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Chromosome Numbers in *Ferocactus* (Cactaceae: Cactoideae)

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The Cactaceae is subdivided into three subfamilies (Pereskioideae, Opuntioideae and Cactoideae) which are traditionally interpreted as monophyletic lineages (Barthlott and Hunt 1993). The Cactoideae include 85% of the species diversity in the family and exhibit the greatest morphological extremes in habit and stem structure. This subfamily includes the tribe Cacteae, which is found mainly in arid or semiarid habitats in North America and contains the genus *Ferocactus* Britton and Rose.

The taxonomy of the genus remains in a somewhat confused state due to several factors. As for most groups within the Cactaceae, the lack of extensive field research to document variability, the relatively high levels of morphological homoplasy, and the use of primarily vegetative morphological characters, make the establishment of species boundaries difficult. The number of species recognized in the genus has been a matter of disagreement and is reflected in several taxonomic treatments that have been produced over the last seven decades. Britton and Rose (1922) proposed a classification in which they included 30 species; subsequently, Lindsay (1955) proposed that the genus was comprised of 25 species. More recently, Taylor (1984) recognized 23 species, while Bravo and Sánchez-Mejorada (1991) accepted 29. Finally, Unger (1992) presented a classification scheme for 20 species based on the previous treatments. Of these classifications, that of Taylor (1984) represents a systematic treatment of the genus *sensu stricto*, and is the most widely accepted by cactus taxonomists because it includes detailed descriptions of floral, fruit and seed coat characters.

The phylogeny of *Ferocactus* is uncertain; however, two major evolutionary lineages [section *Bisnaga* (Orcutt) N. P. Taylor and J. Y. Clark and section *Ferocactus*] have been proposed for the genus. The former probably derived from *F. flavovirens* (Scheidw.) Britton and Rose and the latter from *F. robustus* (Link and Otto) Britton and Rose (Taylor and Clark 1983). These two species are distributed in south-central Mexico, a region that is thought to be the center of origin of the genus (Taylor 1984), and have apparently retained plesiomorphic features that place them as putatively basal species within the genus. On morphological grounds, the two sections are distinguished on the basis of a fruit character: in section *Ferocactus* the fruits dehisce by a basal pore, and in section *Bisnaga* the fruits are juicy and indehiscent, occasionally splitting irregularly (Barthlott and Hunt 1993, Taylor 1984).

This study was undertaken to document chromosome numbers in *Ferocactus* and to discuss the causes of its apparently stable diploid condition.

Materials and methods

Fourteen taxa of *Ferocactus*, representing sections *Bisnaga* and *Ferocactus* were examined in this study. For these species, flower buds and seeds were field collected and fixed from natural populations. When possible the selection of the taxa investigated was based on geographic distribution and their taxonomic position in the two sections as proposed by Taylor (1984).

The preservation of flower buds for meiotic chromosome counts and analyses follows that of Pinkava *et al.* (1977). Buds were fixed in Farmer's solution (3 ethanol : 1 glacial acetic acid, v/v) for at least 24–48 hr, then washed and stored in 70% ethanol under refrigeration. The anthers were dissected from floral buds, squashed in iron-acetocarmine, and mounted in Hoyer's medium following Beeks (1955). For mitotic counts, seedling radicles and chromosome squashes were obtained as described by Cota and Philbrick (1994). Chromosome observations and analyses were conducted with a Leitz phase-contrast microscope and a 100× oil objective. Voucher specimens are deposited in ASU, BCMEX, CANTE, DES, ENCB, HUMO and ISC herbaria as indicated in Table 1. Nomenclature follows that of Taylor (1984) with the exception of *F. rectispinus* and *F. recurvus*, specific epithets that are maintained according to Bravo and Sánchez-Mejorada (1991) and Lindsay (1955).

Results

In this study chromosome numbers are reported for 14 taxa (Table 1). Of these, eight represent first-time counts: *Ferocactus chrysacanthus*, *F. histrix*, *F. macrodiscus*, *F. peninsulae* var. *peninsulae* and var. *townsendianus*, *F. rectispinus*, *F. recurvus* and *F. robustus*. Chromosome counts for *F. cylindraceus* var. *lecontei*, *F. fordii* var. *fordii*, *F. gracilis* var. *gracilis*, *F. latispinus*, *F. viridescens* var. *viridescens* and *F. wislizeni* are confirmed. All chromosome counts reported here are consistent with the basic chromosome number for the family ($x=11$). Our analyses of meiotic figures show no disruption of cell divisions (Figs. 1–6). Mitotic chromosomes arrested in metaphase are homogeneous in overall morphology, from 3–7 μm in length, and mostly metacentric and submetacentric (Figs. 7–14); some species have chromosomes bearing one pair of satellites (Figs. 10, 11, 14).

Discussion

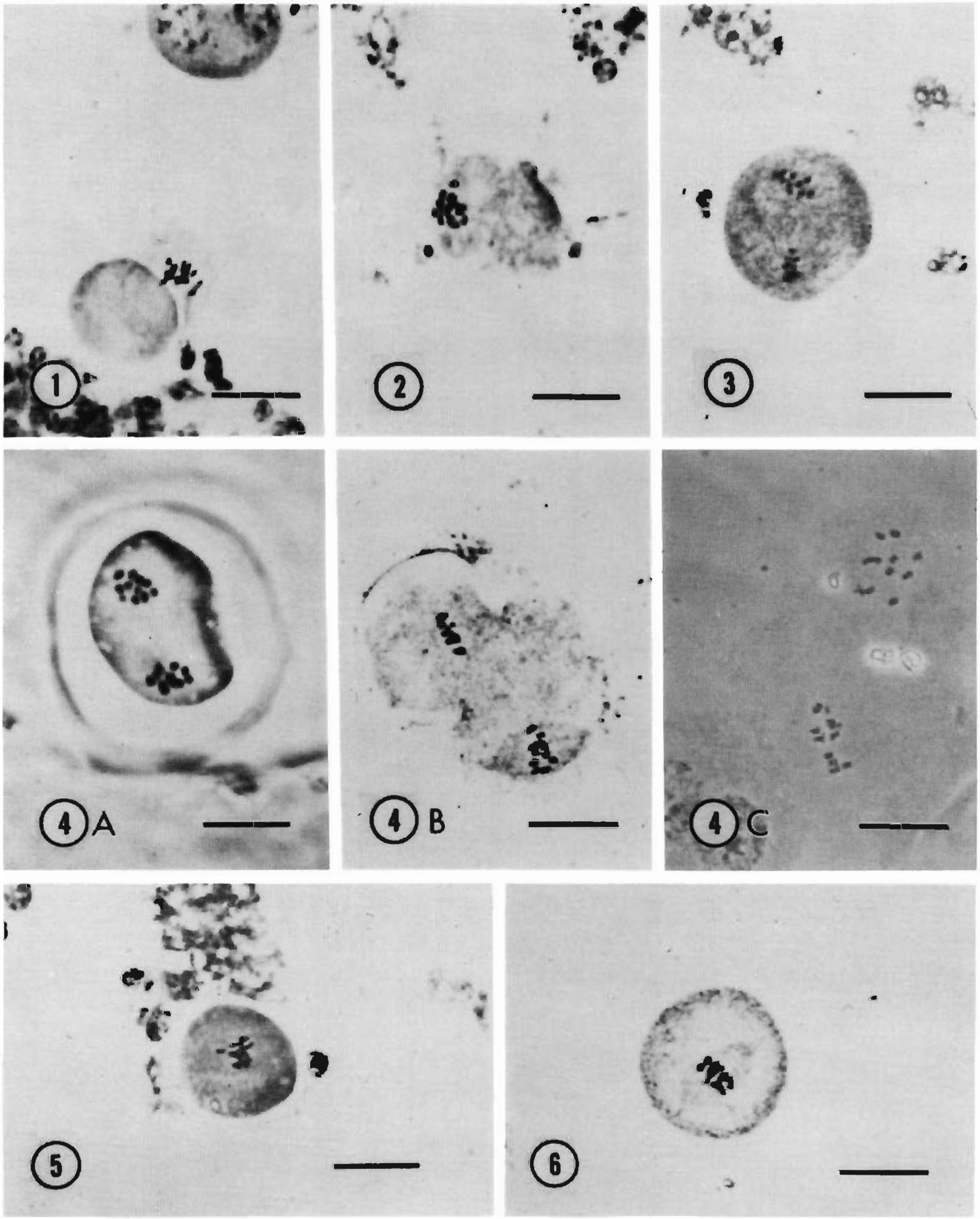
At present, most of the cytological data for *Ferocactus* has been presented as chromosome counts and all previously investigated species are diploid and have the basic number $x=11$ (Beard 1937, Katagiri 1952, Pinkava and McLeod 1971, Pinkava and Parfitt 1982, Pinkava *et al.* 1973, 1977, 1985, Stockwell 1935, Weedin and Powell 1978). No cases of aneuploidy or polyploidy have been documented.

Some of the taxonomic confusion that characterizes *Ferocactus* has been attributed to hybridization and introgression events. Lindsay (1955) and more recently Taylor (1984) have both indicated that Baja Californian species are taxonomically problematic due to probable introgressive processes. This hypothesis is based upon observations of individuals with intermediate morphologies in sympatric areas. Indeed, the possibility of gene exchange between *F. gracilis* and *F. peninsulae* has been suggested (Taylor 1984). Polymorphism in spine shape and color in addition to intermediacy of stem and flower characters are common in individuals of the same population. At present, it is unclear to what degree this variation is genetically and/or environmentally induced. Our analyses of meiotic configurations in different individuals of *F. gracilis* and *F. peninsulae* failed to provide evidence for hybridity at least for the populations investigated. In addition, previous meiotic counts of some Baja Californian and Mexican mainland species (Beard 1937, Pinkava *et al.* 1973, 1977, 1985) exhibited normal pairing. Nonetheless, hybridization should not be ruled out until further cytological analyses are conducted in those central Baja Californian populations of *F. gracilis* and *F. peninsulae* for which gene exchange has been suggested by Taylor (1984).

Table 1. Chromosome counts for *Ferocactus* and voucher information

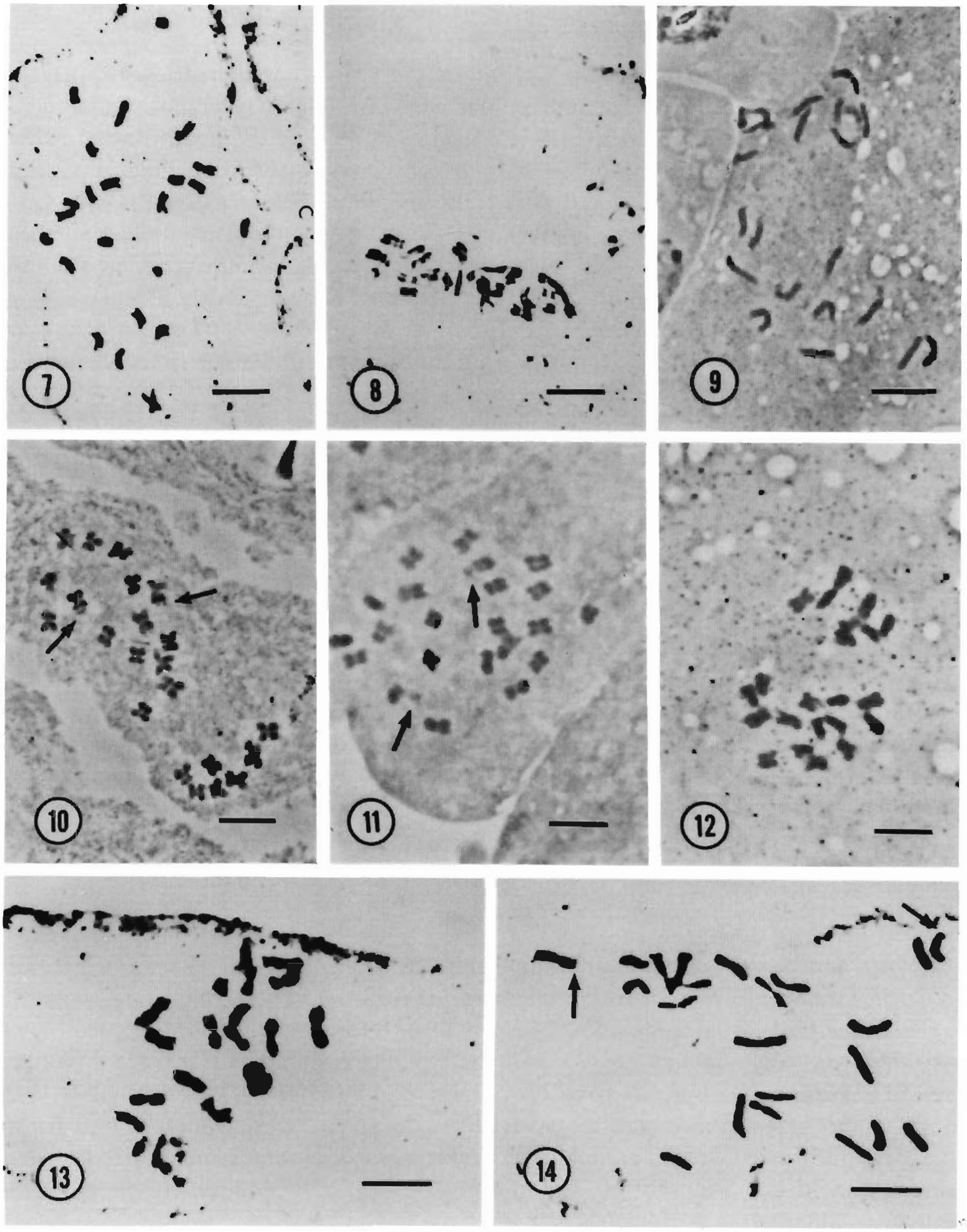
Taxon	Chromosome count	Source, Herbaria
Section *Bisnaga* (Orcutt) N. P. Taylor and J. Y. Clark		
**F. histrix* (DC.) G. E. Lindsay	n=11	MEXICO. Guanajuato: Rancho Alcocer, approx. 5 km SE San Miguel de Allende. 12 Jul 1993, H. Cota 8037 (CANTE)
F. latispinus (Haw.) Britton and Rose	2n=22	MEXICO. Guanajuato: Rancho Alcocer, approx. 5 km SE San Miguel de Allende. 12 Jul 1993, H. Cota 8039 (CANTE)
**F. macrodiscus* (Mart.) Britton and Rose	n=11	MEXICO. Guanajuato: Rancho Alcocer, approx. 5 km SE San Miguel de Allende. 12 Jul 1993, H. Cota 8038 (CANTE)
**F. recurvus* (Miller) G. E. Lindsay	2n=22	MEXICO. Puebla: Tehuacán Valley, between Tehuacán and Zapotitlán Salinas. 17 Jul 1993, H. Cota 8049 (HUMO)
**F. robustus* (Link and Otto) Britton and Rose	2n=22	MEXICO. Puebla: Tehuacán Valley, between Tehuacán and Zapotitlán Salinas. 17 Jul 1993, H. Cota 8045 (HUMO)
Section *Ferocactus*		
**F. chrysacanthus* (Orcutt) Britton and Rose	2n=22	MEXICO. Baja California: Cedros Island, north end of Island. 28 Dec 1985, H. Cota 7441 (ENCB)
F. cylindraceus (Engelm.) Orcutt var. *lecontei* (Orcutt) Britton and Rose	2n=22	U.S.A. California: San Bernardino Co., Clark Mts. May 1992, W. Wisura s.n. (RSA)
F. fordii (Orcutt) Britton and Rose var. *fordii*	n=11	MEXICO. Baja California: Between El Rosario and San Quintin, approx. 10 mi N of El Rosario. 17 May 1994, J. Rebman 2707 (ASU, BCMEX, ISC)
F. gracilis H. E. Gates var. *gracilis*	n=11	MEXICO. Baja California: Rte 1, Km marker 105, between Cataviña-El Rosario. 29 May 1992, H. Cota 8034 (ISC); 1.2 mi NE Rte 1, S of km marker 166. 19 May 1994, J. Rebman 2728 (ASU, BCMEX, ISC); E of El Rosario, along road to Sierra San Borja. 25 Apr 1994, J. Rebman 2635 (ASU, BCMEX, ISC).
**F. peninsulae* (F. A. C. Weber) Britton and Rose var. *peninsulae*	n=11	MEXICO. Baja California: Sierra San Francisco, S of Rancho Los Crestones, E of San Ignacio. 25 Apr 1994, J. Rebman 2629 (ASU, BCMEX, ISC)
* var. *townsendianus* (Britton and Rose) N. P. Taylor	2n=22	MEXICO. Baja California: 0.5 mi E and just S of km marker 89, on road to Todos Los Santos, along Hwy 19, N of Cabo San Lucas. 17 May 1992, H. Cota 8014 (ISC)
**F. rectispinus* (Engelm.) Britton and Rose	2n=22	MEXICO. Baja California: 10 mi from Hwy 1, along road to San Isidro. 25 May 1992, H. Cota 8027 (ISC)
F. viridescens (Torrey and A. Gray) Britton and Rose var. *viridescens*	n=11	MEXICO. Baja California: Arroyo San Carlos, SE of Ensenada. 3 May 1994, J. Rebman, 2643 (ASU, BCMEX, ISC): Punta Morro, S of Ensenada. 26 May, 1994, J. Rebman 2733 (ASU, BCMEX, ISC)
F. wislizeni (Engelm.) Britton and Rose	2n=22	MEXICO. Sonora: 25 km S of the city of Obregón. 12 May 1986, H. Cota 2687 (ENCB) U.S.A. Arizona: Pima Co. 11.1 mi S of junction Interstate 10 and Hwy 83. 19 Aug 1992, L. Slauson 112 (DES)

Symbols: *first report for the species, n=meiotic counts, 2n=mitotic counts, ASU=Arizona State University, BCMEX=Universidad Autónoma de Baja California, CANTE=Jardin Botánico CANTE, DES=Desert Botanical Garden, ENCB=Escuela Nacional de Ciencias Biológicas, HUMO=Universidad Autónoma del Estado de Morelos, ISC=Ada Hayden Herbarium of Iowa State University, RSA=Rancho Santa Ana Botanic Garden.



Figs. 1–6. Photomicrographs of meiotic chromosomes of selected taxa of *Ferocactus*. 1. *F. histrix*, Cota 8037. Metaphase I. 2. *F. macrodiscus*, Cota 8038. Metaphase I. 3. *F. fordii*, Rebman 2707. Metaphase II. 4A–C. *F. gracilis* var. *gracilis*. A. Cota 8034. Metaphase II. B. Rebman 2635. Metaphase II. C. Rebman 2728. Metaphase II. 5. *F. peninsulae* var. *peninsulae*, Rebman 2629. Metaphase I. 6. *F. viridescens* var. *viridescens*, Rebman 2643. Metaphase I. Scale bar $=20\,\mu$m.

In terms of chromosome size, chromosomes in *Ferocactus* are relatively small when compared with other genera, e.g., *Allium* L. however, they appear to be the largest in the Cactaceae. Their relatively uniform morphology and size seem not to be correlated with plant size. Similar patterns in chromosome morphology have been observed previously in unrelated genera of the Cactaceae such as *Echinocereus* Engelm. (Cota and Wallace 1995), *Mammillaria*



Figs. 7–14. Photomicrographs of mitotic chromosomes of selected taxa of *Ferocactus*. 7. *F. latispinus*, Cota 8039. 8. *F. recurvus*, Cota 8049. 9. *F. robustus*, Cota 8045. 10. *F. chrysacanthus*, Cota 7441. 11. *F. cylindraceus* var. *lecontei*, Wisura s.n. 12. *F. peninsulae* var. *townsendianus*, Cota 8014. 13. *F. rectispinus*, Cota 8027. 14. *F. wislizeni*, Slauson 112. Scale bar = 10 μm. Arrows indicate satellites.

prolifera (Miller) Britton and Rose (Johnson 1980) and *Nyctocereus* (Berger) Britton and Rose (Palomino *et al.* 1988). The cytological uniformity in shape and number does not necessarily reflect evidence of close phylogenetic relationship, but the presence of satellites in some northern species, such as *F. chrysacanthus* (Fig. 10), *F. cylindraceus* var. *lecontei* (Fig. 11) and *F. wislizeni* (Fig. 14) indicates that satellites can be used as taxonomic markers, and that further analyses of chromosome morphology might be advantageous to understand the interspecific relationships in the genus.

The frequency of polyploidy in the Cactoideae (12.5%) is considered low (Pinkava *et al.* 1985), and unlike other genera of the subfamily in which polyploidy is relatively common, such as *Echinocereus* (Cota and Philbrick 1994, Pinkava *et al.* 1992, Weedin and Powell 1978), all the species of *Ferocactus* which have been cytologically investigated are diploid. Our findings indicate that *F. robustus*, one of the putatively ancestral species (Taylor 1984) has not experienced changes in chromosome number. The same apparently stable diploid number has been observed in *Ferocactus*' putative sister genera such as *Echinocactus grusonii* Hildm. and *Stenocactus* (*Echinofossulocactus*) *crispatus* (DC.) Berger (Katagiri 1952); *Echinocactus polycephalus* (Engelm.) Bigel. (Pinkava *et al.* 1977); *E. horizonthalonius* Lem. and *E. texensis* Hoppfer (Weedin and Powell 1978); and *S. pentacanthus* (Lem.) Berger (Pinkava and Parfitt 1982).

Based on our findings, it appears that changes in chromosome number do not play a major role in the evolution of *Ferocactus*; it is conceivable that chromosome evolution in the genus may be taking place at the molecular level and, consequently, chromosome rearrangements remain cryptic. Preliminary data on DNA sequences of the chloroplast gene *ndh*F indicate that the rate of nucleotide substitution in the Cactoideae, including *Ferocactus*, is higher than in the Pereskioideae and Opuntioideae (Cota and Wallace, unpublished data). Also, the intron loss within the chloroplast gene *rpo*C1 has been found in the Cactoideae, including *F. flavovirens* (Wallace and Cota 1996). In addition, DNA sequences of non-coding regions of the *rpl*16 and *trn*L-*trn*F genes of the chloroplast genome show sequence divergence (insertions and deletions events) in several species of *Ferocactus* (Cota and Wallace, unpublished data), supporting chromosomal evolution at the molecular level. Further cytological and molecular investigations with a wider taxonomic sampling within *Ferocactus* may be useful to determine the extent of the diploid condition and to detect cases of hybridization in populations where individuals with intermediate morphology are common.

Summary

Meiotic and mitotic chromosome numbers were determined for 14 taxa of *Ferocactus*. Chromosome numbers are reported for the first time for seven species, including two varieties of *F. peninsulae*, and chromosome counts were confirmed for an additional six species. All taxa investigated were diploid and have a basic chromosome number of $x=11$. Within the Cactaceae, *Ferocactus* appear to have the largest chromosomes. Meiotic figures in Baja Californian and Mexican mainland species failed to document hybridization at least for those populations investigated. The morphological homogeneity of chromosomes and the apparently consistent diploid condition throughout the genus suggest that chromosome evolution in *Ferocactus* is taking place at the molecular level.

Key words: Cactaceae, Cactoideae, *Ferocactus*, Diploid.

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