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Karyotypic studies in the genus *Echinocereus* (Cactaceae) and their taxonomic significance

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SUMMARY — Mitotic chromosomes of 11 diploid ($2n = 22$) species of *Echinocereus* and five varieties of the polyploid ($2n = 4x = 44$) *E. engelmannii* included in the seven taxonomic sections of the genus were studied. The genus exhibits relatively symmetric karyotypes, having mostly metacentric chromosomes. Likewise, interspecific and intraspecific variability was observed in terms of genome length, chromosome length, and karyotipic formula. There was also variability in the number of satellites, and they were observed on the short arms of the chromosomes. There is an apparent correlation in decreasing chromosome and genome length in species with more derived morphological characters. In addition, karyotype asymmetry increases as the chromosome and genome length increases. Polyploid taxa investigated had larger chromosomes and genomes than diploid taxa. Robertsonian changes and centric fusion of telocentric chromosomes, are thought to be involved in the existence of homogeneous karyotypes. No evidence of structural chromosomal rearrangements was detected. Overall, the high morphological plasticity that characterizes the genus *Echinocereus* parallels the relatively large karyotypic variability found in the species investigated.

INTRODUCTION

The Cactaceae, a primarily New World family of angiosperms, is characterized by a high level of diversity in habit and vegetative morphology, floral fruit, and seed morphology, and pollination syndromes, which are some of the factors that have contributed to the species richness of the family. With over 1500 species and nearly 100 genera, the determination of systematic relationship at different taxonomic levels has proven to be problematic; the situation is further complicated by extensive parallel evolution in various structures, and the resulting homoplasy makes phylogenetic analyses difficult. Few groups within the family have been adequately studied with respect to their phylogeny, e.g., tribe Pachycereeae (GIBSON and HORAK 1978), *Coryphanta* (Engelm.) Lem. (ZIMMERMAN 1985), Cereeae (TAYLOR and ZAPPI 1989), *Eriosyce* Phil. (WALLACE 1994), and *Echinocactus* Link & Otto *Astrophytum* Lem. (WALLACE, unpublished data). Despite disagreements about generic and tribal

circumscriptions, 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, having approximately 85% of the species diversity, shows the greatest morphological extremes in habit and stem structure. Previous systematic studies of this subfamily (BUXBAUM 1958; GIBSON and NOBEL 1986; BARTHLOTT and HUNT 1993) divide it into ten tribes based upon shared morphological features of vegetative and reproductive structures, as well as biogeographic affinities.

Taxonomic background of the genus Echinocereus *Engelm.*

The tribe Echinocereeae is found primarily within arid and semiarid habitats in North America and cointains the genus *Echinocereus*, which is the subject of this study. As a genus, *Echinocereus* has speciated and radiated into areas of central and northern Mexico and the southwestern Unites States (TAYLOR 1985), and is one of the more specialized genera of the subfamily Cactoideae, with a relatively large number of species (44 to 73, depending upon authority). Previously, *Echinocereus* has been a subject of taxonomic discussion and several taxonomic treatments have been proposed (e.g., BRITTON and ROSE 1922; BRAVO 1937; BACKEBERG 1960; TAYLOR 1985; and BRAVO and SÁNCHEZ-MEJORADA 1991). There is still controversy regarding number of species for the genus, and recent studies evaluating species boundaries are ongoing, (e.g. TAYLOR 1993). The high degree of polymorphism and parallelism in floral attributes and vegetative morphology within and among species, have also made the establishment of species boundaries difficult. Although morphological phylogenetic analyses have not been presented to date, studies of chloroplast DNA restriction site variation confirm this hypothesis by supporting the monophyly of *Echinocereus* (sensu TAYLOR 1985) including *E.* (*Morangaya*) *pensilis* Brandegee (Purpus) (WALLACE and FORQUER 1995).

Cytotaxonomic background of the genus Echinocereus.

Cytological investigations have provided valuable insight into systematic and evolutionary issues in the Cactaceae, however many of the taxonomic and phylogenetic problems remain unsolved. To date the majority of cytlogical studies are related to chromosome counts, in which both euploid and polyploid species have been documented (e.g. PINKAVA *et al.* 1985, 1992). Even though the first cytological studies for members of the family did not consider chromosome morphology, general conclusions are that chromosomes of cacti are characteristically small with no morphological markers other than occasional satellites (STOCKWELL 1935; BEARD 1937; ANGULO 1952; KATAGIRI 1953). SPENCER (1955) and JOHNSON (1978) noted differences in the position of

the centromere and proposed that further investigations of chromosome morphology would be valuable in understanding chromosome evolution in the Cactaceae.

Karyotypic studies have been useful in addressing systematic and evolutionary issues in diverse genera of flowering plants. Several authors (JACKSON 1971; MOSCONE 1989; BERNARDELLO and ANDERSON 1990; RUAS *et al.* 1994; BERNARDELLO *et al.* 1994) have shown the importance of both chromosome numbers and karyotypic analysis in plant systematics. In the Cactaceae, however, few detailed karyotypic studies are available. This is likelyly related to the relatively small chromosome size and the difficulty in preparing them form analysis. Mucilage is usually present in the tissue, which hinders the separation of cells and chromosomes, thus making their observation quite challenging.

To date, only two karyotypic studies have been reported for the Cactaceae. JOHNSON (1980) provided the first karyotype drawings for the Cactaceae based on observations of three varieties of *Mammillaria prolifera* (Mill.) Haw. with different ploidy levels ($2n = 22$, 44, 66). Contrary to expectations, JOHNSON indicated that a small increase in chromosome size occurs as the ploidy level increases. The overall morphology of chromosomes however, was similar in the three varieties investigated: mostly metacentric chromosomes with a few pairs of submetacentric chromosomes. PALOMINO *et al.* (1988) reported karyotypes for two species and one variety of *Nyctocereus* (Berg.) Britt. et Rose, ($2n = 22$), a putatively related genus to *Echinocereus*. The taxa investigated displayed similar patterns in size and morphology of chromosomes relative to *M. prolifera*. At present, a study of intraspecific karyotype variation is being conducted in a Mexican columnar cactus, *Pachycereus weberi* (Coult.) Buxb. (S. GAMA, pers. com.). Preliminary results indicate that chromosome morphology follows the same pattern as in *Mammillaria* Haw. and *Nyctocereus*. Additionally, cytological studies in species of *Ferocactus* Br. & Rose and three *Opuntia* (Tournef.) Mill. species have also revealed that chromosome morphology in these taxa is homogeneous, i.e., mostly metacentric chromosomes (COTA and WALLACE, unpublished data).

In *Echinocereus*, some cytological studies have been presented (ROSS 1981; PARFITT 1987; COTA and PHILBRICK 1994 and references therein). The genus has the base chromosome number for the Cactaceae ($x = 11$), and as in other genera of the family, both diploid and polyploid cytotypes have been documented (PINKAVA and PARFITT 1982; PINKAVA *et al.* 1985, 1992; PARFITT 1987). Recently, COTA and PHILBRICK (1994) have shown the geographic distribution of diploid and polyploid species, and suggested that polyploid cytotypes of *Echinocereus* are distributed at higher latitudes and elevations than their diploid relatives. The present study has been done to cytologically examine twelve species of *Echinocereus*, and to date represents the most extensive investigation of chromosome morphology for the Cactaceae. Its goals are 1) to compare karyotypes of selected taxa, 2) to infer any phylogenetic

relationships from karyotypic data, and 3) to determine patterns of karyotype variation associated with morphological factors.

MATERIALS AND METHODS

Selection of representative taxa. — The taxa used in this study are: *Echinocereus cinerascens* (DC) Lem., *E. engelmannii* (Engelm.) Lem., *E. knippelianus* Liebner, *E. laui* G.R.W. Frank, *E. leucanthus* N. P. Taylor, *E. maritimus* (M.E. Jones) Schum., *E. nicholii* (Benson) Parfitt, *E. pensilis* (K. Brandegee) J. Purpus, *E. pentalophus* (DC) Lem., *E. scheeri* (Salm-Dyck) Scheer, *E. stoloniferus* W. T. Marshal, and *E. triglochidiatus* Engelm. var. *mojavensis* (Engelm & Bigel.) Benson. In addition, five varieties of the *E. engelmannii* (Parry ex Engelm.) Lem., namely, var. *acicularis* Benson, var. *chrysocentrus* (Engelm & Bigel.) Ruempler, var. *engelmanni*, var. *munzii* (Parish) Pierce & Fosb., and var. *variegatus* (Engelm. & Bigel.) Ruempler are included.

These species represent each of the seven taxonomic sections in TAYLOR's 1985 taxonomic treatment (Table 1). Selection of taxa for adequate phylogenetic representation was also based upon the following criteria: morphological and floral characteristics, pollination syndromes, ecology, distribution, and altitudinal and latitudinal ranges. Nomenclature follows that of TAYLOR (1985).

Plant material and squashing routine. — Plant material was either filed collected and/or obtained from the living collection of the Huntington Botanic Garden (HNT) (Table 1). Voucher specimens of field collected taxa are deposited in the herbaria of Rancho Santa Ana Botanic Garden (RSA), Escuela Nacional de Ciencias Biológicas (ENCB) University of Arizona (ARIZ), and Stadusche Sukkulentensammlung (ZSS) (Table 1). The protocol used to obtain mitotic chromosomes from root tips of either germinated seeds and/or adventitious roots followed that of COTA and PHILBRICK (1994). Permanent and semipermanent slides were made using Hoyer's fluid (BEEKS 1955).

Photomicrographs of mitotic chromosomes of examined taxa have been previously documented in COTA and PHILBRICK (1994).

Karyotype analytical method. — Microscopic observations of chromosomes were made using a Leitz phase-contrast microscope at × 100 (oil). Chromosome homology was assigned according to similarities in length, morphology, and centromere position. In addition, satellites were useful in distinguishing homologous pairs. Satellites were classified using the method proposed by BATTAGLIA (1955).

Chromosome asymmetry, or position of the centromere in the chromosomes was determined using a system developed by LEVAN *et al.* (1965) in which the arm ratio (r = long arm/short arm) was calculated for every chromosome. Karyotypes (idiograms) were constructed according to the asymmetry index of the chromosomes, and then grouped from the most metacentric (M, m) to the most submetacentric (sm) (Figs. 4 and 5). The number for each chromosome pair was sequentially assigned following increase in asymmetry.

TABLE 1 - Estimates of chromosome variation in *Echinocereus*. Number of cells measured per population (NC); ploidy level (PL); mean chromosome length (CL), standard deviation in parethesis; total genome length (GL); karyotype formula (KF); number of satellites (NS); intrachromosomal index (A_1); interchromosomal index (A_2). Taxa are arranged by taxonomic sections (Taylor 1985, 1989). Number before species name correspond to taxa in Figures 1, 2, and 3. Number after species name indicates population number when more than one was analyzed. * = Taxa for which idiograms are not provided due to space constraints.

Species	Locality and herbarium	NC	PL	CL	GL	KF	NS	A_1	A_2
Section Morangaya									
1. *E. pensilis*	Mexico. Baja California Sur. *R. Moran 7448* (RSA, HNT)	12	2*n*	3.70 (0.52)	40.76	8m + 3Sm	3	0.31	0.14
Section Erecti									
E. engelmannii									
2. var. *acicularis*	U.S.A CA. *D. Benadom s.n.* (RSA)	7	4*n*	3.56 (0.43)	78.49	7M + 9m + 6sm	1	0.24	0.12
3. var. *chrysocentrus*	U.S.A CA. *D. Benadom 468* (RSA)	11	4*n*	3.75 (0.54)	82.66	4M + 12m + 6sm	3	0.26	0.14
4. var. *engelmanii* (1)*	Mexico. Baja California *H. Cota. 7518* (RSA)	6	4*n*	4.55 (0.82)	100.00	2M + 18m + 2sm	—	0.20	0.18
5. var. *engelmannii* (2)*	Mexico. Baja California *H. Cota. 7513* (RSA)	11	4*n*	3.74 (0.40)	82.30	2M + 13m + 7sm	2	0.30	0.10
6. var. *munzii*	U.S.A. CA. *D. Michener & W. Wisura*, s.n. (RSA)	11	4*n*	4.10 (0.56)	90.53	5M + 12m + 5sm	1	0.23	0.13
7. var. *variegatus*	U.S.A. CA. *D. Benadom, 467* (RSA)	11	4*n*	3.08 (0.42)	67.87	3M + 11m + 8sm	2	0.29	0.13
8. *E. maritimus* (1)	Mexico. Baja California. *H. Cota, 7939* (RSA)	11	2*n*	4.35 (0.60)	47.90	2M + 7m + 2sm	1	0.26	0.13
9. *E. maritimus* (2) *	Mexico. Baja California. *H. Cota, 7940* (RSA)	13	2*n*	4.37 (0.53)	48.10	1M + 7m + 3sm	1	0.28	0.12
10. *E. nicholii*	U.S.A. AZ. *H. Cota & R. Felger, s.n.* (ARIZ)	5	2*n*	4.71 (0.68)	51,90	1M + 9m + 1sm	1	0.23	0.14

Species	Locality and herbarium	NC	PL	CL	GL	KF	NS	A_1	A_2
Section Triglochidiatus									
E. triglochidiatus									
11. var. *mojavensis* (1) *	U.S.A. CA. *P.A. Munz & P.C. Everett 17453* (RSA)	9	2*n*	4.85 (0.46)	53.39	1M + 7m + 3sm	1	0.29	0.09
12. var. *mojavensis* (2) *	U.S.A. CA. *L. Arnseth, 10* (RSA)	13	2*n*	4.21 (0.43)	46.39	2M + 7m + 2sm	1	0.24	0.10
13. *E. scheeri*	Mexico. Chihuahua. *M. Kimnach & Brandt, 982* (HNT)	5	2*n*	3.50 (0.54)	38.53	3M + 5m + 3sm	1	0.23	0.15
Section Echinocereues									
14. *E. cinerascens* (1) *	Mexico. Hidalgo, *E.F. Anderson, 4981* (HNT)	11	2*n*	4.30 (0.74)	47.35	1M + 7m + 3sm	2	0.30	0.17
15. *E. cinerascens* (2)	Mexico. Querétaro. *R. Fernández 1601* (ENCB)	12	2*n*	3.83 (0.37)	42.14	2M + 7m + 2sm	2	0.25	0.10
16. *E. knippelianus*	Mexico. Nuevo Leon. *C. Glass & R. Foster, 3902* (HNT)	4	2*n*	3.74 (0.54)	41.91	2M + 6m + 3sm	1	0.25	0.14
17. *E. pentalophus*	Mexico Tamaulipas. *J. Folsom et al. 1105* (HNT)	6	2*n*	4.17 (0.43)	45.95	1M + 8m + 2sm	1	0.27	0.10
Section Reichenbachii									
18. *E. stoloniferus*	Mexico. Sonora. *Boutin & M. Kimnach 3654* (HNT)	12	2*n*	3.50 (0.40)	38.58	2M + 6m + 3sm	1	0.27	0.11
Section Wilcoxia									
19. *E. leucanthus*	Mexico. Sonora. *Koutnik & J. Tragers, s.n.* (HNT)	10	2*n*	3.67 (0.28)	40.44	2M + 7m + 2sm	1	0.25	0.08
Section Pulchellus									
20. *E. laui*	Mexico. Sonora. *A. Lau* (ZSS)	10	2*n*	3.26 (0.32)	35.91	1M + 7m + 3sm	1	0.28	0.09

When possible, at least ten cells were observed and measured in 11 of the 16 taxa investigated (Table 1). In the remaining five taxa the number of cells observed varied from four to seven; although smaller in sample size, these karyological data are included because they provide insight regarding chromosome variability.

Mean and standard deviations for long and short arms for every homologous pair were calculated. Intrachromosomal (A_1) and interchromosomal (A_2) indices for the karyotypes were calculated following ROMERO-ZARCO (1986). For A_1 the following equation was used:

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

where A_1 is the intrachromosomal index; b_i is the average length for short arms in every homologous chromosome pair; B_i is the average length for long arms in every homologous chromosome pair; and n is the number of homologous chromosome pairs.

For A_2, the formula $A_2 = S/x$ was used. Where: s is the standard deviation of chromosome length for each sample, and x is the mean chromosome length for each sample.

The A_1 value represents the degree of asymmetry in arm length within the chromosomes of the genome, and A_2 is the degree of asymmetry between chromosomes of the genome. This method is useful for analyzing karyotypes of closely related taxa which exhibit little variation in karyotype asymmetry. Both estimates are independent of chromosome size (ROMERO-ZARCO 1986), and the equations provide lower values the more metacentric the chromosomes.

To ascertain patterns of variation between indices and chromosomal variables, an analysis of variance (Microstat Statistical Package) was done using the mean values of five variables of the karyotypes of each taxon. In the analysis the following variables were considered: ploidy level, chromosome size, genome length, and mean intrachromosomal (A_1) and interchromosomal (A_2) index. Raw data are available upon request.

RESULTS

Chromosome number.

Chromosome numbers of all species investigated, with the exception of the varieties of *E. engelmannii* were diploid ($2n = 22$). The five varieties of *E. engelmannii* were tetraploid ($2n = 4x = 44$) (Table 1).

Chromosome lenght.

There is an apparent association between mean chromosome length and degree of specialization among species (for a discussion of primitive versus specialized characters in *Echinocereus* see TAYLOR 1985, 1989, 1993, and COTA and PHILBRICK 1994). Some diploid species with derived characters exhibited

the lowest values of mean chromosome length, e.g., *Echinocereus knippelianus* (3.7), *E. stoloniferus* (3.5), and *E. laui* (3.2) (Table 1; Fig. 1). In contrast, one taxon with relatively primitive vegetative features, *E. engelmannii* var. *engelmannii* (populations three and four, for which data are not included) exhibited the largest mean chromosome length (5.8 and 5.3 respectively). Species with intermediate morphological character had mean chromosome length values that overlapped with those from species with either primitive or derived morphological features.

Values of mean chromosome length were not correlated with variation in altitudinal range for a given taxon. Among diploids, coastal and low elevation species, e.g., *Echinocereus maritimus*, *E. leucanthus*, and *E. nicholii* exhibited similar mean chromosome lengths as species distributed at medium to high elevations, e.g. *E. pentalophus*, *E. cinerascens*, *E. triglochidiatus* var. *mojavensis* (Table 1; Fig. 1). Among the polyploid varieties of *E. engelmannii* mean chromosone length variability was also observed (Table 1). Overall, the amount of variation in mean chromosome length among polyploid taxa was higher than among diploid taxa.

Genome length

The mean genome length in the investigated species of *Echinocereus* ranged from 35 to 129 microns (Table 1; Fig. 2). The smallest genome was represented by the diploid *E. laui* and the largest by the tetraploid *E. engelmannii* var. *engelmannii* (population three). As expected, the total mean genome

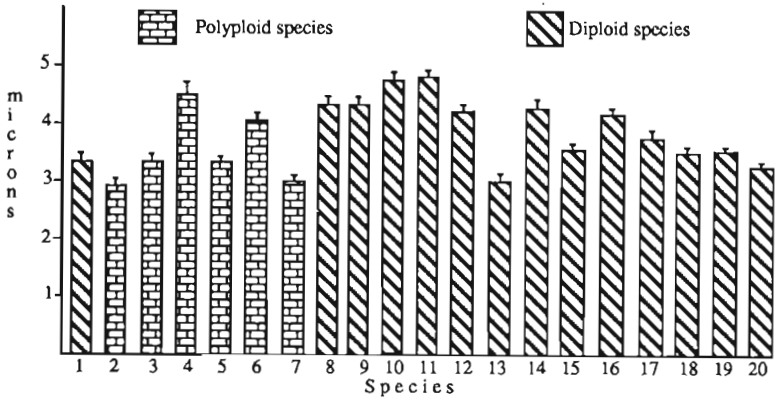

Fig. 1. — Histogram showing the mean chromosome length per species. Small bars represent the standard deviation for the chromosome mean. (see Table 1 for species name).

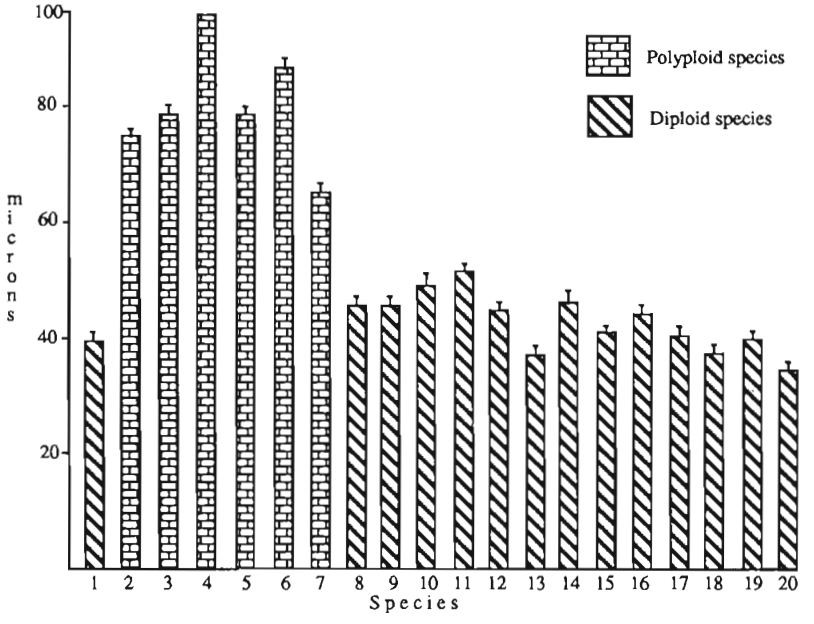

Fig. 2. — Histogram showing the total length of the karyotype (genome) per species. Small bars represent standard deviation. Species names are listed in Table 1.

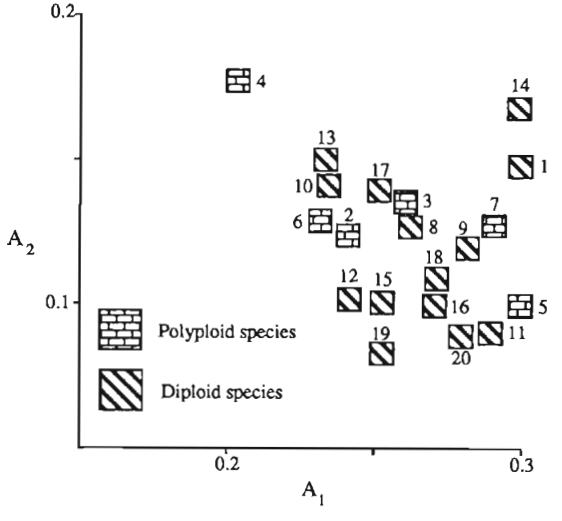

Fig. 3. — Intrachromosomal asymmetry index (A_1) plotted against the interchromosomal index (A_2). Species names are listed in Table 1.

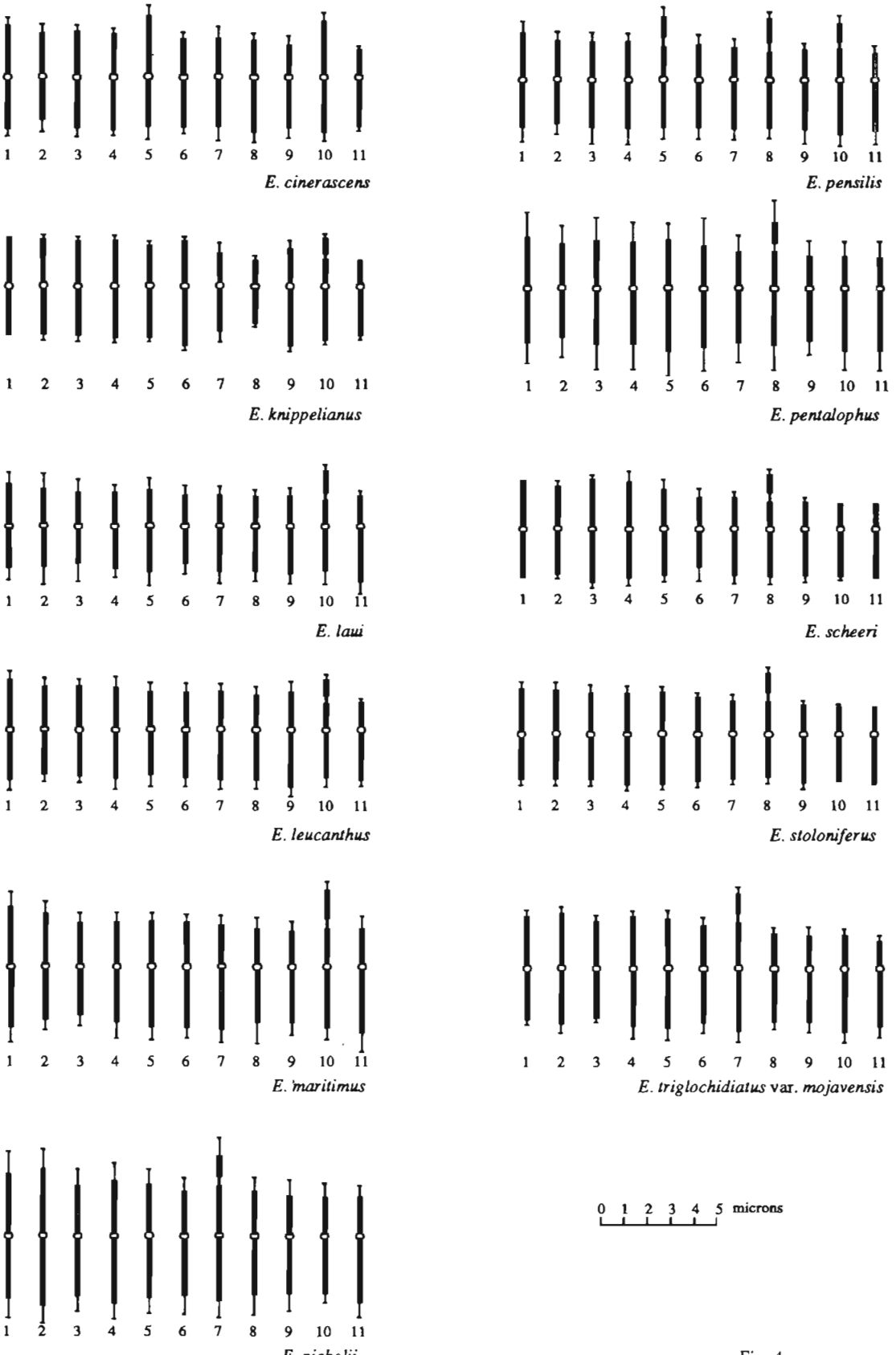
1 2 3 4 5 6 7 8 9 10 11
E. cinerascens
1 2 3 4 5 6 7 8 9 10 11
E. pensilis
1 2 3 4 5 6 7 8 9 10 11
E. knippelianus
1 2 3 4 5 6 7 8 9 10 11
E. pentalophus
1 2 3 4 5 6 7 8 9 10 11
E. lawi
1 2 3 4 5 6 7 8 9 10 11
E. scheeri
1 2 3 4 5 6 7 8 9 10 11
E. leucanthus
1 2 3 4 5 6 7 8 9 10 11
E. stoloniferus
1 2 3 4 5 6 7 8 9 10 11
E. maritimus
1 2 3 4 5 6 7 8 9 10 11
E. triglochidiatus var. mojavensis
1 2 3 4 5 6 7 8 9 10 11
E. nicholii
0 1 2 3 4 5 microns

Fig. 4

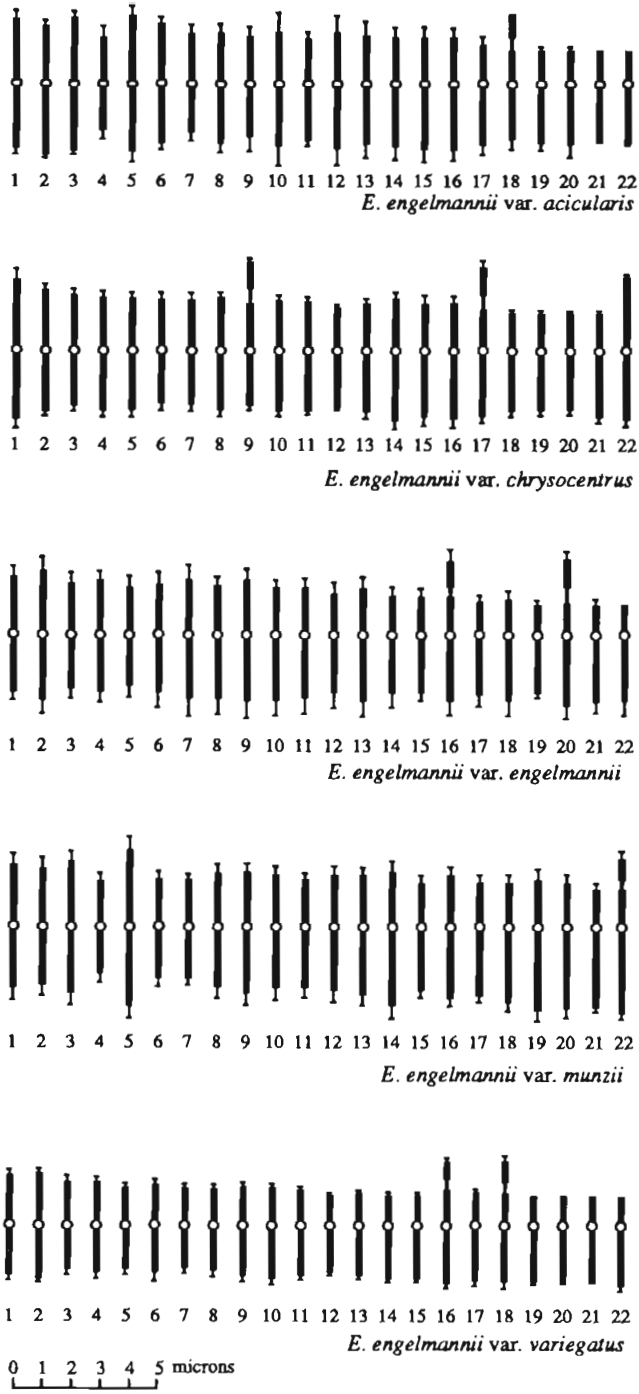

Fig. 5. — Idiograms of polyploid varieties of *Echinocereus engelmanii* showing the haploid set of chromosomes. Circles represent the centromeres, and small bars the standard deviation for each arm. Satellites are indicated at the end of the chromosome arm when observed.

Fig. 4. — Idiograms of representative diploid species of *Echinocereus* showing the haploid set of chromosomes. Circles represent the centromeres, and small bars the standard deviation for each arm. Satellites are indicated at the end of the chromosome arm when observed.

length of diploid taxa was significantly smaller than that for polyploids. The diploid taxa had mean genome lengths ranging from 35 to 53 microns. In polyploid taxa mean genome length ranged from 67 to 129 microns (Table 1; Fig. 2). The amount of variation among polyploids was greater than among diploids; the coefficient of variation (standard deviation/mean) of polyploids was double (0.22) that of diploids (0.11).

In spite of mean genome length variation among taxa, the values were not correlated with TAYLOR's (1985, 1993) taxonomic sectional boundaries. In both diploid and polyploid cytotypes, differences in mean genome lenghts were observed throughout sections, and even in different populations of the same species (Table 1).

Karyotypes.

In general, karyotypes in *Echinocereus* are composed of metacentric (M, m) and submetacentric (sm) chromosomes (Table 1). No single karyotypic formula distinguishes the genus. Due to karyotypic variation among species, the taxonomic sections (TAYLOR 1985) are also not characterized by particular formulae. Karyotypic variation among species involved changes in number and position of secondary constrictions and arm length ratios.

Satellites.

In *Echinocereus*, satellites were of the terminal, linear type. They were located on the distal part of the short arm of metacentric (m) and/or submetacentric (sm) chromosomes. The number and location of satellites differed among diploid and polyploid species, thus there is no consistency in the position of satellites for particular chromosome pairs.

Intrachromosomal (A_1) and interchromosomal (A_2) indices.

As estimated by the A_1 and A_2 indices, the karyotypes of *Echinocereus* were generally homogeneous (Table 1; Fig. 3). No apparent associations were observed between A_1 and A_2 indices. Also, no correlations of asymmetry indices and degree of specialization, pollination syndrome and geographic distribution were revealed. However, there were significant correlations between both asymmetry indices and mean chromosome length (A_1, $r = 0.52$; A_2, $r = 0.66$) and total genome length (A_1, $r = -0.59$; A_2, $r = 0.72$). These correlations indicate that the short to long arm ratio of a chromosome tends to decrease, and the difference in length between chromosomes increases as the mean chromosome length increases.

DISCUSSION

Chromosome length variation has been shown in the genus *Nyctocereus* (PALOMINO *et al.* 1988) where closely related species have the same chromosome number and display variability in chromosome size. In *Echinocereus*, infra- and interspecific chromosome length variability was also observed. These differences in chromosome size probably indicate genome restructuring and may be used for systematic purposes to distinguish closely related species.

Increases in chromosome length have been detected in polyploid species of certain cacti. For instance, JOHNSON (1980) reported a slight increase in the size of the chromosomes of polyploid versus diploid varieties of *Mammillaria prolifera*. No clear patterns are evident in *Echinocereus*, where chromosome length in polyploid taxa was not found to increase or decrease significantly relative to chromosome length in diploids. However, one exception was evident. In the polyploid *E. engelmannii* var. *variegatus* there was a notably smaller mean length of the chromosomes compared to diploid and other polyploid taxa.

Correlations between chromosome length and degree of morphological specialization have been suggested indiverse groups of flowering plants (STEBBINS 1971). Moreover, there has been general agreement taht more derived taxa tend to have smaller chromosomes than do primitive taxa (SWANSON *et al.* 1981). In spite of the lack of an apparent association between chromosome length and degree of specialization among species of *Echinocereus*, some derived taxa were found to have the shortest chromosomes (e.g. *E. laui*, *E. stoloniferus*, *E. scheeri*, and *E. leucanthus*); thus, mean chromosome size tends to be smaller in the more derived species. There is however, an apparent correlation in decreasing chromosome and genome length in species with specialized character states, and karyotype asymmetry increases as the chromosome and genome length increases. Also, the high morphological diversity that characterizes the species of *Echinocereus* parallels the relatively wide karyotypic variability found in the species investigated.

Because of the physical homogeneity of the chromosomes within and between species of *Echinocereus* no major structural differences were detected. Speciation without detectable chromosomal changes has been documented in *Platanus* (SWANSON *et al.* 1981). Furthermore, chromosomal changes such as translocations and inversions were not detected; if chromosomal rearrengements occur they remained cryptic. The use of techniques that allow higher resolution, such as banding would be useful to disclosure chromosomal structural changes in the genus.

In *Echinocereus*, both the diploid and polyploid taxa with larger genomes tend to occupy broader geographic ranges and occupy a wider diversity of ecological habitats. Perhaps the best examples are from the varieties of *E. engelmannii* whose habitats range from near sea level to medium elevations

(var. *engelmannii*) and to high elevations (var. *munzii*). The larger geographic range of the polyploid *E. engelmannii* may be explanied by its capacity to colonize new areas (COTA and PHILBRICK 1994). In *Echinocereus*, the largest genomes were observed in the polyploid taxa. With the exceptions of *E. engelmanni* var. *acicularis* and var. *variegatus*, genome length doubles with increase in ploidy level.

Variation in both genome length and chromosome length was observed among different populations of the same species often located in nearby geographic areas. The varieties of *Echinocereus engelmannii* are a clear example of this variability. Also, the populations of *E. cinerascens*, *E. maritimus*, and *E. triglochidiatus* var. *mojavensis* exhibit some differences in genome and chromosome lenght, similar to the results presented for species of *Nyctocereus* (PALOMINO *et al.* 1988).

Karyotypes in *Echinocereus* tend to be uniform. However, both infraspecific and interspecific variability in karyotypic formula was observed. The predominance of symmetric karyotypes composed mainly of metacentric and few submetacentric chromosomes in the genus *Echinocereus* in particular, and in the Cactaceae in general, may be explained by STEBBINS' (1971) hypotheses on the frequency of chromosome types in plants. Metacentric chromosomes are common in plants; they arise as a result of the fusion of two telocentric chromosomes with little predictable effect in content and gene sequence. Likewise, Robertsonian fusions may also account for the occurrence of metacentric chromosomes (SWANSON *et al.* 1981). The submetacentric chromosomes in *Echinocereus* probably originated through pericentric inversion and unequal translocations between chromosomes and Robertsonian fusions. In addition to *Echinocereus*, examples of karyotypes with mostly metacentric and few submetacentric chromosomes have been documented elsewhere in the Cactaceae, e.g. *Mammillaria* (JOHNSON 1980) and *Nyctocereus* (PALOMINO *et al.* 1988). Both taxa also exhibit a low degree of variability in karyotype morphology. The occurrence of homogeneous karyotypes with mainly metacentric chromosomes has also been reported in other genera of flowering plants: *Nicotiana* (MOSCONE 1989); *Capsicum* (MOSCONE 1990); and *Vernonia* (RUAS *et al.* 1991).

Variability in number, type, and position of satellites provides additional cytotaxonomic characters. The presence of satellites in *Echinocereus* can be used as cytogenetic markers to characacterize particular species, e.g., *E. pensilis* (three satellites) and *E. cinerascens* (two satellites), and one satellite in the remaining diploid cytotypes. In the Solanaceae, polymorphic satellites were useful indistinguishing some species of *Capsicum* (PICKERSGILL 1971; MOSCONE 1990). In contrast to the observations of BERNARDELLO *et al.* (1994) in *Solanum*, satellites in *Echinocereus* were observed at the end of the short arms of chromosomes.

In *Echinocereus* it is not clear whether the plesiomorphic condition is one satellite or three satellites. However, the presence of three satellites in one of

the less specialized species (*E. pensilis*) as well as in *Nyctocereus* (PALOMINO *et al.* 1988) may suggest that three is the plesiomorphic condition, and therefore may serve as a cytological marker that identifies the less derived species of the genus. Also, *N. serpentinus* var. *splendens* has identical karyotipic formula (8m + 3 sm) and same number of satellites (three) (PALOMINO *et al.* 1988) than *E. pensilis*. In addition, molecular evidence generated from cpDNA restriction site analysis indicates that *E. pensilis* is placed phylogenetically as the basal species of the genus since this species shares more restriction site changes with *Echinocereus* species rather than *Nyctocereus* and allied genera (WALLACE and FORQUER 1995). Therefore, this information supports that in part, chromosomal evolution in *Echinocereus* may be related to the loss of satellites and to the gain of mostly metacentric (M) chromosomes that constitute the karyotype of the putatively more derived species, eg., *E. laui*, and *E. leucanthus* (Table 1).

Analysis of karyotype asymmetry is useful in tracing evolutionary trends of karyotype evolution in closely related species, especially when karyotypes tend to be uniform (ROMERO-ZARCO 1986). In *Echinocereus* these indices were difficult to interpret. The apparently close relationship between species, together with the homogeneity in karyotype asymmetry limited the patterns of association. ROMERO-ZARCO (1986) explained a similar situation in terms of relatedness; little if any divergence in karyotype asymmetry was evident among species of grasses.

Conversely, A_1 and A_2 indices have been successfully applied in the Solanaceae in which chromosome size and karyotype asymmetry were correlated among species of *Solanum* (BERNARDELLO and ANDERSON 1990). In spite of the lack of conspicuous differences in asymmetry among species of *Echinocereus* a reduction in chromosome size and genome length was associated with relatively high A_1 index in some of the more derived species in the genus.

The Cactaceae, has been hypothesized to be of relatively recent origin (GIBSON and NOBEL 1986; MAUSETH 1990), and is characterized by small chromosomes with symmetric karyotypes. This family is probably an exception to STEBBINS' (1971) hypothesis concerning increasing karyotype asymmetry in specialized taxa. However, cycles of symmetry-asymmetry can obscure the sequential relationship between karyotypes (JONES 1970). The few Cactaceae that have been examined, in particular *Echinocereus*, show no major changes in chromosome asymmetry; the relatively recent origin of the family may account for this low level of chromosomal changes, as might the generational time since cacti in general are long-lived perennials. Although the majority of the taxa remain unexamined, the available evidence indicates that the genera *Echinocereus*, *Mammillaria* and *Nyctocereus* display homogeneous karyotypes, with no significant differences in asymmetry relative to each other. In *Echinocereus*, the most asymmetric cytotypes occur in species which manifest generally primitive morphological features. MORETTI (1990) has also correlated the existence of

asymmetric karyotypes with primitive morphological and reproductive characters in *Zamia* L. species.

It seems feasible that Robertsonian changes and centric fusion, have been involved for the maintenance of homogeneous karyotypes in *Echinocereus*. PALOMINO *et al.* (1988) reported a similar situation for the symmetric karyotypes of *Nyctocereus*. It appears that interspecific and intraspecific chromosomal variability is associated with morphology and species diversity in *Echinocereus*. In addition, differences in chromosome size probably indicate cryptic chromosomal rearrangements, and speciation in the genus might be a consequence of undetected structural chromosomal changes. Different techniques, such as banding and analysis of meiotic configurations would certainly be useful to document such rearrangements.

Other factors have also been related to species diversity and large geographic range of *Echinocereus*: geographic isolation (TAYLOR 1985), various pollination syndromes (COTA 1993), and differences in ploidy level (COTA and PHILBRICK 1994). Of these, polyploidization may be a major mechanism of rapid speciation. At the diploid level it is possible that speciation is accompanied by structural changes and perhaps gene mutations. Further cytological studies in *Echinocereus* and other genera will be required to assess the value of karyotypic data in determining phylogenetic relationships at various taxonomic levels, as well as to more thoroughly understand chromosomal evolution in the Cactaceae.

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