

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

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**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 ( $0.97 \mu\text{m}$ ) to medium ( $3.46 \mu\text{m}$ ); 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

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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. dillenulhom* 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 interrelationships.

## 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.

- 0.05% aqueous solution of colchicine for 2 hours.
- Saturated aqueous paradichlorobenzene solution with small amount of aesculine for 3 hours.
- Saturated solution of aesculine for 3 hours.
- 0.002 M aqueous solution of 8- hydroxyquinoline for 3 hours. The entire procedure was carried out at  $4^{\circ}$ - $5^{\circ}$  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) HCl for 5 minutes at  $56^{\circ}$ 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. Microphotographs were taken using a Zeiss photographic attachment. The parameters scored were chromosome number, average total chromosome length ( $\bar{m}$ ), 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).

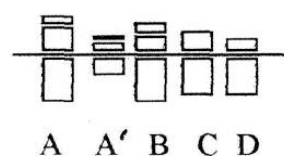


Fig. 1 — Diagrammatic representation of different chromosome types in *Opuntia*.

### 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

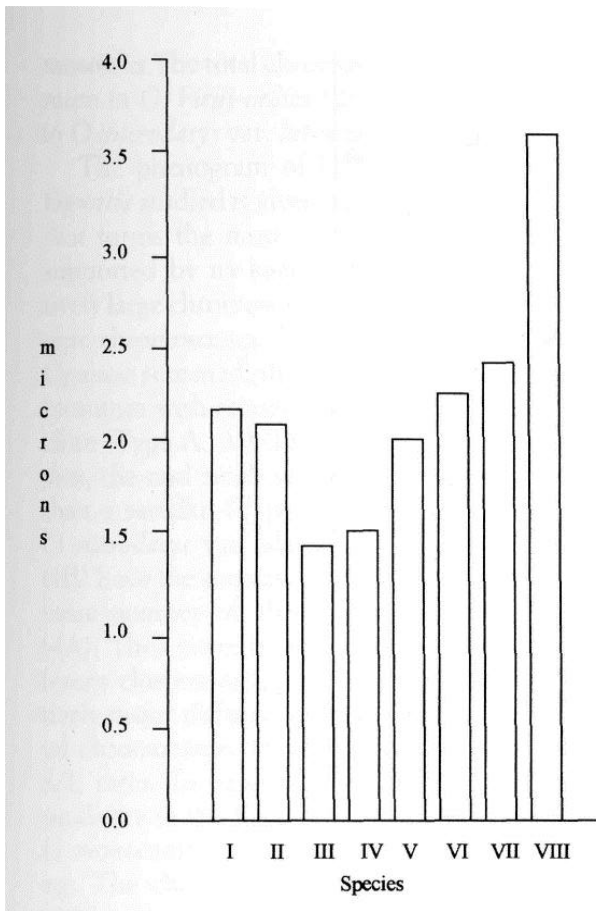


Fig. 2 — Histogram showing the average total chromosome length per species. Species names and codes are listed in Table 1.

the mean chromosome length for each sample (STEBBINS 1971; ROMEROZARCO 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. monacantha 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-

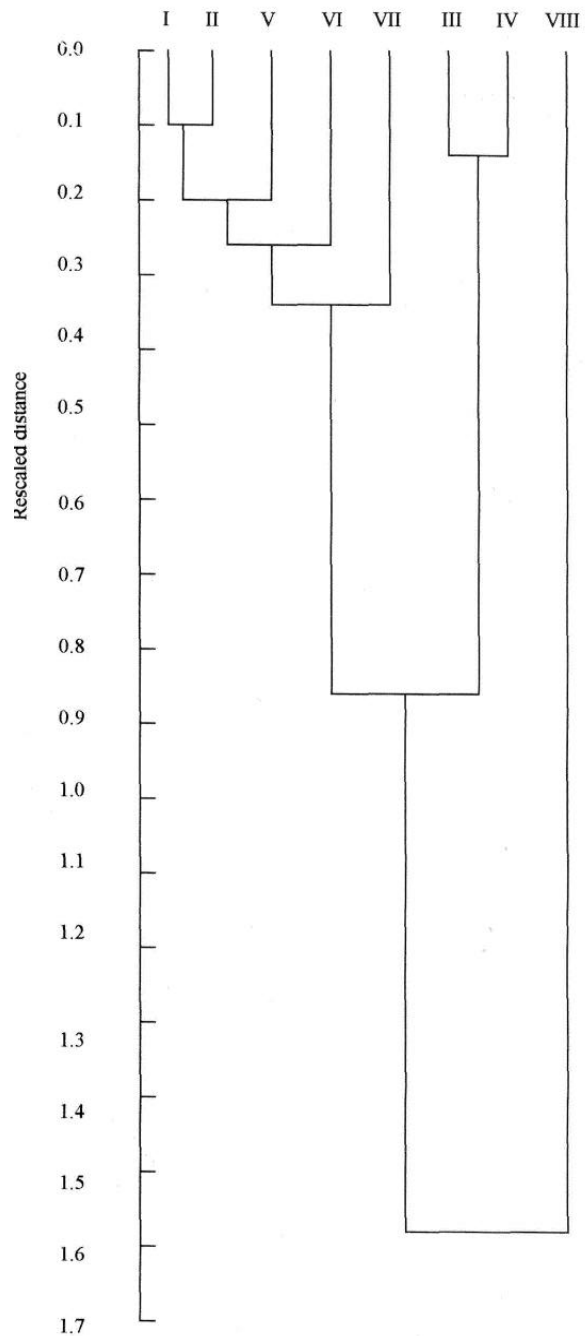


Fig. 3 — The phenogram of UPGMA clustering of the data in Table 1 for *Opuntia* studied.

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<sub>1</sub>), interchromosomal index (A<sub>2</sub>) 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 <sub>1</sub>	A <sub>2</sub>
<i>Opuntia cylindrica</i> (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
<i>O. basilaris</i> 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
<i>O. microdasys</i> (Lehm.) Pfeiff. var. <i>lutea</i>	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
<i>O. stricta</i> var. <i>dillenii</i> (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
<i>O. microdasys</i> (Lehm.) Pfeiff. var. <i>albispina</i>	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
<i>O. monocantha variegata</i> 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
<i>O. robusta</i> 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
<i>O. Ficus-indica</i> (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. microdasys* var. *lutea* ( $2n=22$ ) (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 *O. monacantha variegata* ( $2n=34$ ), a garden variety. The chromosomes range from very small ( $0.97 \mu\text{m}$ ) to medium ( $3.46 \mu\text{m}$ ) in size; the primary constrictions grade from nearly submedian 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.

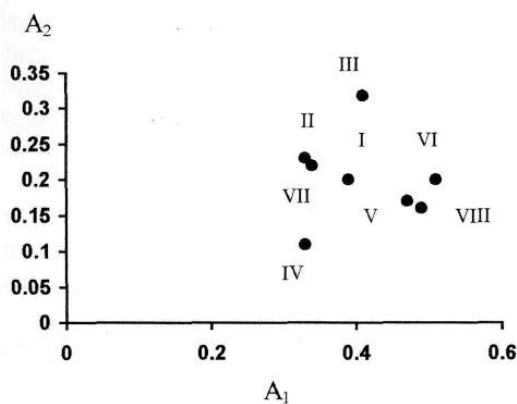


Fig. 4 — Intrachromosomal asymmetry index ( $A_1$ ) plotted against the interchromosomal index ( $A_2$ ). Species names and codes are listed in Table 1.

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).

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