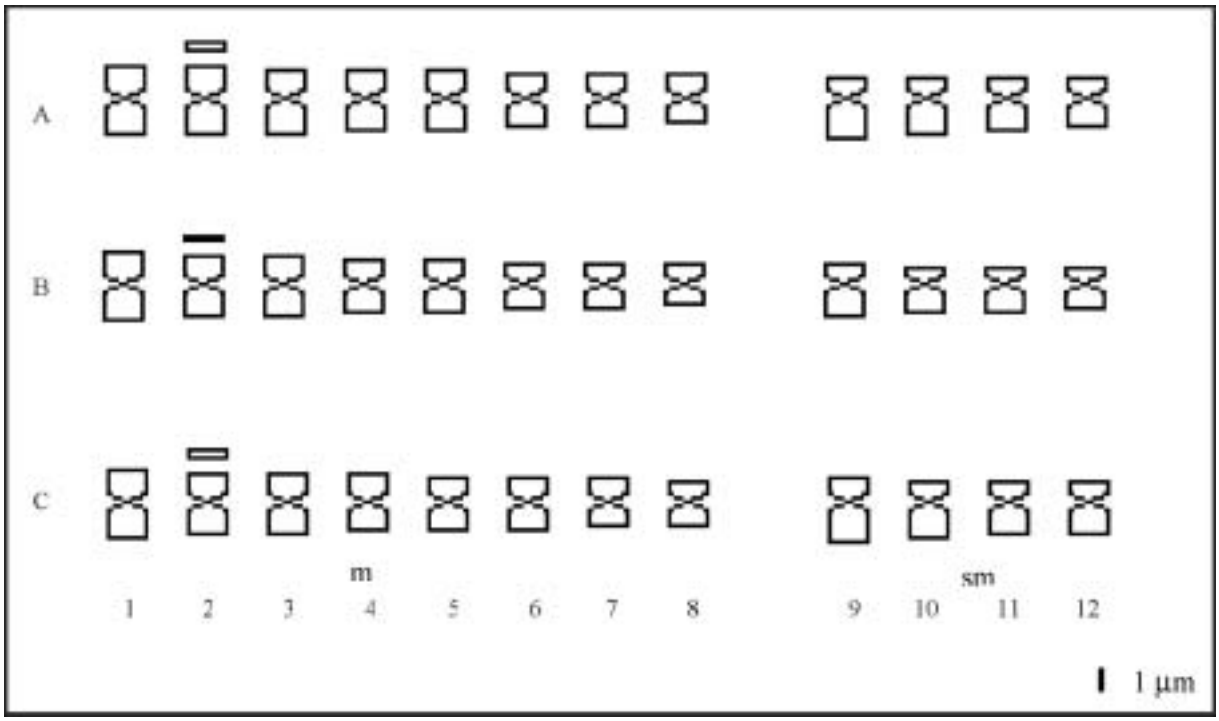

Fig. 3 — Idiograms for *Caesalpinia* species, 2n = 24. A: *C. gilliesii*. B: *C. mimosifolia*. C: *C. paraguariensis*. All at the same scale.

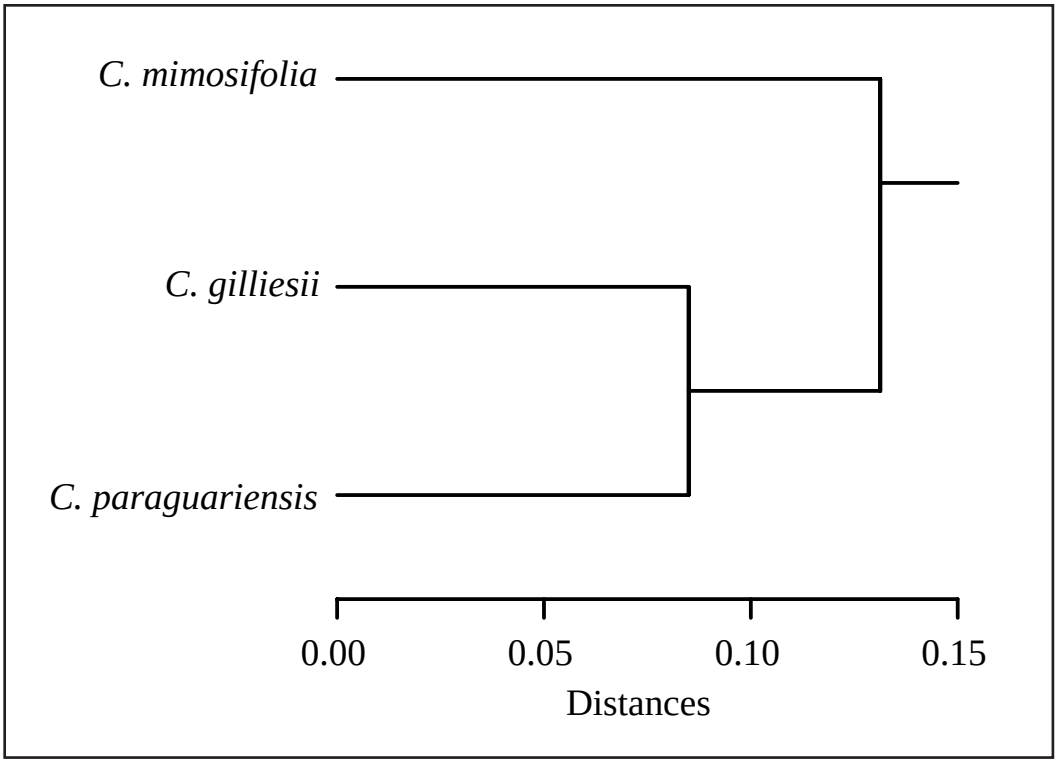

Fig. 4. UPGMA phenogram derived from average taxonomic distance for the *Caesalpinia* species studied based on karyological data.

Table 3 — Comparison of six karyological variables from the studied *Caesalpinia* species by ANOVA (p <0.05). df = degrees of freedom, * = statistically significant differences. A_1: intrachromosomal asymmetry index; A_2: interchromosomal asymmetry index; kl: haploid karyotype length; C: mean chromosome length; R: ratio between the largest and the smallest chromosome of the complement; r: mean arm ratio.

Variables	df	F	p
kl	27	9.30	0.001*
C	27	9.30	0.001*
R	27	14.19	0.004*
A_1	27	8.40	0.001*
A_2	27	17.84	0.000*
r	27	6.86	0.000*

Table 4 — Pairwise comparisons of karyotypic features among the studied *Caesalpinia* species by means of TUKEY's test: A_1= intrachromosomal asymmetry index, A_2 = interchromosomal asymmetry index, kl = haploid karyotype length, C = mean chromosome length, R = ratio between the largest and the smallest chromosomes of the complement, r = mean arm ratio. An asterisk indicates statistically significant differences.

C. mimosifolia	kl (p = 0.001)* C (p = 0.001)* R (p = 0.001)* A_1 (p = 0.631) A_2 (p = 0.000)* r (p = 0.133)	
C. paraguariensis	kl (p = 0.277) C (p = 0.277) R (p = 0.643) A_1 (p = 0.015)* A_2 (p = 0.562) r (p = 0.643)	kl (p = 0.031)* C (p = 0.031)* R (p = 0.000)* A_1 (p = 0.002)* A_2 (p = 0.000)* r (p = 0.003)*
	C. gilliesii	*C. mimosifolia*

other Southern South American genus of the tribe with x = 12 is *Hoffmannseggia* CAV. (GOLDBLATT 1981; ZANIN and CANGIANO 2001); thus, this number may be frequent within the tribe, although data on other genera are lacking.

The studied *Caesalpinia* species have small chromosomes and show a length range in accordance with other explored species of the genus (CHOUDHARY and CHOUDHARY 1988; KUMARI and BIR 1989; SOUZA and BENKO-ISEPPON 2004). Even though satellited chromosomes are not common in Caesalpinioideae as a whole (KUMARI and BIR 1989; SOUZA and BENKO-ISEPPON 2004), all species here examined had a pair.

Overall, woody perennial angiosperms in contrast to annual species have constant, less diversified karyotypes (BRANDHAM 1983; EHRENDORFER 1983), a trend supported by our data. The three species here analysed had a similar karyotype formula, with only m and sm chromosomes. These chromosome types are the most common chromosomes in the subfamily whereas st are rare (KUMARI and BIR 1989; BAIRIGANJAN and PATNAIK 1989). STEBBINS (1971) regarded Caesalpinioideae as primitive within the family, because its species tend to have small chromosomes with relatively symmetrical karyotypes, a trend also found by KUMARI and BIR (1989), SOUZA and BENKO-ISEPPON (2004), and our data. Phylogenetic data suggest that Caesalpinioideae is paraphyletic (DOYLE *et al.* 2000; BRUNEAU *et al.* 2001; WOJCIECHOWSKI *et al.* 2004) and that some of its members are basal for the legumes. On the other hand, *Caesalpinia* is not supported as monophyletic (BRUNEAU *et al.* 2001).

Even though the studied species are morphologically different (ULIBARRI 1996), their speciation was not followed by variation in chromosome number or morphology. This situation was also found in other angiosperm genera (BRANDHAM 1983; STIEFKENS and BERNARDELLO 1996; 2000). However, cryptic structural changes may have occurred, such as paracentric inversions or reciprocal translocations of segments of similar length (STEBBINS 1958). More cytological data on these interesting plants are badly needed to fully understand its karyological evolution.

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