The use of multivariate analysis of karyotypesto determine relationships between species of Opuntia (Cactaceae)

Bulbulbandyopadhyay Biswas & Archana Sharma

The use of multivariate analysis of karyotypes to determine relationships between species of *Opuntia* (Cactaceae)

BULBUL BANDYOPADHYAY (BISWAS) * and ARCHANA SHARMA

Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

Abstract — Somatic chromosomes were studied from roots from seven species and two varieties of the genus *Opuntia* collected from different localities in Eastern India. $2n = 22$ chromosomes were observed in four, $2n = 44$ in two and $2n = 55$ chromosomes in one population. The total chromosome length is maximum in O. *Ficus-indica* ($2n = 55$) and minimum in O. *microdasys* var. *lutea* ($2n = 22$). The karyotypes show a similarity in basic number x = 11, except for the horticultural variety, *O. monocantha variegata* with $2n = 34$. The chromosome sizes range from very small (0.97 µm) to medium (3.46 µm); the primary constriction varies from nearly submedian to median and the numbers of chromosomes with secondary constrictions are from four to six. Numerical analysis of data indicates a general similarity of the karyotypes, with O. *Ficus-indica* occupying a separate position.

INTRODUCTION

The Cactaceae, a primary New World family of angiosperms, is characterised by a high level of diversity in habit and in vegetative, floral, fruit and seed morphology and pollination. The number of taxonomic units, 1500 species and nearly 100 genera, has made problematic the determination of systematic relationship at different taxonomic levels. The situation is further complicated by extensive parallel evolution in various structures and the resultant homoplasy has rendered phylogenetic analyses difficult (CoTA and WALLACE 1995). The principal controversies relate to generic and tribal circumscription. However, it is widely accepted that the Cactaceae is subdivided into three subfamilies: Pereskioideae, Opuntioideae and Cactoideae, which are traditionally interpreted as monophyletic groups.

The subfamily Cactoideae, with approximately 85 % of the species diversity, shows the greatest morphological extremes in habit and stem structure. Systematic studies of this subfamily (BUXBAUM 1958; GIBSON and NOBEL 1986; BARTHLOTT and HUNT 1993) divide it into ten tribes based upon common morphological features of vegetative and reproductive structures, as well as biogeographic affinities. Cytogenetic studies have shown that the Cactaceae have a base number of x=11, and polyploidy is the principal variation (BEARD 1937; REMSKI 1954; PINKAVA and McLEOD 1971). Earliest chromosome counts were n=9 and n=12, as summarized by PINKAVA and McLEOD (1971). Aneuploidy has been reported in meiotic material *of Deamia testudo* (Karw.).

As compared with the vast range of populations and forms and worldwide distribution in arid regions, studies on the chromosomes of *Opuntia* are relatively few. The earliest reports include *2n=22* chromosomes in most of the populations of *Opuntia Ficus-indica* studied (SPENCER 1955; WEEDIN and POWELL 1978). Polyploid races with a basic haploid set of *n=11* chromosomes were reported both in this and other species of the genus. *2n=44* chromosomes were observed in O. *elatior* (SANJAPPA and

* Corresponding author: fax ++91-033-4764419, e-mail: htc@cal3 .vsnl.net.in

SATHYANANDA 1979) from India and *2n=66* in both this species and *O.engelmannii* (Roy and MISHRA 1961). An unusual number of 48 was recorded in *O.dtllenuhom* India (SAMPATHKU-MAR and NAVANEETHAM 1980). High poly-ploids with 2n=88 chromosomes were observed in collections from North America of *O. Ficus-indica* (PINKAVA and McLEOD 1971; McLEOD 1975) and Spain (CARPIO 1952).

The present investigation was undertaken to study and compare the detailed karyotypes of *Opuntia*, which grow extensively in Eastern India, in wild condition. The collections were made from wild populations from different areas of Eastern India.The data were subjected to numerical analysis to trace the interrelation ships.

MATERIAL AND METHODS

Material

Seven species and two varieties of the genus *Opuntia* (Table 1), were collected from different localities in the Eastern India and planted in earthenware pots in suitable mixture of sand and soil.

Somatic chromosomes

They were studied from root-tips, collected within 9 AM. Several pretreating reagents (TJIO and LEVAN 1950; SHARMA and MOOKERJEA 1955; SHARMA and SARKAR 1955; SHARMA and SHARMA 1955) were tried, of which the following were found to be the most suitable.

a) 0.05% aqueous solution of colchicine for 2 hours.

b) Saturated aqueous paradichlorobenzene solution with small amount of aesculine for 3 hours.

c) Saturated solution of aesculine for 3 hours.

d) 0.002 M aqueous solution of 8- hydroxyquinoline for 3 hours. The entire procedure was carried out at 4°-5° C.

After pretreatment, the roots were thoroughly washed and fixed in glacial acetic acid: ethanol mixture (1:3) overnight. Roots were then treated with 1(N) HC1 for 5 minutes at 56°C, thoroughly washed in distilled water, treated with 45% acetic acid for 2-3 minutes, warmed for 2-3 seconds in 2% acetic — orcein staining solution, kept for 4 hours and finally squashed in 45% acetic acid for observation (see SHARMA and SHARMA 1994, 1999).

Karyotype analysis

For karyotype analysis well scattered metaphase plates were drawn from temporary slides with the aid of drawing prisms at different magnifications. Micro-photographs were taken using a Zeiss photographic attachment. The parameters scored were chromosome number, average total chromosome length (m), average long arm (L), average short arm (S), ratio of S/E and total form percent (TF%). Based on relative length and location of constrictions, the chromosomes were classified into five types A, A', B, C and D (Fig. 1).
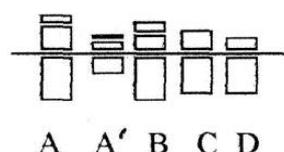

A   A'   B   C   D

Fig. 1 — Diagrammatic representation of different chromosome types in *Opuntia*.

Statistical analysis

It was performed by unweighted pair group method using arithmetic average, UPGMA (CLIFFORD and STEPHENSON 1975; SEBER 1984; DAVIES 1985; SNEATH and SOKAL 1986; HAIR *et al* 1990). In the present investigation, each *Opuntia* species represents an OTU (Operational Unit). Each OTU was scored for four variables (see Table 1) to generate the basic data matrix. The computation has been carried out with squared Euclidean distance. UPGMA cluster analysis was performed on karyotypic features in order to identify species interrelationships. Karyo-type asymmetry for the relationship between the chromosome arms has been estimated for every sample using the following equation:

$$A_1 = 1 - \frac{\sum_{i=1}^{n} b_i/B_i}{n}$$

where A_1 is the intrachromosomal index, ranging from zero to one. The equation is formulated in order to obtain lower values when chromosomes tend to be metacentric, n is the number of homologous chromosome pair or groups, b_i is the average length for short arms in every homologous chromosome pair or group. B_i is the average length for long arms in every homologous chromosome pair or group.

For A_2, the formula $A_2 = S/x$ is used where A_2 is the interchromosomal index, S is the standard deviation of chromosome length for each sample and x is
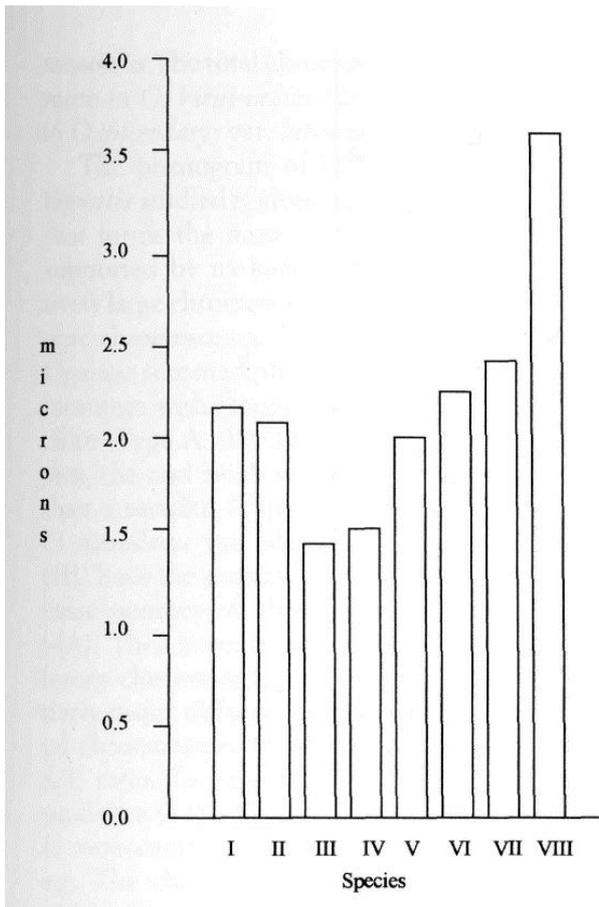
Fig. 2 — Histogram showing the average total chromosome length per species. Species names and codes are listed in Table 1.

the mean chromosome length for each sample (STEB-BINS 1971; ROMERO ZARCO 1984).

RESULTS AND DISCUSSION

Karyotypes of *Opuntia* tend to be uniform. Of the eight populations of *Opuntia* studied here, 2n=22 is present in four, 2n=44 in two and *2n=55* chromosomes in one population. The horticultural variety *O. monocantha variegata* has an unusual number, 2n=34. The chromosomes are divided into five groups on the basis of size and the location of primary and secondary concentrations (Fig. 1). Type A is relatively long to medium sized chromosome with nearly median to median primary constriction and a satellite at the distal end of one arm. Type A' is similar to A but much shorter in size. Type B is relatively long to medium sized chromosome with two constrictions, primary and sec-

ondary, one almost median in position and other located at the distal end of the short arm. Type C is relatively medium sized to short chromosome with median to nearly median primary constriction. Type D is relatively medium sized to short chromosome with nearly submedian to submedian primary constriction. The predominance is of symmetric karyotypes composed of mainly of metacentric and submetacentric chro-
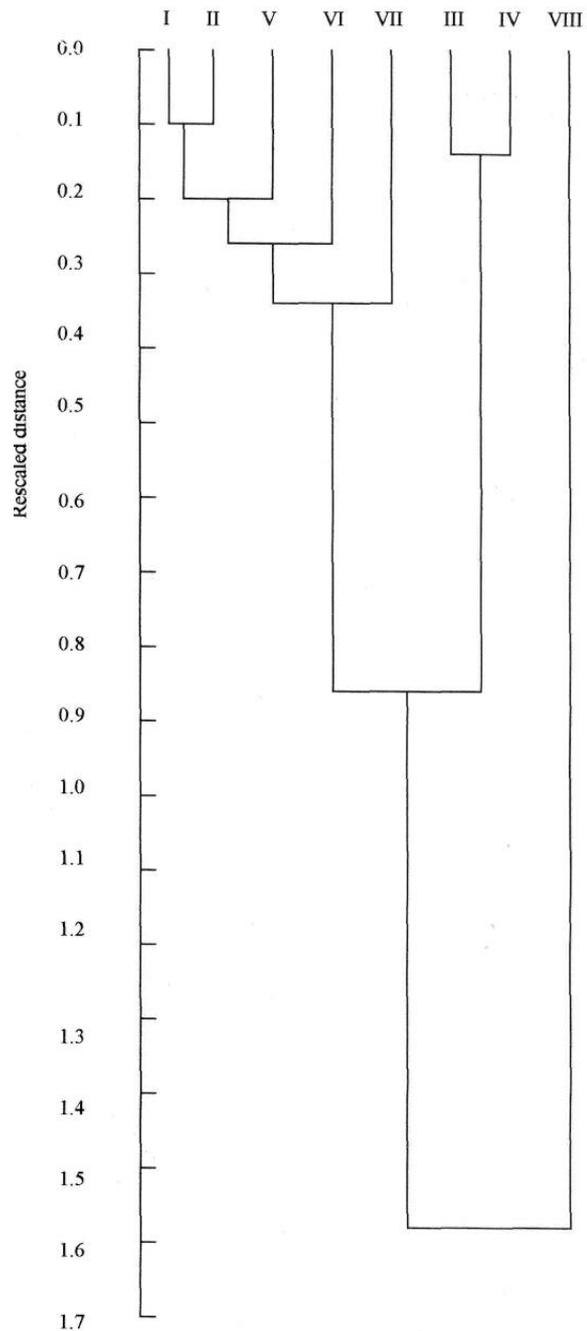

Fig. 3 — The phenogram of UPGMA clustering of the data in Table 1 for *Opuntia* studied.

TABLE 1 — Name of species, codes, chromosome number, average total chromosome length, average long arm (L), average short arm (S), S/L, total form percent (TF%), intrachromosomal index (A_1), interchromosomal index (A_2) of *Opuntia* collected from different areas in the Eastern India.

Name of the species	Code number	Chromosome number ($2n$)	Average total chromosome length (μm)	Average long arm (L) (μm)	Average short arm (S) (μm)	S/L	TF%	Karyotype Formula	A_1	A_2
Opuntia cylindrica (Lam.) DC.	I	44	2.19±0.03	1.38±0.04	0.81±0.02	0.57±0.02	36.67±1.04	4A+2A'+16C+22D	0.39	0.20
O. basilaris Engelm. & Bigel	II	22	2.16±0.03	1.31±0.02	0.85±0.03	0.64±0.03	39.57±1.24	6A+10C+6D	0.33	0.23
O. microdasys (Lehm.) Pfeiff. var. *lutea*	III	22	1.59±0.02	1.02±0.03	0.55±0.02	0.53±0.18	34.89±1.56	4A+10C+8D	0.40	0.28
O. stricta var. *dillenii* (Ker-Gawl) L. Benson	IV	44	1.62±0.02	0.97±0.02	0.64±0.03	0.66±0.03	39.68±1.60	4A+24C+16D	0.33	0.11
O. microdasys (Lehm.) Pfeiff. var. *albispina*	V	22	2.06±0.03	1.36±0.04	0.70±0.03	0.51±0.03	33.81±1.45	4A+6C+12D	0.47	0.17
O. monocantha variegata Haw.	VI	34	2.27±0.04	1.52±0.03	0.74±0.02	0.48±0.01	32.74±0.58	4A+2A'+4C+24D	0.51	0.20
O. robusta Wendl.	VII	22	2.39±0.03	1.44±0.03	0.95±0.01	0.65±0.03	39.59±0.89	4A+10C+8D	0.34	0.22
O. Ficus-indica (L.)Mill.	VIII	55	3.46±0.06	2.32±0.04	1.13±0.07	0.48±0.04	32.47±1.98	6A+2B+16C+31D	0.49	0.16

mosomes.The total chromosome length is maximum in O. *Ficus-indica (2n=55)* and minimum in *O.microdasysvat. lute a (2n=22)* (Fig. 2).

The phenogram of UPGMA clustering of *Opuntia* studied is given in Figure 3. *O.Ficus-indica* forms the most distant cluster (VIII), as supported by its karyotype (2n=55), the relatively large chromosomes and presence of two B type chromosomes. In all other populations of *Opuntia* screened, the end segment in all chromosomes with secondary constrictions is a satellite (Type A, A') (Fig. 1). Only in *O.Ficus-indica,* the end small segment is relatively larger than a satellite (Type B). The two varieties of *O.microdasys* var. *albispina* (V) and var. *lutea* (III) have the somatic number *(2n=22)* and the same number of chromosomes with satellites (4A). They have, however, been placed in different clusters in the phenogram, due to relatively major differences in average length of total chromosome, long arm and short arm and S/L ratio. In general, the karyotypes show a similarity in the base number $x=11$, except for *0. monocantha variegata (2n=34),* a garden variety. The chromosomes range from very small (0.97 μm) to medium (3.46μm) in size; the primary constrictions grade from nearly subme-dian to median and the numbers of chromosomes with secondary constrictions are four and six. As estimated by the A_1 and A_2 indices, the karyotypes of *Opuntia* were generally homogeneous (Table 1, Fig. 4). The scattered diagram showed that II, VII and V, VIII are closer to each other than the remaining taxa indicating a closer association.
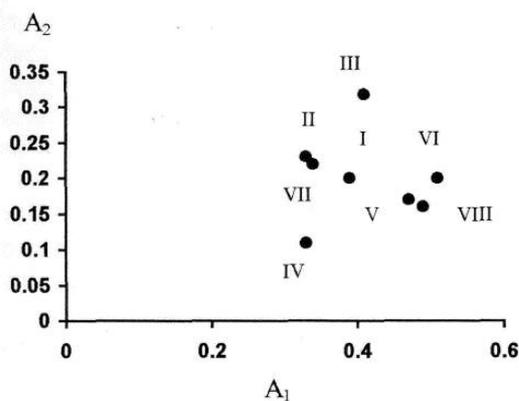

Fig. 4 — Intrachromosomal asymmetry index (A_1) plotted against the interchromosomal index (A_2). Species names and codes are listed in Table 1.

The general similarity in karyotypes indicates that minor chromosomal alterations, aided by polyploidy, may have been responsible for species diversification. Therefore, *Opuntia Ficus-indica* occupies a separate position in the complex with some unusual features in its karyotype. The widespread presence of apomixis in the species and its worldwide distribution may have assisted in the origin and survival of such forms (see SHARMA 1956; SHARMA and SHARMA 1959).

REFERENCES

BARTHLOTT W. and HUNT D.R., 1993 — *Cactaceae.* In: K. Kubitzki, J.G. Rohwer and Bittrich (eds). "The families and genera of vascular plants", vol. II. Springer-Verlag, Berlin.

BUXBAUM P., 1958 — *The phylogenetic division of the subfamily Ceredoidea, Cactaceae.* Madrono,15: 177-206.

CARPIO M.D.A., 1952 — *Nota sobre la cariologia dedos especies del genera Opuntia.* Genet. Iber., 4:47-62.

CLIFFORD H.T. and STEPHENSON W., 1975 —*An introduction to numerical classification.* Academic Press, New York.

COTA J.H. and WALLACE S., 1995 — *Karyotypic studies in the genus Echinocereus (Cactaceae) and their taxonomic significance. C*aryologia, 18: 105-122.

DAVIES R.G., 1985 — *Some problems of numerical taxonomy.* Sci. Progr., 69: 315-339.

GIBSON A.C. and NOBEL P.S., 1986 — *The Cactus Primer.* Harvard University Press. Cambridge.

HAIR J.F. JR., ANDERSON R.E. and TATHAM R.L., 1990 — *Multivariate Data Analysis.* Maxwell Macmillan, New York.

McLEOD M.G., 1975 —*A new hybrid fleshy-fruited prickly pear in California.* Madrono, 23: 96-98.

PINKAVA DJ. and PARFITT B.D., 1988 — *Nomenclatural changes in Chihuahuan Desert, Opuntia (Cactaceae).* Sida, 13: 125-130.

REMSKI M.F., 1954 — *Cytological investigations in Mammillaria and some associated genera.* Bot.Gaz., 116: 163-171.

ROMERO ZARCO C,, 1986 — *A new method for estimating karyotype asymmetry.* Taxon, 35: 526-530.

ROY R.P and MISHRA N.C., 1961 — *Cytological studies in Cactaceae.* Proc. 48th Indian Sci. Congr., Section of Botany, part 3: 300-301.

SAMPATHKUMAR R. and NAVANEETHAM N., 1980 — *Karyomorphological studies in Opuntia.* Proc. 67th Indian Sci. Congr., Section of Botany, part 3:59.

SANJAPPA M. and SATHYANANDA N., 1979 — *IOPB chromosome number reports LXIV.* Taxon, 28: 391-408.

SEBER G.A.F., 1984 — *Multivariate observations.* John Wiley, New York.

SHARMA A.K., 1956 — *A new concept of a means of speciation in plants.* Caryologia, 9: 93-103.

SHARMA A.K. and MOOKERJEA A., 1955 — *Paradichlorobenzene and other chemicals in chromosome work.* Stain Techn., 30: 1.

SHARMA A.K. and SARKAR S., 1955 — *A new technique for the study of chromosome of Palms.* Nature, 176:261.

SHARMA A.K. and SHARMA A., 1956 — *Fixity in chromosome number of plants.* Nature, 177: 335-336.

—, 1959 — *Chromosomal alterations in relation to speciation.* Botanical Review, 25: 514-544.

—, 1994 — *Chromosome Techniques: A manual.* Harwood Academic Publishers, Reading, UK.

—, 1999 — *Plant Chromosomes.* Harwood Academic Publishers, Reading, UK.

SNEATH P.H.A. and SOKAL R.R., 1973 —*Numerical taxonomy.* W.H. Freeman and Co., San Francisco.

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

STEBBINS G.L., 1971 — *Chromosomal evolution in higher plants.* Edward Arnold London.

TJIO, J.H. and LEVAN, A. 1950 — *The use of oxiquinoline in chromosome analysis.* Anal. Estac. Exp. Aula Dei, 2:21-64.

WEEDIN J.F. and POWELL A.M., 1978 — *IOPB chromosome number reports LX.* Taxon, 27: 223-231.