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Chromosome analysis of Mammillaria supertexta, M. crucigera and M. haageana and their comparison with M. san-angelensis (Cactaceae)

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Abstract — This is the first report of chromosome number in *Mammillaria supertexta, M. crucigera* and *M. haageana*, which was the same in the three species: 2n=22, x=11. This number coincides with the number reported for the genus *Mammilaria* and for the Cactaceae family. However, interspecific variation was observed in the karyotypes: *M. supertexta* exhibited longer chromosomes (1.79-3.21 µm) and those of *M. haageana* were shorter (1.51-2.69 µm); in *M. crucigera* the chromosomes were 1.63-2.74 µm. The chromosomes of *M. san-angelensis* (0.80-1.70 µm) are the shortest (PALOMINO *et al*. 1999). Likewise, variation in the length of the haploid genome (LG) was observed: 26.84 µm for *M. supertexta*; of 23.81 µm for *M. crucigera*; and 23.06 µm for *M. haageana.* The LG of *M. san-angelensis* was shorter than that of the above species (13.83 µm). The karyotypic formula for *M. supertexta* and *M. crucigera* was 10m+1sm, and in *M. haageana* it was 9m+2sm. A pair of chromosomes with satellites was observed in *M. supertexta* and *M. haageana*, and in *M. crucigera* two pairs of chromosomes were observed with satellites. Unlike these species, *M. san-angelensis* exhibited no sm chromosome; all were metacentric, and like *M. crucigera*, there were two pairs of chromosomes with satellites. The index of asymmetry calculated for *M. supertexta* was 43.44%, for *M. crucigera* it was 42.55%, and for *M. haageana* it was 42.71%, indicating that the karyotypes were symmetric. The karyotype of *M. haageana* (9m+2sm) was different from that determined for *M. san-angelensis* (11m), thus showing divergence in its genomes. HUNT (1987) considers them to be synonymous, and complementary studies on morphology, hybridization, and cytogenetics are needed to determine the taxonomic category of these plants. *M. haageana* exhibited a total of 11 bivalents, a chiasmata frequency (Fq) = 13.86 and a recombination index (RI) = 24.86. These results are different from those of *M. san-angelensis* whose Fq = 16.74 and RI = 27.74. The higher RI in *M. san-angelensis* indicates that this species has more possibilities of forming new genetic combinations in its progeny and more opportunities of adapting to environmental changes than *M. haageana* with a lower RI. The interspecific variation observed among the four species of *Mammillaria* is possibly due to spontaneous structural changes in their chromosomes.

Key words: chromosome number, karyotype, *Mammillaria.*

INTRODUCTION

Mammillaria is the genus type of the Cactaceae family, and the taxonomic groups that it comprises have structural variability in the stem and flower, but mainly in the seed (BRAVO-HOLLIS and SANCHEZ-MEJORADA 1991).

Evolutionarily, *Mammillaria* is the most recent genus of the Cactaceae family and is widely represented in the Mexican Republic; it is almost endemic in our country. Of a total of 166 species, 160 are found in Mexico, and of these 150 are endemic to this country, including the species *M. supertexta*, *M. crucigera*, and *M. haageana* (HUNT 1992, HERNANDEZ and GODÍNEZ 1994). The first two of these are considered vulnerable or threatened by extinction, according to the International Union for Conservation of Nature (IUCN).

Chromosome number has been reported in 155 species of the genus *Mammillaria*, establishing the basic number at x=11, like that of the Cactaceae family (REMSKI 1954; SOSA and ACOSTA 1966; JOHNSON 1980 and PALOMINO *et al.* 1999). Most of the diploid species are located in the Mexican Republic, where the center of origin and diversity of the genus is located (REMSKI 1954; JOHNSON 1978, 1980).

Within the subgenus *Mammillaria*, chromosome number has been determined in 30 species (REMSKI 1954; PINKAVA and MCLEOD 1971; WEEDIN and

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POWELL 1978; JOHNSON 1978, 1980; ROSS 1981; GALLAGHER and PARFITT 1982; PINKAVA and PARFITT 1982; GILL and GOYAL 1984 and PINKAVA *et al.* 1985).

In the *Supertextae* series, there are reports of chromosome numbers (n and 2n) for four species: *M. ruestii*, tetraploid species (4n=4x=44) (REMSKI 1954), three diploid species (2n=2x=22), *M. vaupelii* (REMSKI 1954), *M. lanata* (GILL and GOYAL 1984) and *M. san-angelensis* (n=11; 2n=22, PALOMINO *et al.* 1999).

The *Supertextae* series, belonging to the genus *Mammillaria*, comprises seven species, according to HUNT (1987). However, BRAVO-HOLLIS and SANCHEZ-MEJORADA (1991) believe that the series comprises 20 species. Of these, *M. haageana* is considered the valid species for the two systems, but HUNT considers *M. san-angelensis* synonymous to *M. haageana*, while for BRAVO-HOLLIS and SANCHEZ-MEJORADA, they are two different species. From this controversy is derived the interest in conducting the present study to contribute cytogenetic information to clarify this taxonomic situation.

This study reports the somatic chromosome number (2n) and karyotype of *M. haageana*, *M. crucigera* and *M. supertexta* and their meiotic behavior of *M. haageana.* A karyotypic comparative analysis between *M. haageana* and *M. san-angelensis* is also presented.

MATERIALS AND METHODS

Plant material - Plants of *Mammillaria supertextae*, *M. crucigera* and *M. haageana* were collected from wild populations in the Mexican states of Puebla and Oaxaca (Table 1). Live plants were transplanted and maintained in a greenhouse at the Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México (JB-IBUNAM). Voucher specimens were deposited at the National Herbarium (MEXU) of the UNAM.

Mitotic chromosome analysis - For the observation of chromosome numbers (2n) and the karyotypes of the three species of *Mammillaria*, 9 to 15 mitotic cells at metaphase stage from 3 plants of each species were observed. Elongating secondary root tip cells were placed in a saturated solution of 1-Bromonaphthalene for 6hr at 18-20ºC in darkness, then fixed in a 3:1 (V/V) Farmer mixture for 24hr. The root tips were hydrolyzed with hydrochloric acid (1N) for 11 min at 60°C and transferred to Feulgen reagent for 2hr, following GARCIA (1990) and CID and PALOMINO (1996), procedures.

Slides were prepared using the squash technique; the best slides were frozen with dry ice (CONGER and FAIRCHIELD 1953) and mounted in Canada balsam. Three of the best cells in each population were photographed with Technical Pan Film using a Zeiss photomicroscope.

Karyotype analysis - The negative film was used to draw and measure chromosome arms and total genome length. Positions of centromeres were determined using a system of LEVAN *et al.* (1964); arm ratio (r= long arm/short arm) was calculated for each chromosome. Index of asymmetry (TF%) was obtained following GUPTA and GUPTA (1978) procedure.

Meiotic chromosome analysis - Meiotic behavior was studied in pollen mother cells (PMC) from fresh anthers squashed in 1% aceto-orcein without prior fixation. A total of 32 MI of PMC derived from three plants of *M. haageana* were analyzed. The following information was recorded: the type of bivalents (IIs), chiasmata frequency (Fq), and recombination index (RI) (WHITE 1973).

Pollen fertility - Estimates were made in samples of pollen stained with cotton blue in lactophenol. Percentages of well-filled stained grains were obtained from samples of 2900 pollen grain derived from three plants from each species.

Statistical analysis - The differences between the means of genome length and asymmetry indices (TF%) in three species of *Mammillaria* were determined using one-way analysis of variance (ANOVA). Means were compared using the test of the minimal significant difference (MSD).

Table 1 — Provenance and karyotype analysis of *Mammillaria supertexta*, *M. crucigera* and *M. haageana* collected by Reyes and Briones.

Species and locality	2n	Karyotype formula	Number of satellites	Range of chromosome length (µm)	Genome length (µm)	Index of asymmetry (TF%)
M. supertexta México. Oaxaca State. 3851	22	10m+1sm	1m	1.79-3.21	26.84	43.44
M. crucigera México. Puebla State.3857	22	10m+1sm	2m	1.63-2.74	23.81	42.55
M. haageana México. Oaxaca State.3834	22	9m+2sm	1m	1.51-2.69	23.06	42.71

RESULTS

Chromosome number - The species *Mammillaria supertexta, M. crucigera* and *M. haageana* exhibited a diploid chromosome number **2n=2x=22, x=11**, in M. haageana, **n = 11** was observed (Table 1; Figs.1 A, B and C).

Chromosome length -In *M. supertexta*, chromosome length of 1.79 to 3.21 µm was observed; the chromosomes of *M. crucigera* exhibited a length of 1.63 to 2.74 µm, and *M.haageana* had the shortest chromosomes, 1.51 a 2.69 µm. (Table 1).

Relative size was 6.67 to 11.96 % for *M. supertexta,* 6.85 to 11.51 % for *M. crucigera*, and 6.55 to 11.67 % for *M. haageana* (Table 2).

Total chromosome length –The size of the chromosomes in the three species studied correlated with the total length of their genomes. The interval for genome length in the species studied was 23.06 µm for *M. haageana,* which had the smallest value, and 26.84 µm for *M. supertexta*, which had the highest. In *M. crucigera* genome length was 23.81µm (Table 1). Genome length was different in the three species ($P<0.05$).

Comparison of the karyotypes of the species, *M. supertexta*, *M. haageana*, *M. crucigera* and *M. san-angelensis,* based on the data of relative size (L%) and the arm ratio (r) of their respective chromosome complements, indicated that, in spite of the differences, a certain uniformity in L% can be observed among said species, with the exception of the chromosome pairs 1 and 4; 9,10 and 11 of *M. san-angelensis*: first two, which had a larger L% and the last three with a smaller L%. In these pairs the differ-

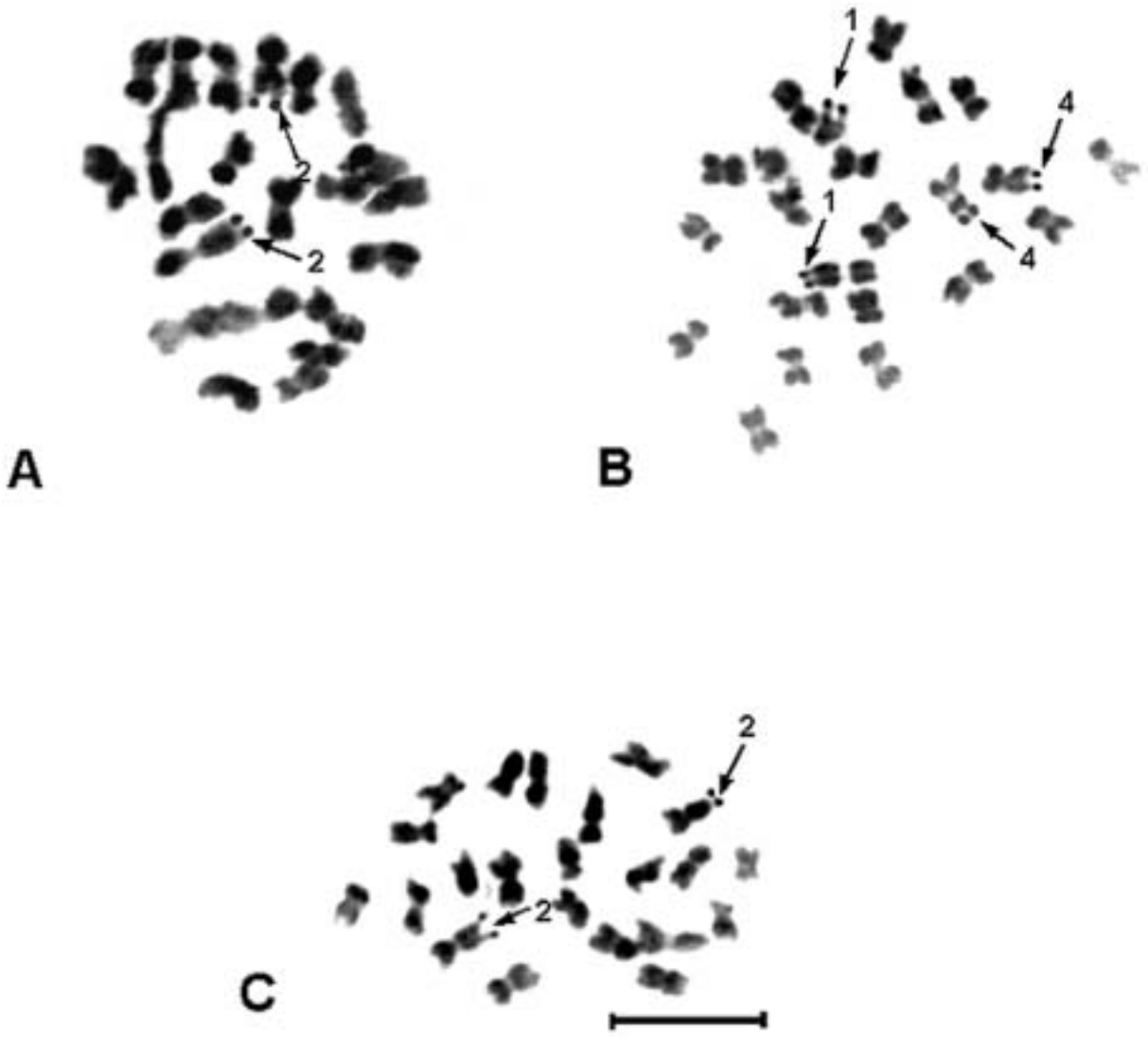

Fig. 1 — Somatic chromosomes, 2n=22, in *Mammillaria:* A) *M. supertexta*, B) *M. crucigera* y C) *M. haageana.* Numbers show chromosomes with satellites. Scale equals 10µm.

Table 2 — Relative length (L%) and arm ratio (r) of somatic chromosomes of *M. supertexta, M. crucigera, M. haageana* and *M. san-angelensis.*

	(L%)				*(r)*			
Chromosome pair	M. supertexta	M. crucigera	M. haageana	M. san-angelensis	M. supertexta	M. crucigera	M. haageana	M. san-angelensis
1	11.96	11.51	11.67	12.30	1.17	1.17	1.12	1.36
2	10.92	11.05	11.53	11.32	1.16	1.17	1.16	1.09
3	10.73	10.16	10.23	10.48	1.18	1.26	1.17	1.08
4	9.99	9.91	9.50	10.48	1.25	1.20	1.96	1.42
5	9.46	9.45	9.50	9.76	1.29	1.21	1.26	1.08
6	8.87	9.03	9.02	9.54	1.22	1.31	1.24	1.13
7	8.31	8.60	8.67	8.46	1.21	1.24	1.25	1.25
8	7.97	8.11	8.11	7.95	2.01	1.22	1.25	1.20
9	7.82	7.73	7.80	7.23	1.23	1.22	2.05	1.00
10	7.30	7.64	7.42	6.65	1.23	1.98	1.19	1.04
11	6.67	6.85	6.55	5.78	1.29	1.29	1.22	1.00

ence in L% is notable, compared with the rest of the species (Table 2; Fig. 2).

Also, a certain homogeneity was observed in terms of arm ratio (r), whose values indicated a predominance of metacentric chromosomes; again the chromosome pairs 1, 4, 9, 10, and 11 of *M. san-angelensis* departed from the behaviora pattern of the rest of the chromosome pairs of the other species. They are, however, also metacentric. The chromosome pairs 4 and 9 of *M. haageana*, 8 of *M. supertexta*, and 10 of *M. crucigera* had higher r values and are submetacentric (Table 2; Fig. 2).

Karyotype – The karyotype formulas of *M. supertexta* and *M. crucigera* were alike, corresponding to 10m+1sm. However, the submetacentric pair matched pair 8 in *M. supertexta* and 10 in *M. crucigera.* Unlike these two species, in *M. haageana* 9m+2sm was observed (Table 1; Figs. 3 A, B y C).

Satellites- In the three species studied, satellites were observed. *M. supertexta* and *M. haageana* exhibited a pair of small spherical satellites measuring 0.40 and 0.33 µm, respectively, located in the short arms of metacentric chromosome pair 2. *M. crucigera* had two pairs of spherical satellites measuring 0.4 µm in the short arms of chromosomes 1 and 4, both metacentric (Table 1; Figs. 1 A, B y C).

Asymmetry indices – The values obtained in the asymmetry indices (TF%) of *M. supertexta, M. crucigera* and *M. haageana* were TF % = 43.44, 42.55 and 42.71, respectively (Table 1). There were no significant differences among the species in TF% values (P>0.05).

Meiotic chromosome behavior- Meiotic behavior in *M. haageana* was normal. There were a total of 11 bivalents (II) (Fig.4) and one Fq per cell of 13.86 and per bivalent of 1.26. The recombination index (RI) was 24.86.

Pollen fertility- By using pollen stainability in cotton blue, all species have very high pollen stainability, *M. supertexta* 99.10%, *M. haageana* 98.59% and *M. crucigera* 97.66%, as expected for meiotic stability.

DISCUSSION

This report is the first communication of chromosome analysis in *M. supertexta*, *M. crucigera* and *M. haageana* (series *Supertextae*). The three species proved to be diploids (**2n=2x=22, x=11**) and **n=11** in *M. haageana*; the same basic chromosome number (**x=11**), indicated chromosome stability in the species. These characteristics coincide with those observed in other species of the same genus and series (*Supertextae*), such as *M. vaupelii* (Remski 1954), *M.lanata* (Gill and Goyal 1984), *M. albilanata* (Mohanty *et al*. 1996), and *M. san-angelensis* (Palomino *et al*. 1999). These results corroborate x=11, for the genus *Mammillaria* and the family Cactaceae (Sosa and Acosta 1966; Johnson 1980 and Palomino *et al*. 1999).

In *M. supertexta*, longer chromosomes (1.79 - 3.21 µm) were observed; the chromosomes of *M. crucigera* were of intermediate length (1.63 - 2.74 µm), and *M.haageana* had the shortest chromosomes (1.51 - 2.69 µm). In the species *M. san-angelensis,* Palomino *et al*. (1999) determined a chromosome length of 0.80 - 1.70 µm (Table 3). This confirms that the species *M. supertexta*, *M. crucigera*, *M. haageana* and *M. san-angelensis*, belonging to the series *Supertex-*

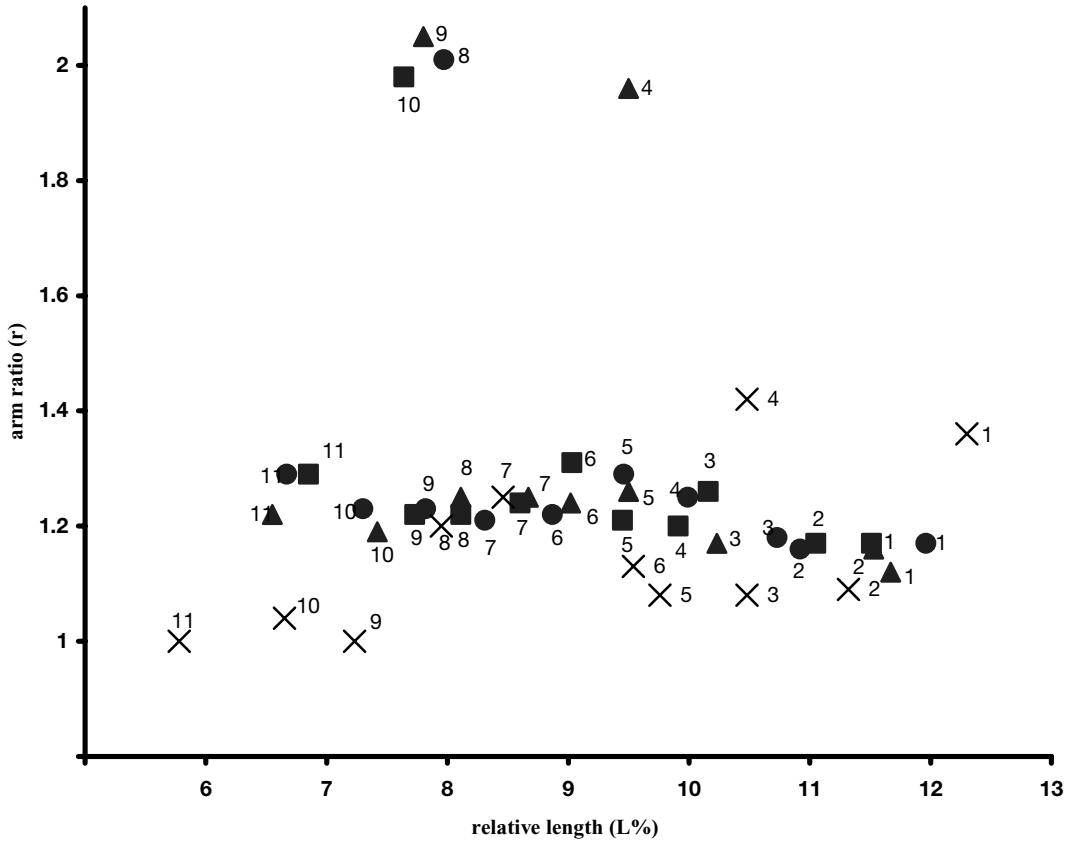

Fig. 2 — Graph of relative length (L%) and arm ratio (r) of chromosome measurement in table 2. • *M. supertexta,* ■ *M. crucigera,* ▲ *M. haageana* y X *M. san-angelensis.*

Table 3 — Karyotype analysis of *Mammillaria supertexta*, *M. crucigera, M. haageana* and *M.san-angelensis**.

Species	2n	Range of chromosome length (µm)	Genome length (µm)	Karyotype formula	Number of satellites	Index of asymmetry (TF%)
M. supertexta	22	1.79-3.21	26.84	10m+1sm	1m	43.44
M. crucigera	22	1.63-2.74	23.81	10m+1sm	2m	42.55
M. haageana	22	1.51-2.69	23.06	9m+2sm	1m	42.71
M. san-angelensis	22	0.80-1.70	13.83	11m	2m	44.39

* karyotype determined by Palomino *et al.* 1999.

tae, vary among their genomes, and therefore there is interspecific variation.

When size of the chromosomes and length of the genome of the three species studied are compared with those of *M. san-angelensis* (Palomino *et al.* 1999), the latter species had smaller chromosomes (0.80 - 1.70 µm) and a LG = 13.83 µm, smaller than those observed in *M. supertexta*, *M. crucigera* and *M. haageana* (Table 3). These variations in genome length of the four species confirm the interspecific variability in the species of *Mammillaria* of the *Supertextae* series, which was determined previously by variation in total length of the pairs of chromosomes.

The differences in chromosome size of different species of the same genus are considered evidence of the restructuring of their genomes and are attributed to rearrangements of the chromosomes, such as deletions, duplications or translocations that occurred in the first stages of their evolution (Palomino *et al.* 1988; Cota and Wallace 1995; Cid and Palomino 1996).

Comparing *M. haageana* with *M. san-angelensis* in terms of L% and r of their respective chromosome complements, minimum differences were observed among chromosomes 2, 7 and 8, in both L% and r, and maximum differences in chromosomes 1,

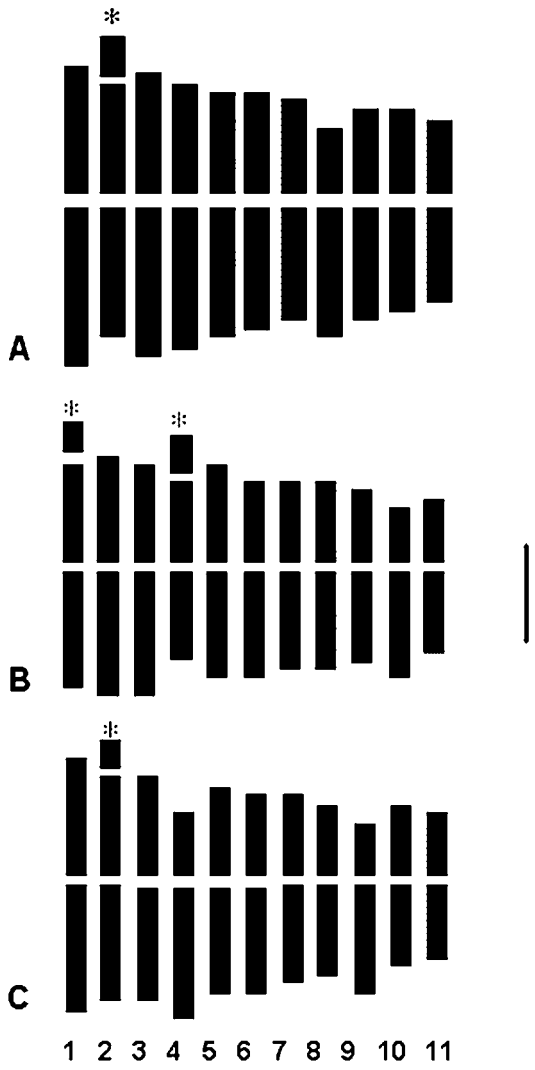

Fig. 3 — Idiograms of three species of *Mammillaria* with 2n=22. The numbers indicate the homologous chromosomes pairs: A) *M. supertexta*, 10m+1sm; B) *M. crucigera,* 10m+1sm y C) *M. haageana,* 9m+2sm. Asterisks show chromosomes with satellites. Scale equals 1μm.

4 and 9. In *M. san-angelensis* the 11 pairs of chromosomes were metacentric and there were no submetacentric pairs, while in *M. haageana* pairs 4 and 9 were submetacentric (Table 3; Fig. 2). These differences in L% and r can be attributed to structural changes, such as deletions, duplications and translocations that have occurred during the evolution of their karyotypes.

The karyotypic formula of 10 pairs of metacentric and one pair of submetacentric chromosomes observed in *M. supertexta* and *M. crucigera* has also been determined in other species of *Mammillaria* such as *M. boolii*, *M. humboldtii*, *M.leucantha* and *M. woodsi* (DAS *et al.* 1998a, 1999 b). The karyotypic formula 9m+2sm that appeared in *M. haageana* has also been reported in *M. bocasana*, *M. collinsii, M. mytax, M. geminispina, M. klisingiana, M. sempervivi* and *M. matudae* (DAS *et al.* 1998a, 1999b and c).

The karyotypes of *M. supertexta, M. crucigera* and *M. haageana* are different from *M. san-angelensis* which, according to PALOMINO *et al.* (1999), exhibited 11 metacentric chromosomes and no submetacentric chromosomes. It is evident that the four species belonging to the *Supertextae* series exhibit variation in the proportion of metacentric and submetacentric chromosomes. This variation has been observed by PALOMINO *et al.* (1988), COTA and WALLACE (1995), and CID and PALOMINO (1996) in other species of cacti, and by DAS *et al.,* (1998a), DAS *et al.,* (1999b and 1999c) in other species of *Mammillaria.*

In the three species studied secondary constrictions were observed. PALOMINO *et al.* (1999), observed in *M.san-angelensis*, as in *M. crucigera*, 2 pairs of satellites on the short arm of the chromosome pairs 1 and 3 and 1 and 4, respectively. This variation could have been due to the occurrence of deletions, duplications, or translocation among the chromosomes (PALOMINO *et al.* 1988; COTA and WALLACE, 1995; CID and PALOMINO 1996; DAS *et al.* 1998a; DAS *et al.*1999b and c).

This variation in the number and position of satellites has also been reported in other species of *Mammillaria* that have 1-3 pairs of satellites on the chromosomes; 1 to 2 pairs are most often observed. Other genera of cacti, such as *Melocactus* (DAS *et al.* 1998 b, c) and *Ferocactus* (DAS *et al.* 1999 d) also have 1 to 3 pairs of chromosomes with satellites.

The values obtained in the asymmetry index (TF %) of *M. supertexta, M. crucigera* and *M. haageana* were TF % = 43.44, 42.55 y 42.71, respectively, similar to those obtained in *M. geminispina* (TF % = 42.55, DAS *et al.* 1998a), *M. klissingiana* (TF % = 42.57, DAS *et al.*1999c), *M. winteriae* (TF % = 43.03, DAS *et al.* 1999c) and *M. bocasana* (TF % = 43.80, DAS e*t al.* 1998a). In other *Mammillaria* species TF% varied in the range of 35.50 to 49.19, within which TF% values were obtained for *M. supertexta, M.crucigera* and *M. haageana;* all of them coincide in having a symmetric karyotype.

Species of the genera *Nyctocereus*, (PALOMINO *et al.* 1988), *Echinocereus* (COTA and WALLACE, 1995) and *Myrtillocactus* (CID and PALOMINO 1996) also have symmetrical karyotypes and TF% values similar to those observed in the genus *Mammillaria.*

Meiotic behavior in *M. haageana* was normal, had a total of 11 bivalents (II), and exhibited a Fq per cell of 13.86 and 1.26 per bivalent. The recombination index (RI) was 24.86. These results differ from those obtained by PALOMINO *et al.* (1999) for *M. san-angelensis,* whose Fq was 16.74 and its RI was

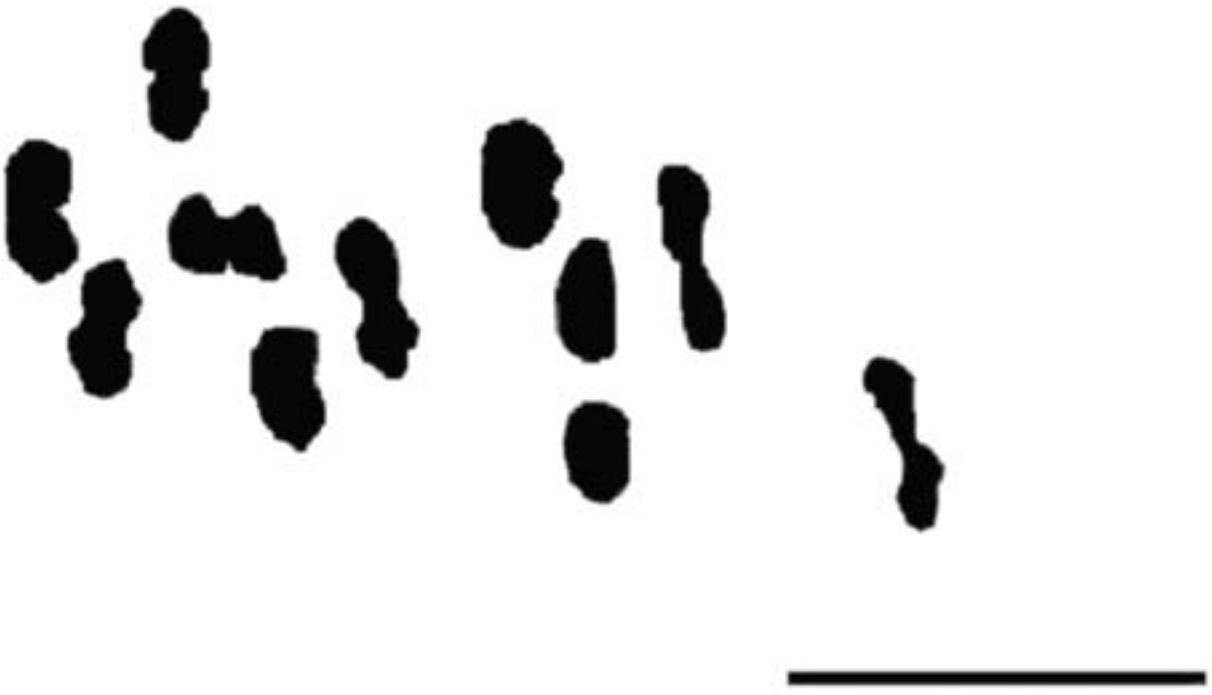

Fig 4. PMCs of *Mammillaria haageana* showing MI with 11 IIs. Scale equals 10μm.

27.74. Being higher, these values mean that *M. san-angelensis*, has more possibilities of new genetic combinations in its progeny and, therefore, more opportunities of adaptation to environmental changes than *M. haageana.* Another species of the same series, *M. albilanata,* had a different Fq, 20.25 per cell and 1.84 per bivalent, which is within the ranges of 17.00-28.80 and 1.54-2.62, observed in other species of *Mammillaria* (Mohanty *et al.* 1996; Das *et al.* 1997; Mohanty *et al.* 1997; Das *et al.* 1998a and Das *et al.* 1999a).

The cytological analysis conducted in this work revealed an interspecific variation in *M. supertexta*, *M. crucigera* and *M. haageana*, as well as in *M. san-angelensis* (Palomino *et al.* 1999), species included in the *Supertextae* series (Table 3). Hunt (1987) considers *M. haageana* to be synonymous to *M. san-angelensis.* According to the cytological analysis of these two species, the two are different in both their karyotypes and their meiotic behavior: *M. haageana* chromosomes were longer (1.51-2.69 μm) than those of *M. san-angelensis* (0.80-1.70 μm). In addition, in *M. san-angelensis* the 11 pairs of chromosomes were metacentric, while in *M. haageana,* 9 metacentric pairs and 2 submetacentric pairs were observed. Differences were also observed in their genomes in terms of the presence of chromosomes with satellites: *M. haageana* had 1, and *M. san-angelensis* had 2. The Fq and RI values were lower in *M. haageana,* Fq=13.86 and RI=24.86, and higher in *M. san-angelensis,* Fq=16.74 and RI=27.74. Based on these results, divergence among the genomes is established, and therefore, complementary studies are needed to clarify the taxonomic category of the two species.

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