

Karyotypic studies of *Cratylia argentea* (Desv.) O. Kuntze and *C. mollis* Mart. ex Benth. (Fabaceae - Papilionoideae)

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Genet. Mol. Res. 6 (3): 707-712 (2007)

Received June 28, 2007

Accepted August 25, 2007

Published September 30, 2007

ABSTRACT. *Cratylia argentea* and *C. mollis* (Fabaceae-Papilionoideae) are legume shrubs native to the Cerrado and Caatinga, respectively. Both species show great resistance to drought and high nutritive value, which makes them a valuable forage resource in tropical regions. Cytogenetic studies were carried out on accessions of *C. argentea* and *C. mollis* from Germplasm Banks of Embrapa Gado de Leite (Juiz de Fora, MG) and Embrapa Semi-Árido (Petrolina, PE), respectively. Root tips were treated with 3 mM 8-hydroxyquinoline and slides were made using the air-dry technique. Karyotype description for each accession took into account the following features: chromosome number; total length, relative length and arm ratio of each chromosome; haploid set length, and degree of asymmetry. Mitotic metaphases in both species showed $2n = 22$ chromosomes, where this is the first report of diploid number for *C. mollis*. Chromosome length was also quite similar for the two species, ranging from 5.08 to 2.50 μm in *C. argentea* and 5.12 to 2.51 μm in *C. mollis*, with haploid sets of equal size, measuring 38.10 and 37.85 μm , respectively. However, they did not show the same karyo-

typic formula, which was $5m + 4sm + 2st$ for *C. argentea* and $7m + 2sm + 2st$ for *C. mollis*. This indicates the occurrence of rearrangements within chromosomes I and VI. Both karyotypes showed a tendency for asymmetry.

Key words: *Cratyliia argentea*, *Cratyliia mollis*, Cytogenetics, Tropical forage, Karyotype, Chromosome evolution

INTRODUCTION

Cratyliia (Fabaceae-Papilionoideae-Phaseoleae-Dioecleinae) is a Neotropical genus found exclusively in South America, mainly east of the Andes mountain range and south of the Amazon River Basin. The origin of this taxon is probably associated with the occupation of the dry habitats formed after cycles of expansion and retraction of savanna vegetation in the Quaternary (Queiroz and Coradin, 1995).

First taxonomic definitions for this genus were accomplished based on the analysis of a herbarium collection and on field research. The five species currently described are: *Cratyliia argentea* (Desv.) O. Kuntze; *C. bahiensis* L.P. de Queiroz; *C. hypargyrea* Mart. ex Benth.; *C. intermedia* (Hassl.) L.P. de Queiroz, and R. Monteiro and *C. mollis* Mart. ex Benth. (Queiroz and Coradin, 1995). According to Argel and Lascano (1998), the taxonomy of this genus is not defined yet, since only morphological features and geographic distribution have been taken into account due to the lack of biological markers.

There has been an increasing interest in *C. argentea* and *C. mollis* due to their potential as forage for tropical regions. Both species are legume shrubs that show tolerance to drought, adaptation to acid soils and relatively high nutritive value (Queiroz et al., 1997; Andersson et al., 2006). These two species are quite similar, considering morphological and phenological characteristics, diverging mainly in geographic distribution. While *C. argentea* is widespread in South America, occurring in different habitats such as the Cerrado and seasonal forests, *C. mollis* is restricted to the Caatinga in the northeast region of Brazil (Queiroz and Coradin, 1995; Argel and Lascano, 1998).

Despite the great potential of these species, there are few studies on their biology. From a cytogenetic point of view, only *C. argentea* has been studied with regard to chromosome number ($2n = 22$) (Andersson et al., 2006), but without any characterization of its karyotype. Therefore, to provide suitable information for taxonomic definitions of *Cratyliia* genus as well as for germplasm preservation and genetic breeding, we describe and compare here the karyotypes of two Brazilian species, *C. argentea* and *C. mollis*.

MATERIAL AND METHODS

Cytogenetic analysis were carried out on 10 accessions of *Cratyliia argentea* and on one accession of *C. mollis* collections from the Germplasm Bank of Embrapa Gado de Leite, Juiz de Fora, MG, and Embrapa Semi-Árido, Petrolina, PE, respectively.

For mitotic analysis, young root tips were pre-treated with 3 mM 8-hydroxyquinoline at 4°C and fixed in methanol:acetic acid (3:1, v/v). The slides were prepared by the air-dry technique with enzymatic maceration based on Carvalho and Saraiva (1997). Briefly, root

tips were submitted to enzymatic maceration with the complex Pectinex[®] NOVO Nordisk[™] for 3 h, at 37°C. The meristematic tissue was dissected and dissociated on a glass slide in the presence of fixative solution to spread the protoplasts. Slides were dried on a hot plate and stained with 20% Giemsa solution.

For each accession, ten metaphases were digitized, using a digital micro-camera (Optronics) attached to an Olympus BX 60 microscope, for counting and measurements. The length of the short (si) or long arm (li) of each chromosome (i) was obtained using Sigma Scan Pro v.1.0 software. These values were used to calculate: total length of chromosome i ($ti = si + li$), haploid set length ($HSL = \sum St_i$), arm ratio ($ri = li / si$) and relative length of chromosome i ($RLi = ti / HSL$). Chromosomes were classified on the basis of arm ratio according to Levan et al. (1964).

The existence of variation among the accessions for RL and for HSL was verified by analysis of variance. Karyotype asymmetry was measured by the criterium proposed by Stebbins (1971).

RESULTS AND DISCUSSION

The most frequent basic numbers described for subfamily Papilionoideae are $x = 8, 11, 7$ and 16 (Souza and Benko-Iseppon, 2004). Considering the basic number $x = 11$, diploid species with $2n = 2x = 22$ represent the majority of some genera, such as *Phaseolus* (Mercado-Ruaro and Delgado-Salinas, 1998), *Vigna* (Sneff et al., 1992) and *Rhynkosia* (Biondo et al., 2003), where they are reported in many other genera in all tribes of Papilionoideae, especially in tribe Phaseoleae.

For subtribe Diocleinae, $2n = 22$ was the main chromosome number reported for five of the eight genera studied: *Canavalia* (Alves and Custódio, 1989; Bairangan and Patnaik, 1989), *Camptosema* and *Pachyrrhizus* (Goldblatt, 1981), *Dioclea* (Goldblatt, 1981; Souza and Benko-Iseppon, 2004), and *Cratylia* (Andersson et al., 2006). In this study, the diploid number $2n = 22$ was confirmed for *C. argentea*, and reported for the first time for *C. mollis* (Figure 1). These countings corroborate the trend for chromosome number conservation in the subtribe and even in the tribe Phaseoleae, with basic number $x = 11$.

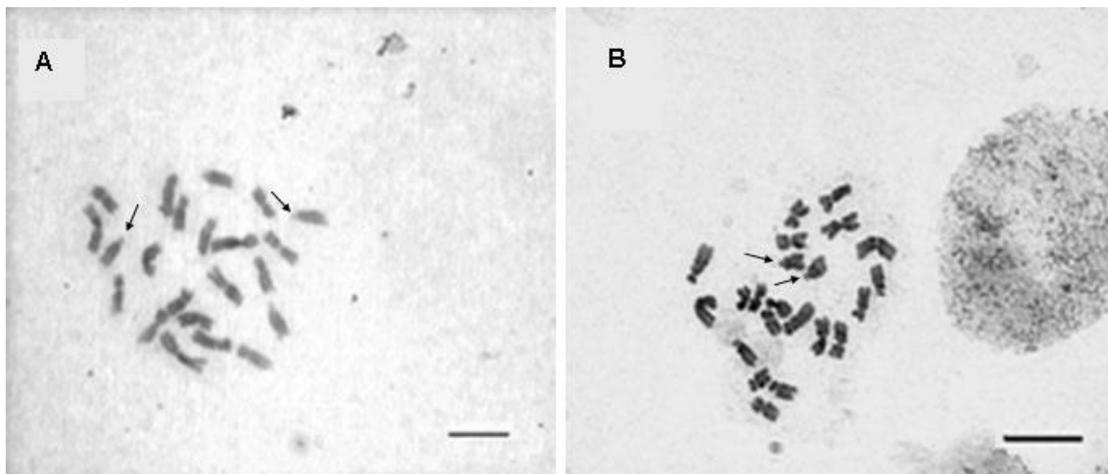


Figure 1. Giemsa-stained metaphase chromosomes of *Cratylia argentea* (A) and *C. mollis* (B), both with $2n = 22$ chromosomes. Arrows indicate the chromosome pairs with satellites. Scale bar = 10 μ m.

Considering karyotype features, the two species were also quite similar. Variation was not detected neither within *C. argentea* (among accessions) nor between *C. argentea* and *C. mollis* for content of DNA, assessed by measuring the HSL and for chromosome RLi. Average values for haploid set length were 38.10 μm in *C. argentea* and 37.85 μm in *C. mollis*. The largest chromosome (chromosome I) of *C. argentea* represented 13.29% of the total genome and the shortest (chromosome XI) represented 6.60%. For the same chromosomes in *C. mollis*, these values were 13.52 and 6.64%. This matching was observed for the other 9 chromosome pairs, with all of them demonstrating very close total length (Table 1).

Table 1. Average values for chromosome features of *Cratylia argentea* and *C. mollis*.

Ci	<i>Cratylia argentea</i>				<i>Cratylia mollis</i>			
	ti (μm)	RLi (%)	ri	Type	ti (μm)	RLi (%)	ri	Type
I	5.08	13.29	1.85	sm	5.12	13.52	1.14	m
II	4.35	11.40	3.60	st	4.27	11.29	3.33	st
III	3.91	10.26	1.19	m	3.90	10.29	1.35	m
IV	3.62	9.52	1.07	m	3.68	9.71	1.56	m
V	3.45	9.05	3.24	st	3.48	9.18	4.25	st
VI	3.30	8.65	1.75	sm	3.36	8.87	1.09	m
VII	3.16	8.31	1.86	sm	2.98	7.84	2.33	sm
VIII	3.04	7.97	1.08	m	2.99	7.90	1.13	m
IX	2.91	7.66	1.22	m	2.93	7.74	1.02	m
X	2.77	7.27	2.01	sm	2.65	7.01	1.89	sm
XI	2.50	6.60	1.40	m ^{sat}	2.51	6.64	1.20	m ^{sat}
HSL	38.10				37.85			

Ci = chromosome; ti = total chromosome length; RLi = relative length; HSL = haploid set length; ri = arm ratio; m = metacentric; sm = submetacentric; st = subtelocentric; ^{sat} = satellite.

The range of chromosome length was 5.08-2.50 μm in *C. argentea* and 5.12-2.51 μm in *C. mollis*, with average sizes of 3.46 and 3.44 μm , respectively. These are high values when compared to the ones found for genera belonging to the same subfamily Papilionoideae. Mercado-Ruaro and Delgado-Salinas (1998) found averages ranging from 0.99 to 1.84 μm in ten species of *Phaseolus*; Souza and Benko-Iseppon (2004) described *Bowdichia nitida* ranging from 1.80 to 0.90 μm and *Diocleia virgata* ranging from 2.50 to 1.10 μm . The latter species, native to the Brazilian Amazon, is the only one belonging to subtribe Diocleinae with its karyotype described, which limits comparisons with related species. However, it is possible to see that the *Cratylia* chromosomes are 2-fold larger than those of the closest taxon described and much larger than in all of the other karyotypes of Fabaceae species analyzed so far.

Karyotypic formulae for the species were 5 m + 4 sm + 2 st for *C. argentea* and 7 m + 2 sm + 2 st for *C. mollis*, with a satellite-like structure on the short arm of chromosome XI in both (Table 1, Figure 2).

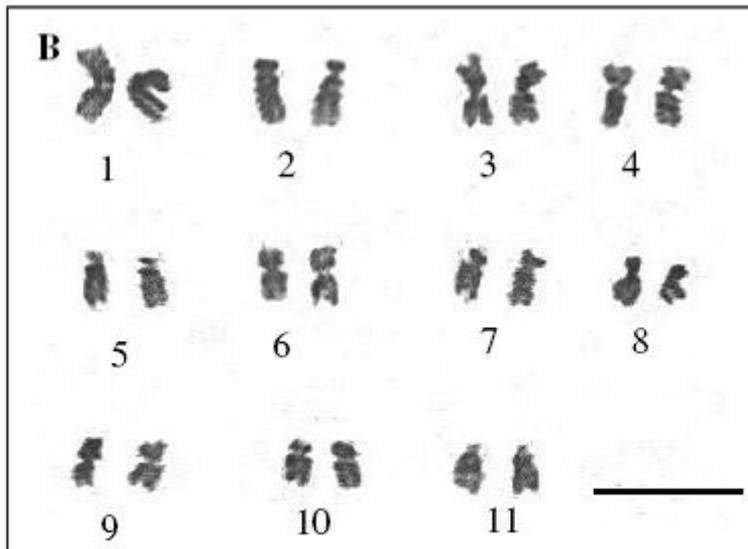
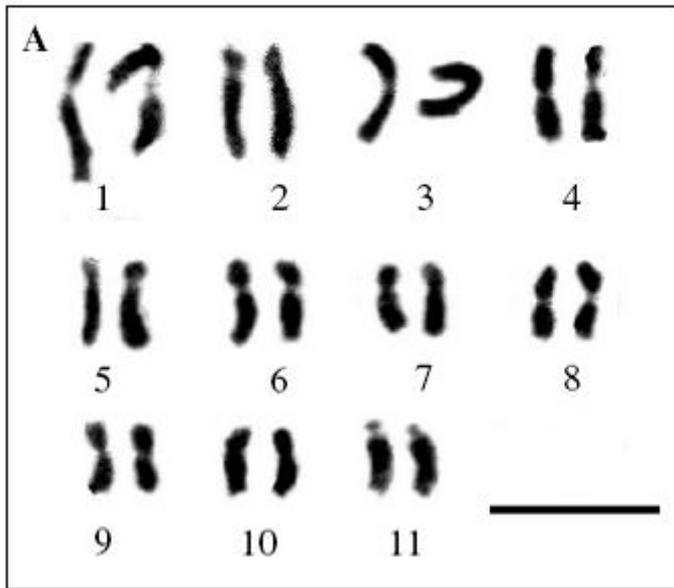


Figure 2. Karyograms of *Cratylia argentea* (A) and *C. mollis* (B). Numbers from 1 to 11 represent each chromosome pair, from longest (1) to shortest (11). Satellites can be observed on chromosome 11 of both species. Scale bar = 10 μ m.

Position of the centromere on chromosomes I and VI turned out to be the only difference between the karyotypes of *C. argentea* and *C. mollis*. While these chromosomes are submetacentric in *C. argentea* they showed a median centromere in *C. mollis*. Considering the results presented above on the HSL and RL, these differences in chromosome morphology can be explained by rearrangement of segments within the chromosome, without gain or loss of genetic material.

While both species studied here show two pairs of subterminal centromeric chromosomes, Kumari and Bir (1989) and Souza and Benko-Iseppon (2004) consider the occurrence of this kind of chromosome in species belonging to Fabaceae uncommon. Among 118 legumes species studied by Bairangan and Patnaik (1989) only 18 show this type of chromosome.

According to Stebbins (1971), the species *C. argentea* and *C. mollis* have a 3A karyotype, a category of karyotypes symmetric in size differences and asymmetric in centromere position. This trend toward asymmetry, along with relatively large chromosomes, can be viewed as evidence that species belonging to subfamily Papilionoideae are the most advanced in Fabaceae (Stebbins, 1971; Souza and Benko-Iseppon, 2004), though the biogeography of the genus indicates that its origin is a recent event (Queiroz and Coradin, 1995). Further cytogenetic investigations of these two species as well as of the other three species of *Cratylia* would provide the information necessary to elucidate the evolutionary and taxonomic relationships within this taxa and among the genus and the others of Phaseoleae-Diocleinae.

ACKNOWLEDGMENTS

The authors thank Martiniano C. de Oliveira and Deise Xavier for the donation of seeds, and Dr. Lyderson Faccio Viccini, Saulo Marçal de Sousa and Suellen Galvino for their assistance during the research. We also thank CAPES for the fellowship to S.M. Vargas and FAPEMIG for financial support.

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