ROSS, ROBERT GRANT, III

STUDIES IN THE CACTACEAE

The University of Oklahoma PH.D. 1980

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs ✓
2. Colored illustrations _____
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Print shows through as there is text on both sides of page _____
6. Indistinct, broken or small print on several pages _____
7. Tightly bound copy with print lost in spine _____
8. Computer printout pages with indistinct print _____
9. Page(s) _____ lacking when material received, and not available from school or author
10. Page(s) _____ seem to be missing in numbering only as text follows
11. Poor carbon copy _____
12. Not original copy, several pages with blurred type _____
13. Appendix pages are poor copy _____
14. Original copy with light type _____
15. Curling and wrinkled pages _____
16. Other _____

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES IN THE CACTACEAE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

ROBERT GRANT ROSS III

Norman, Oklahoma

1980

STUDIES IN THE CACTACEAE

APPROVED BY

Norman Boke

DISSERTATION COMMITTEE

PREFACE

This dissertation is prepared as two chapters. Each chapter will be submitted to American Journal of Botany.

Grateful appreciation is expressed to the members of my committee whose helpful suggestions and encouragement made the completion of this work possible. Special gratitude is given to Dr. Norman H. Boke who provided encouragement, tutelage, and facilities for the various interest which I have pursued. I also extend my thanks to my fellow co-worker Edwin Leuck for his friendship and willingness to work on many aspects of cacti. I thank also my parents for their understanding and support. Finally, the contributions of many individuals who have participated, directly or indirectly, in this research is acknowledged.

A Sigma Xi Grant-in-Aid of Research provided financial support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHROMOSOME COUNTS, CYTOLOGY, AND REPRODUCTION IN THE CACTACEAE

Robert Ross

Department of Botany and Microbiology
University of Oklahoma
Norman 73019

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.

(INTRODUCTION)

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 auto- 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, *et.al.*, 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 two days. After washing in 70% ethanol, the material was stained with alcoholic-carmine-HCl (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

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 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 are 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 have 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. 11). 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. 11). 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 and Stack, 1968) and of interphase nuclei in root tips (Werry, et.al., 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 Schiff's reagent after hydrolysis in 1 N HCl but are visible with phase microscopy after this treatment (Fig. 12). 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 between 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. 10) 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 1.7mu (Fig. 11). Evidence supporting the natural occurence 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* (4X) 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. 8) among diploid microsporocytes in an anther of *Pereskia diaz-romeroana*. Such a meiocyte has the potential for eleven 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, *et.al.* (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 eleven taxa and by cross-pollination in 44 taxa (Table I). Of the eleven 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 (1950) theory that polyploidy is more likely to become established in self-fertile or apomictic taxa. Of the taxa examined in this study (Table I), 66% or 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

Gymnocalycium 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 (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 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. and 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. Ed. 2. Blakiston: McGraw-Hill Book Company, New York, 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. Unpublished Ph.D. Dissertation, University of California.

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 Cactaceae. Cact. Succ. Jour. 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. Unpublished Ph.D. dissertation. 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. Unpublished M.S. Thesis. Memphis State University, Memphis, Tennessee.

Snow, R. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technology 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. Journ. 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.

TABLE 1. CHROMOSOME COUNTS AND MODE OF REPRODUCTION IN THE CACTACEAE

Taxon	Reproduction	Gametic Chromosome Number	Location & 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 liliputana 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) Hagge 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.

TABLE 1 (Continued)

*E. reichenbachii (Terscheck) Haage f. var. perbellus (Br.& R.) L. Benson	S	11	OK: Woods Co., RR 181.
E. reichenbachii (Terscheck) Haage f. var. fitchii (Br.& R.) L. Benson[1]	S	11	TX: Starr Co., RR 152.
E. reichenbachii (Terscheck) 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

TABLE 1 (Continued)

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)

*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.

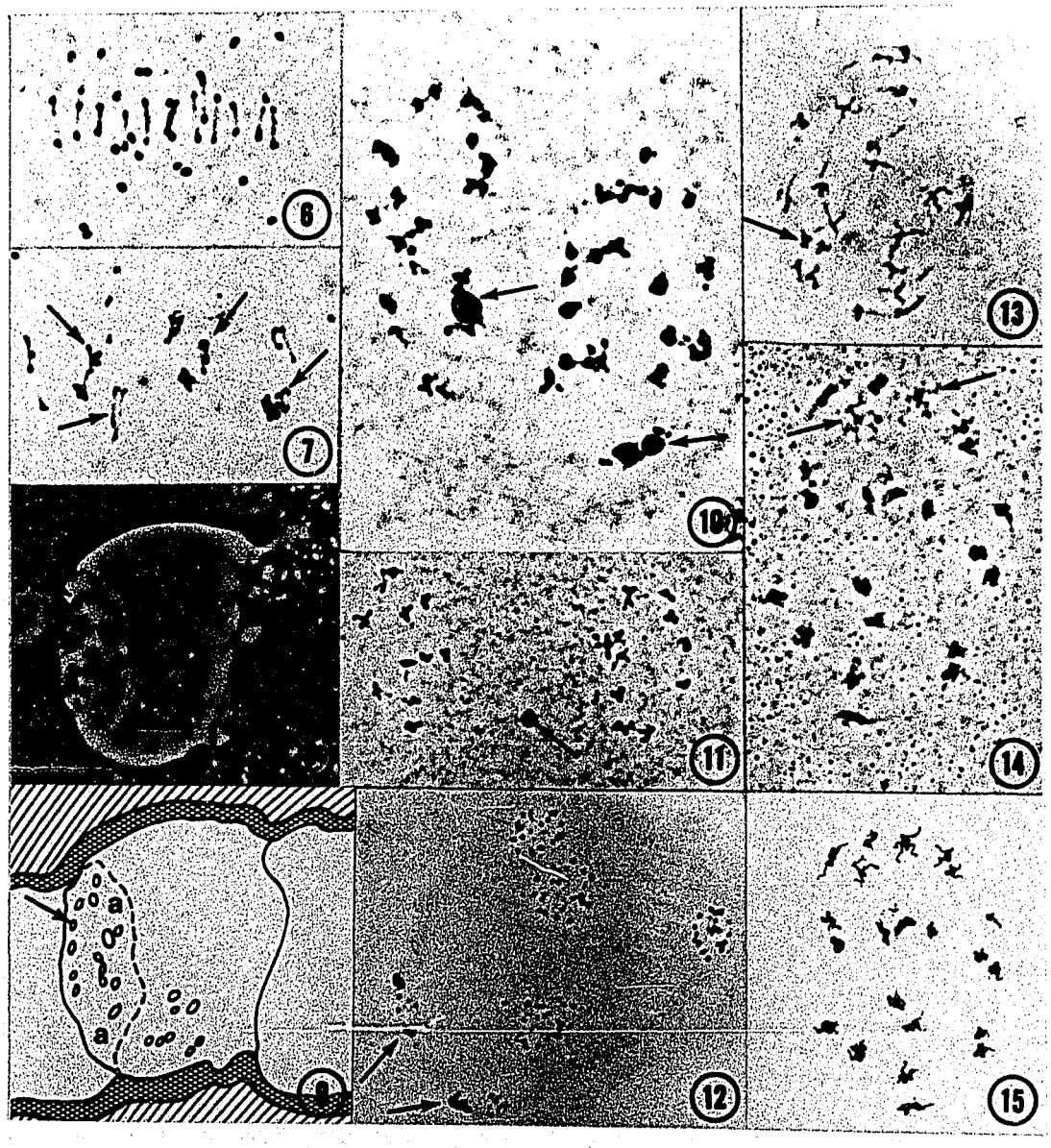
KEY: *First report for a species or infraspecific taxon; **First report for a genus; S-Self-sterile; m- Mitotic count; A- Self-pollination produces seed; CS- Commercial source; 1-Reported by E. C. Beard; 2-Reported by S. Katagiri; 3-Reported by M. F. Remski; 4-Reported by D. J. Pinkava, et.al. either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

FIGURE LINES --1

Fig. 1-7. 1. *Rhipsalis pilocarpa*, pachytene. x 2,000. 2. *Mammillaria candida*, pachytene. x 2,000. 3. *Pereskia diaz-romeroana*, diplotene. Arrows indicate chromosome segments that do not synapse. x 2,000. 4. *Mammillaria prolifera*, diakinesis. Arrow indicates fine chiasma between bivalents that form one of several quadrivalents in this tetraploid. x 2,600. 5. *Mammillaria compressa*, diakinesis. Tetraploid with two quadrivalents, arrows point to quadrivalents. x 2,000. All figures are feulgen stained.

FIGURE LINES --2

Fig. 6-15. 6. Pereskia diaz-romeroana, metaphase I. Tetraploid cell with 22 bivalents. x 2,000. 7. Melocactus matanzanus, metaphase I. Tetraploid with some bivalent pairs indiciated by arrows. x 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. x 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. x 2,000. 11. Echinocereus blanckii var. angusticeps, prophase II. extranuclear body (arrow) between nuclei. x 1,600. 12. Mammillaria compressa, telophase II. Arrows indicate micronuclei. x 1,600. 13. Mammillaria compressa, interkinesis. pair of morphologically similar chromosomes showing secondary association is indicated by the arrow. x 2,600. 14. Mammillaria prolifera, interkinesis. Similarly condensed chromosomes showing secondary association are indicated by the arrows. x 2,000. 15. Interpretive drawing of fig. 14 showing details of chromosomes. Fig. 10 is a Snow's preparation; all others are Feulgen stained.


6
7
8
a
a
10
11
12
13
14
15

INITIATION OF STAMENS, CARPELS, AND RECEPTACLE IN THE CACTACEAE

Robert Ross

Department of Botany and Microbiology
University of Oklahoma
Norman 73019

ABSTRACT

Five taxa representing the three tribes of the Cactaceae have similar patterns of stamen and carpel initiation but display differences in early receptacle development. The first ring of stamens and the carpels arise simultaneously from subsurface layers. The bases of carpels are congenitally connate. Additional stamens are initiated centrifugally. The shape of the floral meristem within the ring created by the first stamens varies. In Pereskia corrugata it remains broadly convex; in Opuntia engelmannii it forms a depression with a small convex central region; in Epiphyllum strictum it forms a broad shallow depression; in Echinocereus reichenbachii var. albispinus it develops a deep depression; and in Mammillaria compressa it develops a depression prior to stamen and carpel initiation. Changes in receptacle shape result from cessation of apical growth and activation of an intercalary ring meristem. These two processes occur earlier in ontogeny in the more advanced of these five taxa.

(INTRODUCTION)

Among the Cactaceae the tropical genus Pereskia, which includes trees and shrubs with broad deciduous leaves and superior ovaries, is considered the most primitive form (Boke, 1963; Gibson, 1975). From the ancestral plexus two broad lines of evolution have arisen (Hunt, 1967): (1) the tribe Opuntiae, with glochids, sympodial habit, and ephemeral, succulent leaves and (2) the tribe Cacteae comparatively little branching and highly reduced leaves, but lacking glochids. Both tribes have inferior ovaries. The Opuntiae include only five genera but are probably the best known of any group of cacti because of their extensive range and weedy nature. The most diverse tribe (77 genera) is the Cacteae, which includes epiphytic forms (e.g. Epiphyllum), "cereoid" or columnar form (e.g. Cereus and Echinocereus), and low-growing succulent forms (e.g. Mammillaria).

Although vegetative diversity allows the characterization of various tribes and series, flowers of cacti are relatively uniform. Most flowers have spirally arranged, free perianth segments and stamens, and a single ring or spiral of carpels, forming a unilocular ovary with a single style. Buxbaum (1953), Tiagi (1955), and Boke (1964) have established that the Cactaceae is one of the few families with a receptacular cup partially or completely enclosing the ovary. Much of the floral variation results from variation in the growth of the receptacle

which produces a superior ovary in Pereskiae, an inferior ovary in Opuntiae, and an inferior ovary with a receptacular floral tube in the Cacteae (Hunt, 1967). The ontogenetic origin of these ovary forms is poorly understood, as are details concerning the initiation of floral organs. One of the first studies in cacti of floral development (Payer, 1852) established that perianth segments are initiated spirally and centripetally, stamens centrifugally. Recent works confirm these observations but have not determined whether carpel initiation is acyclic (Buxbaum, 1953; Tiagi, 1955) or cyclic (Boke, 1964). Studies of early ontogeny have been concerned with Pereskia (Boke, 1963, 1964) and Opuntia (Payer, 1852; Huber, 1929; Buxbaum, 1953), whereas floral development in the largest and most diverse tribe, Cacteae, has only briefly been examined (Tiagi, 1957; Boke 1964).

In view of the investigations by Buxbaum, Tiagi, and Boke on developing and mature flowers, I investigated the pattern of stamen and carpel initiation and the early development of the receptacle. Examinations of one representative of the Pereskiae, one of the Opuntiae, and three of the Cacteae were conducted and comparisons were made with respect to patterns of development.

MATERIALS AND METHODS--Materials for this study came from a cactus collection assembled by N. H. Boke at the University of Oklahoma and from field-collected specimens of Echinocereus reichenbachii (Terscheck) Haage f. var. albispinus (Lahman) L. Benson growing in Commanche Co., Oklahoma. Pereskia corrugata Cutak, Opuntia engelmanii Salm-Dyck, Epiphyllum strictum (Lemaire) Britton and Rose, and Mammillaria compressa DeCandolle were obtained from various commercial

sources. Voucher specimens of each taxon are in the Robert Bebb Herbarium (OKL).

Fresh buds were prepared by removing most outer perianth segments with a razor blade. Exposed meristems were then excised and floated on a droplet of water in a depression slide. Illumination and photographic methods were modified from an oblique illumination technique employed in studies of areole development (Boke, 1955). Little detectable distortion results from this technique except for small droplets which appeared on the meristem surface. After photography floral meristems were immediately fixed in Craf V (Sass, 1940). Paraffin sections 10-15 mu thick were stained with safranin and fast green (Boke, 1952). All photomicrographs of longisections were arranged with the floral axis parallel to the edge of the page.

RESULTS--General Organography--Each of the examined taxa has a distinctive habit and floral appearance and is briefly described below. More detailed descriptions are found in the taxonomic treatments by Britton and Rose (1919-1923) and Backeberg (1970).

Pereskia corrugata is one of the more easily grown representatives of the tribe Pereskiae, but the species regretably is described from a single specimen of unknown origin (Cutak, 1951). In cultivation it grows as a shrub with leaves 10-15 cm long which are slightly crenate or undulate. Reddish-orange, rotate flowers are borne throughout the spring and summer from apical meristems on short shoots or from areoles near the terminal bud. The superior ovary of the flower at anthesis rests inside a broad receptacular depression with perianth segments and stamens attached around the upper perimenter of the receptacle (Fig. 11).

The ovary roof, consisting of dorsal carpellary tissue, is free of other adnate tissues and is therefore considered superior (Boke, 1964). Ovules and their placentae are supplied from the ventral bundles of the carpel and are adnate to the invaginated receptacle. A nectary is present at the base of the stamens. Fruit morphology is unknown for this species because it is self-incompatiable and only one biotype exists.

Opuntia engelmannii Salm-Dyck is one of many species commonly called prickly pear. It is a large species with flattened, ovoid stems 25-35 cm in diameter and sympodial branching, forming a shrub nearly two meters tall. Areoles cover each stem but only those of upper stems normally bear flowers. Initially, buds appear like short cylindrical shoots with numerous spirally arranged areoles. Inside this stem or receptacle is the ovary (Fig. 21). Flowers are rotate with 20-30 yellow perianth segments and numerous thigmotropic stamens. Perianth segments, stamens, and style abscise as a unit early in fruit development. Near maturity the fruit turns reddish-purple and the flesh becomes slightly sweet.

The third tribe is represented by Epiphyllum strictum (Lemaire) Britton and Rose, Echinocereus reichenbachii (Terscheck) Haage F. var. albispinus (Lahman) L. Benson, and Mammillaria compressa DeCandolle. These taxa, like most Cacteae, are cortical succulents with microscopic leaves. Epiphyllum strictum is a sympodially branching epiphyte of tropical central America with flattened stems up to two meters long and five to eight cm wide. As seedlings, areoles of this plant produce five to seven spines, but adult plants lack spines. Early in summer, flowers are borne which are actinomorphic except for the receptacle tube (Fig. 31).

The receptacular floral tube bears numerous bracts externally, scattered stamens internally, and a ring of stamens just below the perianth segments. Nocturnal flowering, strong sweet odor, white floral parts, horizontally oriented floral tube, stigma and anthers presented at mouth of the tube, and nectar deep in the floral tube are associated with moth pollination in this genus. Epiphyllum strictum is well known in cultivation for its large nocturnal flowers and is commonly called "Night Blooming Cereus."

Echinocereus reichenbachii var. albispinus is a small caespitose cactus occurring primarily in the Wichita Mountains in Oklahoma. Areoles and their leaf bases are arranged in vertical rows, forming 13-20 ribs per stem. Normally, flowers originate in the spring from areoles initiated the previous year, but they also develop from the apical meristem (Fig. 39). Spine-bearing areoles, arranged in a continuous spiral, cover the receptacle and the outer floral tube. Attached distally on the tube are 35-60 magenta perianth segments. Green stigma lobes on a single style project above the stamens, which line the inner floral tube. These floral parts wither, but do not abscise as the fruit matures. Fruits dehisce by one to four sutures developing irregularly in the receptacle wall 45-50 days after cross-pollination.

Mammillaria compressa is a low-growing, clumping plant with up to 50 stems in a cluster. Areoles and their leaf bases (tubercles) are spirally arranged on the plant and bear four to six spines per areole (Fig. 51). From the floral meristems of two- or three-year-old areoles a ring of flowers develops on each stem. The receptacle enclosing the ovary and forming the floral tube is usually free of areoles and spines

(Fig. 50). Pinkish-red perianth segments are attached distally, forming a flower one centimeter in diameter. Stamens line the receptacle tube, with a nectary at the base of the stamens adjacent to the style. Fruits at maturity elongate by intercalary growth. Dried persistent floral parts are attached to a bright red fruit about two centimeters long.

Floral Meristems and Tepal Initiation--Flowers originate laterally from areole meristems in most cacti, but may also terminate branches. In P. corrugata the flower is either terminal on a short branch (Fig. 11) or arises from axillary buds (areoles). Opuntiae may have terminal flowers, as in Pereskiopsis and Opuntia, subgenus Cylindriopuntia, or lateral flowers, as in O. engelmannii (Fig. 21), a member of the subgenus Opuntia. In Cacteae terminal flowers are rare and anomalous; for example, E. reichenbachii var. albispinus normally has flowers originating from lateral areoles but may sometimes have a terminal one (Fig. 39).

Buds useful for the study of floral initiation range from 1.5 mm long in P. corrugata and M. compressa to 5.0 mm long in E. strictum and O. engelmannii. In many cacti young buds are short pedicillate, as in Pereskia, or sessile, as in Opuntia and Epiphyllum. However, floral buds may be hidden by tubercles and trichomes during early development or partially covered by epidermal tissues of the tubercle. Floral meristems of Mammillaria are at the bases of tubercles and have numerous trichomes associated with them (Fig. 51). Echinocereus buds typically burst through the epidermis after the initiation of floral organs and are covered with spines borne by areoles of the receptacle and floral

tube. Submergence of the areole meristem during tubercle ontogeny creates the illusion of an endogenous origin.

From earliest development, perianth segments cover a floral meristem. Initiation of perianth is by periclinal divisions of subsurface layers. Among these taxa intergradation between reduced leaves and perianth segments can be seen in the mature flowers (Fig. 21, 31). Areole and tepal primordia frequently form a continuous spiral during initiation, as in Echinocereus (Fig. 32). Tepal primordia resemble leaf primordia (Ross, in press) except for the abscence of an axillary meristem (Fig. 9,30,46). Perianth initiation is completed as other floral organs begin to appear (Fig. 33). Floral meristems are initially convex and range in diameter from approximately 350 mu in Mammillaria and Pereskia to 730 mu in Epiphyllum. The height above the perianth primordia ranges from 300 mu in Opuntia to 30 mu in Mammillaria. Meristems of a particular stage show little variation in size on a given plant but may vary on different plants of the same species. In Echinocereus several specimens provided buds which were at the end of perianth initiation. These were 425 mu to 560 mu in diameter. Size variation also occurs at other stages of development and may explain the variable number of floral organs that is characteristic of this genus and others. The pattern of floral development in Echinocereus is consistent within those specimens studied even thought there is variation in size.

Stamen Initiation--Stamens in cacti are initiated in centrifugal sequence. Generally a ring of stamens appears almost simultaneously (Fig. 13,23,33,41) and additional stamens appear basipetally from this

primary ring (Fig. 4,16,25,43). A deviation from the nearly simultaneous initiation of the primary stamen ring occurs in Pereskia, where five to eight stamens appear at equally spaced points, forming a circle (Fig. 1). Others later complete the perimenter of this circle (Fig. 2,3). There is another variation subsequent to initiation of the primary stamen ring. Normally stamens radiate from the central ring in a symmetrical fashion, creating a larger circle of new primordia. In Echinocereus, however, stamens in certain regions appear before others on adjacent radii (Fig. 34). As initiation advances, these clusters of stamens form an irregular border (Fig. 35). Stamens formed after the primary ring are generally not in an obvious phylotactic spiral, nor is there evidence of centripetal initiation, even though there is sometimes tissue between the primary stamen ring and carpel primordia (Fig. 13). Stamens continue to arise on the outer margins of the meristem, leaving little meristematic tissue between the stamen and perianth primordia. Elongation of the stamens occurs only after all are initiated.

Carpel Initiation--Carpel primordia arise almost simultaneously in a circle (Fig. 1,13,23). Initially separate, they soon become united by a meristematic ring (Fig. 2,34,44). Intercalary concrescence is precocious in Pereskia, Echinocereus, and Mammillaria such that individual primordia are recognizable only as small mounds of tissue at initiation (Fig. 1,33,42). In Opuntia and Epiphyllum the tips of the primordia become crescentic (Fig. 16,25) whereas their bases are congenitally united. The sinus of each primordium represents a carpel locule, but these individual locules also are soon united, forming a single locule. Neither eccentric nor acyclic initiation was observed.

In surface views and sections floral meristems appear to initiate carpel and stamen primordia during the same brief period of time. In Mammillaria and Echinocereus carpel initiation is partially obscured in the surface photographs by the receptacle concavity, but rotating the meristems revealed that primordia were formed at the same time. Stamen and carpel initiation occurs by periclinal divisions in the sub-surface layer of the meristems (Fig. 8,18,28). These meristematic regions are typically darker staining than adjacent vacuolated cells. Elongation of stamen primordia is initially more rapid than that of carpels, sometimes giving a false impression of earlier formation.

Early Receptacle Development--Floral meristems are initially convex, but the persistance of this shape varies with the taxon. Near the end of perianth initiation increased meristematic activity in the flank portions of floral meristems (over that of the central region) causes a broadening or truncation of the floral apex. Most of this activity is in arcuate files of cells which occur in all taxa studied (Fig. 18,19). However, the time and degree of activity in this meristematic ring vary with the taxa. In Pereskia corrugata the floral meristem gradually changes from convex to flat by the end of floral initiation (Fig. 10). Opuntia, Epiphyllum, and Echinocereus initially have a convex meristem which becomes truneated, but after carpel and stamen initiation begins each developes a central depression or cup. In Mammillaria the convex meristem flattens (Fig. 45) and then a ring of tissue grows up around and above a central region that is meristematically inactive (Fig. 4b). In each taxon the androecium meristem, which initially slopes downward, is displaced outward and upward into a more

horizontal position as centrifugal initiation of stamens begins (Fig. 10, 20,30,38,49). In the Opuntiae and Cacteae this displacement continues raising stamen and tepal primordia above the carpel primordia. The intercalary meristem finally produces a floral tube in most members of the Cacteae. The floral apex declines in meristematic activity during stamen and carpel initiation. Cells of the central apical region become vacuolated and lighter staining relative to radial portions. On the basis of developmental studies (Boke, 1963, 1964, 1966) it has been determined that this residual tissue is not carpellary and forms the floor of the ovary locule. At anthesis the locule floor is concave but the precocity of change from convex to concave varies among the taxa examined. In P. corrugata a change in shape occurs near the end of stamen initiation (Fig. 10). A convex meristem remains through stamen initiation in O. engelmannii (Fig. 20). In members of the Cacteae examined, concavity develops before stamen and carpel initiation. Epiphyllum (Fig. 27) and Echinocereus (Fig. 36) are only slightly concave at this stage, but Mammillaria is markedly concave (Fig. 46). Changes in receptacle shape are a result of cessation of apical activity and growth of an intercalary ring meristem consisting of arcuate files of cells.

DISCUSSION--Patterns of carpel and stamen initiation have been resolved by examining floral meristems with both surface and section photomicrography. Carpel and some stamen primordia originate simultaneously as two concentric circles, but their simultaneous appearance is obscured by rapid stamen development and invagination of the receptacle. From the primary ring of stamens, more stamens arise centrifugally, utilizing

the remaining meristem. Stamen primordia are tightly packed but no obvious spiral phyllotacty is evident in any taxon studied. Some cluster patterns of stamens, particularily in Echinocereus, are similar to those in reports of stamen development for the Dilleniidae (Leins, 1975). This developmental pattern and the presence of stamen trunk bundles in many taxa (Buxbaum, 1953; Tiagi, 1963; Boke, 1963, 1960) suggests that the cactus androecium consists of a variable number of stamen fasicles and that a lower number of stamens than is presently found in most taxa is the ancestral condition. An increase in stamen numbers by centrifugal initiation rather than centripetal development is probably related to the lack of apical growth and to the presence of radial growth. Cessation of apical growth limits the addition of androecial tissue apically and radial expansion creates meristematic tissue outside the initially formed primary stamen ring (Stebbins, 1974).

Formation of a receptacle cup surrounding the ovary occurs in all cacti (Boke, 1964). At anthesis Pereskia pititache (Boke, 1963), P. aculeata (Boke, 1966), and P. diaz-romeroana (Boke, 1968) have superior ovaries that become pseudoinferior by extensive receptacular growth, forming berry-like fruits. This ontogenetic shift during fruit maturation from superior to pseudoinferior (Boke, 1964) occurs during early floral initiation in Opuntiae and Cacteae. Typically, stamens and carpels arise in nearly the same horizontal plane but thereafter the carpels are shifted to a lower level. In M. compressa, however, receptacle deformation is such that carpels are initiated below the stamens. The earlier cessation of apical growth is correlated with an increasing prococity of meristem activity in the files of arcuate cells and therefore results in the

increasingly earlier shift from a convex to a concave floral apex.

The trend of earlier receptacle cup formation generally agrees with views of phylogeny in the Cactaceae. More derived taxa have earlier meristem activation or increasing precocity of the gene (Stebbins, 1974). For example, receptacle meristem activity is triggered during fruit development in Pereskia (Boke, 1963, 1966, 1968), during carpel and stamen initiation in Opuntia, Epiphyllum and Echinocereus, and during perianth initiation in Mammillaria. Mammillaria is considered by Hunt (1967) and Backeberg (1970) as the most derived of the five genera examined, and, consistent with the ideas of Stebbins concerning increasing precocity of the gene, the meristem forms the receptacular cup earlier in this genus than in the other four. Britton and Rose (1919-1923) placed Epiphyllum and other epiphytes after Mammillaria in their classification scheme, but early floral ontogeny does not support this position. Epiphyllum, which has one of the longest floral tubes in the family, initiates extensive receptacle growth only after all primordia have been initiated and ceases apical growth slightly before stamen initiation. In this respect it resembles Opuntia more closely than other members of the Cacteae. This late development of the floral tube supports the views of Hunt and Backeberg that epiphytes are one of the early forms of Cacteae, being derived in their habit but maintaining a large number of relic characteristics.

LITERATURE CITED

Backeberg, C. 1970. Das Kakteen Lexikon. Gustav Fischer, Jena.

Boke, N. H. 1952. Leaf and areole development in Coryphantha. Amer. J. Bot. 39: 134-145.

_____. 1955. Dimorphic areoles of Epithelantha. Amer. J. Bot. 42: 725-733.

_____. 1963. Anatomy and development of the flower and fruit of Pereskia pititache. Amer. J. Bot. 50: 843-858.

_____. 1964. The cactus gynoecium. a new interpretation. Amer. J. Bot. 51: 598-610.

_____. 1966. Ontogeny and structure of the flower and fruit of Pereskia aculeata. Amer. J. Bot. 53: 534-542.

_____. 1968. Structure and development of the flower and fruit of Pereskia diaz-romeroana. Amer. J. Bot. 55: 1254-1260.

Britton, N. L., and J. N. Rose. 1919-1923. The Cactaceae. Carnegie Inst. Washington. Publ. 248.

Buxbaum, F. 1953. Morphology of cacti. section II. flower. Abbey Garden Press, Pasadena, Calif.

Cutak, L. 1951. A new scarlet Pereskia. Cact. Succ. Jour. 23: 171-173.

Gibson, A. C. 1975. Another look at the cactus research of Irving Widmer Bailey, pp. 76-85. In, C. Glass and R. Foster (eds.), 1975 Yearbook, Cact. Succ. J. (Los Angeles) Suppl.

Huber, J. A. 1929. Blüten und Samenentwicklung der Kakteen und ihre Bebeutung für deren systematische Stellung. Monatschr. Deutsch. Kakteen-Gesellsch. 1: 175-190.

Hunt, D. R. 1967. Cactaceae. In Hutchinson, J. The genera of flowering plants, Vol. 2. Oxford: Clarendon Press.

Leins, Peter. 1975. Die Beziehungen zwischen multistaminaten und einfachen Androeceen. Bot. Jahrb. Syst. 96: 231-237.

Payer, J. B. 1852. Organogénie de la classe des Cactoidées (Cactées Ficoides et Têtragoniees), et de celle des Berbérinées (Berbéridées, Ménispermées). Ann. Sci. Nat. Ser. 3, 18: 233-256.

Sass, J. E. 1940. Elements of botanical microtechnique. McGraw-Hill Book Co., New York.

Stebbins, G. L. 1974. Flowering plants evolution above the species level. Belknap Press of Harvard University Press, Cambridge.

Tiagi, Y. D. 1955. Studies in floral morphology. I. Vascular anatomy of the flower of certain species of the Cactaceae. Jour. Indian Bot. Soc. 34: 408-428.

_____. 1957. Studies in floral morphology. III. A contribution to the floral morphology of Mammillaria tenuis DC. Jour. Univ. Sauger (India) Pt. II. (B), 7-31.

_____. 1963. Studies in floral morphology. VIII. A further study of the vascular anatomy of the flower of certain species of the Cactaceae. Jour. Indian Bot. Soc. 42: 559-573.

FIGURE LINES --1

Fig. 1-11. Pereskia corrugata. 1-6. Darkfield photomicrographs of living specimens showing stamen and carpel initiation. Some tepals remain; other were removed for visibility. 1. Floral meristem during early initiation. Some stamen members more prominent; carpels barely discernable (arrows). x 90. 2. Early stamen primordia encircle meristem; carpel meristem ring forming. x 90. 3. Centrifugal initiation of stamens beginning; five carpels are present (arrow pointing to carpel). x 90. 4. and 5. Centrifugal stamen initiation; carpel meristem ring present. x 90 and x 55, respectively. 6. Stamen and carpel initiation complete; central black region with five points results from cutting off the bottom of the ovary locule; the five points are carpel sinuses or small individual locules. x 50. 7-10. Median longisections of floral meristems. all x 150. 7. Floral meristem, showing tepal formation. 8. Meristem in Fig. 1. Carpel initiation shown by periclinal divisions in subepidermal layer (arrow); androecium meristem present. 9. Meristem in Fig. 3. Carpel primordia distinguishable and androecium meristem active. 10. Meristem in Fig. 5. Residual meristem convex; numerous stamen primordia; two carpel primordia in middle. 11. Flowering shoot with flower split longitudinally. x 1.0. t- tepal; s- stamen; c- carpel.

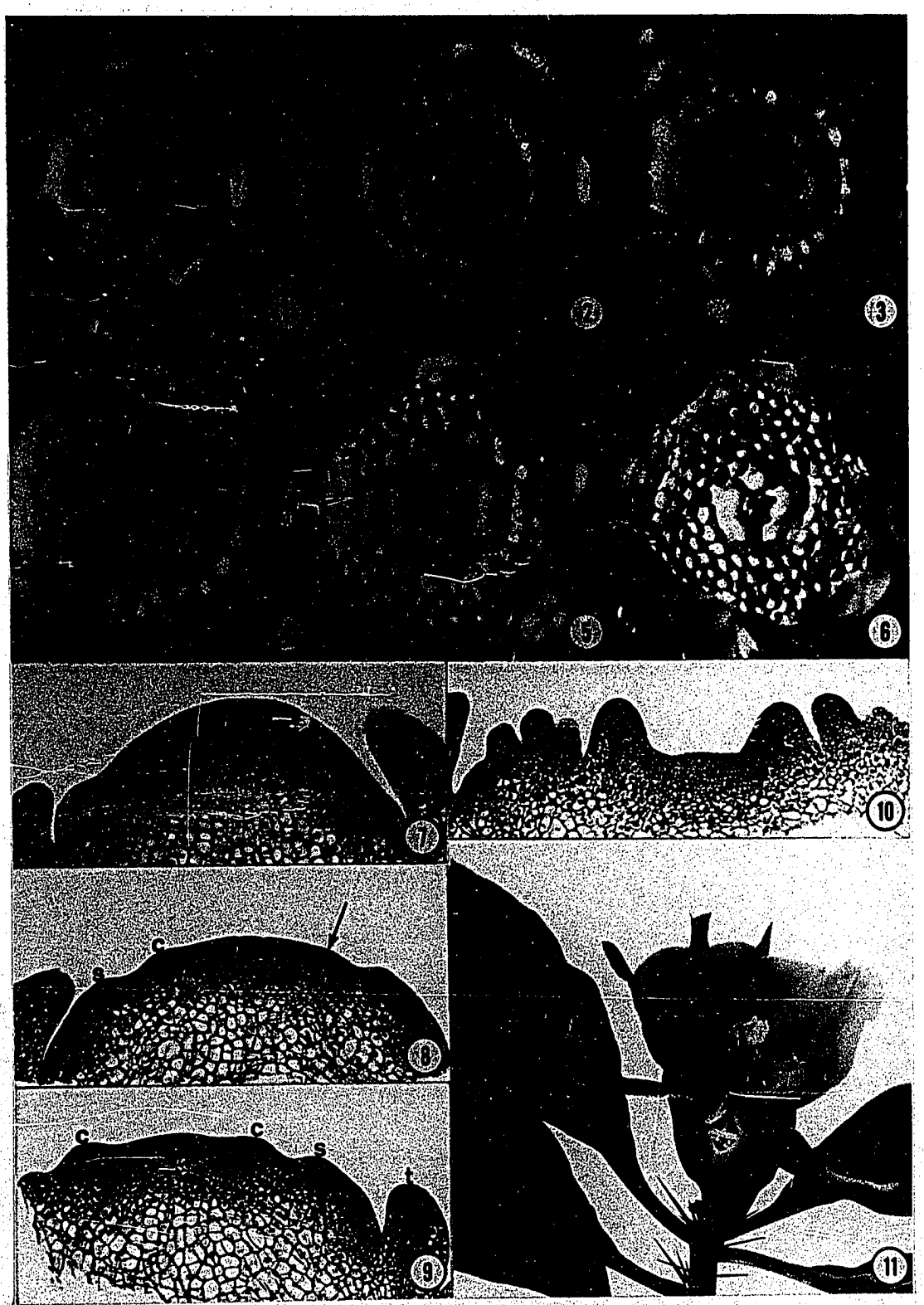

4
3
5
6
7
10
S
C
8
C
C
S
9
11

FIGURE LINES --2

Fig. 12-21. Opuntia engelmannii. 12-16. Darkfield photomicrographs of living specimens, showing sequence of stamen and carpel initiation. 12. Floral meristem prior to stamen and carpel initiation is nearly flat with slightly convex center. x 55. 13. Nearly simultaneous initiation of carpel primordia and of first stamens. x 100. 14. Later stage, primordia more pronounced. Note region (arrow) free of primordia between stamens and carpels. x 55. 15. Beginning of centrifugal stamen initiation (arrow). x 70. 16. Centrifugal stamen initiation progressing, carpels crescentic. x 95. 17-20. Median longisections of floral meristems. 17. Meristem in Fig. 12. Mantel three to four cell layers thick. x 150. 18. Meristem in Fig. 14, showing stamen and carpel primordia originating from subsurface layers. x 150. 19. Meristem in Fig. 15, showing elevation of stamen and carpel primordia by files of arcuate cells. x 150. 20. Meristem in Fig. 16. Residual meristem convex; androecium further elevated. x 110. 21. Portion of stem with bud and flowers. Ovary locule deep inside receptacle, stamens line a conical receptacle with perianth segments around upper portion. x 1.0. f- arcuate files of cells; c- carpel; s- stamen; t- tepal.

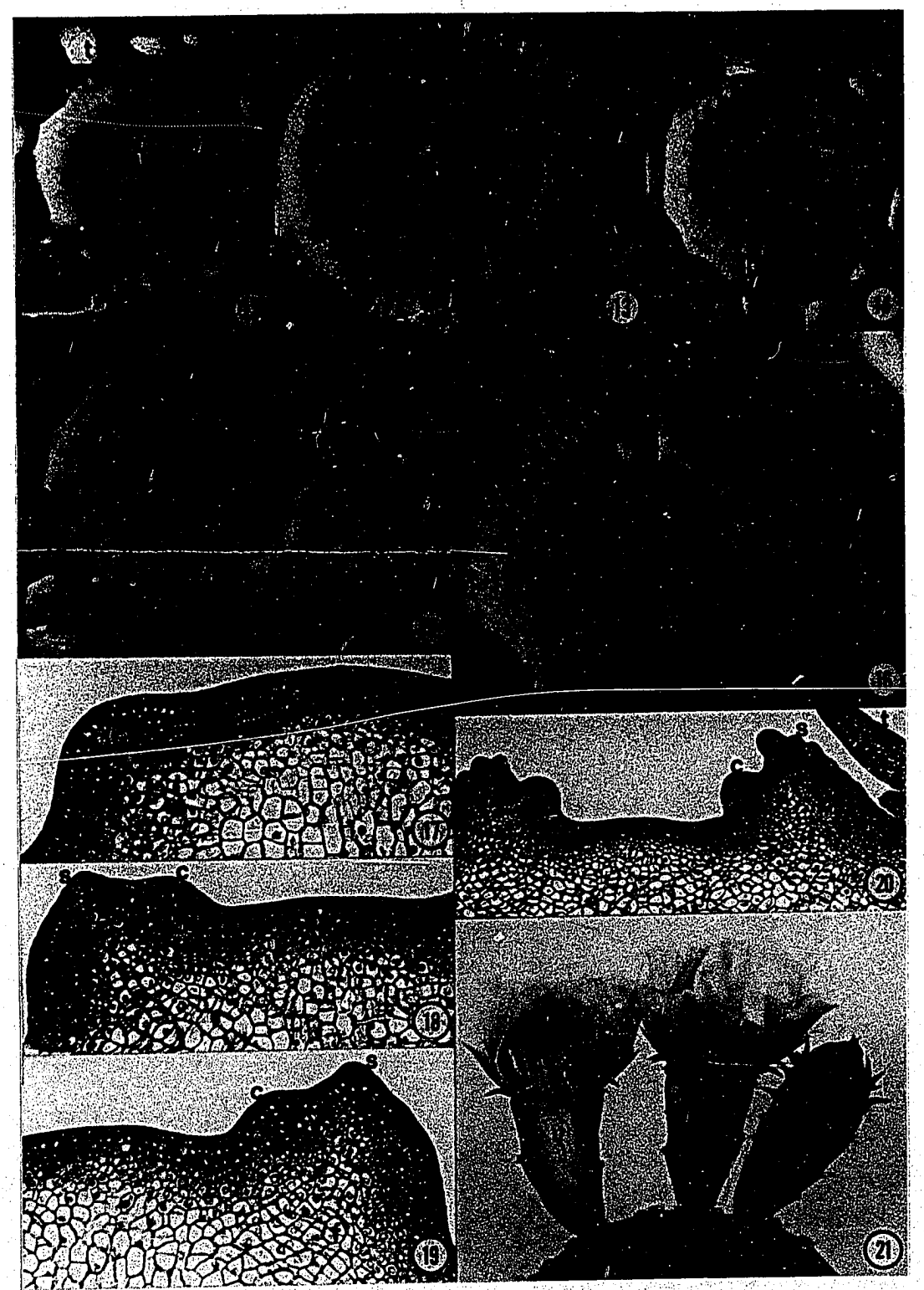


FIGURE LINES --3

Fig. 22-31. Epiphyllum strictum. 22-25. Darkfield photomicrographs of living specimens, showing stamen and carpel initiation. 22. Tepal initiation nearly complete; stamen primordia forming at edge of flattened meristem; first of carpel primordia discernable (arrow). x 90. 23. First stamens encircle flattened meristem; several carpel primordia discernable. x 90. 24. Centrifugal stamen initiation beginning; carpel whorl clearly visible, each separate carpel developing a sinus (arrow). x 90. 25. Centrifugal stamen initiation proceeding. x 55. 26-30. Median longisections of floral meristems. 26. and 27. Meristems prior to stages represented in Fig. 22. Apparent primordia (arrow) represent edge of floral meristem. No primordia are present during conversion from convex to concave surface. x 100. 28. Meristem in Fig. 22. Differential staining and cell division in subsurface layers represent carpel initiation (arrow) and the androecium meristem. x 150. 29. Meristem in Fig. 24. Carpel and stamen primordia. x 150. 30. Meristem in Fig. 25. Centrifugal stamen initiation; files of arcuate cells below stamen meristem. x 150. 31. Stem and flower with long receptacular floral tube. Ovary at base (arrow). x 0.4. f- arcuate files of cells; t- tepal; c- carpel; s- stamen.

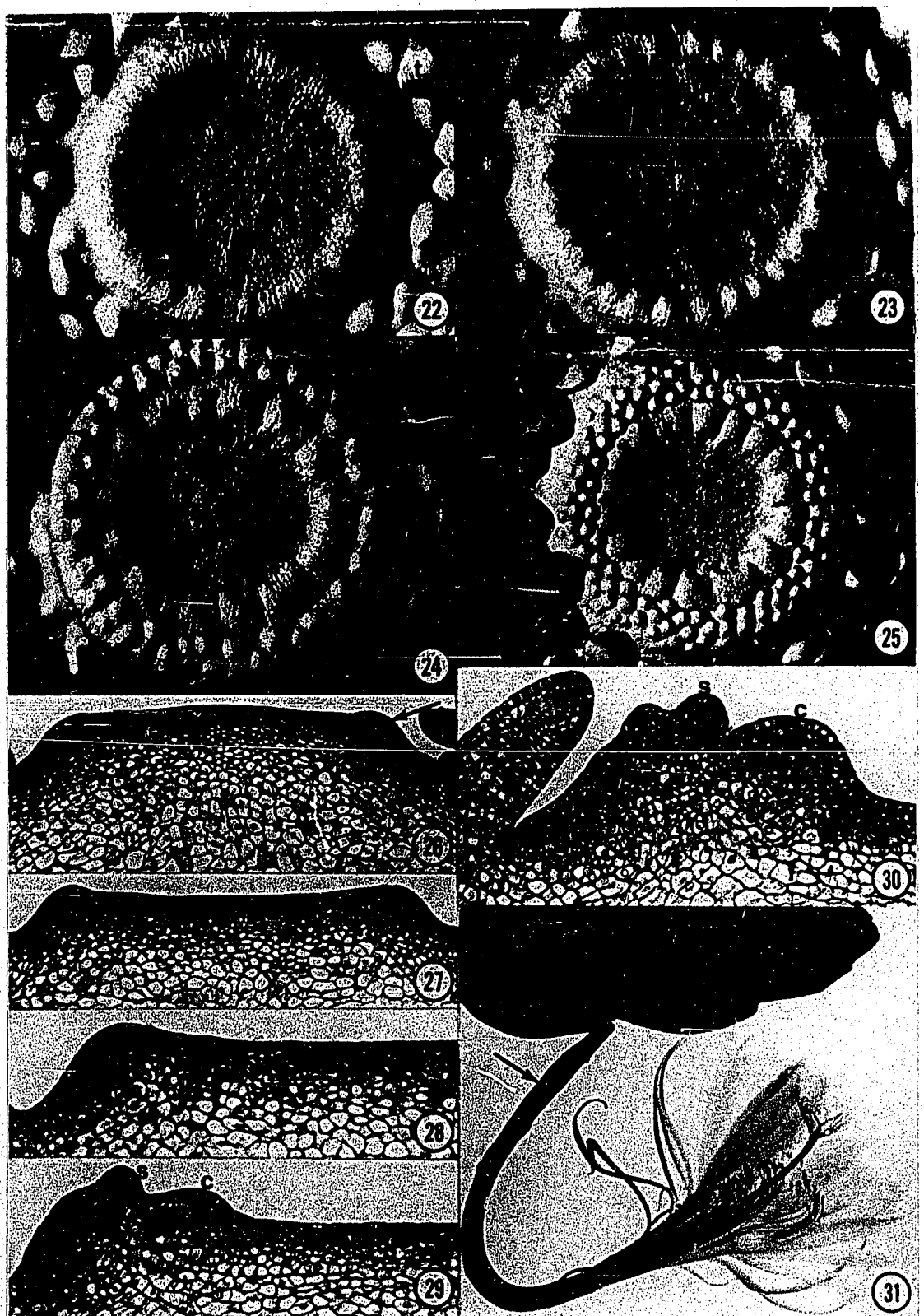

22
23
24
25
26
27
28
29
30
31
S
C
S
C

FIGURE LINES --4

Fig. 32-39. Echinocereus reichenbachii var. albispinus. 32-35. Darkfield photomicrographs of living specimens, showing tepal, stamen, and carpel initiation. 32. Areole primordia to outside (arrow) intergrade with tepal primordia. x 55. 33. Floral meristem. Initiation of first ring of stamens; incomplete carpel ring barely discernable (arrow points to one carpel). Droplets are exudation from meristem cells and are an artifact. x 80. 34. Centrifugal stamen initiation; note earlier stamen initiation along certain radii (arrows); carpel primordia form a continuous inner ring but are obscurred by receptacle cup (cf. Fig. 37). x 80. 35. Centrifugalstamen initiation proceeding in some areas more rapidly than in tohers (arrow); individual carpel primordia visible. x 55. 36-38. Median longisections of floral meristems. 36. Meristem in Fig. 33. Stamen primordia and files of arcuate cells. x 150. 37. Meristem in Fig. 34. Carpel primordia on inner lining of receptacle cup. x 150. 38. Meristem in Fig. 35. Concave residual meristem; carpel and stamen primordia. x 150. 39. Anomalous apical flower with areoles on floral tube, tepals at apex, stamens lining floral tube, and inferior ovary. x 1.0. f- arcuate files of cells; t- tepal; s- stamen; c- carpel.

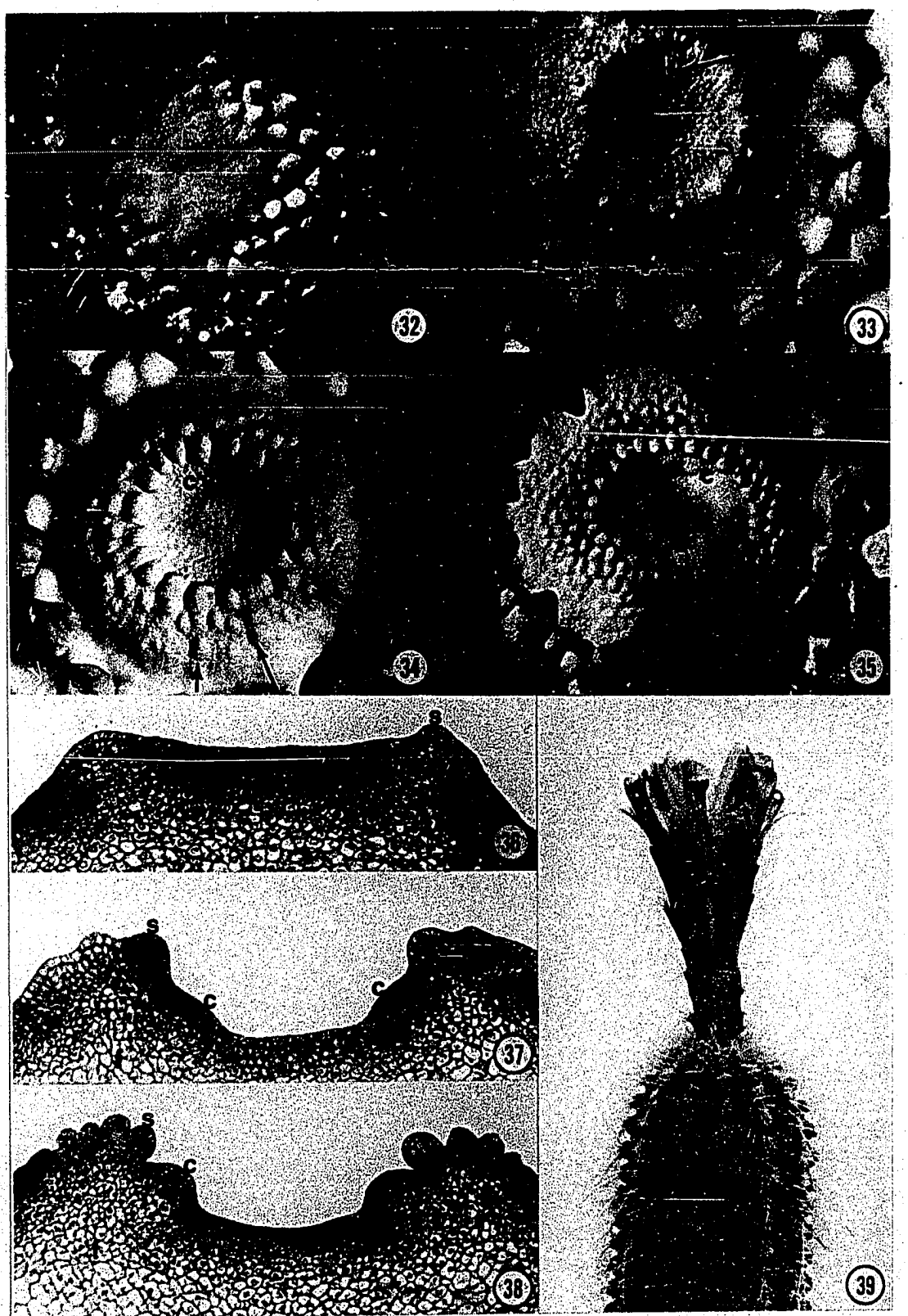
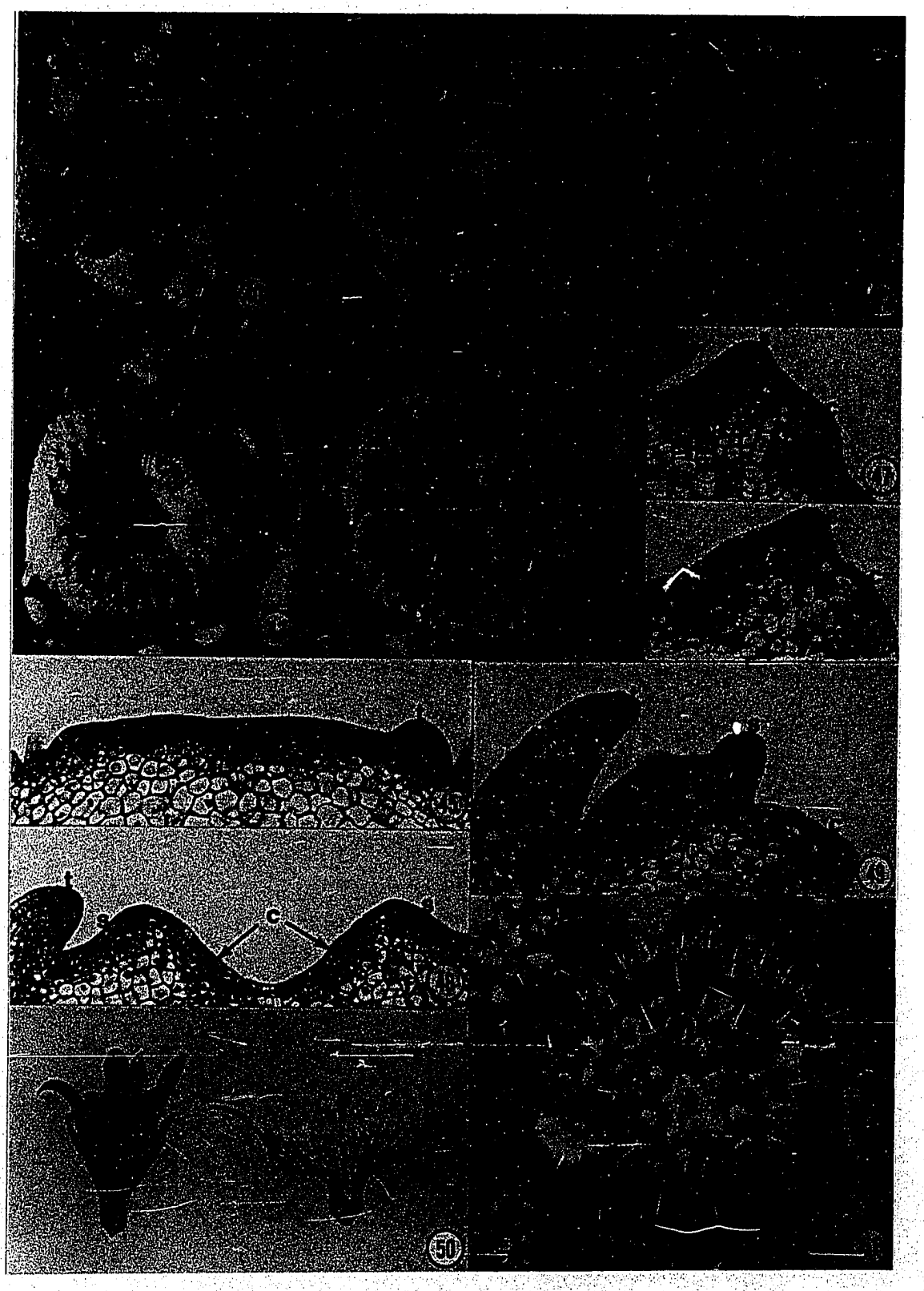

32
33
34
35
S
C
36
37
38
39

FIGURE LINES --5

Fig. 40-51. Mammillaria compressa. 40-44. Darkfield photomicrographs showing tepal, stamen, and carpel initiation. 40. Floral meristem. Tepal initiation on a broadly convex surface. x 90. 41. Meristem invaginated in center; upper rim bears early stamen primordia (arrow). x 90. 42. First stamen ring complete; carpel primordia forming inside receptacle cup (cf. Fig. 47). x 90. 43. Centrifugal stamen initiation beginning; five carpel primordia present (arrow). x 90. 44. Stamen initiation continues; congential fusion of carpels evident (arrow); central black region results from removal of residual central meristem. x 90. 45-49. Median longisections of floral meristems. 45. Meristem in Fig. 40; tepal initiation and single-layered tunica. x 150. 46. Meristem in Fig. 41; files of arcuate cells; darker staining areas represent early stamen and carpel primordia (arrows). x 150. 47. and 48. Meristem in Fig. 42 and 43, respectively. Further carpel and stamen development. x 200. 49. Meristem in Fig. 44, showing tepal, stamen, and carpel primordia. x 200. 50. External view of flower to left. Ovary region of receptacle free of areoles and tepals. Median sectin of flower to right. Note attachment of stamens and tepals and inferior ovary. x 2.5. 51. Plants with flowers and tubercles in a phylotactic spiral. x 1.0. f- arcuate files of cells; t- tepal; c- carpel; s- stamen.


S
C
50