New chromosome counts and evidence of polyploidy in Haageocereus and related genera in tribe Trichocereeae and other tribes of Cactaceae

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Abstract. Chromosome numbers for a total of 54 individuals representing 13 genera and 40 species of Cactaceae, mostly in tribe Trichocereeae, are reported. Five additional taxa examined belong to subfamily Opuntioideae and other tribes of Cactoideae (Browningieae, Pachycereeae, Notocacteae, and Cereeae). Among Trichocereeae, counts for 35 taxa in eight genera are reported, with half of these (17 species) for the genus *Haageocereus*. These are the first chromosome numbers reported for 36 of the 40 taxa examined, as well as the first counts for the genus *Haageocereus*. Both diploid and polyploid counts were obtained. Twenty nine species were diploid with $2n=2x=22$. Polyploid counts were obtained from the genera *Espostoa, Cleistocactus, Haageocereus,* and *Weberbauerocereus*; we detected one triploid ($2n=3x=33$), nine tetraploids ($2n=4x=44$), one hexaploid ($2n=6x=66$), and three octoploids ($2n=8x=88$). In two cases, different counts were recorded for different individuals of the same species (*Espostoa lanata,* with $2n=22$, 44, and 66; and *Weberbauerocereus rauhii*, with $2n=44$ and 88). These are the first reported polyploid counts for *Haageocereus, Cleistocactus,* and *Espostoa*. Our counts support the hypothesis that polyploidy and hybridization have played prominent roles in the evolution of *Haageocereus*, *Weberbauerocereus*, and other Trichocereeae.

Key words: *Cleistocactus*, karyology, Peru, polyploidy, *Weberbauerocereus*.

The Trichocereeae are tree-like, columnar, or globular cacti found in arid and semiarid biomes in South America south of the equator and the Galapagos Islands. As currently recognized (Anderson, 2001), the tribe comprises 26 genera and 413 species. *Haageocereus* Backeb., one of the most taxonomically complex genera in Trichocereeae, is a shrubby columnar cactus largely restricted to the western slopes of the Peruvian Andes, with one species that extends into northern Chile. As is true of most genera in Trichocereeae, *Haageocereus* is poorly understood and relationships among its members have been historically controversial (Buxbaum, 1958; Barthlott & Hunt, 1993; Hunt, 1999; Anderson, 2001). Systematic studies of the genus are badly needed as evidenced by the proliferation of names and descriptions. There are approximately 120 named species plus subspecies of *Haageocereus* but only 20 are accepted by Anderson (2001). In large part, the taxonomic difficulty found in members of the Cactaceae is the result of extensive morphological variability. This variability has been attributed to environmental gradients (Gibson & Nobel, 1986), as well as to changes associated with hybridization and genome doubling (polyploidy).

Polyploidy has been suggested to be a prominent process during angiosperm evolution (Tate et al., 2005; and references therein).

Brittonia, 59(2), 2007, pp. 290–297. ISSUED: 27 September 2007
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In Cactaceae, studies in *Opuntia* (Grant, 1971; Grant & Grant, 1979; Grant, 1980; Rebman & Pinkava, 2001; Baker, 2002) and *Mammillaria* (Katagiri, 1953; Remski, 1954) show that polyploidy, as well as hybridization, are major evolutionary forces in the family, but perhaps previously underappreciated. Patterns of relationships in both genera were found to be complex due to polyploidy, interspecific hybridization, and vegetative propagation. These processes may also be frequent in other cacti (Pinkava et al., 1985; Anderson, 2001). For example, Ross (1981) conducted cytological, morphological, and reproductive studies on 55 species of Cactaceae. His observations on modes of reproduction showed a correlation between polyploidy and self-fertility, vegetative reproduction, adventive embryony, and profuse branching. Nonetheless, the relative importance of hybridization and polyploidy in this family (of about 1800 species) remains uncertain because so few cacti have been examined in detail.

In spite of the fundamental importance of chromosome number, counts have been concentrated in only a few genera of Cactaceae, mainly from North America (Goldblatt & Johnson, 1978–2006), including *Opuntia* Mill. (Remski, 1954; Pinkava & McLeod, 1971; Pinkava et al., 1973, 1977; Ross, 1981; Pinkava & Parfitt, 1982; Pinkava et al., 1985; Baker, 2002), *Mammillaria* Haw. (Katagiri, 1953), *Echinocereus* Engelm. (Cota & Philbrick, 1994; Cota & Wallace, 1995), and *Selenicereus* Britton & Rose (Lichtenzveig et al., 2000). The work of Lambrou and Till (1993) is the only survey of an entire genus, *Gymnocalycium* Pfeiff. ex Mittler, plus some hybrids. Polyploidy has been reported to be absent in Pereskioideae, widespread in Opuntioideae and sporadic in Cactoideae, occurring mostly at the tetraploid level (Pinkava et al., 1985). Significantly, only about 15% of Trichocereeae (Cactoideae) have published chromosome counts and there are no published chromosome counts for the large genus *Haageocereus* (Goldblatt & Johnson, 2006). Polyploids in Trichocereeae have been reported in 27 species: *Trichocereus spachianus* (Lem.) Riccob. with $2n=44$ (Katagiri, 1953), *Gymnocalycium bruchii* (Speg.) Hosseus with $2n=44$, *Rebutia kupperiana* Boed. with $2n=44$, *Rebutia spegazziana* Backeb. with $2n=44$ (Ross, 1981), 20 species of *Gymnocalycium* with $2n=44$ and two species with $2n=66$ (Lambrou & Till, 1993); and *Weberbauerocereus weberbaueri* (K. Schum. ex Vaupel) Backeb. with $2n=44$ (Sahley, 1995). We therefore undertook cytogenetic studies in the Trichocereeae (with a focus on *Haageocereus*, a large genus for which no counts have been reported), plus other tribes in the Cactoideae, to provide the first chromosome counts for most of these groups and to assess whether polyploids are present.

Materials and Methods

Stem sections and seeds were collected from natural populations (Table I). Vouchers were deposited in Herbario San Marcos, Lima (USM) and Herbario San Agustín, Arequipa (HUSA), Peru.

Somatic chromosomes were counted using root tips. Root tips were obtained in two different ways. We germinated seeds for a few taxa on moist filter paper and then removed root tips from them. For most taxa, we used stems of plants collected from natural populations. Stems were used to propagate plants that were maintained in the University of Florida Botany Department greenhouse, and induced to develop adventitious roots, which are several times larger than roots from seedlings and hence much easier to use in chromosome squashes.

General cytogenetic methods followed Soltis (1980) and Speranza et al. (2003). Root tips from seedlings or those collected in the greenhouse were collected during the early hours of the morning (between 7:00 and 9:00 am), when cell division has been observed to be most active (Cota & Philbrick, 1994), and placed in a solution of 2 mM 8-hydroxyquinoline for 4 to 6 hours at room temperature and 4 hours to overnight at 4° C. After this treatment, roots were rinsed in distilled water and fixed in a solution of 3:1 absolute ethanol and glacial acetic acid, for at least 24 hours at room temperature. If not used immediately, roots were stored in 70 % ethanol at 4° C. Fixed roots were rinsed in buffer (40 mM citric acid, 60 mM sodium citrate), digested at 37° C with a combination

of 3 % (w/v) Cellulysin® (Calbiochem, San Diego, CA), 1 % (w/v) cellulase "Onozuka" RS (Yakult Pharmaceutical, Japan), and 4 % (v/v) pectinase (Sigma-Aldrich, St. Louis, MO). The digestion time varied and had to be adjusted for each individual. Most roots were digested after 30 to 90 min. Root tips were dissected in 60 % acetic acid, stained with 2 % lacto-propionic (1:1) orcein, squashed, and sealed. Two to ten cells per individual were examined in each case and separate counts were made for different individuals of the same taxon or individuals of the same taxon obtained from different sources. Initial microscopic observations were made under a Nikon Alphaphot-2 microscope and photographs taken under a Zeiss Axioplan microscope with a Kodak MDS 290 digital camera.

Results and Discussion

Chromosome numbers for a total of 54 individuals, representing 13 genera and 40 species are reported (Table I). Naming and classification is based on Anderson (2001) except for the recognition of *Haageocereus fulvus* (Rauh & Backeb.) F. Ritter (Hunt, 1999), *H. multangularis* (Willd.) F. Ritter (Ritter, 1981), *H. multicolorispinus* Buining (Ritter, 1980), and *H. pacalaensis* subsp. *repens* (Rauh & Backeb.) Ostolaza (Ostolaza, 2000). Like other Cactaceae (Lewis, 1980; Pinkava et al., 1985), the basic chromosome number for all the taxa examined was $x=11$. The diploid number $2n=2x=22$ was found in most species of *Haageocereus* examined, as well as other Trichocereeae we analyzed (e.g., the genera *Cleistocactus* Lem., *Matucana* Britton & Rose, *Mila* Britton & Rose). Previously reported diploid counts that are confirmed in the present study are: *Cleistocactus acanthurus* (Vaupel) D. R. Hunt (Diers, 1961) and *Echinopsis eyriesii* (Turpin) Pfeiff. & Otto (Katagiri, 1953). Importantly, polyploid counts were also obtained for some species (Fig. 1), including triploid ($2n=3x=33$), tetraploid ($2n=4x=44$), hexaploid ($2n=6x=66$), and octoploid ($2n=8x=88$) numbers. No aneuploids were recorded. Six polyploids were detected for *Haageocereus*: *H. acranthus* (Vaupel) Backeb. subsp. *acranthus, H. acranthus* subsp. *ollowinskianus* (Backeb.) Ostolaza, *H. chalaensis* F. Ritter, *H. fulvus* (Rauh & Backeb.) F. Ritter, *H. multicolorispinus* Buining ($2n=4x=44$); and *H. tenuis* F. Ritter ($2n=3x=33$). Among other Trichocereeae, polyploids were detected in six species belonging to the genera *Cleistocactus* Lem., *Espostoa* Britton & Rose and *Weberbauerocereus* Backeb.: *C. sepium* (Kunth) F. A. C. Webber ($2n=4x=44$), *E. lanata* (Kunth) Britton & Rose ($2n=4x=44$, and $2n=6x=66$), *W. johnsonii* F. Ritter ($2n=8x=88$), *W. rauhii* Backeb. ($2n=4x=44$, and $2n=8x=88$), *W. weberbaueri* ($2n=4x=44$), and *W. winterianus* F. Ritter ($2n=8x=88$). In two cases, a single species contained different cytotypes: *Espostoa lanata,* with $2n=22$, 44 and 66; and *Weberbauerocereus rauhii*, with $2n=44$ and 88. Three varieties of *Cleistocactus sepium* from Ecuador (var. *morleyanus*, var. *sepium*, and var. *ventimigliae*) were reported to be diploid by Baker (2002). We report a tetraploid *Loxanthocereus jajoianus* (Backeb.) Backeb., now placed in synonymy with *C. sepium* (Hunt, 1999; Anderson, 2001).

Chromosomes were very small in size in all taxa examined (3 µm to 5 µm). They were all observed to be of similar size, metacentric or submetacentric, and were not clearly distinguished morphologically. It has been argued that speciation has occurred rapidly and relatively recently in the Cactaceae and may have been accompanied by very little (or at least cryptic) chromosomal change (e.g., in *Mammillaria*, Remski, 1954; 55 taxa of Cactaceae, Ross, 1981; *Echinocereus*, Cota & Philbrick, 1994). Some authors also suggest that the high similarity in chromosome morphology and number observed would explain in part, the ease with which cacti can cross and produce fertile intergeneric hybrids even between morphologically divergent genera (Remski, 1954; Gibson & Nobel, 1986). But other than work focused on a relatively small group of Cactaceae, very little is known about karyotypes in the group.

These are the first chromosome counts for *Haageocereus* and the first reports of polyploidy in *Haageocereus, Espostoa* and *Cleistocactus*. Polyploidy in Cactaceae can occur through premeiotic abnormalities (Ross, 1981) or somatic doubling in the meristems, as observed in *Mammillaria* (Remski, 1954). These events can lead to the establishment of

TABLE I
SOMATIC CHROMOSOME NUMBERS FOR TAXA IN THE TRICHOCEREEAE AND OTHER TRIBES OF CACTACEAE

Taxon	Provenance and voucher specimens [a]	Chromosome [b] number ($2n$)
TRIBE TRICHOCEREEAE		
Cleistocactus acanthurus (Vaupel) D. R. Hunt *	PE. Lima: Huarochirí, *MA 1629* (USM)	22
Cleistocactus acanthurus (Vaupel) D. R. Hunt *	PE. Lima: Huarochirí, *MA 1630* (USM)	22
Cleistocactus fieldianus (Britton & Rose) D. R. Hunt	PE. Cajamarca: San Marcos, *MA 1699* (USM)	22
Cleistocactus sepium (Kunth) F. A. C. Weber ex Rol. Goss. *	PE. Arequipa: Arequipa, *MA 1606* (HUSA, USM)	**44**
Cleistocactus serpens (Kunth) F. A. C. Weber ex Rol. Goss.	PE. La Libertad: Otuzco, *MA 1714* (USM)	22
Cleistocactus sp.	PE. Amazonas: Utcubamba, *MA 1671* (USM)	22
Echinopsis eyriesii (Turpin) Pfeiff. & Otto *	AR. Formosa, *KK 1474* (MG)	22
Espostoa blossfeldiorum (Werderm.) Buxb.	PE. Amazonas: Chachapoyas, *MA 1691* (USM)	22
Espostoa lanata (Kunth) Britton & Rose	PE. La Libertad: Trujillo, *MA s/n* (USM)	22
Espostoa lanata (Kunth) Britton & Rose	PE. Lambayeque: Lambayeque, *MA 1659a* (USM)	**44**
Espostoa lanata (Kunth) Britton & Rose	PE. Lambayeque: Lambayeque, *MA 1656* (USM)	**66**
Espostoa senilis (F. Ritter) N. P. Taylor	PE. Cajamarca: San Marcos, *MA 1704* (USM)	22
Haageocereus acranthus (Vaupel) Backeb. subsp. *acranthus*	PE. Lima: Huarochirí, *MA 1628* (USM)	**44**
Haageocereus acranthus (Vaupel) Backeb. subsp. *acranthus*	PE. Lima: Lima, *CO s.n.* (MG)	**44**
Haageocereus acranthus (Vaupel) Backeb. subsp. *ollowinskianus* (Backeb.) Ostolaza	PE. Lima: Huaura, *MA 1644* (USM)	**44**
Haageocereus australis Backeb.	PE. Ica: Nazca, *MA 1616* (USM)	22
Haageocereus chalaensis F. Ritter	PE. Arequipa: Caravelí, *MA 1600* (HUSA, USM)	**44**
Haageocereus decumbens (Vaupel) Backeb.	PE. Arequipa: Islay, *MA 1578* (HUSA, USM)	22
Haageocereus decumbens (Vaupel) Backeb.	PE. Arequipa: Islay, *MA 1579* (HUSA, USM)	22
Haageocereus decumbens (Vaupel) Backeb.	PE. Arequipa: Caravelí, *MA 1588* (HUSA, USM)	22
Haageocereus fulvus (Rauh & Backeb.) F. Ritter	PE. Ancash: Huaraz, *MA 1650* (USM)	**44**
Haageocereus icosagonoides Rauh & Backeb.	PE. Cajamarca: San Pablo, *MA 1707* (USM)	22
Haageocereus icosagonoides Rauh & Backeb.	PE. Cajamarca: San Pablo, *MA 1711* (USM)	22
Haageocereus icosagonoides Rauh & Backeb.	PE. No locality information, *KK 1639* (MG)	22
Haageocereus multangularis (Willd.) F. Ritter	PE. Ancash: Huarmey, *MA 1616* (USM)	22
Haageocereus multicolorispinus Buining	PE. Ica: Nazca, *MA 1617* (USM)	**44**
Haageocereus pacalaensis Backeb.	PE. Lambayeque: Chiclayo, *MA 1652* (USM)	22
Haageocereus pacalaensis Backeb. subsp. *repens* (Rauh & Backeb.) Ostolaza	PE. La Libertad: Trujillo, *MA 1539* (USM)	22
Haageocereus pacalaensis Backeb. subsp. *repens* (Rauh & Backeb.) Ostolaza	PE. La Libertad: Trujillo, *MA s.n.* (USM)	22
Haageocereus platinospinus (Werderm. & Backeb.) Backeb.	PE. Arequipa: Arequipa, *MA s.n.* (USM)	22
Haageocereus platinospinus (Werderm. & Backeb.) Backeb.	PE. Arequipa: Arequipa, *MA 1614* (HUSA, USM)	22
Haageocereus pseudomelanostele (Werderm. & Backeb.) Backeb.	PE. Ancash: Huaraz, *MA 1651* (USM)	22
Haageocereus pseudoversicolor Rauh & Backeb.	PE. No locality information, *KK 1380* (MG)	22
Haageocereus tenuis F. Ritter	PE. Lima: Huaura, *MA 1635* (USM)	**33**
Haageocereus versicolor (Werderm. & Backeb.) Backeb.	PE. Lambayeque: Lambayeque, *MA 1658* (USM)	22
Haageocereus sp.	PE. Arequipa: Caravelí, *MA 1596* (HUSA, USM)	22
Lasiocereus fulvus F. Ritter	PE. Amazonas: Chachapoyas, *MA 1684* (USM)	22
Lasiocereus rupicola F. Ritter	PE. Cajamarca: San Marcos, *MA 1698* (USM)	22
Matucana haynei (Otto ex Salm-Dyck) Britton & Rose subsp. *haynei*	PE. Lima: Huarochirí, *KK 1548* (MG)	22

(*continued*)

TABLE I (*continued*)

Taxon	Provenance and voucher specimens [a]	Chromosome [b] number ($2n$)
Matucana haynei (Otto ex Salm-Dyck) Britton & Rose subsp. *herzogiana* (Backeb.) Mottram	PE. Ancash: Huaraz, *MA 1647* (USM)	22
Mila caespitosa Britton & Rose	PE. Lima: Huarochirí, *MA 1627* (USM)	22
Mila caespitosa Britton & Rose	PE. Lima: Huaura, *MA 1638* (USM)	22
Mila caespitosa Britton & Rose	PE. Lima: *KK 243* (MG)	22
Weberbauerocereus johnsonii F. Ritter	PE. Cajamarca: San Pablo, *MA 1708* (USM)	**88**
Weberbauerocereus rauhii Backeb.	PE. Arequipa: Caravelí, *MA 1592* (HUSA, USM)	**44**
Weberbauerocereus rauhii Backeb.	PE. Ica: Nazca, *CO 82173* (MG)	**88**
Weberbauerocereus weberbaueri (K. Schum. ex Vaupel) Backeb. *	PE. Arequipa: Arequipa, *MA 1613* (HUSA, USM)	**44**
Weberbauerocereus winterianus F. Ritter	PE. La Libertad: Otuzco, *MA 1713* (USM)	**88**
Weberbaureocereus winterianus F. Ritter	PE. La Libertad: Otuzco, *MA s.n.* (USM)	**88**
TAXA OUTSIDE TRICHOCEREEAE		
Austrocylindropuntia pachypus (K. Schum.) Backeb. [Opuntioideae]	PE. Lima: Huarochirí, *MA 1631* (USM)	22
Browningia microsperma (Werderm. & Backeb.) W. T. Marshall [Browningieae]	PE. Lambayeque: Lambayeque, *MA 1655* (USM)	22
Corryocactus aureus (Meyen) Hutchison ex Buxbaum [Pachycereeae]	PE. Arequipa: Arequipa, *MA 1603* (HUSA, USM)	22
Eriosyce islayensis (Förster) Katt. [Notocacteae]	PE. Arequipa: Caravelí, *MA 1591* (HUSA, USM)	22
Praecereus euchlorus (F.A.C. Weber) N. P. Taylor [Cereeae]	PE. Cajamarca: Jaén, *MA 1663* (USM)	22

[a]Abbreviations: AR: Argentina; PE: Peru; CO: Carlos Ostolaza; KK: Karel Knize; MA: Mónica Arakaki; USM: Herbario San Marcos, Lima, Peru; HUSA: Herbario Universidad San Agustín, Arequipa, Peru; MG: Mesa Garden, New Mexico, USA.

[b]Polyploid numbers are in bold.

*Previously counted.

polyploids when they occur in conjunction with self-fertility or asexual reproduction (Ross, 1981). Polyploidy has been suggested as an important evolutionary mechanism in plants (Tate et al., 2005; and references therein), and Cactaceae are not an exception. It has been suggested that some of the major changes occurring in this group are related to chromosome doubling (Gibson & Nobel, 1986). In specific genera, like *Espostoa* and *Weberbauerocereus*, the prevalence of high ploidy levels indicates that polyploidy has played an important role in their diversification. No diploids have been detected in *Weberbauerocereus* and the genus may have an allopolyploid origin given that only tetraploid ($2n=4x=44$) and octoploid ($2n=8x=88$) cytotypes have been detected. In spite of the above, the presence of diploids in almost every species in the rest of the genera indicates that differentiation within the Trichocereeae has been occurring mostly at the diploid level.

Previous polyploid counts in Trichocereeae reported only tetraploids and hexaploids. It is shown here that, as in the case of *Opuntia* (Baker & Pinkava, 1987), uneven ploidy levels are not only present (most probably as a result of sexual polyploidization), but also fixed by asexual reproduction. In this case the triploid microspecies *Haageocereus tenuis*, propagates by apomixis (Arakaki et al., in prep.).

Haageocereus polyploids thrive in extremely arid and severe environments compared to most diploid species in the genus. For example, several populations of the polyploid *Haageocereus acranthus* are found in disturbed areas, usually in dry steep rocky slopes. They receive water only during the short rainy season (December to March). The only existing population of the polyploid *H. tenuis* is

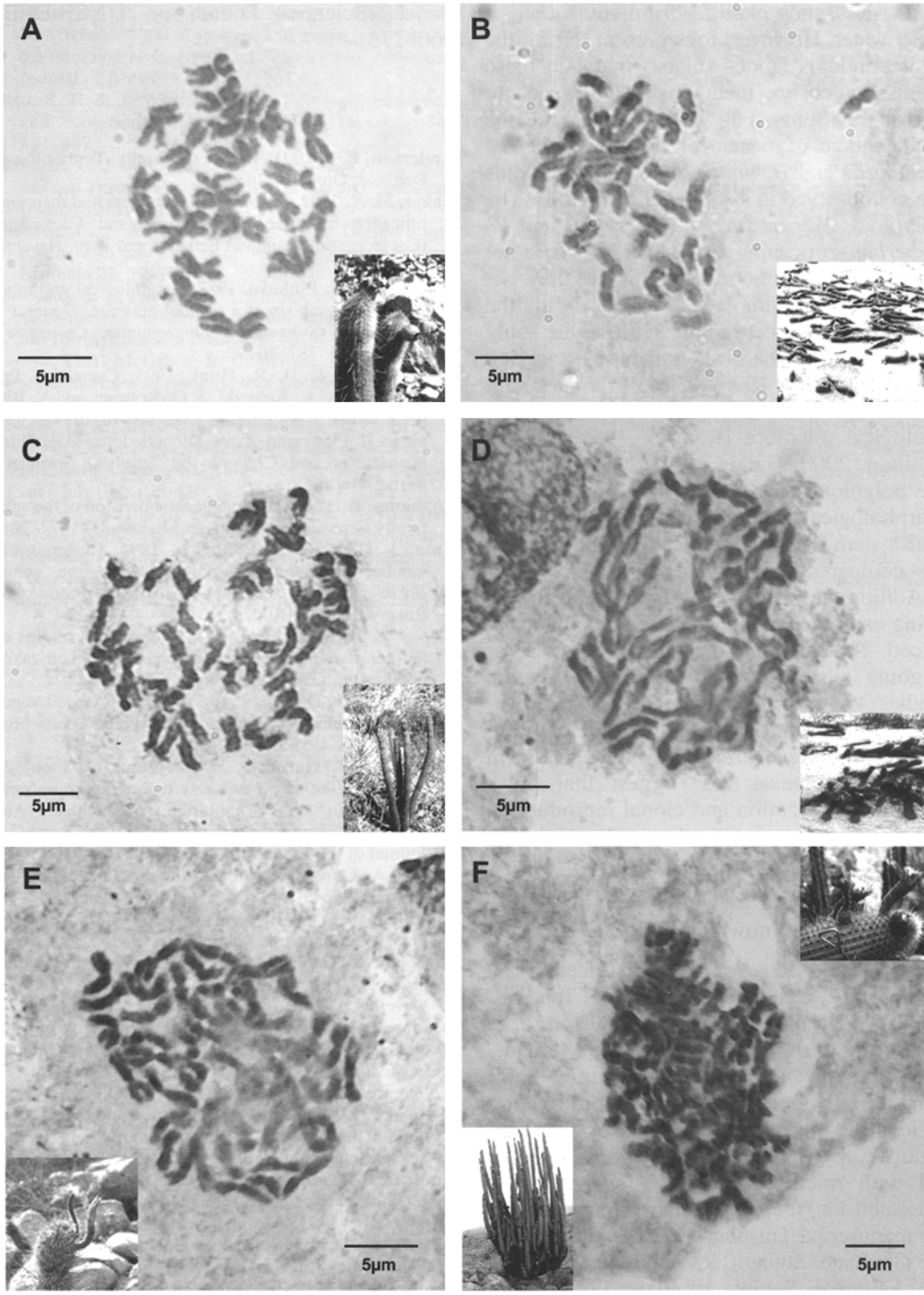

FIG. 1. Somatic chromosomes and habit of actual plants from diploid and polyploid species representing the Trichocereeae. **A.** *Haageocereus pseudomelanostele* ($2n=22$). **B.** *H. tenuis* ($2n=33$). **C.** *H. fulvus* ($2n=44$). **D.** *H. multicolorispinus* ($2n=44$). **E.** *Cleistocactus sepium* ($2n=44$). **F.** *Weberbauerocereus rauhii* ($2n=88$).

found in a sandy plain far from any source of fresh water. However, fogs present during the winter months (June–August) maintain these plants, which are facing habitat loss due to human pressures. Fog also seems to be the main source of water for the polyploids *H. chalaensis* and *H. multicolorispinus*. Diploids are not observed in such harsh conditions. The polyploids *Weberbauerocereus rauhii* and *W. weberbaueri* occupy very dry areas where not many other plants survive. Since they set flower and fruit almost year round, during the dry season they become almost the only source of food for birds and bats occupying the area (Sahley, 1995). These examples support the idea that polyploidy confers greater ecological tolerance (Remski, 1954; Otto & Whitton, 2000; Garcia et al., 2006). Most of the polyploids also show some characteristic morphological features, such as a dark greenbluish stem color and reduced number of stem ribs compared to diploids.

Additional species of Trichocereeae are being examined and chromosome counts produced. This information will be valuable for ongoing systematic and population genetic studies. We want to evaluate further the prevalence and evolutionary significance of polyploidy in *Haageocereus* and other genera in the Trichocereeae. We suggest that polyploidy, hybridization and clonal reproduction have played prominent roles in the evolution of some groups within the Trichocereeae.

Acknowledgments

We thank R. Aguilar, F. Cáceres, N. Calderón, A. Cano, N. Cieza, M. La Torre, B. León, L. Martin, J. Mauseth, C. Ostolaza, F. Peláez, Y. Ramírez, E. Rodríguez, J. Roque, and P. Sandoval for assistance during fieldwork. Some seed material and confirmation of identifications were provided by C. Ostolaza. We also thank B. Hauser and K. Vliet for aid with microscopy; and V. Simmons, J. Tate, and M. Vaio for valuable comments on the manuscript. This work was partially funded by Graduate Student Research Grants from the American Society of Plant Taxonomists (ASPT), the Botanical Society of America (BSA), the Cactus and Succulent Society of America (CSSA), the International Association for Plant Taxonomy (IAPT), and the National Science Foundation (NSF-DEB-0608273).

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