CARYOLOGIA Vol. 54, no. 2: 147-154, 2001

Karyotypic studies in *Opuntia cochinera, O. hyptiacantha,* and *O. strepthacantha* (Cactaceae)

GUADALUPE PALOMINO* and HUMBERTO M. HERAS

Laboratorio de Citogenética, Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70614, México, D.F. 04510, México.

Abstract – Plants of *Opuntia cochinera*, *O. hyptiacantha,* and *O. streptacantha* species, collected in the Valley of Mexico, were consistently octoploids with a chromosome number $2n=8x=88$, confirming a base chromosome number $x=11$ for the genus *Opuntia*.

Karyotypes of the three species were homogeneous with 88 metacentric chromosomes. Interspecific variation of the genome was shown in chromosome size, genome length and chromosomes with satellites. The smallest chromosomes were found in *O. hyptiacantha* (1.07-2.50 µm), the intermediate size in *O. cochinera* (1.19-2.71 µm) and the largest in *O. streptacantha* (1.55-3.57 µm). Genome length was as follow: *O. hyptiacantha* had the smallest value (152.49 µm), *O. cochinera* an intermediate value (166.79 µm), and *O. streptacantha* the longest (210.99 µm). *Opuntia cochinera* presented 16 chromosomes with a satellite, this fact suggested an auto-octoploid origin. There were 6 chromosomes with a satellite in *O. hyptiacantha* and 20 in *O. streptacantha*, it indicated that both species had an allo-octoploid origin; so it is possible to call these *Opuntia* species with genomes of partial homology.

Key words: chromosome number, karyotype, *Opuntia*, polyploidy.

INTRODUCTION

The genus *Opuntia* (Cactaceae: Opuntioideae Schum, tribe Opuntieae (Brtt. and R.) Backbg., series Streptacantha Britt. and R.) comprises more than 200 especies. It is distributed from Canada to Chile (HUNT and TAYLOR 1986). BRAVO-HOLLIS (1978), recognized 65 *Opuntia* species of Mexican arid and semiarid habitats. For the Valley of Mexico only 15 wild species have been reported (SCHEINVAR 1982).

All *Opuntia* species are perennial, arborescent, and proliferate in tropical thorn forest (RZEDOWSKY 1988). In Mexico species of this genus are found wild and as cultivars; these are used as food, forage and medicinally.

Within the series Streptacantha, *Opuntia cochinera* Griff., *O. hyptiacantha* Web., and *O. streptacantha* Lem. stems (nopales) and fruits (tunas) are widely used as food and forage (PIMIENTA 1990). Mesoamerican tribes have used *Opuntia* stems against inflammation and diabetes (PIMIENTA 1990). In some instances *Opuntia* is used also to regenerate areas affected by erosion (BRAVO-HOLLIS and SÁNCHEZ-MEJORADA 1991). There are reports of *Opuntia* being used for the elaboration of paints and cosmetics (BRAVO-HOLLIS and SCHEINVAR 1995). The genus *Opuntia* has a base chromosome number $x=11$, a common number in most genera of Cactaceae. In the subfamily Opuntioideae most species are polyploids. There are 125 taxa of subfamily Opuntioideae in North America, of which 60% are polyploid (PINKAVA *et al*. 1985). Within the genus *Opuntia* there are chromosome counts (either n or 2n) for 136 species; 63.13% are polyploid (3x to 30x) and of these, tetraploids (4x) are the most frequent. The remaining 36.87% are diploids (BOWDEN 1945; CARPIO 1952; KATAGIRI 1952, 1953; SPENCER 1955; SOSA and ACOSTA 1966; GERALD

* Corresponding author: fax: ++52 5 622 9046; e-mail: hasbach@servidor.unam.mx.

1973; MCLEOD 1975; PINKAVA and MCLEOD 1971; PINKAVA and PARFITT 1982; PINKAVA *et al.* 1973, 1977, 1985, 1992; PARFITT 1978, 1980; WEEDIN and POWELL 1978, 1980; GRANT and GRANT 1979; SANJAPPA 1979; SAMPATHKUMAR and NAVANEETHAM 1980; WARD 1984; BAKER and PINKAVA 1987). Nearly 6% of polyploid species of *Opuntia* are 8x. YUASA *et al.* (1973), reported for *O. streptacantha* diploids (2*n*=2x=22) and octoploids (2*n*=8x=88). Octoploids have been recorded by PINKAVA and PARFITT (1982). In addition it has been pointed out that the presence of polyploids and the high level of hybridization in the subfamily Opuntioideae has played an important role in its evolution (PINKAVA *et al.* 1985). So far, more than 24 taxa have been produced by inter- and intraspecific breeding (PINKAVA and MCLEOD 1971; PINKAVA *et al.* 1985).

The present study analyzes the chromosome numbers (2*n*), karyotypes, and ploidy levels of *Opuntia cochinera*, *O. hyptiacantha* and *O. streptacantha* from plant populations in Mexico City and the State of Mexico.

MATERIAL AND METHODS

Plant material

Plants of *Opuntia cochinera*, *O. hyptiacantha* and *O. streptacantha* were collected from three wild populations in Mexico City and the State of Mexico, Mexico (Table 1). Live plants were transplanted to the cacti living collectins of the Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). For each of the species, 8 cladodes (stems) from five plants were used to obtain and observe the chromosomes. Branches were planted in pots containing a mixture of peat, then maintained in a greenhouse at the Jardín Botánico, IBUNAM. Voucher specimens were deposited at the National Herbarium (MEXU) of the UNAM.

Mitotic chromosome analysis

For the observation of chromosome numbers (2*n*) and the karyotypes of the three species of *Opuntia*, 20 mitotic cells in metaphase stage from 5 plants of each species were observed. Elongating secondary root tip cells were placed in a saturated solution of 1-Bromonaphthalene for 5 hr at 18°C in darkness. Then the root tips were hydrolyzed with hydrochloric acid (IN) for 10 min at 60°C and transferred to Feulgen reagent for 1 hr, following PALOMINO and VÁZQUEZ (1991). 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

Photographs of the best 3 cells of each species were digitalized in black and white images using a scanner ScanJet 3P HP (300 dpi), with the aim of obtaining an image in a personal computer. The files

Table 1 – Provenance and karyotype analysis of three species of *Opuntia*.

Species, locality and collection number	2*n*	Karyotype formula	Number of satellites	Range of chromosome length (µm)	Genome length (µm) $\bar{X}$±SE		Index of asymmetry (TF%) $\bar{X}$±SE	
O. cochinera México. México State. Guadalupe Mountains. L. Scheinvar, 1991	88	88 m	16 m	1.19-2.71	166.79*	0.04	45.31	0.11
O. hyptiacantha México. México State Tepotzotlán. L. Scheinvar, 1405-a	88	88 m	6 m	1.07-2.50	152.49*	0.03	45.60	0.17
O. streptacantha México. México City. Vicente Guerrero Mountains. H. Heras, 1	88	88 m	20 m	1.55-3.57	210.99*	0.05	44.68	0.42

* Statistically significant at $P < 0.05$.

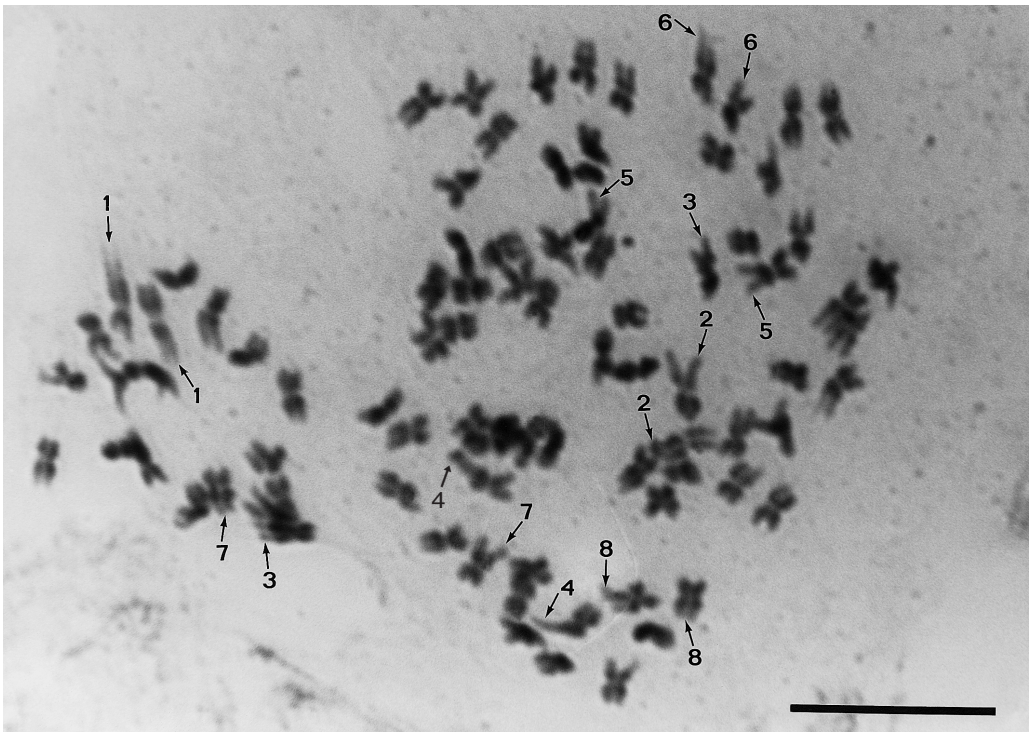

Fig. 1 – Chromosomes of *Opuntia cochinera* with 2*n*=88. Numbers show chromosomes with satellites. Scale equals 10µm.

were transferred to Corel Draw software package. These images were used to draw and measure short and long chromosome arms and total genome length. Positions of centromeres were determined using a system developed by LEVAN *et al.* (1964); arm ratio (r=long arm/short arm) was calculated for every chromosome. Karyotypes were constructed according to the asymmetry index of the chromosomes, then grouped. Index of asymmetry (TF%, or TF index) of the karyotypes was obtained following GUPTA and GUPTA (1978).

Statistical analysis

The differences between the means of genome length and asymmetry indices (TF%) in three species of *Opuntia* were determined applying the Student pairwise "t" test. Statistical analysis was performed in the computer using Statsoft CSS (1991) software package.

RESULTS

Chromosome number and ploidy level – Plants of populations of *Opuntia cocchinera*, *O. hyptiacantha,* and *O. streptacantha* were consistently octoploid with 2*n*=8x=88 (Table 1; Figs. 1, 2, 3, 4, 5, 6).

Chromosome length – Based on the relative percentage length, the chromosomes were organized in descending order for each species. Therefore the three species studied showed chromosome number 88 as the shortest in size and chromosome number 1 as the longest. There were similar relative percentage lengths: 0.70-1.63 µm for *O. hyptiacantha*, 0.71-1.63 µm for *O. cochinera* and 0.71-1.67 µm for *O. streptacantha*. The absolute chromosome length presented differences among the species. The smallest chromosomes were those in O. *hyptiacantha* ranging between 1.07-2.50 µm. A smaller chromosome length was observed in *O. cochinera* it was from 1.19-2.71 µm. Finally, *Opuntia streptacantha* had the largest chromosomes ranging between 1.55-3.57 µm (Table 1).

Genome length – Differences between chromosome length of the 3 species were correlated with their mean genome length. The genome length in the investigated species of *Opuntia* ranged from 152.49-210.99 µm (Table 1). This correlation indicated that the smallest genome length was that of *O. hyptiacantha* (152.49 µm) and the largest by *O. streptacantha* (210.99 µm). *O. cochinera* showed an intermediate genome length value of 166.79 µm compared with the other two species (Table 1). Genome length from

the three species of *Opuntia* differed significantly ($P<0.05$).

Karyotype analysis – Table 1 contains the karyotypic characterization for plants of the three species. All of them shown 88 metacentric chromosomes (Table 1, Figs. 2, 4, 6). No single karyotypic formula distinguished the 3 species of *Opuntia*. Karyotype variation among species involved variation in the chromosome size, genome length, and the number and position on their satellites.

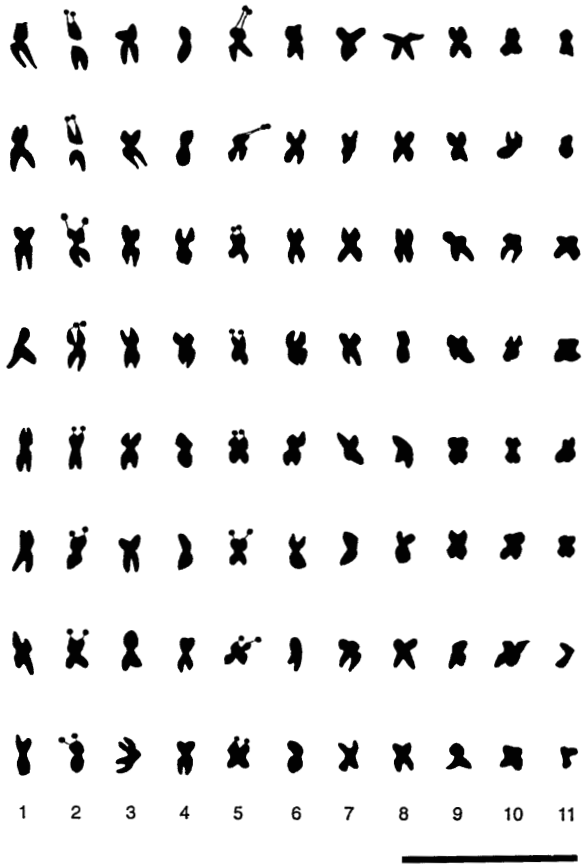

Fig. 2 – Karyotype of *Opuntia cochinera*, 2*n*=88. Numbers indicate groups of homologous chromosomes. Scale equals 10 µm.

Fig. 4 – Karyotype of *Opuntia hyptiacantha* with 2*n*=88. Numbers indicate groups of homologous chromosomes. Scale equals 10 µm.

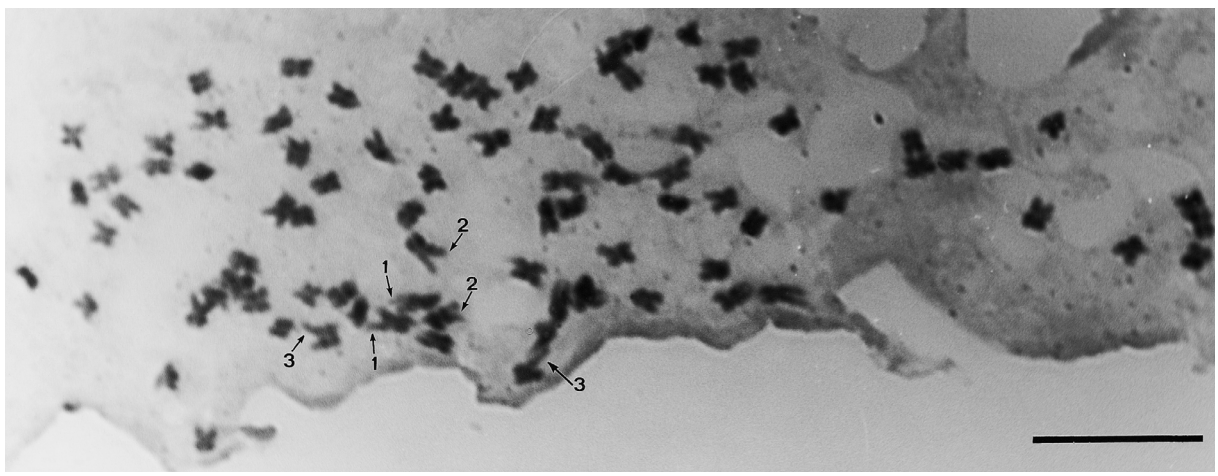

Fig. 3 – Chromosomes of *Opuntia hyptiacantha* with 2*n*=88. Numbers show chromosomes with satellites. Scale equals 10 µm.

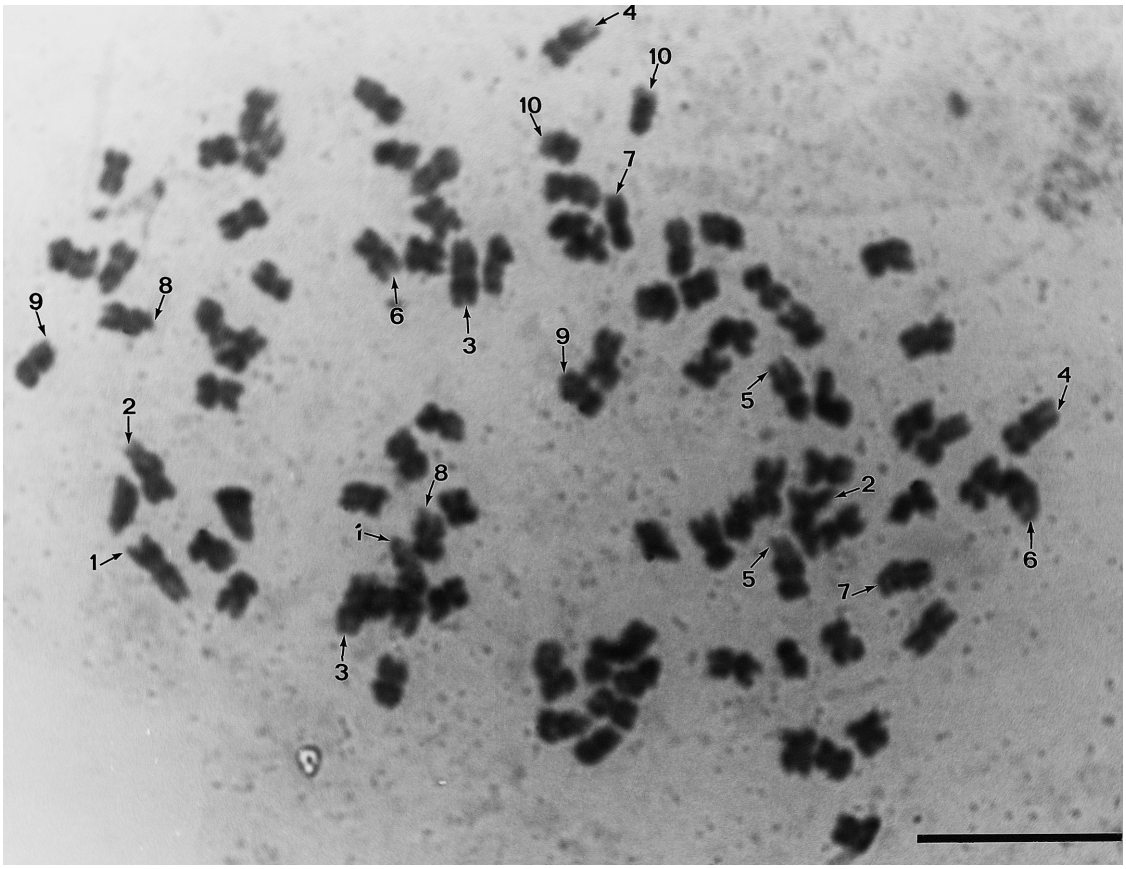

Fig. 5 – Chromosomes of *Opuntia streptacantha* with $2n$=88. Numbers show chromosomes with satellites. Scale equals 10 µm.

Satellites – For the three species of *Opuntia* satellites were of terminal linear type. Differences were found in number and position of satellites among chromosome complements (Figs. 1, 2, 3, 4, 5, 6; Table 1). For *Opuntia cochinera* there were 16 metacentric chromosomes with a satellite on chromosome numbers: 2, 4, 5, 7, 10, 12, 14, 19, 20, 24, 30, 36, 40, 41, 57 and 64 (Figs. 1 and 2). For *O. hyptiacantha* only 6 chromosomes with a satellite were found, these were 3, 6, 10, 14, 19 and 30 (Figs. 3 and 4). *O. streptacantha* showed 20 chromosome with a satellite, these corresponded to the numbers 1, 2, 3, 4, 5, 6, 8, 9, 12, 15, 17, 21, 23, 26, 31, 34, 36, 42, 47 and 71 (Figs. 5 and 6).

Asymmetry indices – As estimated by TF% asymmetry indices, the karyotypes of the three species of *Opuntia* were generally homogeneous. The TF% values among the species were very similar and they did not differ significantly ($P>0.05$). TF% value for *O. hyptiacantha* was TF%=45.60. *O. cochinera* showed TF%=45.31, and for *O. streptacantha* a TF%=44.68 was observed (Table 1).

DISCUSSION

Populations of *Opuntia cochinera*, *O. hyptiacantha* and *O. streptacantha* were consistently octoploids with $2n=8x=88$. For both *O. cochinera* and *O. hyptiacantha* it is the first time that a chromosome number is reported. In the case of *O. streptacantha* there were previous records of diploid plants with $2n=2x=22$ and octoploid plants with $2n=8x=88$ (YUASA *et al.* 1973). PINKAVA and PARFITT (1982), observed $n=44$ in anaphase I of meiosis in octoploid plants of *O. streptacantha* that were collected in Mexico, from San Luis Potosi and Zacatecas. Their results were similar to ours, confirming that a population of *O. streptacantha* from the Mexican Valley is octoploid. Previous reports of chromosome counts (n and $2n$) in *Opuntia* supported our study and confirmed that *O. cochinera*, *O. hyptiacantha* and *O. streptacantha* had a base chromosome number x=11, and $2n=8x=88$.

Karyotypes here observed for *Opuntia cochin-*

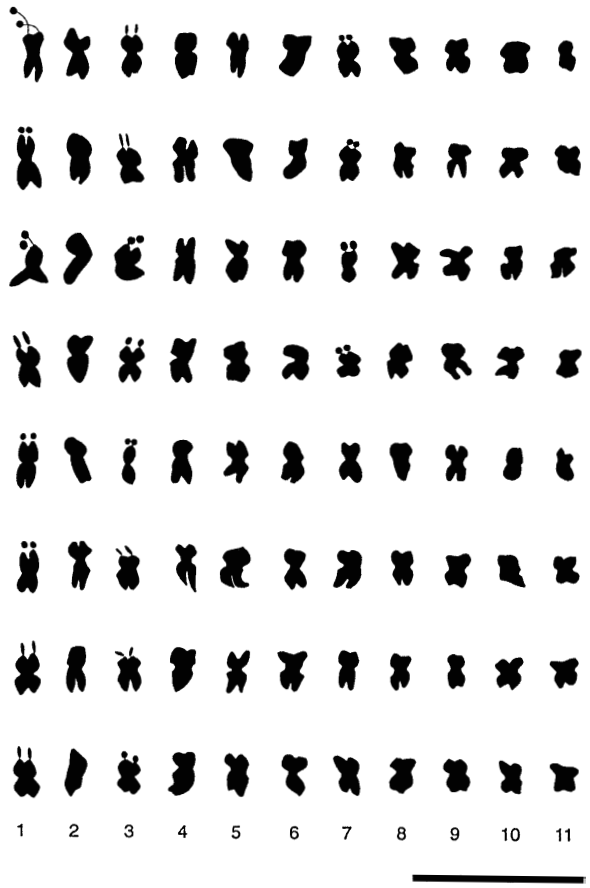

Fig. 6 – Karyotype of *Opuntia streptacantha* with 2*n*=88. Numbers indicate groups of homologous chromosomes. Scale equals 10 µm.

era, *O. hyptiacantha* and *O. streptacantha* showed small chromosomes (1.07-3.57 µm, Table 1), and all three species with 88 metacentric chromosomes (Figs. 1, 2, 3, 4, 5, 6; Table 1). So, it is possible to say that these are species cytologically homogeneous. This is common within the family Cactaceae. For example, as in the genera *Mammillaria* (JOHNSON 1978), *Nyctocereus* (PALOMINO *et al.* 1988), *Echinocereus* (COTA and WALLACE 1995), *Myrtillocactus* (CID and PALOMINO 1996) and *Ferocactus* (COTA *et al.* 1996).

Although *Opuntia cochinera*, *O. hyptiacantha,* and *O. streptacantha* showed similar karyotypes (2*n*=88 metacentric chomosomes), it is possible to distinguish differences among the three species. The most conspicuous is the variation in chromosome size, genome length, and the number of chromosomes with satellites. *O. hyptiacantha* showed the smallest chromosomes (1.07-250 µm), *O. cochinera* had chromosomes of intermediate size (1.19-2.72 µm) and *O. streptacantha* the largest (1.55-3.57 µm, Figs. 1, 2, 3, 4, 5, 6; Table 1). A correlation of genome length and chromosome size of *Opuntia* species here studied were found (Table 1). *O. hyptiacantha* had the smallest genome length (152.49 µm), *O. cochinera* showed an intermediate length (166.79 µm) and *O. streptacantha* (210. 99 µm) had the largest ($P<0.05$; Table 1). This indicates that each species has a different genome length and genome structure. Although there are few karyological studies of this type in Cactaceae, it has been shown that there is interspecific variation in groups closely related based in genome length, indicating correlation between genome length and chromosome size , for example, in species of *Nyctocereus* (PALOMINO *et al.* 1988) and *Echinocereus* (COTA and WALLACE 1995).

The number and position of secondary constrictions in *Opuntia* species was variable. *Opuntia hyptiacantha* had six chromosomes with a satellite, *O. cohinera* 16, and *O. streptacantha* 20. Position of satellites in *O. hyptiacantha* and *O. streptacantha* were similar in two chromosomes, and the first had four in common with *O. cohinera*. Between *O. cochinera* and *O. streptacantha* there are in common five satellites in the same chromosomes (Figs. 1, 2, 3, 4, 5, 6). Variation in satellite position could have been a result of pericentric inversions, as they have been proved in *Opuntia leptocaulis* (PINKAVA *et al.* 1985), and in *Mammillaria prolifera* (JOHNSON 1978). This could also be due to structural cryptic rearrangements on their chromosomes that were not evident during the microsporogenesis, as happens in *Echinocereus* species (COTA and WALLACE 1995).

Variation in chromosome satellites in the three species of *Opuntia* may indicate their auto-or allo-polyploid origin. *O. cochinera* had 16 chromosomes with a satellite, which could suggest an auto-octoploid origin; if it is hypothesized as an euploid species where the chromosome complement is constituted by a duplication of a multiple number of the basic chromosome number. This was observed in *O. robusta* (SOSA and ACOSTA 1966). The presence of 6 chromosomes with a satellite in *O. hyptiacantha* and 20 chromosomes with a satellite in *O. streptcantha* suggests that both species have an allo-octoploid origin. Species with partial genome homology have been shown to have an auto-allo-octoploid origin; *O. amyclea* and *O. megacantha*, when SOSA and ACOSTA (1966), observed during the meiosis of these species the for-

mation of multivalent chromosomes (tetravalents, hexavalents and an octavalent ring) in metaphase I, although in metaphase II, there was a formation of 44 normal dyads.

Acknowledgements – This study was supported by Jardín Botánico of the IBUNAM and CONACyT, project No. 1107P-N. We thank Javier Martínez for his photographic work, Jorge Saldivar for help in preparing the table and L. Rico and L. Kelly for critical review of the manuscript.

REFERENCES

BAKER M.A. and PINKAVA D.J., 1987 – *A cytological and morphometric analysis of a triploid apomictic, Opuntia x Kelvinensis (Subgenus Cylindropuntia, Cactaceae).* Brittonia, 39: 387-401.

BOWDEN W.N., 1945 – *A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae.* Amer. J. Bot., 32: 81-92.

BRAVO-HOLLIS H., 1978 – *Las Cactáceas de México. 2ª* ed., Vol. 1, en colaboración con SÁNCHEZ-MEJORADA H. México. Univ. Nacional Autónoma de México, México.

BRAVO-HOLLIS H. and SÁNCHEZ-MEJORADA H., 1991 – *Las Cactáceas de México.* Univ. Nacional Autónoma de México, México, Vol. 3.

BRAVO-HOLLIS H. and SCHEINVAR L., 1995 – *El interesante mundo de las Cactáceas.* Consejo Nacional de Ciencia y Tecnología, México.

CARPIO M.D.A., 1952 – *Notas sobre la cariología de dos especies de Opuntia.* Genética Ibérica, 4: 47-62.

CID R. and PALOMINO G., 1996 – *Cytotypes behavior in Mexican populations of Myrtillocactus geometrizans var. geometrizans (Cactaceae).* Cytologia, 61: 343-348.

CONGER A.D. and FAIRCHIELD L.M., 1953 – *A quick freeze method for making smear slides permanent.* Stain Tech., 28: 281-283.

COTA J.H. and WALLACE R.S., 1995 – *Karyotypic studies in the genus Echinocereus (Cactaceae) and their taxonomic significance.* Caryologia, 48: 105-122.

COTA J.H., REBMAN J.P. and WALLACE R.S., 1996 – *Chromosome numbers in Ferocactus (Cactaceae: Cactoideae).* Cytologia, 61: 431- 437.

GERALD K., 1973 – *IOPB. Chromosome number reports XXXIX.* Taxon, 22: 115-118.

GRANT V. and GRANT K.A., 1979 – *Systematics of the Opuntia phaeacantha group in Texas.* Bot. Gaz., 140: 199-207.

GUPTA R. and GUPTA P.K., 1978 – *Karyotypic studies in the genus Crotalaria L. Cytologia*, 43: 357-369.

HUNT D. and TAYLOR N., 1986 – *The genera of the Cactaceae: towards a new consensus.* Bradleya, 4: 64-78.

JOHNSON M.A.T., 1978 – *Diploid cytotypes in Mammillaria prolifera and three other Mammillaria species.* Cactus Succul. J. (Great Britain), 40: 9-12.

KATAGIRI S., 1952 – *Studies on the chromosome number in some Cactaceae species.* Jap. J. Breed., 1: 233.

–, 1953 – *Chromosome numbers and polyploidy in certain Cactaceae.* Cactus. Succul. J. (U.S.), 25: 141-143.

LEVAN A., FREDGA K. and SANDBERG A., 1964 – *Nomenclature for centromeric position on chromosome.* Hereditas, 52: 201-220.

MCLEOD M.G., 1975 – *A new hybrid fleshy-fruited prickly-pear in California.* Madroño, 23: 96-98.

PALOMINO G., ZULETA S. and SCHEINVAR L., 1988 – *Estudios citogenéticos de dos especies y una variedad del género Nyctocereus (Cactaceae).* Bol. Soc. Bot. México, 48: 75-80.

PALOMINO G. and VÁZQUEZ R., 1991 – *Cytogenetic studies in Mexican populations of species of Crotalaria L. (Leguminosae-Papilionoideae).* Cytologia, 56: 343-351.

PARFITT B.D., 1978 – *IOPB. Chromosome number reports LIX.* Taxon, 27: 53-61.

–, 1980 – *Origin of Opuntia curvospina (Cactaceae).* System. Bot., 5: 408-418.

PIMIENTA B.E., 1990 – *El nopal tunero.* Universidad de Guadalajara, México.

PINKAVA D.J., BAKER M.A., PARFITT B.D., MOHLEBROCK M.W. and WORTHINGTON R.D., 1985 – *Chromosome numbers in some cacati of western North America V.* System. Bot., 10: 471-483.

PINKAVA D.J., MC GILL L.A., REEVERS T. and MCLEOD M.G., 1977 – *Chromosome numbers in some cacti of western North America III.* Bull. Torrey Bot. Club, 104: 105-110.

PINKAVA D.J. and MCLEOD M.G., 1971 – *Chromosome numbers in some cacti of western North America.* Brittonia, 23: 171-176.

PINKAVA D.J., MCLEOD M.G., MC GILL L.A. and BROWN R.C., 1973 – *Chromosome numbers in some cacti of western North America II.* Brittonia, 25: 2-9.

PINKAVA D.J. and PARFITT B.D., 1982 – *Chromosome numbers in some cacti of western America IV.* Bull. Torrey Bot. Club, 109: 121-128.

PINKAVA D.J., PARFITT B.D., BAKER M.A. and WORTHINGTON R.D., 1992 – *Chromosome numbers in some cacti of western North America VI, with nomenclature changes.* Madroño, 39: 98-113.

RZEDOWSKY J., 1988 – *Vegetación de México.* Limusa 4a. reimpresión. México.

SAMPATHKUMAR R. and NAVANEETHAM N., 1980 – *Karyomorphological studies in Opuntia*. Proc. Indian Sci. Congr. Assoc. (III, C), 67: 59.

SANJAPPA M., 1979 – *IOPB. Chromosome number reports LXIV.* Taxon, 28: 393-995.

SCHEINVAR L., 1982 – *Las cactáceas del Valle de México.* Ph D. Thesis. Facultad de Ciencias, Universidad Nacional Autónoma de México, México.

SOSA R. and ACOSTA A., 1966 – *Poliploidía en Opuntia spp.* Agrociencia,1: 100-106.

SPENCER J.L., 1955 – *A cytological study of the Cactaceae of Puerto Rico.* Bot. Gaz., 117: 33-37.

WARD D.E., 1984 – *Chromosome counts from New Mexico and Mexico Cactaceae.* Phytologia, 56: 55-60.

WEEDIN J.F. and POWELL A.M., 1978 – *Chromosome number in Chihuahuan desert Cactaceae. Trans-Pecos Texas.* Amer. J. Bot., 65: 531-537.

WEEDIN J.F. and POWELL A.M., 1980 – *Chromosome number reports LXIX.* Taxon, 29: 716-718.

YUASA H., SHIMIZU H., KASHIWAI S. and KONDO N., 1973 – *Chromosome numbers and their bearing on the geographic distribution in the subfamily Opuntiodeae (Cactaceae).* Rep. Inst. Breed. Research. Tokio Univ. Agric., 4: 1-10.

Received January 24, 2001; accepted March 10, 2001