Amer. J. Bot. 68(4): 463–470. 1981.

CHROMOSOME COUNTS, CYTOLOGY, AND REPRODUCTION IN THE CACTACEAE[1]

ROBERT ROSS

Department of Botany, University of Rhode Island, Kingston, Rhode Island 02881

ABSTRACT

Chromosome counts and observations of reproduction for 55 taxa of Cactaceae indicate that polyploidy is correlated with self-fertility, adventive embryony, profuse branching, and vegetative reproduction. Six genera (*Blossfeldia, Cleistocactus, Frailea, Pelecyphora, Rebutia,* and *Strombocactus*) and 35 species or varieties are reported here for the first time. Preliminary observations of pachytene and diplotene indicate that these stages may be more useful in chromosome recognition than mitotic stages. Secondary association at metaphase I and II is interpreted as a retention of homologue association at interphase I and II (interkinesis). During meiosis of certain species, Feulgen negative bodies are present. The production of an abnormal premeiotic division is suggested as a mechanism for polyploid origin.

SEVERAL cytogenetic studies establish that the Cactaceae have a base number of $x = 11$, and polyploidy is the principle variation (Beard, 1937; Remski, 1954; Pinkava and McLeod, 1971). Earlier counts of $n = 9$ and $n = 12$, as summarized by Pinkava and McLeod (1971), were in error, but aneuploidy has been reported in meiotic material of *Deamia testudo* (Karw.) Britt. & Rose, $n = 12$ (Bhattacharyya, 1970). Either autopolyploidy or allopolyploidy have been reported in ten genera, including the large, well-surveyed *Mammillaria* and *Opuntia* (Katagiri, 1953; Remski, 1954). The significance of polyploidy, however, has not been related to the biology of the plants, particularly the mode of reproduction. Data from this study and from earlier works on embryology (Maheshwari and Chopra, 1955; Engleman, 1960; Tiagi, 1970), systematics (Philbrick, 1963; Fischer, 1971), and pollination ecology (Alcorn, McGregor and Olin, 1962) of the family, allow an initial comparison between reproductive mode and ploidy level.

There are a number of cytological features reported in the literature on Cactaceae which were reinvestigated during the examination of meiotic material to determine ploidy level. In the first chromosome report for the family, cytomixis in a species of *Mammillaria* was noted and illustrated (Ishii, 1929). The only illustrated study of cactus meiosis (Beard, (1937) does not show this phenomenon nor is it reported by other authors. Beard, however, found extra-nuclear bodies in *Echinocereus papillosus* Linke (=*E. blanckii* (Poselger) F. Palmer *var. blanckii*) and other unspecified taxa. These bodies were also observed in *Hylocereus undatus* (Haw.) Britt. & Rose (Banerji and Sen, 1954). Finally from Beard's work is the interesting description of tetraploid *Mammillaria compressa*—"pollen mother cells at interkinesis show twenty-two pairs of chromosomes." Similar pairing of chromosomes at metaphase I and II is reported by Lawrence (1931) and Darlington (1937) as secondary association.

The cactus collection at the University of Oklahoma provided meiotic material for the examination of the above mentioned cytological features and for the determination of chromosome numbers in many unreported taxa. Flowering and fruiting of plants in the collection also permitted study of reproductive modes and their relation to polyploidy in the family.

MATERIALS AND METHODS—South American plants obtained from commercial sources and field-collected Mexican and United States plants were grown in University of Oklahoma greenhouses for floral and meiotic material. Buds and roots were fixed in Carnoy's solution (3 ethanol:1 glacial acetic acid, V:V) between 9:00 a.m. and 11:00 a.m. and stored for 2 days. After washing in 70% ethanol, the material was stained with alcoholic-carmine-HC1 (Snow, 1963) or Feulgen's stain (Jensen, 1962). Squashing in 45% acetic acid and immediately photographing with a Leitz phase contrast microscope and high contrast copy film produced the best results. Material was mounted either in Hoyer's medium or air dried and

[1] Received for publication 29 January 1980; revision accepted 18 November 1980.

This investigation represents a portion of a dissertation submitted for the Ph.D. degree, University of Oklahoma, Norman, Oklahoma. The author is grateful for the assistance and suggestions offered by Drs. N. H. Boke, J. Estes, Edwin Leuck, D. J. Pinkava and Mr. Jim Weedin in preparation of this manuscript.

mounted in Clearmount. Callose wall observations were enhanced by mounting a meiocyte wall in Hyrax because of the medium's higher refractive index. Stages of embryo development were isolated in Herr's clearing solution (Herr, 1971).

Greenhouse plants were artifically self-pollinated and fruit and seed production investigated. Plants that produced fruit following selfing were excluded from pollinators the following year in order to verify original observations.

Pollination is generally agreed to be essential for endosperm initiation in cacti, a prerequisite for the development of either sexual or asexual embryos (Maheshwari and Chopra, 1955). To determine whether self-pollinating taxa are autogamous (zygotic embryos) or apomictic (adventive embryos), embryo development was observed in those taxa where ten fruits of varying age were available. A series of stages was required because, although zygotic embryos are produced initially, in later stages adventive embryos may also develop (Philbrick, 1963).

Voucher specimens were deposited in the Robert Bebb Herbarium (OKL).

OBSERVATIONS AND DISCUSSION—*Pachytene and diplotene stages*—During mitosis and meiosis, cactus chromosomes have few distinctive morphological characters. Mitotic chromosomes occasionally show a pair of satellites (Remski, 1954) but otherwise appear similar. Karyotyping of only one species, *Hylocereus undatus,* has been attempted (Banerji and Sen, 1954). Pachytene and diplotene, however, reveal chromosomes with a chromomere pattern (Fig. 1, 2). Usually, recognition of particular chromosomes is not possible; however, in *Pereskia diaz-romeroana,* two bivalents at diplotene are marked by regions adjacent to the telomeres which do not synapse (Fig. 3). These pictures show that karyotyping studies are more profitable using meiotic material than mitotic.

Multivalent formations—Most taxa were examined at diakinesis for bivalents and multivalents. Polyploids form bivalents, except in *Rebutia spegazziana, R. cv. nivea* and *Mammillaria prolifera* (Fig. 4), each of which has three to five quadrivalents. The number of quadrivalents is possibly higher, for chiasmata are frequently lost during diakinesis (Fig. 4). Observations of numerous multivalents in *M. prolifera* are similar to those by Remski (1954), but findings in *M. compressa* differ. Remski reports that in *M. compressa* meiosis is very irregular, with microspores rarely being produced. In most plants I investigated, microspores are produced and there are very few quadrivalents (Fig. 5).

Secondary association—Bivalents at metaphase I and chromosomes at metaphase II occasionally appear in pairs. Beard (1937) noted that "pollen mother cells at interkinesis show twenty-two pairs of chromosomes" in tetraploid *M. compressa.* This phenomenon is termed secondary pairing, or secondary association, by Darlington (1937). He observed that chromosomes may not associate at diakinesis but may become secondarily paired at metaphase I and metaphase II; however, they may rarely form quadrivalents (Lawrence, 1931). The secondary association is interpreted by Darlington as revealing the presence of some homology. Another interpretation (Heilborn, 1936) is that secondary pairing of homologues results not from "attraction between homologous parts of chromosomes" but from a "differential grouping of chromosomes of different size and mass."

The indistinguishable chromosomes of the Cactaceae sometimes form pairs of bivalents at metaphase I in tetraploids (Fig. 7). Such a situation may be a chance association of bivalents in a tetraploid or interpreted as an example of secondary association. During prophase II, chromosomes of similar morphology (Fig. 13) or chromosomes with similar degrees of condensation (Fig. 14, 15) appear to be associated. Because individual chromosomes lack distinctive features, many associations remain questionable. To determine if the associations are actually between homologous chromosomes, the nucleoler organizing regions were analyzed. In the cactus material of this study there is one nucleolus per genome, and therefore one nucleolus organizer per genome. In *Rebutia* cv. 'nivea' (4N), the nucleolus was used as a marker. *Rebutia* cv. 'nivea' produces one or two nucleoli in each interkinesis nucleus (Fig. 10). In each instance where one nucleolus was observed, two chromosomes were attached to the nucleolus; when two nucleoli were present, each nucleolus has a single chromosome attached and the two nucleoli were closely associated (Fig. 10). I interpret this to mean that the two nucleoli are forming in close association during the short period of interkinesis because the genes for their formation are in close proximity. The two nucleolus organizers are on either homologous or homeologous chromosomes.

The concept that homologues are associated at times other than prophase I is supported by observations of premeiotic divisions (Brown

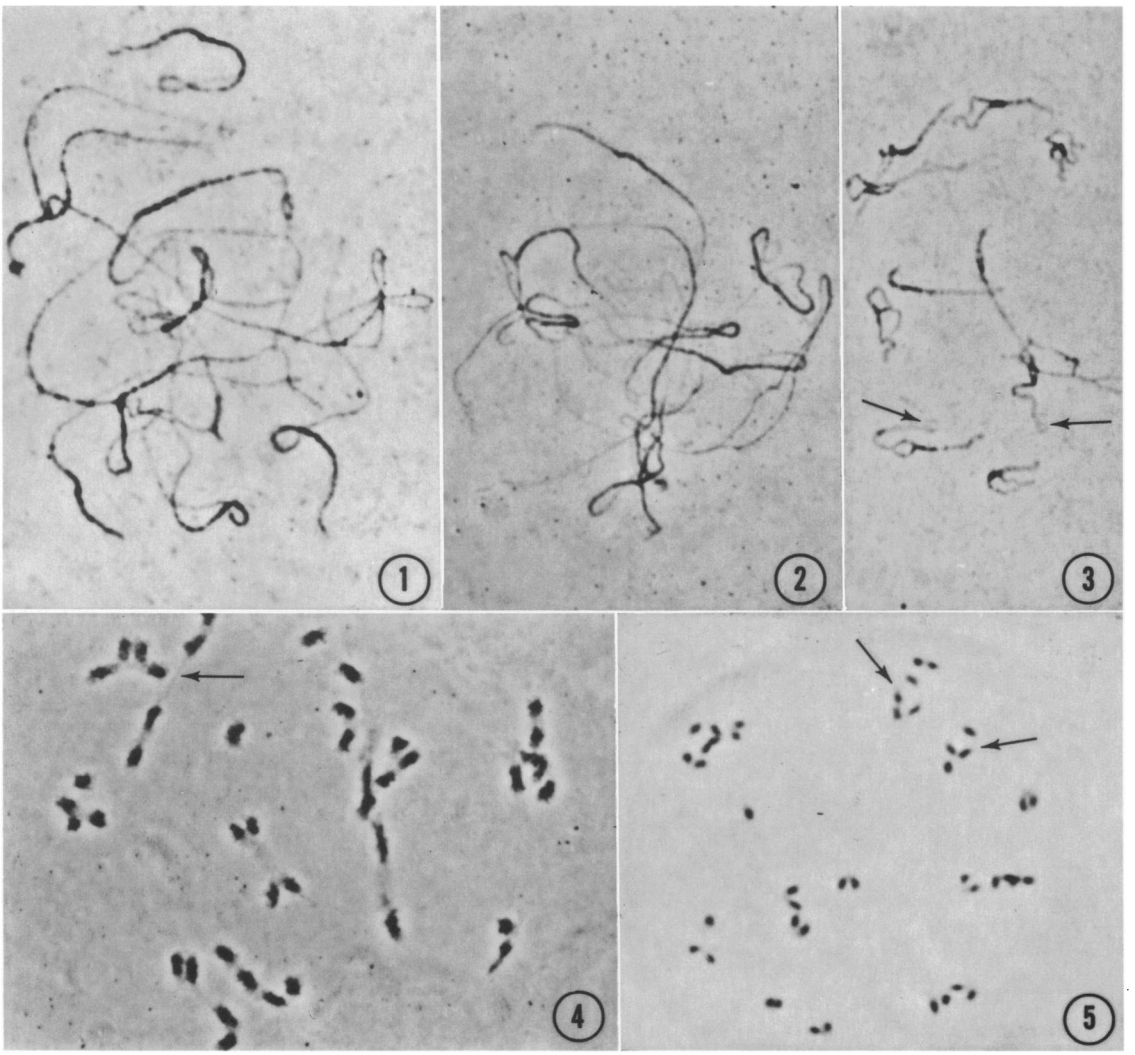

Fig. 1–5. **1.** *Rhipsalis pilocarpa,* pachytene. ×2,000. **2.** *Mammillaria candida,* pachytene. ×2,000. **3.** *Pereskia diaz-romeroana,* diplotene. Arrows indicate chromosome segments that do not synapse. ×2,000. **4.** *Mammillaria prolifera,* diakinesis. Arrow indicates fine chiasma between bivalents that form one of several quadrivalents in this tetraploid. ×2,600. **5.** *Mammillaria compressa,* diakinesis. Tetraploid with two quadrivalents, arrows point to quadrivalents. ×2,000. All figures are Feulgen-stained.

and Stack, 1968) and of interphase nuclei in root tips (Werry, Stoffelson, Engels, van der Lan, and Spanjers, 1977). The mechanisms for the association of homologues at these stages probably also function at interkinesis. Secondary associations at metaphase I and II are, possibly, a retention of homologue associations from interphase and interkinesis, respectively.

Extranuclear bodies—Extranuclear bodies are reported by Beard (1937) in many taxa from early diakinesis to telophase II, but she refers specifically only to *Echinocereus papillosus* Linke. Of 45 other taxa which Beard studied, only *Hylocereus undatus* is also reported to have similar bodies (Banerji and Sen, 1954). In my study, extranuclear bodies were found in *E. blanckii var. angusticeps, E. knipleanus,* and *Mammillaria wildii.* In these taxa, the bodies do not stain with periodic Shiff's reagent after hydrolysis in 1 N HCl, but are visible with phase microscopy after this treatment (Fig. 11). In *Hylocereus undatus,* the bodies were also found to be Feulgen negative (Banerji and Sen, 1954). These observations indicate that the extranuclear bodies do not contain DNA.

Cytomixis—Even though cytomixis has been investigated for over 50 years, interpretations still vary. Most workers agree in defining cytomixis as the transfer of chromatin be-

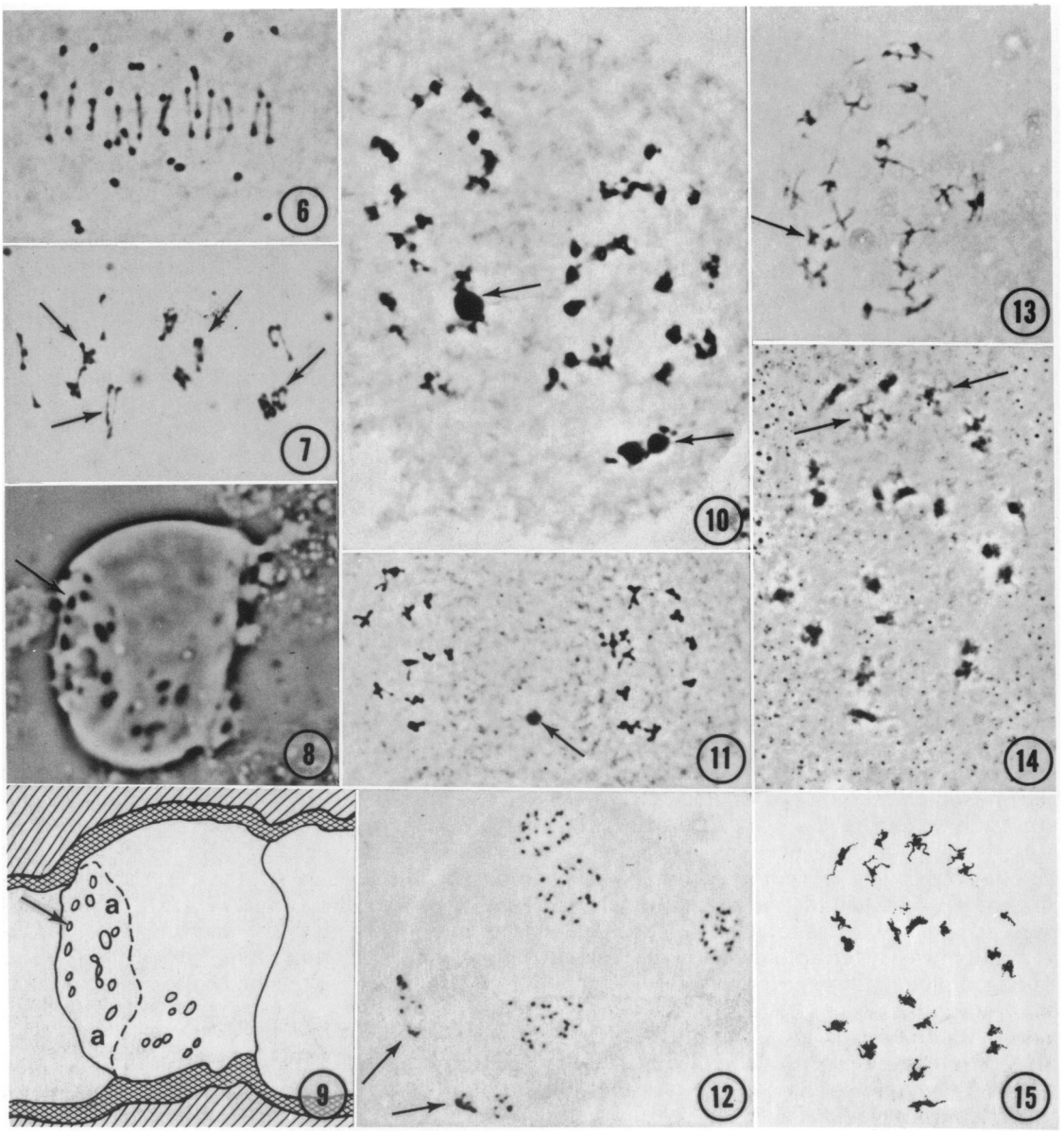

Fig. 6–15. **6.** *Pereskia diaz-romeroana*, metaphase I. Tetraploid cell with 22 bivalents. ×2,000. **7.** *Melocactus matanzanus*, metaphase I. Tetraploid with some bivalent pairs indicated by arrows. ×2,000. **8.** Callose wall of *Opuntia bigelovii* with arrow near pores (dark spots). Dark spots and dark halo around callose wall is a phase effect. ×1,400. **9.** Interpretive drawing of Fig. 8 showing the outline of adjacent cells removed in preparation. Surface "a" is cell wall between cells. **10.** *Rebutia sp.* shows cell in interkinesis with nucleus on left having a single nucleolus (left arrow) and other nucleus having two nucleoli (right arrow) closely associated. ×2,000. **11.** *Echinocereus blanckii* var. *angusticeps*, prophase II. extranuclear body (arrow) between nuclei. ×1,600. **12.** *Mammillaria compressa*, telophase II. Arrows indicate micronuclei. ×1,600. **13.** *Mammillaria compressa*, interkinesis. pair of morphologically similar chromosomes showing secondary association is indicated by the arrow. ×2,600. **14.** *Mammillaria prolifera*, interkinesis. Similarly condensed chromosomes showing secondary association are indicated by the arrows. ×2.000. **15.** Interpretive drawing of Fig. 14 showing details of chromosomes. Fig. 10 is a Snow's preparation; all others are Feulgen-stained.

tween microsporocytes, but they differ on whether or not it is an artifact. Heslop-Harrison (1966) reported that cytoplasmic channels between cells result in the microsporocytes functioning as a coenocyte whose nuclei develop and divide in synchrony. Cytoplasmic channels connect meiocytes through pores in the callose wall and allow the exchange of small organelles but not nuclear material. In his opinion experiments and observations indicate

that nuclear transfer is caused by physical and osmotic pressure in preparing the tissue; i.e., cytomixis is induced in vitro and does not occur in vivo. A different interpretation was made by Whelan (1974) in a survey of pores in callose walls. He considered the pores "to be indicative of cytoplasmic connections between the meiocytes; the exchange of cytoplasmic organelles should be possible, and in extreme cases, the exchange of nuclear material."

Pores in the callose wall of cactus meiocytes are common (Fig. 8) and similar to those illustrated by Whelan (1974). Normally pores are restricted to regions adjacent to microsporocytes and range in size from 0.3 to 1.7 μm (Fig. 9). Evidence supporting the natural occurrence of cytomixis could not be found, but the phenomenon is frequently present in cactus material as an artifact. The appearance of cytomixis is probably produced by applying pressure, either physical or osmotic, to anthers during fixation (Heslop-Harrison, 1966).

Premeiotic abnormalities—Abnormal premeiotic divisions are rare but occasionally produce meiocytes containing additional chromosomes. One tetrad of microspores from *Mammillaria compressa* (4×) had additional chromosomes in two micronuclei which had not been incorporated into the products of meiosis (Fig. 12).The normal complement of chromosomes was present in each microspore nucleus ($n = 22$), and the base number was present in each micronucleus. A second abnormality was a tetraploid meiocyte (Fig. 6) among diploid microsporocytes in an anther of *Pereskia diaz-romeroana*. Such a meiocyte has the potential for 11 quadrivalents, but 22 bivalents were present. Therefore, meiosis would likely yield unreduced gametes and consequently polyploids. Through such gametes polyploidy may arise by the production of an intermediate triploid plant (Harlan and DeWet, 1975). Pinkava, McGill, Reeves, and McLeod (1977) have found a diploid population of *Opuntia basilaris* var. *treleasei* which included a triploid individual which was hypothesized to have arisen from an unreduced plus reduced gametes.

Polyploidy and reproductive mode—Observations on the reproduction of the 55 taxa show that seeds are produced upon self-pollination in only 11 taxa and by cross-pollination in 44 taxa (Table 1). Of the 11 self-pollinating taxa, seven are autogamous and *Mammillaria prolifera* is apomictic by adventive embryos. *Rebutia kupperiana, R. spegazziana, R.* cv. 'nivea' lacked crucial developmental stages for determination.

Most of the taxa requiring cross-pollination were not examined for embryo development because of the paucity of seed material. Eleven *Echinocereus* taxa of this study were examined and found to be allogamous. Previous studies also report zygotic embryos in taxa of *Astrophytum, Thelocactus,* and *Pediocactus* (Engleman, 1960) which my observations indicate are self-incompatible. Therefore, most taxa requiring cross-pollination are reproducing sexually. However, some primarily allogamous taxa, *Mammillaria zeilmanniana* (Ross, 1974) and *M. tenuis* DC. (*M. elongata var. tenuis* (DC.) Schumann) (Tiagi, 1970), are also partially apomictic by adventive embryos after endosperm formation.

A comparison of the ploidy level with the mode of reproduction in the Cactaceae agrees with Stebbins's (1950) theory that polyploidy is more likely to become established in self-fertile or apomictic taxa. Of the taxa examined in this study (Table 1), 66% of polyploids (6 taxa) are self-fertile but only 11% (5 taxa) of the diploids. Self-sterile polyploids of this study, *Mammilaria compressa, M. parkinsonia,* and *Gymnocalcycium bruchii,* have extensive vegetative branching. *Opuntia,* which has a high frequency of vegetative propagation, adventive embryos, and self-fertility (Philbrick, 1963), has extensive polyploidy. Forty-eight percent of the *Opuntia* taxa examined by Weedin & Powell (1978) and Pinkava et al. (1977) are polyploids. In contrast, Remski (1954) reports only 8% polyploidy in *Mammillaria,* which has few of the reproductive characteristics favoring polyploids (Craig, 1945; Tiagi, 1970).

Remski (1954) hypothesized that somatic doubling, which occurs in root tips, may also occur in the apical meristem and thereby produce autopolyploids in *Mammillaria*. She considered the extensive quadrivalent formation evidence for autoploidy. Such quadrivalent formation would also occur, however, in interracial hybrids (Stebbins, 1950). Supporting a hybrid origin for polyploids, even self-fertile taxa, is the presence of mechanisms favoring cross-pollination. Large, showy flowers for which the cacti are noted occur in most of the self-fertile polyploids. At first glance, two exceptions in this study are *Blossfeldia* and *Melocactus. Blossfeldia liliputiana,* the smallest of the cacti, has a small flower, but it is not readily self-pollinating, even though it is self-fertile and nectar is produced. In *Melocactus matanzanus* the flowers are inconspicuous, but the subtending spines of the flowers form a

TABLE 1. *Chromosome counts and mode of reproduction in the Cactaceae*[a]

Taxon	Reproduction	Gametic chromosome no.	Location and voucher
Ancistrocactus scheeri (SD.) Br. & R.*	S	11	TX: Starr Co., RR 151.
Astrophytum capricorne (Dietr.) Br. & R.$_2$	S	11	MEXICO: Coahuila, NB sn.
Blossfeldia liliputiana Werd.**	A	33	CS, RR 210.
Cleistocactus baumannii (Lem.) Lem.**	S	11	Univ. of Calif. 53.1221, RR 201.
Coryphantha cornifera (DC.) Br. & R. var. *echinus* (Engelm.) L. Benson$_5$	S	11	TX: Terrell Co., NB sn.
C. ottonis (Pfeiff.) Lem.*	S	11	CS, RR 215.
Echinocereus blanckii var. *angusticeps* (Clover) L. Benson$_1$	S	11	TX: Duval Co., RR 190.
E. pectinatus (Scheidw.) Engelm. var. *pectinatus**	S	11	CS, RR 216.
E. pectinatus (Scheidw.) Engelm.) var. *rigidissimus**	S	11$_m$	NM: Hidalgo Co., RR 132.
E. pectinatus (Scheidw.) Engelm. var. *wenigeri* L. Benson*	S	11	CS, RR 224.
E. reichenbachii (Terscheck) Haage f. var. *albertii* L. Benson*	S	11	TX: Jim Wells Co., RR 175.
E. reichenbachii (Terscheck) Haage f. var. *albispinus* (Lahman) L. Benson*	S	11	OK: Comanche Co., RR 140.
E. reichenbachii (Terscheck) Haage f. var. *perbellus* (Br. & R.) L. Benson*	S	11	OK: Woods Co., RR 181.
E. reichenbachii (Terschek) Haage f. var. *fitchii* (Br. & R.) L. Benson$_1$	S	11	TX: Starr Co., RR 152.
E. reichenbachii (Terschek) Haage f. var. *reichenbachii*$_1$	S	11	OK: Murray Co., RR 139.
E. reichenbachii (Terscheck) Haage f. var. *chisoensis* (Marshall) L. Benson*	S	11	TX: Brewster Co., RR & J. Weedin 146.
E. viridiflorus Engelm. var. *viridiflorus*$_4$	S	11	TX: Randall Co., RR 180.
Echinofossulocactus sp.*	S	11	CS, RR 217.
Epithelantha bokei L. Benson$_5$	A	11	TX: Brewster Co., NB & J. Massey 488.
Escobaria tuberculosa (Engelm) Br. & R.$_{4,5}$	S	11	TX: Brewster Co., RR 147.
Frailea colombiana (Werd.) Backbg.**	A	11	CS, RR 197.
Gymnocalycium bruchii (Speg.) Hoss.*	S	22	CS, RR 202.
G. damsii Br. & R.*	S	11	CS, RR 203.
Mammillaria bocasana Pos.$_2$	S	11	CS, RR 112.
M. candida Scheidw.$_3$	S	11	MEXICO: San Luis Potosi, NB sn.
M. compressa DC.$_{1,3}$	S	22	CS, RR 218.
M. melaleuca Karw. ex SD.*	S	11	MEXICO: Tamaulipas, C. Glass & R. Foster #666
M. parkinsonia Ehrenbg.$_3$	S	22	CS, RR 219.
M. pectinifera Weber*	S	11	CS, RR 209.
M. pennispinosa Krainz	S	11	CS, RR 196.
M. spinossisima Lem.$_3$	S	11	CS, RR 221.
M. prolifera (Miller) Haw. var. *texana* (Poselger) Borg$_3$	A	22	TX: Duval Co., RR 191.
M. uncinata Zucca.$_3$	S	11	CS, RR 222.
M. wildii Dietr.$_3$	S	11	CS, RR 110.
M. zeilmanniana Böd.$_3$	S	11	CS, RR 111.
Melocactus matanzanus Leon*	A	22	CUBA: Matanzas, NB sn.
Myrtillocactus geometrizans (Mart.) Cons.$_2$	S	11	MEXICO: Queretaro, NB sn.
Neolloydia erectrocentra (Coulter) L. Benson*	S	11	AZ: Pima Co., NB sn.
Notocactus haselbergii Berger*	S	11$_m$	CS, RR 204.
Pelecyphora aselliformis Ehrenberg**	S	11	MEXICO: San Luis Potosi, NB sn.
P. strobiliformis (Werd.) Fric. & Schelle*	S	11	CS, RR 206.
Pereskia corrugata Cutak*	S	11	Mo. Bot. Garden 19913, RR 220.

TABLE 1. *Continued*

Taxon	Reproduction	Gametic chromosome no.	Location and voucher
P. diaz-romeroana Cardenas*	A	11	BOLIVIA: Seeds from Cardenas, RR 195.
Rebutia kupperiana Bod.**	A	22	CS, RR 213.
R. minuscula K. Sch.*	A	11	CS, RR 214.
R. spegazziana Backbg.*	A	22	CS, RR 199.
R. steinbachii Werd.*	S	11	CS, RR 194.
R. violaciflora Backbg.*	A	11	CS, RR 205.
R. sp. (unidentified cultivar—"Nivea")	A	22	CS, RR 198.
Rhipsalis pentaptera Pfeiff.*	S	11_m	CS, RR 193.
R. pilocarpa Loefgr.*	S	11	CS, RR 192.
R. salicornioides (Haw.) Br. & R.*	S	11	CS, RR 211.
Thelocactus valdezianus (Moller)*	S	11	MEXICO: Coah., C. Glass & R. Foster, 2996.
Strombocactus disciformis (DC.) Br. & R.**	S	11	CS, RR 208.
S. klinkeranus Backbg. & Jacobs*	S	11	MEXICO: San Luis Potosi, E. Anderson, 1626.

[a] KEY: * First report for a species or infraspecific taxon; ** First report for a genus; S—self-sterile; m—Mitotic count; A—Self-pollination produces seed; CS—Commercial source; 1—Reported by E. C. Beard; 2—Reported by S. Katagiri; 3—Reported by M. F. Remski; 4—Reported by D. J. Pinkava et al. either 1971, 1973, or 1977; 5—Reported by J. Weedin & A. M. Powell; Abbreviations for names of collectors: RR—Robert Ross, NB—Norman Boke.

bright red structure, a cephalium, and the individual flowers have abundant nectar for the pollinator.

Polyploidy in the Cactaceae originates through premeiotic abnormalities such as those observed in *Pereskia diaz-romeroana* or somatic doubling in the meristems as hypothesized by Remski. These rare events probably occur in all types of plants, but lead to the establishment of polyploid taxa when they are present in conjunction with self-fertility or apomictic mechanisms.

LITERATURE CITED

ALCORN, S. M., S. E. MCGREGOR, AND G. OLIN. 1962. Pollination and pollinating agents of the saguaro. Ecology 43: 259–267.

BANERJI, E., AND S. SEN. 1954. A contribution to the cytology and embryology of *Hylocereus Undatus* (Haw.) Br. & R. Bull. Bot. Soc. Bengal 8: 18–23.

BEARD, E. C. 1937. Some chromosome complements in the Cactaceae and a study of meiosis in *Echinocereus papillosus*. Bot. Gaz. 99: 1–21.

BHATTACHARYYA, P. K. 1970. Cytological study of *Deamia testudo* (Karw.) Britt. & Rose. A cactus growing wild in west Bengal. Sci. Cult. 36: 108–109.

BROWN, W. V., AND S. M. STACK. 1968. Somatic pairing as a regular preliminary to meiosis. Bull. Torrey Bot. Club 95: 369–378.

CRAIG, R. T. 1945. The *Mammillaria* Handbook. Abbey Garden Press, Pasadena, California.

DARLINGTON, C. D. 1937. Recent advances in Cytology. Second edition. Blakiston: McGraw-Hill Book Company, New York.

ENGLEMAN, E. M. 1960. Ovule and seed development in certain cacti. Amer. J. Bot. 47: 460–467.

FISCHER, P. C. 1971. Taxonomical and ecological relationship of the *Coryphantha vivipara* complex in the Cactaceae. Ph.D. thesis, University of California, Berkeley.

HARLAN, J. R., AND J. M. DEWET. 1975. On O. Winge and a prayer: The origins of polyploidy. Bot. Rev. 41: 361–390.

HEILBORN, O. 1936. The mechanics of so-called secondary association between chromosomes. Hereditas 22: 167–188.

HERR, J. M. 1971. A new clearing-squash technique for the study of ovule development in angiosperms. Amer. J. Bot. 58: 785–790.

HESLOP-HARRISON, J. 1966. Cytoplasmic connections between angiosperm meiocytes. Ann. Bot. 30: 221–230.

ISHII, T. 1929. Pollen development in a *Mammillaria sp.* Bot. Mag. Tokyo. 43: 560–561.

JENSEN, W. A. 1962. Botanical Histochemistry. W. H. Freeman, San Francisco.

KATAGIRI, S. 1953. Chromosome numbers and polyploidy in certain Cacataceae. Cact. Succ. J. 25: 141–142.

LAWRENCE, W. J. C. 1931. The secondary association of chromosomes. Cytologia 2: 352–384.

MAHESHWARI, P., AND R. M. CHOPRA. 1955. The structure and development of the ovule and seed of *Opuntia dillenii* Haw. Phytomorphology 5: 112–122.

PHILBRICK, R. N. 1963. Biosystematic studies of two Pacific coast *Opuntia*. Ph.D. thesis, Cornell University, Ithaca, New York.

PINKAVA, D. J., AND M. G. MCLEOD. 1971. Chromosome numbers in some cacti of western North America. Brittonia 23: 171–176.

———, M. G. MCLEOD, L. A. MCGILL, AND R. C. BROWN. 1973. Chromosome numbers in some cacti of western North America. II. Brittonia 25: 2–9.

———, L. A. MCGILL, T. REEVES, AND M. G. MCLEOD. 1977. Chromosome numbers in some cacti of western North America. III. Bull. Torrey Bot. Club 104: 105–110.

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

ROSS, R. G. 1974. Embryology of selected species of the Cactaceae. M.S. thesis, Memphis State University, Memphis, Tenn.

SNOW, R. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technol. 38: 9–13.

STEBBINS, G. L., JR. 1950. Variation and evolution in plants. Columbia University Press, New York.

TIAGI, Y. D. 1970. Cactaceae. *In* symposium on the comparative embryology of the angiosperms. J. Indian Nat. Sci. Acad.: 30–35.

WEEDIN, J. F. AND A. M. POWELL. 1978. Chromosome numbers in Cactaceae, Trans Pecos Texas. Amer. J. Bot. 65: 531–537.

WERRY, P. A. TH. J., K. STOFFELSON, F. M. ENGELS, F. VAN DER LAN, AND A. W. SPANJERS. 1977. The relative arrangement of chromosomes in mitotic interphase and metaphase in *Haplopappus gracilis*. Chromosoma (Berl.) 62: 93–101.

WHELAN, E. D. P. 1974. Discontinuities in the callose wall, inter-meiocyte connections, and cytomixis in angiosperm meiocytes. Can. J. Bot. 52: 1219–1224.