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VOLUME XLII A

MAHESHWARI COMMEMORATION VOLUME

Editor

T. S. SADASIVAN

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PROFESSOR P. MAHESHWARI

DEDICATION

IN the summer of 1962, a suggestion came forward from Professor B. G. L. Swamy to organize and edit a Festschrift to Professor P. Maheshwari on his entering the sixtieth year in November, 1963. This was enthusiastically accepted by the Members of the Council of the Indian Botanical Society. The result was that a Working Group was formed with four morphologists and embryologists: Prof. J. Venkateswarlu, Andhra University, Waltair, Prof. B. G. L. Swamy, Presidency College, Madras, Prof. V. Puri, School of Plant Morphology, Meerut, Dr. B. M. Johri, University of Delhi, and myself sandwiched between morphologists. Invitations were immediately issued to Indian botanists for liberal donations towards this project and simultaneously many distinguished botanists at home and abroad were invited to send articles for this Commemoration Volume. The response on both counts was most gratifying and we have much pleasure in releasing this excellent collection of able writings in many branches of botany from all corners of the world. I wish to record here, on behalf of the Working Group (Commemoration Committee) and Council of this Society, our grateful thanks to all those who co-operated in this joint endeavour, particularly my colleagues here and in Delhi who spared time in editorial matters.

A detailed account of Professor Maheshwari's career and achievements has been kindly prepared by Dr. B. M. Johri and follows immediately. There are, however, certain aspects of the professor which perhaps are better covered by a person who did not have the privilege of being his pupil but had the honour of being examined at the Master of Science Examination in 1936, and subsequently been an admirer, friend and close associate. At the time I met him first, Professor Maheshwari was in his thirties and already his reputation in his chosen field was high and we still vividly remember the sharp and yet kindly *viva voce* we were subjected to in our examination. This made a deep and lasting impression on the examinees.

From 1950, my association with Professor Maheshwari has been more intimate than before and I have had many opportunities of discussing varied problems of teaching and research and, truly, these occasions have been most pleasant, stimulating and profitable.

Professor Maheshwari is to be regarded as father, mother and attending gynaecologist for the subject of plant morphology and embryology in India. Not content with these three major roles, he has also played the part of pediatrician and the result is, and has been, that a most vigorous school developed in his good care at Delhi and, indeed, his former colleagues trained in that field are holding their own in many parts of India, in the universities and in research institutes.

As a teacher his thoroughness and erudition are hardly excelled in this sub-continent and, in fact, his lectures and writings are a model, exact but not exacting and punctuated with a rare sense of humour.

In the words of *Muṇḍaka Upanishad:*

पुरुष एवेदं विश्वं कर्म तपो ब्रह्म परामृतम्।
एतद्यो वेद निहितं गुहायां सोऽविद्याग्रन्थि विकिरतीह सौम्य॥

"The person (Purusha) himself is everything here:
Work (*karman*) and austerity (*tapas*) and Brahma, beyond death
He who knows that, set in the secret place (of the heart)—
He here on earth, my friend, rends asunder the knot of ignorance".

Professor Maheshwari, the teacher and humanist, to be what he is, must have drunk deep at the fountain-head of Indian Philosophy—the Vedanta.

In the field of embryology, Professor Maheshwari's researches are so well known that it needs no particular reference here. Nonetheless, I would like to mention that ever since the discovery of the indole compounds, gibberellins, kinetin and other auxins and anti-auxins, many well-known physiologists have shifted their interests to the border line field of 'growth and cell proliferation'. The diehard embryologists were none too slow to grasp this new situation and they entered this domain in great numbers all over the world. Professor Maheshwari was again a pioneer in this regard in this country. With his customary gusto he plunged into morphogenesis and embryo culture while many in this country still hesitate to do so. In an ultimate analysis, it is the alert mind and the creative work that it accomplishes that matters. I may be permitted to quote the *Kaṭha Upanishad:*

इन्द्रियेभ्यः पराह्यर्था अर्थेभ्यश्च परं मनः।
मनसस्तु परा बुद्धिर्बुद्धेरात्मा महान्परः॥
....अव्यक्तात्पुरुषः परः।
पुरुषान्न परं किंचित् ....

"Higher than the senses are the objects of sense.
Higher than the objects of sense is the mind (*manas*);
And higher than the mind is the intellect (*buddhi*).
Higher than the intellect is the great Self (Ātman)."

"Higher than the Unmanifest is the Person.
Higher than the Person there is nothing at all."

The *Chāndogya Upaniṣad* has an interesting reference to the seed (a field in which Professor Maheshwari is so vitally interested) which I wish to quote here. This relates to discussions between

Śvetaketu and his father Uddālaka concerning the key to all knowledge. Śvetaketu under advice from his father spent twelve years studying the Vedas and returned to his father's home at the age of twenty-four, conceited, thinking himself learned, proud. Uddālaka theorized that out of nothing, nothing can come. In the causeless beginning there must have been a first cause: *Sat* meaning, Being with consciousness. *Sat* willed that it may expand and multiply, so it produced *Tejas* (light) and then water and it produced food and all the living things of the world. Śvetaketu asked, most pertinently, then how can this vast universe with its multitudinous variety be produced in this simple way? Uddālaka, in turn, asked Śvetaketu to fetch a fruit of the big banyan tree (*Nyagrodha*—sacred fig) and break it.

"What do you see there?"
"These little seeds."
"Break one of the little seeds."
"What do you see there?"
"Nothing at all" said the son.

Said Uddālaka: "Yet in that subtle substance inside, which your eye does not even perceive, existed all this big branching *Nyagrodha* tree. Do you wonder at it? Likewise, all that exists, the universe, was in that *Sat* which thou art."

"If the *Sat* is the all-pervading cause of all, why is it not perceived clearly?" As a lump of salt is dissolved in water and disappears, so too is the *Sat* lost from the view of the world. But it is everywhere in the universe, as the salt is present in water.

I have not mentioned hitherto an aspect of Professor Maheshwari's personality which those of us who have known him for years have learnt to respect and admire. It is his devotion to Science and his friendly and kindly feelings to all those who have had the privilege of knowing him. I am tempted to quote again from *Brihad-Āranyaka Upanishad:*

यस्यानुवित्तः प्रतिबुद्ध आत्मा····।
····तस्य लोकाः स उ लोक एव॥

"He who has found and has awakened to the Soul
The world is his: indeed, he is the world itself."

University Botany Laboratory
MADRAS
T. S. SADASIVAN

August 17, 1963

PROFESSOR P. MAHESHWARI
BIOGRAPHY AND CONTRIBUTIONS TO BOTANY

BORN at Jaipur on the 9th of November, 1904, the young Maheshwari had his schooling in his home town. Then, in 1921, he joined the Ewing Christian College, Allahabad, from where he passed the Intermediate Examination in 1923, and took the Bachelor's degree in Science in 1925. At this institution he came under the influence of the late Dr. Winfield Dudgeon, an American missionary teacher, and this association lasted until Dudgeon died after a brief illness in 1932 at Chicago in the U.S.A.

The M.Sc. and D.Sc. degrees were taken from the University of Allahabad in 1927 and 1931, respectively. For his doctoral thesis Maheshwari worked under the inspiring guidance of Dudgeon on the morphology, anatomy and embryology of some angiosperms. This was the beginning of a career which brought him international recognition.

While a research student at the University of Allahabad, Maheshwari simultaneously started his teaching career at the Ewing Christian College in 1928. After submitting his doctoral thesis in 1930, he joined the Agra College, Agra, and continued on the staff of the Botany Department at first as Lecturer and then as Associate Professor until 1936 when he left for his first European tour. Soon after his return he joined the University of Allahabad (1937–39) and for a short period (July–September, 1939) also served at the Lucknow University. In November 1939, he joined the University of Dacca (then in undivided Bengal before the partition of India in 1947) as Reader and Head of the Department of Biology. He again left for a tour of Europe and the U.S.A. in 1945 and returned in 1947 when he was promoted to a Professorship and also served the University as Dean of the Faculty of Science. At Dacca he started the B.Sc. courses in Biology in 1939 and post-graduate classes in Botany in 1947. In 1949, the late Sir Maurice Gwyer, then Vice-Chancellor of the University of Delhi, invited him to join as the Professor and Head of the Department of Botany which he did in March 1949.

Professor Maheshwari has travelled very widely. During his first European tour (1936–37) he worked at the University of Kiel (Germany) and visited several Universities in the continent and in England. One of the persons who inspired him most was Professor Karl Schnarf of the University of Vienna (Austria). It was during this brief visit that the idea of writing a book on the embryology of angiosperms took root. Having published in *Current Science* (1935) a brief review on embryological studies in India, he now wrote a comprehensive review on the types of embryo sacs in angiosperms which appeared in the *New*

Phytologist in 1937. This was completed during his stay in Europe and England.

During his second visit abroad in 1945, he devoted most of his time at the Harvard University in completing his projected book entitled *An Introduction to the Embryology of Angiosperms* which was published in 1950 by McGraw-Hill, New York. This book has since been reprinted twice. It was during a visit to the Smith College, Northampton, Mass., that the late Professor A. F. Blakeslee aroused his interest in experimental embryology. Thus, a chapter under this title found place in the above book, and his interest in this field has led to the establishment of a large section of experimental morphology and embryology at the University of Delhi. Another book entitled *Recent Advances in the Embryology of Angiosperms*, edited by him, is now in press.

Other visits abroad include those in 1950, 1954 and 1959, to attend the International Botanical Congresses at Stockholm, Paris, and Montreal, respectively. At the invitation of UNESCO he visited Indonesia in 1952, and Egypt in 1954. In 1958, he paid a short visit to the U.S.S.R. as a member of a Scientific Delegation sponsored by the Government of India. It was during this trip that he was officially presented a copy of the Russian translation of his book on the *Embryology of Angiosperms*. In the summer of 1959, he was a Visiting Professor at the University of Illinois, Urbana, where he delivered a course of lectures on the Embryology of Angiosperms. At Urbana he also continued to work on a new book on gymnosperms which will shortly go to press. Earlier, in 1956, he paid a short visit to the U.S.A. as a member of the study group for studying the courses in General Education in American Universities. This project was jointly sponsored by the Government of India and the Ford Foundation for introducing the teaching of this subject in Indian Universities.

In 1961, at the invitation of the Federal Republic of Germany, he visited several West German Universities and also attended the annual session of the German Botanical Society at Halle.

The numerous visits abroad provided opportunities for establishing personal contacts with colleagues in various parts of the world. They were also utilized in collecting plant materials for embryological studies at home.

In 1934, Professor Maheshwari was elected a Fellow of the Indian Academy of Sciences; in 1935 a Fellow of the National Institute of Sciences of India; in 1947 a Corresponding Member of the American Botanical Society and Honorary Foreign Fellow of the American Academy of Arts and Sciences; in 1959 he was made Foreign Member of the Kaiserlich Deutsche Akademie der Naturforscher Leopoldina, Halle; and in 1959 he received the honorary doctorate of the McGill University, Montreal. In recognition of his contributions to botany, in 1959 he was awarded the Birbal Sahni Medal of the Indian Botanical Society.

In 1950, he presided at the Botany Section of the Indian Science Congress at Poona, and later in the year he was one of the Vice-Presidents of the International Botanical Congress held at Stockholm. In 1951, he was elected the President of the Indian Botanical Society. In 1954, he was President of the Embryology Section of the International Botanical Congress held at Paris, and in 1959 Vice-President of the Morphology Section of the International Botanical Congress at Montreal. From 1956–62 he served as the Biological Secretary of the National Institute of Sciences of India. He is President of the National Academy of Sciences, India, for the current year.

With the excellent technique which Dr. Dudgeon learnt under the famous Professor C. J. Chamberlain at the University of Chicago, Chicago, he trained Maheshwari to excel him. This will be borne out by his several publications on Microtechnique, and the microscopic slides prepared by him have found a place in innumerable institutions in India and abroad. He possesses what is probably the world's largest collection of materials imbedded in paraffin and microscopic slides on all groups of plants owned by any single individual. His phenomenal success is due not only to his own hard work but also that of his wife, Shanti Maheshwari, who was his close collaborator in microtechnique in the earlier years of their married life.

Under Maheshwari's guidance there was started a school of plant morphology at Agra College, Agra, as early as 1931. Some of the students trained there are Dr. Bahadur Singh, Assistant Director, National Botanic Gardens, Lucknow; Professor V. Puri, School of Plant Morphology, Meerut; Dr. S. P. Capoor, Virus Pathologist, Plant Virus Research Laboratory, Poona; and Mr. H. R. Bhargava, Saugar University, Sagar. Among his other pupils now occupying important positions in Indian botany are Dr. R. V. Sitholey, Assistant Director, National Botanic Gardens, Lucknow; Professor K. S. Bhargava, University of Gorakhpur; Dr. R. Khan, Reader in Botany, Muslim University, Aligarh; and Dr. D. D. Pant, Assistant Professor of Botany, University of Allahabad.

A much bigger school came up at the University of Delhi with his joining the Department of Botany in 1949. Since 1952, as many as 467 papers have been published, and 54 Ph.D. theses approved by the University of Delhi. He has himself published 134 papers from 1929 to 1963. A list of his publications is appended at the end which will serve to show the wide interest of Professor Maheshwari. From 1929–63, over 600 papers have been published by him and his collaborators.

Soon after his return from the International Botanical Congress at Stockholm (1950), he started a departmental bulletin of a semi-technical nature, the *Botanica*, which is now being subscribed for by almost all Indian Universities and Colleges that teach botany. Simultaneously, he sounded numerous friends and collaborators in India and abroad if it was time to start a journal devoted exclusively to plant

morphology—a branch of botany in which the largest number of contributions are published annually in India. This led to the establishment of the International Society of Plant Morphologists of which he was elected the first President. He is now one of the two Vice-Presidents. The Society is mainly concerned with the diffusion of knowledge in the fields of plant morphology, anatomy and embryology. The chief purpose of establishing this Society was to publish a journal of plant morphology and, therefore, it was christened *Phytomorphology*. This is a truly International Journal of Plant Morphology in which almost every issue contains articles from both India and outside. Professor Maheshwari is the Founder-Editor of the Journal and with the appearance of the first issue in 1951 the cause of plant morphology was further advanced. He is continuing with the editorship and the journal is now in its 13th year. *Phytomorphology* is widely circulated in India and abroad, and is one of the premier scientific journals in India.

Some other publications from this department include *Modern Developments in Plant Physiology* (1958); *Proceedings of the Summer School of Botany*, Darjeeling (1962); *Plant Embryology—A Symposium* (1962); *Plant Tissue and Organ Culture—A Symposium* (1963) and *Fifty Years of Science in India : Progress of Botany The Dictionary of Economic Plants of India* is now in press. He has recently been commissioned by the National Council of Educational Research and Training, Government of India, to prepare a *Text Book of Biology for the Higher Secondary Classes*. This book is being written by 17 teachers from various Indian Universities and in due course it would be translated into different regional languages.

Professor Maheshwari is an excellent teacher, technician, research worker, and organizer. He aims at nothing but the very best and does not tolerate poor standards. He is ever ready to help whomsoever he can and takes a broad interest in the general advancement of botany at all levels.

In closing this biographical note, a word may be said about his family. His father, Mr. Bijey Lal, was employed in the Office of the Medical Department in Jaipur. He was very keen to give the best possible education to his son and in 1921 sent young Maheshwari to the University of Allahabad which was then considered to have a top place in the academic field. Throughout his life (he died in 1939) he did whatever he could to help his son. Similarly, his mother made many sacrifices to advance his career; she died in 1961 at the ripe age of 80 years. Professor Maheshwari has a sister who is settled in Calcutta. He married Shanti in 1923, and they have three sons and three daughters some of whom are themselves making significant contributions to botany.

I have known Professor Maheshwari since 1931, when I was a student at the Agra College, Agra, and took my doctorate under him in 1936. He is held in high esteem by his colleagues all over the world which will also be amply borne out by the response of so many friends who have contributed articles to this Commemoration Volume.

CONTRIBUTIONS TO BOTANY

This general account is not intended to be a review of the work done by Professor Maheshwari but will present some of the more significant achievements resulting from his own work and that carried out with his collaboration and under his direction.

CRYPTOGAMS

Considerable experimental work has been done on the moss, called *Physcomitrium coorgense*[1] (1963). Mature gametophytes, cultured on semi-solid media, produced apogamous sporogonia (50% were fertile) and callus masses from the stem and venter of the archegonium. The development of sporophytes in darkness far exceeded that in light. A large number of sterile sporogonia differentiated from the callus in cultures kept in darkness. In light, the same callus produced both leafy gametophytes and apogamous sterile sporogonia. In a liquid medium containing coconut milk the callus dissociated into single cells and cell aggregates which first organized into short filaments and later produced sterile sporophytes. Although the sporophytes developed beyond the confines of archegonia, they passed through the normal stages of embryogeny. The lack of meiotic divisions in the sporogonia suggests that the callus as well as the resulting sporogonia are haploid.

GYMNOSPERMS

In 1935, P. Maheshwari published an account of the development of gametophytes of *Ephedra foliata*[2] and cleared the confusion on the terminology for designating the constituents of the male gametophyte. It was also demonstrated that the nucellus is of dual origin—partly from the derivatives of the epidermis and partly from the parietal cell. The later development was also worked out but published[3] in 1944. An interesting feature is the occurrence of "double fertilization" which was reported earlier from Vienna in *E. campylopoda*.

The gymnosperms worked out at Delhi include *Biota orientalis*, *Cedrus deodara*, *Cephalotaxus drupacea*, *Cryptomeria japonica*, *Cupressus funebris*, *Cycas circinalis*, *Ephedra campylopoda*, *E. foliata*, *E. gerardiana*, *Fitzroya cupressoides*, *Gnetum gnemon*, *G. ula*, *Pilgerodendra uviferum*, *Pinus gerardiana*, *P. insularis*, *P. roxburghii* and *P. wallichiana*. Some observations have also been made on aspects of the life history of *Acmopyle pancheri* and are in course of publication.

P. Maheshwari and Vimla Vasil [4] (1961) made a detailed developmental study of the stomates of *Gnetum gnemon* and *G. ula* from epidermal peels of young leaves, collars (cupules), axes of male and female

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[1] M. Lal, In *Plant Tissue and Organ Culture—A Symposium*. Int. Soc Plant Morphol., Delhi.

[2] *Proc. Indian Acad. Sci.* **1 B.**

[3] R. Khan, *Proc. nat. Acad. Sci. India* **13**;

[4] *Ann. Bot., Lond.* **N. S. 25.**

cones, and the two outer envelopes of the ovule. These are invariably of the haplocheilic type, *i.e.*, the guard cells and the subsidiary cells have an independent origin. Sometimes, the epidermal cells next to the guard cells lie parallel to them but are not derived from the stomatal mother cells as reported earlier by H. Takeda[5] (1913). In other gymnosperms the stomatal structure is based on the study of fossils or mature leaves of living plants leading to the classification of this group into two categories—those with syndetocheilic and others with haplocheilic types of stomata[6] (1931). In most gymnosperms the stomates were considered to be haplocheilic; the syndetocheilic condition being confined to the Cycadeoidales, *Welwitschia* and *Gnetum*. The studies of P. Maheshwari and Vimla Vasil are of considerable significance since they show that the mere position of the subsidiary cells with respect to the guard cells does not lead to a correct interpretation.

The monograph on *Gnetum*[7] (1961) brings together, for the first time, the scattered information available in existing literature, and the detailed anatomical and embryological studies on *G. ula*[8] (1959) and *G. gnemon*[9] (1962) carried out in this laboratory. The pollen grains show a distinct prothallial cell, the development of the female gametophyte is tetrasporic, there is complete absence of archegonia, the embryo lacks the free nuclear phase, and has long, uninucleate, suspensor tubes. The mature embryo develops from the 'peculiar cell' derived from the suspensor tube. A special structure called the "feeder' serves to transfer food materials from the 'endosperm' to the embryo.

A quantitative survey of some nitrogenous substances and fats in the developing female gametophyte and embryo of *Pinus roxburghii*[10] (1958) was undertaken to follow the changes that take place during their development. At the end of October the percentage of alcohol-soluble nitrogen in the gametophyte is rather low. It gradually increases and reaches a peak at the end of November but declines again. Simultaneously, with the fall in the alcohol-soluble nitrogen, there is a rise in the protein content. Fat accumulation starts when the seed is about to mature.

It has been possible to culture the male cones of *Pinus roxburghii*[11] (1963) from the microspore mother cell to the shedding stage of pollen. The microsporangia dehisced and the pollen grains germinated *in situ* forming a loose mass of tubes some of which branched profusely. In some cultures the antheridial cell produced the stalk and body cells but the sperm nuclei were not formed.

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[5] *Ann. Bot., Lond.* N. S. **27.**

[6] R. Florin, *K. Vetensk. Akad. Handl.* **10.**

[7] P. Maheshwari and Vimla Vasil, *Gnetum*, C.S.I.R., New Delhi.

[8] Vimla Vasil, *Phytomorphology* **9.**

[9] Madhulata Sanwal, *Ibid.* **12.**

[10] R. N. Konar, *Ibid.* **8.**

[11] ———. In *Plant Tissue and Organ Culture—A Symposium.* Int. Soc. Plant Morphol., Delhi.

So far there are only three reports of the development of a callus mass from the pollen grains of gymnosperms in aseptic cultures. A haploid tissue ($n = 7$), capable of continuous subculturing, has recently been obtained from the mature pollen grains of *Ephedra foliata* (Konar, in press). Soon after germination supernumerary divisions occur in the pollen grain giving rise to a tissue in about 60% of the cultures.

From the hypocotyledonary callus of *Pinus gerardiana* (Konar, in press) single cells and cell aggregates have been obtained in liquid synthetic media containing tyrosine and tryptophan. The cell aggregates initially grew 2-dimensionally but later assumed a 3-dimensional pattern.

ANGIOSPERMS

TAXONOMY.—Duthie's (1903-20) "Flora of the Upper-Gangetic Plain and of the adjacent Siwalik and Sub-Himalayan tracts" is out of date and incomplete with regard to the State of Delhi. A recent study has brought out several interesting features; J. K. Maheshwari (in press) has described 531 plants which fall under 120 families. The current botanical names have been given and, wherever possible, the vernacular names have also been included. There are notes on the habit and habitat, time of flowering, and economic importance.

A few of the less known plants of this region are *Juncus bufonius* (Juncaceae), *Zeuxine strateumatica* (Orchidaceae), *Argemone ochroleuca* (Papavaraceae), *Basella rubra* (Basellaceae), *Cayratia carnosa* (Vitaceae), *Glossostigma spathulatum* (Scrophulariaceae), and *Ichnocarpus frutescens* and *Rauvolfia serpentina* (both of Apocynaceae). About 42% of the total number of species are pantropical (cosmopolitan in the tropics of the Old World), 21% constitute the African element, and 17% are indigenous.

ANATOMY.—For his doctoral thesis, in addition to other investigations, P. Maheshwari worked on the stem anatomy of *Rumex crispus*[12] (1929) and *Boerhaavia diffusa*[13] (1930), and gave a detailed description of the anomalous secondary growth in these plants. He explained that in *Rumex* the medullary bundles arise from the inner ends of the same provascular strands which produce the normal bundles. This finding contradicted the earlier supposition that the medullary bundles developed from the pith cells.

EMBRYOLOGY.—The following families have been studied with special emphasis on those genera which have a disputed taxonomic position: Acanthaceae, Aizoaceae, Alismaceae, Amaranthaceae, Anacardiaceae, Apocynaceae, Araceae, Aristolochiaceae, Begoniaceae, Berberidaceae, Bixaceae, Boraginaceae, Butomaceae, Cactaceae, Campanulaceae, Cannabinaceae, Capparidaceae, Commelinaceae, Compositae, Cornaceae, Crossosomataceae, Cruciferae, Cucurbitaceae, Cuscutaceae, Cyperaceae, Dilleniaceae, Dipsacaceae, Droseraceae, Eriocaulaceae,

[12,13] *J. Indian bot. Soc.* **8**, **9**.

Euphorbiaceae, Flacourtiaceae, Fumariaceae, Garryaceae, Gentianaceae, Gramineae, Haloragidaceae, Hydrocharitaceae, Juncaceae, Labiatae, Leguminosae, Lemnaceae, Lentibulariaceae, Liliaceae, Limnanthaceae, Loasaceae, Lobeliaceae, Loranthaceae, Magnoliaceae, Malvaceae, Molluginaceae, Moraceae, Moringaceae, Myrtaceae, Nyctaginaceae, Nymphaeaceae, Olacaceae, Onagraceae, Orchidaceae, Orobanchaceae, Papaveraceae, Piperaceae, Podostemaceae, Polemoniaceae, Ranunculaceae, Rhamnaceae, Rutaceae, Salicaceae, Salvadoraceae, Santalaceae, Schisandraceae, Scrophulariaceae, Selaginaceae, Stackhousiaceae, Tamaricaceae, Theaceae, Trapaceae, Ulmaceae, Umbelliferae, Verbenaceae, Winteraceae, Zingiberaceae and Zygophyllaceae.

The families Loranthaceae and Santalaceae are considered to be unusually difficult for embryological investigation. However, during the last 13 years, it has been possible to study 15 genera and 23 species of the Loranthaceae, and 10 genera and 17 species of the Santalaceae.

B. M. Johri and S. P. Bhatnagar[14] (1960) have reviewed the embryology of the Santalales. R. Narayana[15] (1958) observed a distinct vascular supply in the calyculus of *Nuytsia floribunda* and concluded that this structure is a true calyx. Ovules, in the usual sense, are absent and the megaspore mother cells differentiate in the lobes (comparable with rudimentary ovules) of the mamelon (placenta), or in the mamelon, or even in the ovarian tissue. The tips of the gametophytes may remain limited to the mamelon, or extend into the style up to various lengths. In some genera (*Barathranthus* and *Tupeia*) the upper ends of the embryo sacs invade the stigma and in *Helixanthera* they may even reach up to the stigmatic epidermis. The lower end of the embryo sac extends downward up to the base of the saucer-shaped or tubular hypostase. The endosperm is Cellular and normally all the endosperms developing in an ovary fuse to form a single composite structure. The zygote divides longitudinally followed by repeated transverse divisions. The biseriate proembryo is distinguishable into a terminal embryonal tier and the suspensor. The elongation of the suspensor pushes the embryonal tier into the ovary where endosperm is already well advanced. Further development of the embryo takes place here. The mature fruit is usually a pseudoberry with a vase-shaped endosperm enclosing a dicotyledonous embryo. The latter lacks a true radicle and in many species the cotyledons are closely appressed to each other producing a pseudo-monocotyledonous appearance. Polyembryony is quite common. The first connected account of the life history was given by P. Maheshwari and B. Singh[16] (1952) for *Macrosolen cochinchinensis*, and B. Singh[17] (1952) for *Dendrophthoe falcata*.

The embryology of the Santalaceae also shows many variations.[14] In *Leptomeria billardierii*[18] (1959) the sporogenous cells of the anther

[14] *Proc. nat. Inst. Sci. India* **26** B Suppl.

[15] *Phytomorphology* **8.**

[16] *Bot. Gaz.* **114.**

[17] *J. Linn. Soc. Lond.* **53.**

[18] Manasi Ram, *Nature, Lond.* **184.**

give rise to embryo sac-like structures. The ovules may be bi-, uni- or ategmic and the placental-ovular complex shows a reduction series. The outline of the embryo sacs is often tortuous, and the tip may extend up to the base of the style while the chalazal end may invade the pedicel. In *Quinchamalium*[19] (1961) the tips of the synergids form extensive, tubular haustoria which reach up to one-third the length of the style. These are the largest synergid haustoria so far reported in angiosperms. The endosperm may be Cellular or Helobial with primary as well as secondary haustoria. The endosperm consumes the integument, placenta and endocarp so that it comes to lie next to the sclerotic mesocarp.

The achene-bearing genera of the Ranunculaceae are usually considered to possess uniovulate carpels. However, in addition to the single fertile ovule, some genera like *Adonis annua*[20] (1962) and *Clematis gauriana*[21] (1962) have 2–4 accessory sterile ovules. The occurrence of a bisporic embryo sac in the Ranunculaceae has been established for the first time in *Adonis annua*. In *C. gauriana* the embryo sacs in the sterile ovules follow a tetrasporic development and the mature gametophyte has only four nuclei which usually organize into an egg, two polar nuclei, and one antipodal cell.

Following the studies of S. B. Kausik[22] (1938) on the elongated, tubular and coiled chalazal endosperm haustoria in the Proteaceae, similar structures have been observed in several other families especially the Cucurbitaceae[23] (1958) and Leguminosae[24] (1959). In both families the endosperm is of the Nuclear type and the seeds are exalbuminous. Soon after fertilization the lower end of the embryo sac elongates appreciably, and contains dense cytoplasm and free endosperm nuclei. It digests the reserve food materials from the adjoining tissue and this activity is at its maximum at the heart-shaped stage of the embryo. In the upper broader part of the embryo sac the endosperm becomes cellular and as it grows in size the haustorium becomes more and more coiled, ceases to be aggressive, and gets closely appressed.

The haustorium usually remains coenocytic but it becomes cellular in *Benincasa*, *Citrullus* and *Coccinea* although the cells are multinucleate. The tip is dilated in *Cucumis* and it also shows a free nuclear zone at the junction of the endosperm proper and the haustorium. Besides the haustorium, the peripheral basal region of the cellular endosperm produces mound-like projections which increase the absorptive surface. The length of the haustorium varies from 333 μ in *Citrullus fistulosus*

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[19] Saroj Agarwal, *Nature, Lond.* **192.**

[20] N. N. Bhandari, *Phytomorphology* **12.**

[21] M. R. Vijayaraghavan, *Ibid.* **12.**

[22] *See* P. Maheshwari, 1950. *An Introduction to the Embryology of Angiosperms*, McGraw-Hill, New York.

[23] R. N. Chopra and Saroj Agarwal, *Phytomorphology* **8.**

[24] *See* B. M. Johri and Sudha Garg, *Ibid.* **9.**

to 12,000 μ in *Cucurbita ficifolia*; the longest haustorium, 16,165 μ was observed in *Echinocystis lobata* (unpublished).

In the Leguminosae the haustorium becomes cellular in *Desmodium laburnaefolium*. At the globular stage of the proembryo the length of the haustorium varies from 400 μ in *Acacia senegal* to 18,400 μ in *Delonix regia*. It usually reaches right up to the base of the ovule; further extension being checked by the barrier tissue.

In the Loasaceae[25] (1962) the endosperm is of the Cellular type and there are well-developed micropylar and chalazal haustoria. In *Loasa bergii* the micropylar haustorium is coenocytic with hypha-like extensions which ramify inside the ovule, funiculus and sometimes even the placenta. In *Blumenbachia hieronymi* the micropylar haustorium is a conspicuous multi-celled structure with hypertrophied nuclei and it persists in the mature seed; the chalazal haustorium remains uninucleate and produces numerous branches which digest the surrounding integumentary cells.

We may now turn our attention to the study of the embryo. In 1937, F. H. Billings[26] reported that in *Isomeris arborea* (Capparidaceae) the embryo sac contains only two synergids and a single polar nucleus, but the egg, lower polar nucleus and antipodal cells are absent. He added that the embryo does not arise from the zygote but from one of the central endosperm nodules. This finding has always been open to doubt, and P. Maheshwari and R. Khan[27] (1953) and R. C. Sachar[28] (1956) undertook a detailed study of this plant. They showed that the zygotic embryo is formed as usual from a Polygonum type of embryo sac and the Nuclear endosperm does not show any nodules as observed by Billings. However, the embryo is peculiar in having a conspicuous, coiled and uniseriate suspensor composed of large coenocytic cells. It is these cells which appear like nodules in oblique sections and Billings misinterpreted them as of endosperm origin.

Yet another unusual situation was described in *Paeonia* (Paeoniaceae) where M. S. Yakovlev and M. D. Yoffe[29] (1957) claimed that the zygote undergoes a free nuclear division. The nuclei are said to become peripherally disposed. After wall formation has taken place, some of the peripheral groups of cells become meristematic and give rise to embryonal primordia one of which produces the mature embryo. This is a gymnospermous pattern of development and is not known in any other angiosperm. M. S. Cave *et al.*[30] (1961) confirmed the findings of Yakovlev and Yoffe. However, Prem Murgai[31] (1962),

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[25] V. Garcia, In *Plant Embryology—A Symposium*, C.S.I.R., New Delhi, *Phytomorphology* **12**.

[26] *New Phytol.* **36**.

[27,28] *Phytomorphology* **3, 6.**

[29] *Ibid.* **7**.

[30] *Amer. J. Bot.* **48**.

[31] In *Plant Embryology—A Symposium*, C.S.I.R., New Delhi.

who investigated several species of *Paeonia*, has shown that the division of the zygote *is* followed by a wall as is common in other angiosperms. The subsequent development is not yet clear but there is certainly nothing comparable with the condition in gymnosperms.

The success in tracing these peculiarities in the organization of the embryo sac and endosperm in the Loranthaceae and Santalaceae, of the endosperm in the Cucurbitaceae and Leguminosae, and of the embryo in *Isomeris* and *Paeonia* is due to the study of dissections. Before the advent of microtomy, both hand sections and dissections formed a routine procedure in all embryological studies. When microtomy became more prevalent, dissections were unfortunately given up. However, such techniques are by no means to be ignored; and it is a combination of sections and dissections which often gives a true picture rather than an examination of the sections alone. Professor Maheshwari has added many refinements to staining procedures as well as the technique of dissections which are now widely followed.

PHYLOGENETIC EMBRYOLOGY.—The taxonomic assignment of several disputed genera and families has been checked and some reassignments suggested on the basis of embryological data. A few selected examples are given below.

The genus *Butomus*, along with *Butomopsis*, *Hydrocleis* and *Limnocharis*, was included in the Butomaceae. However, *Butomus* shows the Polygonum type of embryo sac, while the gametophyte in the other three genera is of the Allium type—a condition characteristic of the Alismaceae.[32] On the basis of morphology and cytology also *Butomus* differs markedly from the other genera. Therefore, *Butomus* alone should be retained in the Butomaceae and the other genera transferred to the Alismaceae.[33]

Most taxonomists assign the family Lemnaceae to the Spathiflorae (Arales), but, on the basis of a supposedly Helobial endosperm in *Lemna*, A. Lawalrée[34] (1945) concluded that the Lemnaceae should be included in the Helobiales. This has been contradicted by S. C. Maheshwari[35] (1956) who has shown that Lawalrée misinterpreted the Cellular endosperm of *Lemna* as Helobial. Thus, there is no justification for transferring the Lemnaceae from the Arales to the Helobiales. This has been further confirmed by studies on *Wolffia* and *Spirodela*[36] (1959).

Gagnepain and Boureau[37] (1947) pointed out that in *Exocarpus* the cladodes are similar to those of *Phyllocladus*, the fleshy pedicel is

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[32] *See* R. B. Roper, 1952. *Phytomorphology* **2**; S. C. Maheshwari, 1955. *Ibid.* **6.**

[33] *See* B. M. Johri, 1963. In *Recent Advances in the Embryology of Angiosperms*, Int. Soc. Plant Morphol., Delhi.

[34] *Bull. Soc. Roy. Bot. Belgium* **71.**

[35] *Phytomorphology* **6.**

[36] S. C. Maheshwari, *Proc. 9th Int. bot. Congr. Montreal.*

[37] *Bull. soc. bot. Fr.* **94.**

like that of *Acmopyle* and *Podocarpus*, the ovule is naked, and the 'fruits' resemble those of the Taxaceae. They, therefore, concluded that *Exocarpus* should be assigned to a separate family, the Exocarpaceae, to be placed near the Taxaceae in the gymnosperms. Manasi Ram[38] (1959) made a thorough study of several species of this genus and discussed its affinities. She found that the floral structure is typically angiospermous—the pollen is 2-celled, the embryo sac is of the Polygonum type with haustorial extensions, the endosperm is Cellular with prominent chalazal haustoria, the division of the zygote is followed by a wall, and the embryo has two cotyledons. There can thus be no doubt that *Exocarpus* is a true angiosperm. The anatomy of the node, vascular anatomy of flower, structure of placental-ovular complex, development of embryo sac, endosperm and embryo, and structure of the fruit all point to a close relationship with the Santalaceae.

B. M. Johri and H. Singh[39] (1959) have produced arguments against C. E. B. Bremekamp's[40] (1953) view that *Elytraria* should be removed from the Acanthaceae-Nelsonioideae and placed in the Scrophulariaceae-Rhinanthoideae. The embryological features common between the Nelsonioideae and Rhinanthoideae are: presence of integumentary tapetum, Polygonum type of embryo sac, formation of micropylar and chalazal endosperm haustoria, and an albuminous seed. However, the asymmetrical growth of the central endosperm chamber and presence of a rudimentary jaculator in the Nelsonioideae, in contrast to the symmetrical growth of the central endosperm chamber and absence of a jaculator in the Rhinanthoideae, speak against the transfer of *Elytraria* from the Acanthaceae to the Scrophulariaceae. This has been further confirmed in another member of the Nelsonioideae—*Nelsonia*[41] (1963).

The family Loranthaceae is divided into two subfamilies—Loranthoideae and Viscoideae—but there are important differences in their embryology. The Loranthoideae show triradiate pollen, Polygonum type of embryo sac which may extend to various lengths in the style and stigma, composite endosperm formed by the fusion of all the endosperms in an ovary, and vertical division of the zygote. In the fruit the viscid layer is situated outside the vascular supply to the corolla. In the Viscoideae, on the other hand, the pollen is spherical, embryo sac is of the Allium type and shows a characteristic U-shaped curvature, the endosperms develop individually in each embryo sac but they do not fuse, and the division of the zygote is usually transverse. The viscid layer of the fruit is surrounded by vascular strands on either side. In view of these differences P. Maheshwari, B. M. Johri and S. N. Dixit[42] (1957) suggested that both the subfamilies should be raised to the rank of families and designated as Loranthaceae and Viscaceae, respectively.

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[38] *Phytomorphology* **9**.

[39] *Bot. Notiser* **112**.

[40] *Proc. Koninkl. Nederl. Akad. Van Wetenschappen-Amsterdam* **56**.

[41] H. Y. Mohan Ram and Pushpa Masand, *Phytomorphology* **13**.

[42] *J. Madras Univ.* **27 B**.

EXPERIMENTAL EMBRYOLOGY.[43]—The reproductive organs of a number of plants have been grown on synthetic nutrient media in aseptic cultures. These include the anthers of *Allium*, *Cajanus* and *Rhoeo*; ovaries of *Aerva*, *Althaea*, *Anethum*, *Foeniculum*, *Hyoscyamus*, *Iberis*, *Linaria*, *Ranunculus*, *Trachyspermum*, *Triticum*, *Tropaeolum* and *Zephyranthes*; ovules of *Abelmoscus*, *Argemone*, *Citrus* (also excised nucelli), *Gossypium*, *Nicotiana*, *Opuntia* (also placenta), *Papaver* and *Zephyranthes*; endosperm of *Exocarpus*, *Gossypium*, *Ricinus* and *Santalum*; and embryos of *Amyema*, *Amylotheca*, *Brassica*, *Cassytha*, *Citrus*, *Cuscuta*, *Dendrophthoe*, *Gossypium*, *Urena*, *Santalum*, *Scurrula* and *Trigonella*.

The anthers of *Allium* and *Rhoeo*[44] (1959), excised at an early stage of meiosis I, developed up to the microspore stage. In *Allium*[45] (1963) and *Iberis*[46] (1961) the fruits developed satisfactorily only if pollinated flowers were cultured. Here, as well as in *Althaea*[47] (1958) and *Hyoscyamus* (unpublished) better growth was obtained if the calyx was retained. However, the ovaries of *Ranunculus*[48] (1962) and *Zephyranthes*[49] (1959) produced normal fruits without the retention of any accessory floral whorls. The pollinated ovaries of *Anethum*[50] (1963) developed into normal fruits but in many of them supernumerary embryos were formed which burst through the fruit wall. Germination occurred *in situ* and the seedlings produced flowers in due course. The excised ovules from the pollinated flowers of *Abelmoscus* (unpublished), *Nicotiana* (unpublished), *Papaver*[51] (1961) and *Zephyranthes*[52] (1959) gave rise to fertile seeds which germinated *in situ*. The excised nucelli from the pollinated ovules of *Citrus*[53] (1961, 1962) produced a callus which differentiated into regenerants (pseudobulbils) and germinated into normal seedlings.

The mature endosperm of *Ricinus communis* and *Santalum album* forms a callus, particularly when the entire endosperm with the embryo

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[43] A detailed account of these studies, by B. M. Johri and Sipra Guha, appears elsewhere in this Volume under the title "The technique of *in vitro* culture in the study of physiology of reproduction" (*see* p. 58).

[44] I. K. Vasil, *J. exp. Bot.* **10**.

[45] B. M. Johri and Sipra Guha, In *Plant Tissue and Organ Culture—A Symposium*, Int. Soc. Plant Morphol., Delhi.

[46] Nirmala Maheshwari and M. Lal, *Phytomorphology* **11**

[47] R. N. Chopra, In *Modern Developments in Plant Physiology*, Univ. Delhi, Delhi.

[48] R. C. Sachar and Sipra Guha, In *Plant Embryology—A Symposium*, C.S.I.R., New Delhi.

[49] R. C. Sachar and Manju Kapoor, *Phytomorphology* **9**.

[50] B. M. Johri and C. B. Sehgal, In *Plant Tissue and Organ Culture—A Symposium*, Int. Soc. Plant Morphol., Delhi.

[51] Nirmala Maheshwari and M. Lal, *Phytomorphology* **11**.

[52] Manju Kapoor, *Ibid.* **9**.

[53] N. S. Ranga Swamy, *Ibid.* **11**; P. S. Sabharwal, In *Plant Tissue and Organ Culture—A Symposium*, Int. Soc. Plant Morphol., Delhi.

intact, is cultured on White's medium containing 2, 4-D (unpublished data).

The ovoidal embryos of *Cuscuta*[54] (1962) and globular proembryos of *Dendrophthoe*[55] (1963) produced polyembryonal masses which repeated this behaviour in subcultures. The mature embryos responded differently. In *Cuscuta* the stem tip formed a shoot whereas the basal end behaved like the ovoidal embryos. The mature embryo of *Dendrophthoe* formed a normal seedling with a series of holdfasts. When it was cultivated with the endosperm intact, besides the seedling a number of accessory leaves and foliar buds also developed from the callused radicular end.

Following successes in intra-ovarian pollination in *Eschscholzia* and *Papaver*[56] (1962), the most important achievement is the test-tube fertilization in *Papaver*[57] (1962) by growing the mature pollen and ovules simultaneously on nutrient media. Not only is the development of endosperm and embryo quite normal, but the seeds also germinate *in situ* producing healthy seedlings. Similar results have been obtained in *Argemone*, *Eschscholtzia* and *Nicotiana* (unpublished data).

METABOLIC STUDIES ON DEVELOPING OVULES.—Some physiological and biochemical changes occurring in the pollinated ovules during their development into seeds have also been studied. In *Papaver somniferum*[58] (1963) the oxygen uptake shows four peaks. The first occurs 0–3 days after pollination and coincides with fertilization, the second 4–6 days after pollination when free nuclear endosperm is formed and the zygote divides transversely, the third 7–9 days after pollination at the time of wall formation in the endosperm, and the fourth 20–24 days after pollination when the cotyledons elongate. On the basis of fresh weight the maximum rate of uptake is attained just after pollination and is maintained at a high level for 48–72 hours thereafter.

During the four peaks the rate of respiration is high and polyphenol oxidases seem to mediate final electron transfer. The activities of α- and β-amylases, acid phosphatases and succinic dehydrogenase are maximal 6–8 days after pollination. During the first 10 days following pollination the activity of glutamic-alanine transaminase is rather low, and peak activity occurs 16–17 days after pollination. The alcohol-insoluble nitrogen increases continuously till maturity. The maximum level of RNA per ovule is reached after 11 days, and of DNA 15 days after pollination.

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[54] P. Maheshwari and B. Baldev, In *Plant Embryology—A Symposium*, C.S.I.R., New Delhi.

[55] B. M. Johri and Y. P. S. Bajaj, In *Plant Tissue and Organ Culture—A Symposium*, Int. Soc. Plant Morphol., Delhi.

[56] P. Maheshwari and Kusum Kanta, In *Plant Embryology—A Symposium*, C.S.I.R., New Delhi.

[57] Kusum Kanta, N. S. Ranga Swamy and P. Maheshwari, *Nature, Lond.* **194.**

[58] S. C. Maheshwari and M. M. Johri, *Naturwissenschaften* (in Press).

The acidic fraction of growth substances has been analysed in the ovules of *Gossypium hirsutum*, var. Indore-2, at 3, 8, 15, 22 and 36 days after pollination (unpublished data). The chromatograms showed two spots with growth-promoting activity. One of these has been identified as that of IAA while the identity of the other is under investigation. On the basis of fresh weight, the maximal concentration of IAA is reached three days after pollination and thereafter it gradually declines. There is again a slight but distinct rise in the amount 22 days after pollination during the growth of the embryo.

Besides the emphasis on morphology and embryology, considerable attention is also being paid to the cytology of endosperm, induction of flowering, structure of style and stigma in relation to pollen tube growth, and allied topics.

In conclusion, it may be stated that Professor Maheshwari has repeatedly drawn attention to the importance of embryological studies in botanical research and teaching. This was the subject of his presidential address to the Indian Botanical Society at Nagpur (1945). A Spanish translation of this article appeared in the *Bul'etin of the Argentina Botanical Society* in 1951. This was also the topic of his presidential address to the Embryology Section at the International Botanical Congress at Paris in 1954. His keen interest in phylogenetic embryology is borne out by a series of publications which have appeared from the University of Delhi. In 1950, he spoke on this subject at the International Botanical Congress at Stockholm, and a few months later before the British Association for the Advancement of Science. The same subject was the topic of a symposium at the International Botanical Congress at Montreal in 1959. The importance of experimental embryology has also been stressed similarly. As President of the Botany Section of the Indian Science Congress at Poona in 1950, he gave an address on " Contacts between embryology, physiology, and genetics " and this theme has been expounded in other articles published in 1958, 1959, 1962 and 1963. At the International Symposium on the 'Physiology of pollen and fertilization', held at Nijmegen (The Netherlands) in August 1963, he discussed the various problems concerning the "Control of fertilization in flowering plants".

Fresh fields of study are being explored day-by-day, and I have no doubt that in the remaining years of his active career, Professor Maheshwari will further advance the cause of botany.

University of Delhi, Delhi B. M. JOHRI

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CONTRIBUTORS

A. ABRAHAM, *Department of Botany, Kerala University, Trivandrum, India* (pp. 1–3).

CHESTER A. ARNOLD, *Department of Botany, University of Michigan, Ann Arbor, Michigan, U.S.A.* (pp. 4–9).

N. L. BOR, *Royal Botanic Gardens, Kew, Surrey, England* (pp. 10–15).

T. V. DESIKACHARY, *Department of Botany, University of Madras, Madras, India* (pp. 16–26).

A. E. DIMOND, *The Connecticut Agricultural Experiment Station, New Haven, Connecticut, U.S.A.* (pp. 46–57).

TH. ECKARDT, *Institut für Systematische Botanik und Pflanzengeographie der Freien Universität Berlin, Berlin-Dahlem, Germany* (pp. 27–34).

G. ERDTMAN, *Palynological Laboratory, Nybodagatan* 5, *Stockholm-Solna, Sweden* (pp. 35–38).

A. FAHN, *Department of Botany, The Hebrew University, Jerusalem, Israel* (pp. 39–45).

SIPRA GUHA, *Department of Botany, University of Delhi, Delhi, India,* (pp. 58–73).

A. C. HILDEBRANDT, *Department of Plant Pathology, College of Agriculture, University of Wisconsin, Madison, Wisconsin, U.S.A.* (pp. 216–224).

JAMES G. HORSFALL, *The Connecticut Agricultural Experiment Station, New Haven, Connecticut, U.S.A.* (pp. 46–57).

B. M. JOHRI, *Department of Botany, University of Delhi, Delhi, India* (pp. 58–73).

T. N. KHOSHOO, *Department of Botany, Jammu and Kashmir University, Srinagar, India* (pp. 74–82).

HITOSHI KIHARA, *National Institute of Genetics, Misima, Japan* (pp. 83–101).

A. E. DE MAGGIO, *College of Pharmacy, Rutgers, The State University, Newark, N. J., U.S.A.* (pp. 306–320).

T. S. MAHABALE, *Department of Botany, University of Poona, Poona, India* (pp. 102–109).

MARION O. MAPES, *Department of Botany, Cornell University, Ithaca, New York, U.S.A.* (pp. 237–247).

P. N. MEHRA, *Department of Botany, Panjab University, Chandigarh, India* (pp. 110–121).

L. MEIßNER, *German Academy of Sciences at Berlin Institute of Biochemistry of Plants, Halle/Saale, Germany* (pp. 130–134).

Y. MERCHANT, *St. Xavier's College, Bombay, India* (pp. 213–215).

G. MOREL, *Station Centrale de Physiologie Végétale, C.N.R.A., Versailles (S. and O.), France* (pp. 306–320).

YUJI MORIMURA, *Institute of Applied Microbiology, University of Tokyo and The Tokugawa Institute for Biological Research, Tokyo, Japan* (pp. 122–129).

K. MOTHES, *German Academy of Sciences at Berlin Institute of Biochemistry of Plants, Halle/Saale, Germany* (pp. 130–134).

DEVENDRA DATT NAUTIYAL, *Department of Botany, University of Allahabad, Allahabad, India* (pp. 150–158).

Y. OGURA, *Botanical Institute, University of Tokyo, Tokyo, Japan* (pp. 135–141).

B. P. PAL, *Indian Agricultural Research Institute, New Delhi, India* (pp. 142–149).

DIVYA DARSHAN PANT, *Department of Botany, University of Allahabad, Allahabad, India* (pp. 150–158).

GEORGE F. PAPENFUSS, *Department of Botany, University of California, Berkeley, California, U.S.A.* (pp. 159–166).

W. R. PHILIPSON, *Department of Botany, University of Canterbury, Christchurch, New Zealand* (pp. 167–179).

V. A. PODDUBNAYA-ARNOLDI, *The Main Botanical Garden, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.* (pp. 180–184).

J. PROSKAUER, *Department of Botany, University of California, Berkeley, California, U.S.A.* (pp. 185–188).

V. PURI, *School of Plant Morphology, Meerut College, Meerut, India* (pp. 189–198).

A. J. RIKER, *Department of Plant Pathology, College of Agriculture, University of Wisconsin, Madison, Wisconsin, U.S.A.* (pp. 216–224).

T. S. SADASIVAN, *Department of Botany, University of Madras, Madras, India* (pp. 199–212).

H. SANTAPAU, *Botanical Survey of India, Calcutta, India* (pp. 213–215).

HARVEY W. SPURR, JR., *Department of Plant Pathology, College of Agriculture, University of Wisconsin, Madison, Wisconsin, U.S.A.* (pp. 216–224).

T. A. STEEVES, *Department of Biology, University of Saskatchewan, Saskatoon, Canada* (pp. 225–236).

F. C. STEWARD, *Department of Botany, Cornell University, Ithaca, New York, U.S.A.* (pp. 237–247).

C. V. SUBRAMANIAN, *Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India* (pp. 248–258).

D. SUBRAMANIAN, *Department of Botany, University of Madras, Madras, India* (pp. 199–212).

K. SUBRAMANYAM, *Botanical Survey of India, Calcutta, India* (pp. 259–275).

M. S. SWAMINATHAN, *Division of Botany, Indian Agricultural Research Institute, New Delhi, India* (pp. 276–282).

HIROSHI TAMIYA, *Institute of Applied Microbiology, University of Tokyo and The Tokugawa Institute for Biological Research, Tokyo, Japan* (pp. 122–129).

R. N. TANDON, *Department of Botany, University of Allahabad, Allahabad, India* (pp. 283–289).

KOICHIRO TSUNEWAKI, *National Institute of Genetics, Misima, Japan* (pp. 83–101).

J. VENKATESWARLU, *Department of Botany, Andhra University, Waltair, India* (pp. 290–300).

S. C. VERMA, *Department of Botany, Panjab University, Chandigarh, India* (pp. 110–121).

C. W. WARDLAW, *Department of Botany, University of Manchester, Manchester, England* (pp. 301–305).

R. H. WETMORE, *Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A.* (pp. 306–320).

ROSALIE WUNDERLICH, *Botanical Institute, Rennweg* 14, *Vienna, Austria* (pp. 321–330).

CONTENTS

CHROMOSOME CONSTITUTION AND OIL CONTENT IN THE COCONUT ENDOSPERM

BY A. ABRAHAM

Botany Department, Kerala University, Trivandrum, India

(Received for publication on May 27, 1963)

THOUGH the chief source of human food is provided by the endosperm of the seeds of various plants, we are still without adequate information on several aspects of the cytology and development of this tissue. In a recent investigation conducted in this laboratory on the development of the endosperm of a tall variety of *Cocos nucifera* L. (coconut) very interesting facts have been revealed (Abraham and Mathew, 1963). The points relevant to the present paper may be summarised as follows:

(*a*) The nuclei in the young coconut embryos are diploid ($2n = 32$) and no aberrant type of division has been observed in the hundreds of dividing cells examined.

(*b*) Visible formation of cellular endosperm on the inner face of the endothelium commences about the sixth month after opening of the inflorescence. This jelly-like solid or semi-solid endosperm tissue is thicker at the antipodal end (irrespective of the position of the nut on the bunch).

(*c*) Nuclei possessing three different chromosome numbers have been observed in the endosperm. The majority of these, especially the endosperm tissue adjacent to the endothelium, show the normally expected triploid condition ($3n = 48$). The next in frequency are the hexaploid ($6n = 96$). A smaller number of $12n$ nuclei with 192 chromosomes have also been observed in the tissue towards the inside.

(*d*) The increase in chromosome number is the result of a *c*-mitotic type of division (Plate I). Further observations showed that by and large the endosperm can be roughly divided into three layers, the outermost (nearest the endothelium) being triploid in chromosome constitution, the middle layer being hexaploid and higher levels of polyploidy ($12n$ and even $24n$) being present in the innermost layer (in contact with the 'milk'). The proportion of these layers in the endosperm has not been accurately determined and it may be expected that depending on conditions which promote *c*-mitotic divisions this may vary; consequently it is possible that variation in this respect may occur from variety to variety as well as between different bunches on the same plant.

It is known from various investigations that in polyploids there are significant differences in the metabolism of cells of the same organism depending on the chromosome constitution (Swanson, 1960). This fac indicated the desirability of finding whether there exists differences in the chemical composition of different parts of the endosperm which may be related to the varying chromosome constitution. An analysis was made of the oil content from different layers of the endosperm taken from mature nuts. The endosperm was divided arbitrarily into three layers of approximately equal thickness. It may be noted that the endosperm in coconut is a uniform tissue and no 'layers' as such are recognisable. The following results were obtained:

Part of the endosperm	Percentage of oil on dry basis
1. Outermost layer (nearest the endothelium)	75·68
2. Middle layer	54·11
3. Inner layer (towards the embryo sac cavity)	41·11

From the observations on mode of development of the endosperm it is now tentatively concluded that these three 'layers' have cells with chromosome constitutions which are different. Present indications are that the outermost layer with the highest oil content of 75·68% is predominantly triploid, the middle layer with 54·11% oil content has more of hexaploid cells and the inner layer with relatively the lowest oil content (41·11%) has cells of higher ploidy (12*n* and upwards). Apparently, beyond the triploid level increase in chromosome number tends to reduce the oil content of the cells. These observations are of great practical significance in coconut breeding and emphasise the need for further careful studies on the cytology and development of the endosperm in coconut.

It is indeed striking that the endosperm, which is the most important part of the coconut, has not been adequately investigated. Even in a recent authoritative monograph on the coconut palm (Menon and Pandalai, 1960) there is but casual reference to the endosperm. This only shows the paucity of published data on the subject.

In Professor P. Maheshwari's (1950) most valuable book on the *Embryology of Angiosperms*, whose great contributions to botanical science are being recognised through this Commemoration Volume, the chapter on "Endosperm" ends as follows: "More likely, endosperm nuclei with deviating chromosome numbers arise because of disturbed

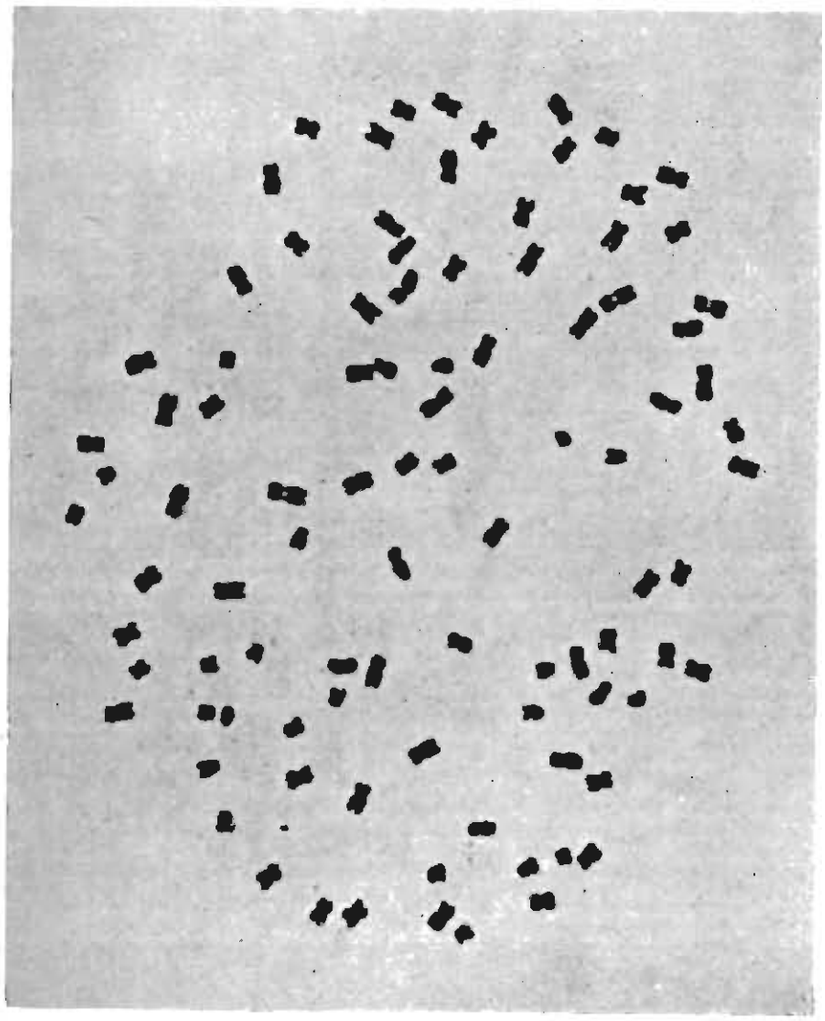

FIG. 1

A. Abraham

mitoses. The most reasonable explantion for mosaic endosperm would, therefore, lie either in aberrant behaviour of the chromosomes or possibly in somatic mutations (*cf.* Clark and Copeland, 1940)''. The present study has clearly shown that in coconut, layers of endosperm tissue with differing ploidy arise through c-mitotic type of divisions, and the oil content of the endosperm tissue varies with the ploidy.

This work was done under a scheme of research wholly financed by the Indian Central Coconut Committee. My thanks are due to Shri P. Gopinath, Research Assistant, for the excellent cytological preparation shown in Plate I, and to Shri N. Chit aranjan Nair, Chemical Assistant, for the analysis of oil content of the endosperm.

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EXPLANATION OF PLATE I

FIG. 1. C-mitotic division in a hexaploid endosperm cell. Only 95 'double chromosomes' are seen at metaphase in this cell, though in several divisions examined the normally expected 96 chromosomes were seen.

CORDAITES-TYPE FOLIAGE ASSOCIATED WITH PALM-LIKE PLANTS FROM THE UPPER TRIASSIC OF SOUTH-WESTERN COLORADO

BY CHESTER A. ARNOLD

University of Michigan, Ann Arbor, Michigan, U.S.A.

(Received for publication on February 15, 1963)

IN 1956 the late Dr. R. W. Brown described some fossilized palm-like plants from the Late Triassic Dolores formation of south-western Colorado, and created for them the genus *Sanmiguelia*. The discovery of these plants was of unusual significance because of its relation to the problem of the origin and the pre-Cretaceous history of flowering plants. If it could become definitely established that *Sanmiguelia* is a palm, it would be the oldest known angiosperm, antedating by more than a whole period the oldest previously unquestioned remains of this class of plants. Moreover, it would place the monocotyledons in advance of the dicotyledons in the chronological order.

The fossils that Brown described were impressions of rather large, ovate, lengthwise pleated leaves, and a few stem casts with leaf portions still attached. Attachment was alternate by what appears to have been sheathing bases. No cuticle or tissue of any kind was preserved.

Although Brown was completely aware of the problematic nature of the fossils and carefully refrained from jumping at conclusions or making any brash or dogmatic statements concerning the significance of this discovery, he does say (1956, p. 203) that "*Sanmiguelia lewisi* is regarded tentatively but credibly as a primitive palm". There is scarcely any doubt but had it come from some Late Cretaceous or younger deposit, it would be accepted as a palm without question. But coming from a horizon assigned to the Upper Triassic, doubts have arisen and may continue to arise in the minds of some whether the plant is correctly identified and whether there might be an error in the age determination of the bed.

After making due allowance for the limitations imposed by preservation in the form of impressions and casts, the author agrees with Brown that *Sanmiguelia* resembles a palm more than any other kind of plant. It is true that additional evidence such as palm-like fructifications or structurally preserved stems will have to be obtained before the determination can be considered final.

The question of the existence of angiosperms during pre-Cretaceous times is still and has long been one of the major problems in paleobotany. Although they have been the dominant class of land plants from Late Cretaceous to Recent times, they diminish rather abruptly when traced in the opposite direction, and approach the vanishing point at the base of the Cretaceous system. Reported occurrences of pre-Cretaceous angiosperms, of which there have been several, have been reviewed and appraised by Scott *et al.* (1960) who emphasize the highly uncertain status of all of them. They do admit that *Sanmiguelia* is the best example ever discovered of a possible pre-Cretaceous angiosperm, but they reiterate its questionable nature and claim that because of the wide range of structure displayed among Mesozoic cycadophytes, *Sanmiguelia* could be a member of that group. They, therefore, choose to regard it tentatively as such. Hughes (1961) gives a similar analysis of pre-Cretaceous angiosperm evidence. He says that *Sanmiguelia* shows no conclusively angiosperm characters, and that it could just as well be some undescribed cycadophyte or ginkgophyte as an angiosperm.

Simple pleated leaves are certainly not characteristic of cycadophytes. *Ginkgo* and its fossilized relatives have simple leaves, but it would require considerable stretching of the concept of a ginkgoalean leaf to embrace *Sanmiguelia*. A torn or fragmented leaf could bear some deceptive resemblance to *Baiera*, but that is about as far as the comparison can be carried.

The claim that *Sanmiguelia* does not show enough characters to justify calling it a palm seems to fail to give due consideration to its palm-like appearance. It would be difficult or impossible to point to a character revealed in almost any angiosperm leaf that does not have a parallel in some other group. The characters shown by a fossil oak leaf, for example the venation pattern, its shape, type of margin, or anything else might be matched in some fern, cycad, or gnetalean leaf. Yet one would not for those reasons refuse to identify a fossil leaf as an oak.

Even though one admits the possibility that *Sanmiguelia* might be some heretofore unrecognized cycadophyte or ginkgophyte, this does not seem to justify complete exclusion of it from the angiosperm complex on the basis of evidence now available. After all, are not resemblances the ultimate criteria of affinity? No one has shown any real cycadophytic or ginkgophytic characters in it. Bock (1962) does say that its petiole is lens-like elliptical in cross-section like a cycadophyte, but he shows no such petiole, and apparently misinterpreted Brown's figure of the stem cast bearing an attached leaf for the petiole itself. Virtually all of the resistance to inclusion of *Sanmiguelia* in the Palmales develops from its Triassic age. It is this more than anything shown by its form or surface features or any morphological characters which would indicate affinity with some other group.

In 1961 the author visited the locality in the San Miguel canyon in Colorado where the plant fossils in question were originally found,

Along a road that leads up the north side of the canyon to a mine the stratigraphic sequence described at this place is plainly seen. First above the floor of the canyon are the dark red shales of the Permian Cutler formation. Then one sees the Dolores formation composed of bright red, calcareous, massive and thin-bedded cliff-forming sandstones with a few shales and conglomerates. This formation extends to an elevation of about 750 feet above the canyon bottom. Its upper limit is clearly shown by the overlying whitish Jurassic age Entrada sandstone.

At a height of 420 feet above the San Miguel River (as determined by altimeter reading) and which is specified by Brown as the level at which *Sanmiguelia* occurs, plant remains consisting of fragments of *Sanmiguelia* and *Cordaites*-like leaves were found. The fragments of the former were from leaves somewhat smaller than those that Brown figured. The pleats are only 10 mm wide at the apical end and 6 mm at the other. However, this size difference does not seem to be sufficient for assuming a different species than the one Brown named.

Bock (1962) disputes the Triassic age determination of the bed containing *Sanmiguelia*, and claims that the plants came from the Entrada sandstone, and are therefore of Jurassic age. It is not understandable why Bock thinks the plants came from the Entrada sandstone when this formation is so distinct from the Dolores formation, and the plants were found several hundred feet below it.

Two portions of finely striate, linear-ovate, *Cordaites*-like leaves were collected near the place where the *Sanmiguelia* material was found. Originally these leaves must have been at least 30 cm long and possibly longer. They resemble *Cordaites* leaves, being parallel-striate, slender without midrib, and with no evidence of attachment by a broadened basal sheath. The larger specimen (Plate I, Fig. 2) is 20 cm long, and tapers from 3·4 cm at the forward end to 2·8 cm at the other which was toward the base. The other specimen which is slightly smaller shows the surface striations more distinctly (Plate I, Fig. 3). Neither specimen shows any thickening along the median line and no cuticle is preserved. If these leaves were found in Pennsylvanian or Permian age strata, they would be identified as *Cordaites* without any hesitation.

These two leaves are similar to and probably identical with *Yuccites poleoensis* (Daugherty, 1941, p. 70, Plate 13, Fig. 1) from the Upper Triassic Poleo sandstone of New Mexico. Leaves of this kind are rather widely scattered throughout Triassic and Jurassic strata but they are rarely abundant. Unassociated as they are with fructifications, opinions differ as to their affinities. The older authors regarded them as monocotyledons. Later they were classified with the Cordaitales or as *incertae sedis*. They have been referred to such genera as *Yuccites*, *Krannera*, *Phyllotenia*, and *Pelourdea*. The last name seems to be the most suitable one. It was proposed by Seward (1917, p. 277) as a substitute for *Yuccites* of Schimper and Mougeot on grounds that

the latter bears misleading implications of affinities. Although the rules of nomenclature do not allow name changes for that reason, *Pelourdea* does seem to be a valid substitution because *Yuccites* was preoccupied when Schimper and Mougeot proposed it in 1884. First in 1822 and again in 1825 De Martius used the name *Yuccites microlepis* for a plant which he described as follows: "A woody stem, simple or branched above, with branches nearly equalling the stem in thickness, covered with plain or gibbous scales, crenulated or erose at the margin, imbricated inward, and thence not distinct beneath, destitute of cicatrices". De Martius apparently had a branched *Lepidodendron* stem, and the change Seward made was justified, though for a different reason than the one he gave. *Yuccites* is not a suitable genus for any cordaite-type or monocot leaf of any age.

Since *Yuccites* is not an appropriate genus for the Triassic leaves from Colorado and New Mexico, the name *Pelourdea poleoensis* (Daugherty) comb. nov. is proposed for them. The amended diagnosis is as follows:

Leaves slender, linear-ovate, up to 4·2 cm in width and 65 cm or more in length, gradually tapering distally to a rounded apex; narrowing somewhat toward base but no indication of leaf-sheath; ribs about 20 per cm, rarely if at all forked.

Holotype.—University of California, Museum of Paleontology, Paleobotanical Series No. 1568.

Hypotype.—University of Michigan, Museum of Paleontology, Collection No. 46197.

Reeside *et al.* (1957) place the Dolores formation at the level of the upper part of the Chinle formation and the Huggett sandstone. In a more generalized correlation chart Oriel and Craig (1960) place the Dolores on par with the Chinle formation and line it up with the Jelem formation and the Dokum group. The Dolores is therefore in the upper Keuper but below the Rhaetic in the standard European section. It is built up of sediments derived from the nearby ancestral Uncomphagre highland. Equivalence with the upper part of the Chinle is indicated by the fauna and the lateral extension of the beds.

Daugherty (1941) has described the Chinle flora, which consists of ferns, cycadophytes, conifers, and other plants. In a personal communication he has called attention to the resemblance between *Sanmiguelia lewisi* and a fragment he described from the Chinle as *Baiera arizonica* (1941, p. 85, Plate 18, Fig. 4). The latter looks like a fragment of a pleated leaf. The pleats are narrower but otherwise the similarity is close. Aside from this the Dolores flora is too small for comparison with the Chinle flora. Of course, the most striking feature of the latter is the large logs of *Araucarioxylon* in the Petrified Forest member of the Chinle formation. Logs of this kind have not been found in the Dolores formation because the special conditions necessary for their preservation did not exist at the place where the sediments were deposited.

Most of the *Cordaites*-type leaves found in the Early Mesozoic that have been assigned to *Pelourdea* and *Yuccites* are probably gymnosperms. In the Cretaceous, however, the chances are considerably greater that some or all of them are monocots. This introduces problems in their classification and requires use of form genera. The early paleobotanical writers were impressed by the superficial resemblances of these fossils to leaves of *Typha*, *Yucca*, and other monocots, and there was a tendency to classify all such leaves under Monocotyledonae regardless of their age.

Pelourdea-type leaves have been reported a few times from the North American Mesozoic in addition to the instances mentioned above. Fontaine (1905) figured a plant of uncertain identity from the Jurassic of Oregon as *Yuccites hettengansis*. King (1937) includes *Pelourdea* sp. in a short list of plants from the Bissett conglomerate of Texas, but no information is given about it. No doubt many leaves of this type have escaped notice or have been ignored as unidentifiable.

The reason for reporting the occurrence of two kinds of leaves in the same formation when we are so uncertain about the affinities of either calls for comment. If *Sanmiguelia* is a monocot, *Pelourdea poleoensis* might also be one because if there were palms during the Triassic period there could have been other monocots too. On the other hand, both could belong to categories of lower rank as Scott *et al.* (1960) and Hughes (1961) think most likely. However, the best evidence seems to indicate that *Sanmiguelia* is a palm and that *Pelourdea poleoensis* is a cordaite. If future discoveries should support these possibilities which at present are based on inference, we would have a co-mingling in the fossil record of early angiosperms with holdovers of the more ancient coal swamp flora, which is usually assumed to have disappeared long before flowering plants evolved. Thus, the occurrence of "pro-angiosperms" in carboniferous floras becomes less improbable than heretofore assumed.

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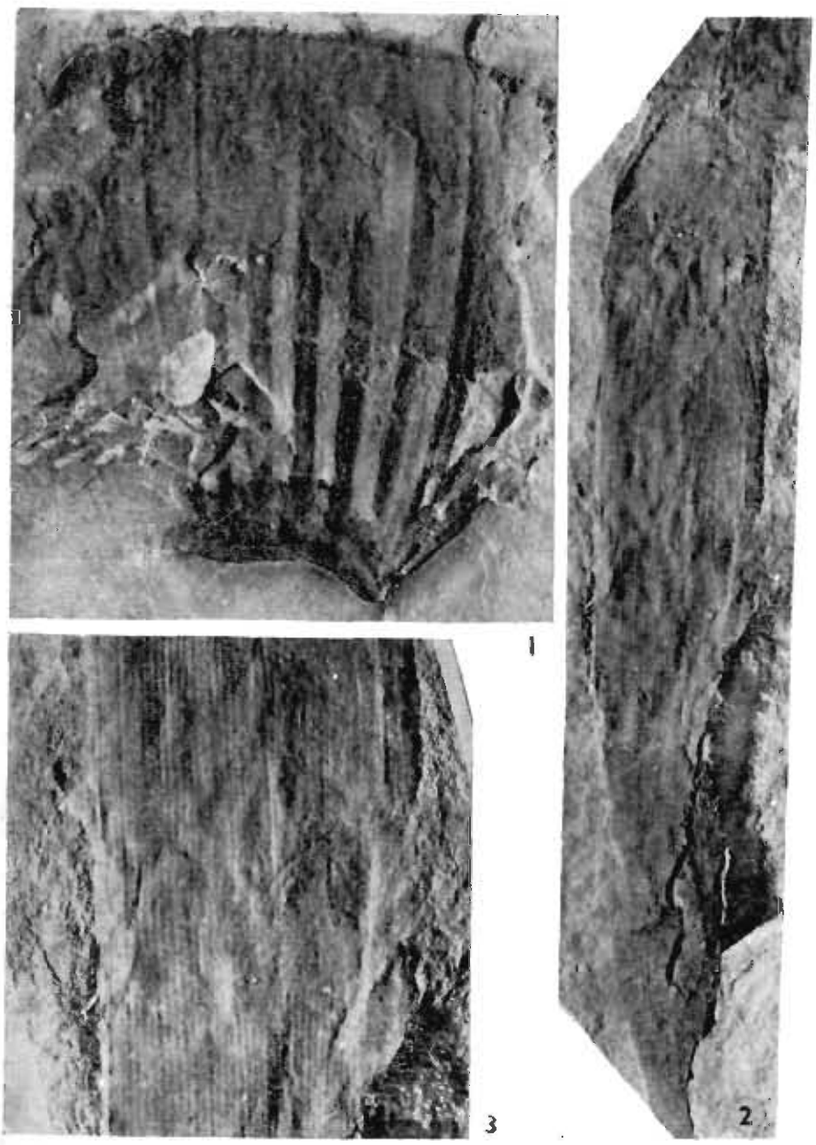


FIGS 1-3

Chester A. Arnold

NEW ASIATIC GRASSES

By N. L. Bor

Royal Botanic Gardens, Kew, Surrey, England

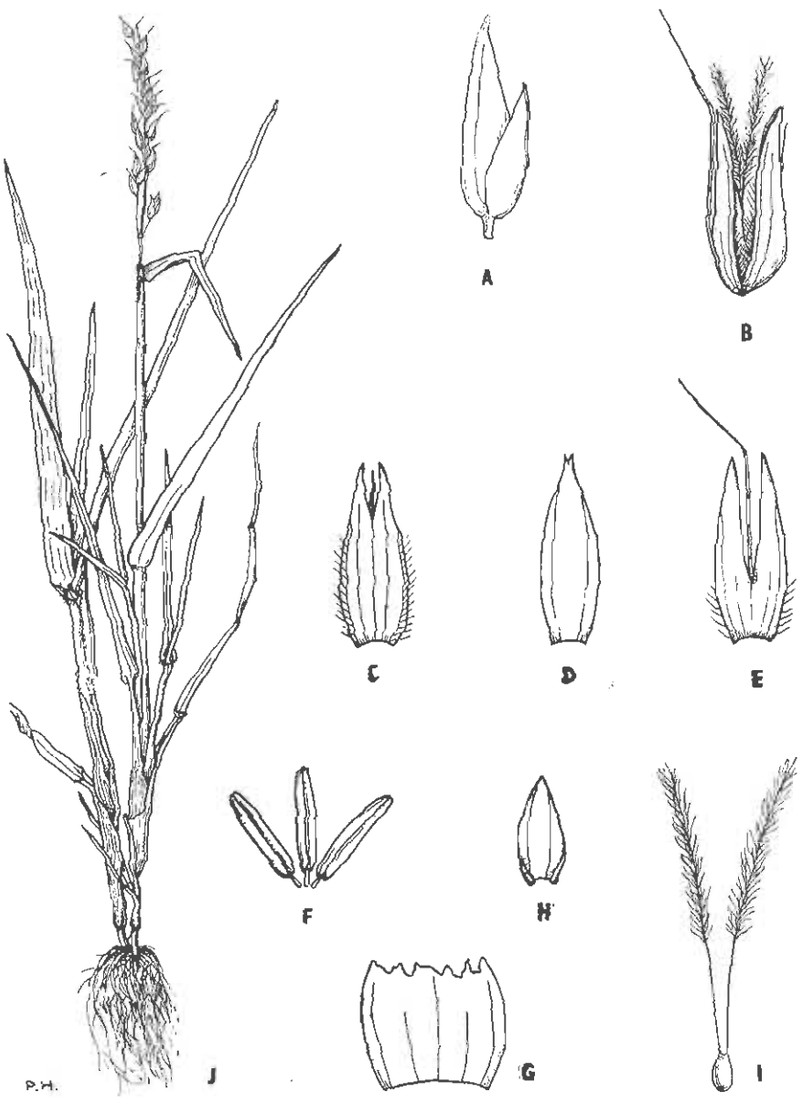
(Received for publication on March 29, 1963)

Anthoxanthum siamense **Bor** sp. nov. cum *A. horsfieldii* (Kunth) Mez comparanda sed ab ea lemmatibus inferioribus dorso glabris, longioribus, lemmate superiore chartaceo, exaristato, omnino glabro satis recedit.

Gramen perenne, paludicola, culmis usque 25 cm. altis, glabris-levibusque, rhizomate fibroso. Foliorum laminae basin versus rotundatae, oblongae, in apicem acuminatum attenuatum, planae, nonnunquam complicatae, usque 12 cm. longae, 7–8 mm. latae, supra villis sparsis brevibus caducis obtectae, infra glabrae; vaginae laxae, glabrae levesque; ligulae membranaceae, 2·5 mm. longae, laceratae. Panicula simplex usque 4 cm. longa; axis ramique omnino glabri; spiculae 6 mm. longae, 3-flores; gluma inferior 4 mm. longa, ovato-acuta, glabra, 1-nervia, valde compressa; gluma superior 6 mm. longa, elliptico-acuta, 3-nervia, glabra, valde compressa. Anthoecium primum masculinum; lemma 5·5 mm. longum, 2 mm. latum, oblongo-truncatum, apici fissum, 5-nervium (fissura 2 mm. longa), dorso glabrum, marginibus media parte inferiore barbato-ciliatum, brevissime aristatum; arista 1·5 mm. longa, recta; palea angusta, lemmati aequilonga; stamina 3; antherae 3 mm. longae. Anthoecium secundum vacuum; lemma lemmati primo simillimum sed fissura lemmatis tres quadrantes aequans; arista ex fissura perfecta, 7 mm. longa; palea nulla. Anthoecium tertium hermaphroditum; lemma 3–3·5 mm. longum, 4 mm. latum, omnino glabrum leveque, explanatum late elliptico-truncatum, 5-nervium, exaristatum; palea oblongo-acuta, 2-nervia; stamina 3; antherae 3 mm. longae; styli duo; stigmata plumosa.

A perennial grass with a fibrous rhizomatose base, growing in swampy places. Culms smooth and glabrous, up to 25 cm. tall. Leaf-blades linear, ending in a long acuminate tip, rounded at the base, flat, sometimes folded, up to 12 cm. long, 7–8 mm. wide, covered on the upper surface with rather short caducous white hairs, glabrous below; sheathes lax, smooth and glabrous, striate, ciliate on one margin; ligule lacerate, membranous, 2·5 mm. long.

Inflorescence a simple panicle up to 4 cm. long; axis and branches completely glabrous; spikelets few in number, 3-flowered; lower glume 4 mm. long, ovate acute, glabrous, 1-nerved, strongly compressed; upper glume elliptic-acute, 6 mm. long, 3-nerved, glabrous, firmly compressed. Lowest floret masculine; lemma 5·5 mm. long, 2 mm. broad, elliptic-oblong, 5-nerved, split at the apex for 2 mm., 5-nerved, ciliate on the margins at the base, shortly awned in the cleft; awn 1·5 mm. long,



TEXT-FIG. 1. *Anthoxanthum siamense* Bor. A, spikelet; B, spikelet with glumes removed; C, lower lemma; D, its palea; E, second lemma; F, androecium; G, upper lemma; H, its palea; I, gynoecium; All, × 60. J, habit, × ⅓.

straight, palea elliptic, as long as the lemma; stamens 3; anthers 3 mm. long. Second floret empty; lemma similar to the lowest lemma but cleft for three quarters of its length; awn in the cleft perfect, 7 mm. long; palea absent. Third floret hermaphrodite; lemma 3–3·5 mm. long, 4 mm. broad, completely smooth and glabrous, when flattened broadly elliptic-truncate, 5-nerved, awnless; palea elliptic-acute, 3·5 mm. long; stamens 3; anthers 3 mm. long; styles 2; stigmas plumose.

Thailand.—Chiengmai, Doi Inthanond, 15–4–1960, alt. 2580 m. *Smitinand* and *Alsterlund* 6668; a common grass growing in water. Typus in Herb. Kew. (Text-Fig. 1).

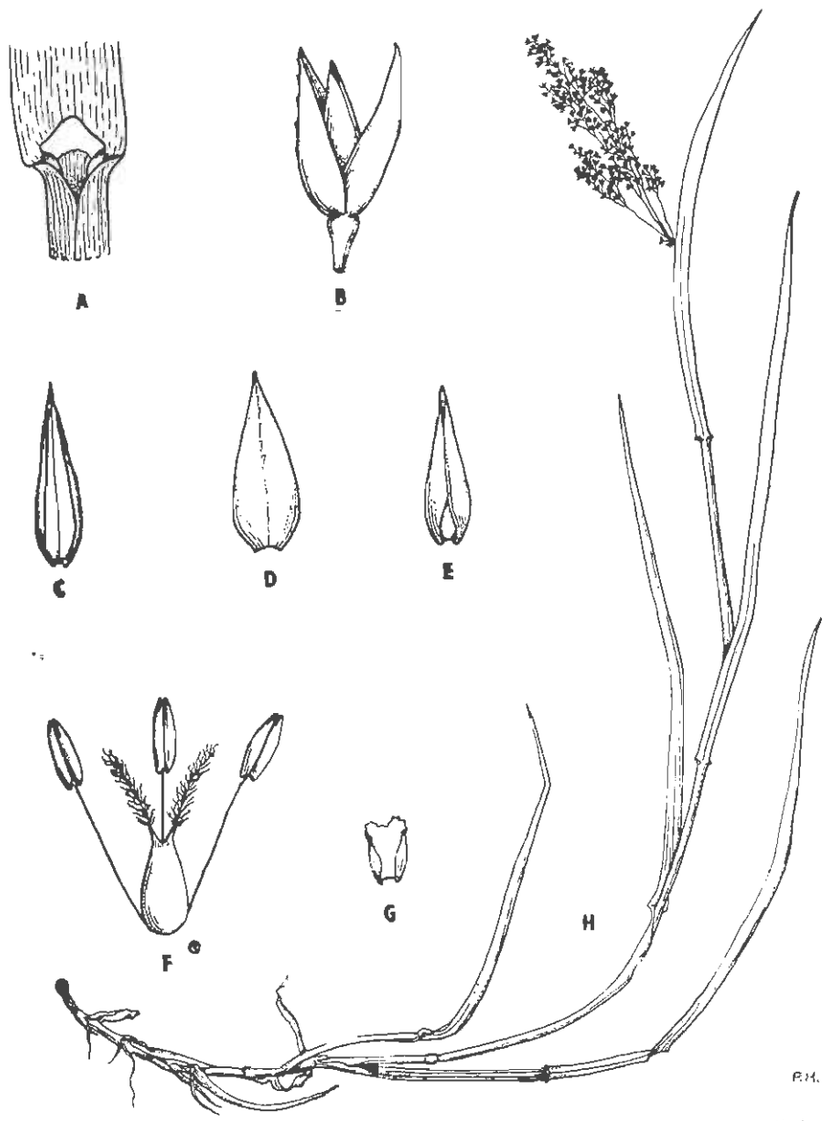
Agrostis clemensorum **Bor** sp. nov. ab aliis speciebus hujus ditionis Asiae austro-orientalis spiculis minutis (1·5–2 mm. longis) acuminati. distincta.

Gramen perenne (?) basi repens, radicans. Culmi usque 45 cms alti, vaginis obtecti, erecti, basin versus sparse ramosi, glabri levesque. Foliorum laminae usque 16 mm. longae, 5 mm. latae, lineari-acuminatae, utrinque scabridae, glabrae, valide nervatae, planae, rigidae; culmorum vaginae arcte complectentes, glabrae levesque, inferiores a culmis solutae; ligulae membranaceae, truncatae, circa 1 mm. longae, demum laceratae. Panicula erecta, densa, stricta, usque 6 cm. longa, 10 mm. lata, multi-spiculata; rami ramulique scaberuli. Spiculae ambitu lanceolatae, purpureae, 1·5–2 mm. longae. Glumae aequales, elliptico-acuminatae, 1·5 mm. longae, 1-nerviae, post anthesin hiantes, glabrae, inferior carina apicem versus scabra, superior levis; lemma 1·4 mm. longum, elliptico-acutum, haud truncatum, hyalinum, 5-nerve, callo glabro; palea bractea hyalina circa 0·7 mm. longa; stamina 3; antherae 0·75–0·9 mm. longae; styli 2, breves; stigmata brevia, plumosa.

A perennial grass, creeping and rooting at the base. Culms up to 45 cm. tall, erect, smooth and glabrous, covered by the sheaths, sparsely branched towards the base. Leaf-blades linear-acuminate, up to 16 cm. long, 5 mm. wide, glabrous, scabrous on both surfaces, flat, rigid, strongly nerved; culm-sheaths closely clasping, smooth and glabrous, the lower falling away from the base; ligules membranous, truncate, about 1 mm. long, lacerate with age.

Inflorescence an erect, strict, dense panicle up to 6 cm. long, 10 mm. broad, of many spikelets; branches and branchlets scaberulous. Spikelets lanceolate in outline, purplish in colour, 1·5 mm. long. Glumes equal, elliptic-acuminate, 1·5 mm. long, 1-nerved, gaping after flowering, glabrous, the lower scabrid on the back in the upper half, the upper smooth and glabrous. Lemma 1·4 mm. long, elliptic-acute, not truncate, hyaline, 5-nerved, glabrous on the callus; palea a hyaline scale about 0·7 mm. long; stamens 3; anthers 0·75–0·9 mm. long; styles 2, short; stigmas short, plumose.

Java; Mt. Gedeh, circa 3,000 m., 7–9–1932, *J. and M.S. Clemens* 30438; under the structure (sic) on the summit; leaves and stem rigid, Typus in Herb. Kew. (Text-Fig. 2).



TEXT-FIG. 2. *Agrostis clemensorum* Bor. A, ligule, × 6; B, spikelet; C, lower glume; D, upper glume; E, lemma; all, × 16; F, floret, × 20; G, palea, × 16. H, habit, × 1.

This species has been confused with *Agrostis infirma* Buese, under which name it was received at Kew. It is, however, an altogether much more robust plant with a very dense panicle and minute spikelets. I have named it in honour of the two collectors whose travels in Melanesia have done so much to elucidate the remarkable flora of those parts.

Tripogon siamensis **Bor** sp. nov. cum *T. wardii* comparanda sed partibus spiculae antherisque minoribus recedit. Gramen perenne, caespitosum. Culmi graciles, teretes, simplices, leves glabrique, usque 45 cm. alti. Foliorum laminae angustissimae, lineares, filiformes, usque 25 cm. longae, convolutae, 2–3 mm. latae, supra scaberulae, infra leves; vaginae culmos complectentes, leves glabrique, pilis paucis longis instructae; ligulae angustae, membranaceae, ciliatae.

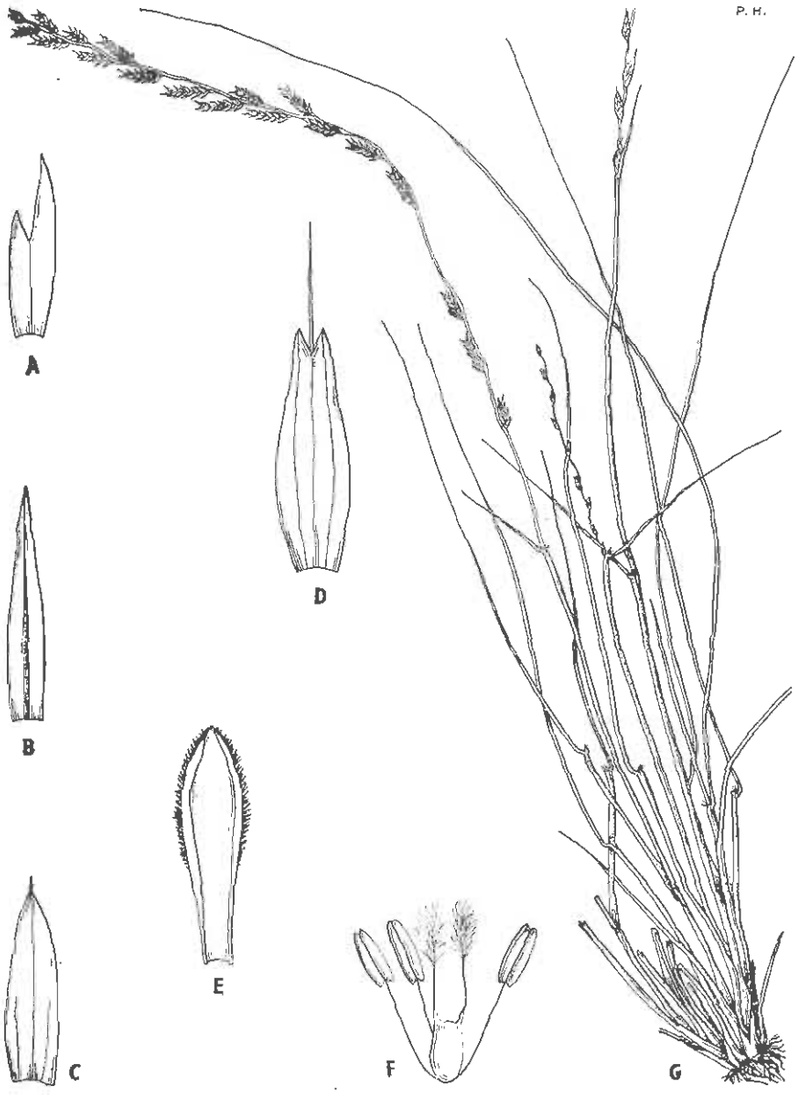
Inflorescentia spica terminalis, usque 20 cm. longa. Spiculae 8–10-flores, sessiles vel brevissime pedicellatae, rhachin continuam secus 2-seriatim dispositae; rhachilla supra glumas et inter flores articulata, floribus inferiore vacuo excepto hermaphroditis. Gluma inferior 5 mm. longa, 1-nervia, subulata lobo laterale instructa; gluma superior 4·5 mm. longa, lanceolato-acuta, 1-nervia, glabra. Lemma inferius vacuum, oblongum, 1-nervium, brevissime aristatum; palea nulla. Lemma secundum 4·45 mm. longum, 3-nervium, apici bilobatum, inter lobos aristatum; arista 3 mm. longa, recta; palea 2·5 mm. longa, bicarinata; stamina 3; antherae 1 mm. longae; styli 2; stigmata longa, plumosa; caryopsis non visa.

A caespitose perennial grass. Culms slender, terete, simple, smooth and glabrous, up to 45 cm. tall. Leaf-blades very narrow, linear, filiform, convolute, up to 25 cm. long, 2–3 mm. wide, scaberulous on the upper surface, smooth below, erect, overtopping the inflorescence; sheaths clasping the culms, smooth and glabrous, except for a few long hairs at the mouth; ligule narrow, membranous, ciliate.

Inflorescence a terminal spike of spaced spikelets up to 25 cm. long. Spikelets 8–10-flowered, sessile or very shortly pedicelled, seated on a continuous rhachis in two rows; rhachilla disarticulating above the glumes and between the florets; with the exception of the lowest, the florets hermaphrodite. Lower glume 5 mm. long, 1-nerved, with a lateral tooth on one margin; upper glume 4·5 mm. long, lanceolate-acute, 1-nerved, glabrous. Lowest lemma empty, oblong acute, shortly awned, 1-nerved, without a palea. Second and succeeding lemmas 4–4·5 mm. long, 3-nerved, 2-lobed at the tip, with a 3 mm. long awn issuing from the cleft; palea 2·5 mm. long, 2-keeled; stamens 3; anthers 1 mm. long; styles 2; stigmas plumose; caryopsis not seen.

Thailand.—Loei, Phu Krading, 14–7–1959, 1300 m.; *Tem Smitinand* 6074; tufted grass, common on rocky ground, along edge of evergreen forest. Typus in Herb. Kew. (Text-Fig. 3).

This species is distinguished from the other species of *Tripogon* so far described, with one exception, by the presence of an empty lowest lemma. The exception is *Tripogon wardii* Bor, which also has the lowest



TEXT-FIG. 3. *Tripogon siamensis* Bor. A, lower glume; B, upper glume; C, lowest lemma; D, second lemma; E, its palea; all, × 8; F, floret, × 10; G, habit, × 3.

lemma empty without a palea, but in that species the spikelets are closely crowded along the rhachis.

STATUS OF THE ORDER CHAETANGIALES (RHODOPHYTA)

BY T. V. DESIKACHARY

University Botany Laboratory, Madras 5, India

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IN 1958, in an article dealing with the Taxonomy of Algae I had considered the possibility of erecting an order, Chaetangiales (*nomen nudum*) but had refrained from doing so because of two factors: firstly the implication of the carpogonium in the production of the gonimoblast in *Scinaia* (Svedelius, 1915), and secondly the occurrence of fusions between the fertilized carpogonium and the supporting cell in *Dermonema* while the gonimoblast arises from the fertilized carpogonium itself (Svedelius, 1939). These two were thought to be somewhat against a clear-cut demarcation of the Chaetangiales from the rest of the Nemalionales. I had intended, therefore, to deal with this problem in a later paper. Recently I investigated the genus *Dermonema* and have found that in no case I could observe the fusion of the supporting cell and the fertilized carpogonium (Desikachary, 1962). It would also be significant to note here that according to Svedelius himself (1942, p. 86) this fusion does not necessarily take place in *Dermonema*. Similarly it is now known that in a number of members which resemble *Scinaia* the origin of the gonimoblast initials does not completely agree with that described for *Scinaia* by Svedelius. Thus, it would appear there is a greater uniformity with regard to the post-fertilization development of the Chaetangiales than has been so far recognized. I would, therefore, deal with this problem in the present paper.

Chaetangiaceae include *Chaetangium*, *Scinaia*, *Pseudoscinaia*, *Gloiophloea*, *Pseudogloiophloea*, *Galaxura*, *Actinotrichia* and *Whidbeyella*. The post-fertilization changes of these genera are now well known with the exception of *Actinotrichia* and I shall deal with these genera below in a very brief way.

Chaetangium.—The carpogonial branches arise laterally from one of the cortical filaments and are 4-celled. The three sterile cells below the carpogonium cut off several lateral branches of one to three cells each. The cell below the carpogonium, the hypogynous cell, remains in pit connection with the carpogonium. The zygote nucleus appears to migrate into this hypogynous cell and undergoes meiosis therein. The gonimoblast originates from the hypogynous cell as a small cell, and it produces several chains of gonimoblast filaments (Martin, 1939; Levring, 1945).

Scinaia.—The carpogonial branch is 3-celled. The hypogynous cell cuts off four cruciately arranged cells. After fertilization the zygote nucleus migrates into one of the hypogynous cells which functions as an auxiliary cell and undergoes meiosis therein. The gonimoblast initial though arising from the hypogynous cells pierces through the empty carpogonium and comes out (Svedelius, 1915; Levring, 1953).

Galaxura.—The carpogonial branch is 3-celled and the sterile cells below the carpogonium develop lateral branches at a very early stage. After fertilization the zygote nucleus (or its products) migrates into the hypogynous cell. Open pore connections are now formed between the hypogynous cells and the lateral branches. The gonimoblast filaments are formed either through the basal part of the carpogonium, directly from the hypogynous, or its laterals, or even from the cell below the hypogynous cell, *i.e.*, the first sterile cell of the carpogonial branch (Svedelius, 1942 *a* and *b*).

In *Gloiophloea* (Levring, 1953) the carpogonial branches are 3-celled. The hypogynous cell divides into four and after fertilization branched filaments are developed from the third cell of the carpogonial branch and these form a thin wall around the gonimoblast. The gonimoblasts arise from the carpogonium in association with the hypogynous cells, in the same way as in *Scinaia.*

In *Pseudoscinaia* (Levring, 1953) and *Pseudogloiophloea* (Levring, 1955) the gonimoblast filaments arise from the fertilized carpogonium. Later a large fusion cell is formed involving the four hypogynous cells and the carpogonium. Svedelius (1956) has observed that in *Pseudoscinaia* several gonimoblasts are formed from the fusion cell which is formed after fertilization. Similarly in *Pseudogloiophloea* (Svedelius, 1956) the hypogynous cell of the carpogonial branch and its laterals grow rapidly. The zygote nucleus migrates into one of the hypogynous cells immediately after fertilization and undergoes reduction division therein. Desikachary and Singh (1958) have described the post-fertilization stages in *Pseudogloiophloea fascicularis.* According to them the cruciately arranged hypogynous cells are really compactly arranged one-celled and two-celled laterals of the hypogynous cells, thus bringing it closer to other genera where the hypogynous cells similarly form sterile laterals. The gonimoblast initial is formed from the hypogynous cell and may emerge out directly.

Whidbeyella.—The carpogonial branch is 3-celled. The hypogynous cell divides to form four cells. The zygote nucleus apparently migrates from the fertilized into one of the hypogynous cells, as the primary gonimoblast initial is formed from one of them (Scagel, 1962).

Thus it would appear the Chaetangiaceae show a greater degree of uniformity in post-fertilization development than has hitherto been considered and are essentially characterized by the fusion of the fertilized carpogonium with a hypogynous cell, the transference of the zygote

nucleus or its products to that cell, and lastly the formation of the gonimoblast initials from the hypogynous cell. This last character seems to be basic though variations have been reported. There is need for studying variations in the earliest stages of the gonimoblast formation. In fact, Svedelius himself (1915, p. 38; 1933, p. 52) reports cases in *Scinaia* in which the gonimoblast initials arise from the hypogynous cell directly and he does attach significance to these observations. This recognition has its taxonomic repercussion, which is the purpose of the present paper.

In any consideration of the taxonomic status to be given to the genera of the Chaetangiaceae or the validity of a separate order, the Chaetangiales, attention is naturally turned to the taxonomic prinicples or criteria on which orders are at present based in the Florideophycideae (Rhodophyta). Schmitz, and later Kylin, the two persons to whom we owe so much in the evolution of the Red Algal classification, have based it on the post-fertilization stages and more particularly on the auxiliary cells. It would be to no purpose to discuss at length the evolution of the classifications as, at the present day, it is Kylin's that is generally accepted. Kylin (*see* 1956) defined the different orders: Typical auxiliary cells absent: Nemalionales and Gelidiales; Typical auxiliary cells present: Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales. Thus, Nemalionales with which we are presently concerned are said to have no auxiliary cells and are further differentiated by their haplobiontic life cycles from the Gelidiales with diplobiontic life cycles. The last character is not any more valid in differentiating the Nemalionales and the Gelidiales (*see also* Drew, 1957) but I would not discuss this here.

It would be relevant therefore to discuss the "auxiliary cell" as such. The term auxiliary cell was used by Schmitz (1883) but it was Kylin and Svedelius who brought the term to a great debating point. As pointed out earlier the definition of an auxiliary cell has at the present day a great significance or consequence in taxonomic considerations, though recognized, not yet fully given effect to so far as the Nemalionales are concerned.

Schmitz (1883; *see also* Kylin, 1935) pointed out that the sporogenous filaments of the Rhodophyceae connect themselves with nutritive cells in the mother plant and he calls these auxiliary cells. Kylin (1923) distinguishes two types, one in which it functions only as a nutritive cell such as in the Gelidiales and the second in which it not only functions as a nutritive cell but also as the starting point of the gonimoblast as in many Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales. Kylin would like to apply the term auxiliary cell only to the last category. In 1928, he uses the term typical auxiliary cell to denote the auxiliary cells from which the gonimoblast arises and stresses this character should be a starting point of Floridean systematics. He also mentions that typical auxiliary cells are absent in two Orders, the Nemalionales and the Gelidiales. He considers the procarpic forms as a rule at a higher

evolutionary level than the non-procarpic forms. His article in 1935 elucidates the same view-point. Thus, Kylin amended the definition of the word and reoriented its significance. According to him in Florideae with typical auxiliary cells direct communication is established between the gonimoblast and the mother plant and the carpogonial branch plays no part in the transportation of the nutritive material. Thus, the food-supply becomes more direct. According to Kylin the typical auxiliary cell never occurs in the carpogonial branch. "Typical auxiliary cells never lose their function of being the starting points of development of gonimoblasts but they can lose their nutritive function. Sometimes they have a nutritive function only during the very first stage of the development of the gonimoblasts but are of no importance later on and seldom do not disappear totally. In such cases there are other cells which succeed them in acting as food-supplying medium."

Svedelius (1933; *see also* 1942*a*, p. 82-87) was the first to disagree with Kylin. In his opinion "It is a matter of secondary importance whether or not an auxiliary cell belongs to the carpogonial branch proper, and also whether the gonimoblast is developed directly from the latter or not. It should be pointed out that all such cells that fuse directly or indirectly with the carpogoniumand that, though temporarily, have received and housed future gonimoblast nuclei, must be designated auxiliary cells. When Schmitz chose the term 'auxiliary cell' he certainly had in mind the fact that these cells were supporting not only as food storing cells but also as housing the fertilized nucleus. An auxiliary cell must have received and housed the fertilized female nucleus or nuclei originating therefrom. This quality distinguishes the auxiliary cell sharply from the cells that are only food storing, which often accompany the carpogonial branches, the so-called 'sterile cells' in the carpogonial branch system. Such cells are to be regarded as storage tissue and are easily distinguishable from auxiliary cells."

Martin (1939) agrees with Svedelius in designating the hypogynous cells of the Chaetangiaceae as the auxiliary cells.

Fritsch (1945) seems to regard only those cells producing the gonimoblast filaments as the auxiliary cells. In a foot-note on p. 601 he says: In certain Florideae the connecting filaments first fuse with cells resembling auxiliary cells, but these *nutritive cells* do not provide a stimulus for carpospore formation. Comparable phenomena occur in some Nemalionales.

Papenfuss (1951) distinguishes two types of auxiliary cells, the generative auxiliary cell which is perhaps the same as the typical auxiliary cell of Kylin—and he includes the supporting cells or other cells of the Nemalionales which furnish nutritive materials and generate the gonimoblast—and the nutritive auxiliary cells for the cells which on fusion furnish nutritive materials but do not serve as starting points of the gonimoblast. These latter fusions are yet considered essential by Papenfuss and as examples he gives Gelidiales, some Cryptonemiales and Gigartinales.

Drew (1953) discusses the terminology of the auxiliary cell. She feels a distinction must be made, however, between genuine nutritive auxiliary cells, *i.e.*, specified cells with which the carpogonium or gonimoblast filaments fuse either in the case of the carpogonium before the formation of the gonimoblasts or in the case of the gonimoblasts, before the formation of the spore-producing filaments and nutritive cells and nutritive tissue with which no fusion takes place. She gives the following definition: An auxiliary cell is a specified cell of the gametophyte with which the carpogonium fuses before the formation of the gonimoblast or a cell with which a primary gonimoblast fuses. Auxiliary cells have a purely nutritive function (nutritive auxiliary cells) in those cases where no fertilization nucleus is transferred or combined nutritive and generative functions (generative auxiliary cells) in those cases where the fertilization nucleus or its derivative is transferred to that cell and there initiates the development of secondary gonimoblasts.

Thus, it would appear that the problem centres round what function denotes an auxiliary cell. All fusions after the fertilization of the carpogonium involving either the carpogonium or the products of the fertilized carpogonium (connecting filaments) must be presumed to be nutritive. In fact a whole series of forms exist in the Nemalionales which show various patterns of non-specific post-fertilization fusions leading to the formation of large fusion cells, and similarly in the Gelidiales which are essentially nutritive and in a number of Gigartinales specific fusions do occur with differentiated cells which signify similar nutritive functions. Simultaneous with such fusions we see, as Drew has ably analyzed, a gradual transference of the site of gonimoblast formation to sites closer to the region of nutrition, *i.e.*, these auxiliary cells, and ultimately a segregation of these sporogenous filaments to the central cells which are nutritive. In other words it is the gradual shifting of the gonimoblast formation from the fertilized carpogonium to the auxiliary cells. It appears these fusions are essential for purposes of nourishment of the spore-producing mechanisms and in this evolution of the nourishment of the parasitic sporophyte there is a definite and effective segregation of the spore-producing capacity to definite centra. This view that auxiliary cells are essentially nutritive in function does go in well with Schmitz's conception of the auxiliary cells involving forms like *Naccaria*. Thus all fusions whether with a large number of indefinite or definite cells near or away from the fertilized carpogonium are primarily for nutrition and in the highly evolved forms all these also happen to be or become sites of gonimoblast production too.

This conception of the function of the auxiliary cells does not minimise the association of the generative function in the evolved auxiliary cell in the classification of the red algae.

The second objection to the application of the term auxiliary cell to specific cells comes from Kylin according to whom the auxiliary cells should not be carpogonial. This restriction seems to go beyond the original definition of the word as intended by Schmitz and it would be right to apply the term more easily to all cells which are centra of

gonimoblast production and which receive the fertilization nucleus or its products. In other words, Svedelius was right in applying this term to the Nemalionalen members which form gonimoblasts from cells of the carpogonial branch, hypogynous cells, which form primary centra of gonimoblast formation. This does not of course include large fusion cells, formed by the non-specific fusions of more than one cell of the carpogonial branch from which primarily or secondarily gonimoblasts arise. This analysis brings out that strangely in both the currently understood advanced forms (or ancestral forms according to some) and in primitive (or derived according to some) forms we see the formation of the gonimoblasts from auxiliary cells which are derived from the cells of the carpogonial branch or the supporting cells of the carpogonial branch and probably with it a segregated or condensed pattern of gonimoblast production with distinct auxiliary cells. Thus it would appear that from a non-specific pattern of nutrition there has been an evolution to specific pattern involving the carpogonial branch and in this various auxiliary cell types have evolved though finally there is a reversion to carpogonial pattern.

One feels that Kylin's hesitancy in accepting the carpogonial auxiliary cells seems to be probably borne out of taxonomic changes entitled by such a recognition, as advocated by Svedelius. Svedelius himself, while arguing for the Nemalionalen auxiliary cell, did not advocate any taxonomic change in the conception of Orders.

It would seem appropriate that the term auxiliary cell should be applied to any cell other than the fertilized carpogonium belonging to the carpogonial branch or not, from which the gonimoblast filaments or their precursors, the ooblastema filaments, make their appearance. Use of Papenfuss' terminology has the advantage of retaining the original significance given to it by Schmitz while at the same time helping to stress the added generative function. How then should the Chaetangiaceae and the Bonnemaisoniaceae be taxonomically arranged ? In the Chaetangiaceae the gonimoblast filaments arise from a modified hypogynous cell which should be considered as a generative auxiliary cell. Thus, it would appear that the Chaetangiaceae are more or less uniform in their post-fertilization changes and would seem distinct from the rest of the Nemalionales. The order Chaetangiales may, therefore, be created to include the family Chaetangiaceae.

The only other family of the Nemalionales which very nearly resembles the Chaetangiaceae is the Bonnemaisoniaceae and in a lesser way the Naccariaceae. In the Naccariaceae the gonimoblast filaments develop directly from the fertilized carpogonium though the carpogonium may fuse with a cell in the carpogonial branch (Kylin, 1928). Fan (1961) has drawn comparison between this family and the Gelidiales and very correctly suggests that it may be better to retain this family in the Nemalionales.

Bonnemaisoniaceae includes *Bonnemaisonia*, *Asparagopsis*, *Delisea*, *Ptilonia* and *Leptophyllis*. We know very little of the post-fertilization

development in the last genus. But of the others we know fairly well. The following is a short account of the genera.

In *Bonnemaisonia* the carpogonial branches arise from the short secondary laterals. The terminal cell of the fertile branch cuts off lateral cells which are involved in the formation of the cystocarpic wall. The subterminal cell cuts off three pericentral cells one of which becomes the supporting cell of the carpogonial branch. The hypogynous cells give rise to laterals which show enlargement and further division after fertilization. The zygote nucleus remains in the carpogonium in which the reduction division occurs (*cf.* Magne, *1960*). The fertilized carpogonium forms the gonimoblast initial, while the hypogynous cell and the nutritive cells fuse together and serve nutritive purposes (Svedelius, 1933; Kylin, 1916). The gonimoblast initial forms first a basal disc made of several cells which later fuse with the nutritive cells and form a large fusion cell which occupies the floor of the cystocarp.

Ptilonia (Levring, 1955) agrees very closely with *Bonnemaisonia*. In this genus the carpogonial branches are three-celled, and are formed near the growing tips from the pericentral cells which become the supporting cells. The hypogynous cell forms a small number of sterile cells. After fertilization a group of cells is formed from the fertilized carpogonium. The primary gonimoblast is connected with the hypogynous cell, nutrition is delivered from the accessory cells of the hypogynous cell, and a fusion cell is gradually formed. From the fusion cell a number of branchlets arise which form the carpospores. The development of the gonimoblast is thus very similar to *Bonnemaisonia*.

Asparagopsis and *Delisea* agree very closely in their post-fertilization development. In *Asparagopsis* (Kylin, 1928; Svedelius, 1933) the carpogonial branches arise as secondary fertile laterals. The penultimate cell cuts off the supporting cell. The carpogonial branch is three-celled. The zygote nucleus migrates from the fertilized carpogonium to the hypogynous cell and the carpogonium degenerates. Reduction division takes place in the hypogynous cell. The cells below in the carpogonial branch show wider protoplasmic connections and ultimately a large fusion cell is formed at the base of the cystocarp. The wall of the cystocarp is formed by the activity of the cells of the fertile lateral branch below the carpogonium. The gonimoblast arises from the hypogynous cell which is thus an auxiliary cell. Two or more gonimoblast branches develop from the auxiliary cell. These primary gonimoblast cells form a central group of cells from which originates, by means of active cell divisions in different directions, a rather large tissue, the placenta, in the centre of the cystocarp. Cell fusions occur in the older parts of the gonimoblast itself as well as in the surrounding parts of the tissue of the female branch.

In *Delisea* (Levring. 1953) the carpogonial branches are three-celled and are developed opposite normal sterile branchlets near the growing tips of the thallus. The hypogynous cell cuts off a few cells serving as nutritive cells. The fertilized carpogonium is obviously connected

with the hypogynous cell and together with the accessorial nutritive cells of the hypogynous cell, a fusion cell is formed. From this fusion cell a number of fertile branches are developed.

Feldmann J. *et* G. (1942) established the Bonnemaisoniales and it has been accepted only by a few workers so far. They stress the existence of a haploid tetrasporophyte which is morphologically different from the gametophyte as of sufficient importance in separating the order Bonnemaisoniales. In 1952, Feldmann confirmed his earlier views on Bonnemaisoniales that it represents algae with highly evolved vegetative organization which may be considered to be derived from the diplobiontic forms by a morphological reduction of the tetrasporophyte which is still independent producing apomeiotic tętraspores (*see*, however, Magne, 1960). Chadefaud (1940) and Feldmann (1952) seem to feel that the Bonnemaisoniales may be considered to have been derived retrogressively from the diplobiontic forms probably on similar lines as we see in the Gigartinales. The cytology of the vegetative thallus and their construction, as also in a lesser way the post-fertilization development, according to Feldmann, appear to bring into closer relationship the Bonnemaisoniaceae and the Ceramiales especially the rhodomeloid forms.

Chadefaud (1960) has followed up his earlier ideas on the vegetative organization and cytology of the Rhodophyceae and classified these into three categories or groups: *(1) Eufloridées (with a single* order, Nemalionales)—carposporophyte purely carpogonial (not auxiliary) with florideoside associated with trehalose and with mucilage rich in mannonates, plants without a cladome (Acrochaetiales) and plants with uni- or multiaxial cladomes (Eunemalionales). He considers these as archaic characters; (2) Mesofloridées with evolved characters (Chaetangiales, Gelidiales, Gigartinales, Cryptonemiales and Rhodymeniales); (3) Metafloridées with highly evolved characters (Bonnemaisoniales and Ceramiales). Thus, he considers that Bonnemaisoniales have certain vegetative or cytological characters which bring it closer to the Ceramiales, *viz.*, a rather progressively evolved cladome, semi-rhodomeloid with refractive cellular secretions, gonimoblast filaments sunk in conceptacles with a bunch of nutritive filaments, and a trigenetic life-cycle. He considers the Bonnemaisoniales and the Ceramiales are interrelated by such characters as the tendency for the disappearance of the primordial thallus which is practically absent in the Ceramiales, the evolution of the cladome towards a rhodomeloid structure, procarps lodged in conceptacles, and the existence of cellular secretions.

As against these characters there are not many characters that would permit the Chaetangiaceae or the Chaetangiales and the Bonnemaisoniales to be merged together. In fact, in the Bonnemaisoniales the post-fertilization changes are not uniform in all the members and the vegetative characters are different from that of the Chaetangiales. I would therefore consider that the Chaetangiales are distinct from the Bonnemaisoniales. Chadefaud (1960) establishes the order Chaetangiales

(*nomen nudum*; *see also* Desikachary, 1958), as characterized by multiaxial cladomes, with distinctly multilayered cortex, primitive stellate chromatophores with pyrenoids as in *Galaxura* resembling the Nemalionales. Chadefaud does not recognize the auxiliary cells. I would, therefore, add the presence of auxiliary cells which give rise to the gonimoblast initials. A Latin diagnosis is added at the end.

Chadefaud (1960) puts both the Naccariaceae and the Bonnemaisoniaceae under the Bonnemaisoniales. The purpose of this paper is to delineate the characters and justify the establishment of the Chaetangiales. Therefore, it would be beyond my intention to discuss the Naccariaceae. Nevertheless it may be pointed out that both in certain vegetative characters and in the post-fertilization development there are differences between the Naccariaceae and the Bonnemaisoniaceae. I have pointed out earlier that Naccariaceae have certain resemblances with the Gelidiales as has been well pointed out by Fan (1961). I would support Fan in placing this family in the Nemalionales until future investigations reveal a better taxonomic placement.

In a similar way I am opposed to the inclusion of the Chaetangiaceae in the Bonnemaisoniales as has been suggested by Fan (1961) merely on the basis of the presence of an auxiliary cell. Fan (1961) considers the Nemalionales (*sensu stricto*, *sans* Bonnemaisoniaceae, *sans* Chaetangiaceae) as mainly haplobiontic in the majority and the Gelidiales as diplobiontic. In this he does not take note of the Acrochaetiaceae, which Feldmann (1952) likes to elevate into an order Acrochaetiales. and the general opinion that the Gelidiales are not so distinct from the Nemalionales *sensu lat.* (*see* Drew, 1957). Only in one aspect the Gelidiales seem distinct that is they represent a distinct stage in the evolution of the red algal carposporophyte.

Dixon (1961) analyses the characters of Nemalionales and considers that the different families are closely interrelated and there are no adequate criteria by which they can be separated. He suggests that the Gelidiaceae be returned to the Nemalionales. As Dixon has pointed out there is a growing urge for a revision of Kylin's system and a reversion to Nemalionales sensu Schmitz does not serve the purpose.

There is a need for basing taxonomy on more than one character. In single character taxonomy in the words of Svedelius (1933, p. 53), "We see from this the risk of basing the system of the Rhodophyceae too one-sidedly on one character only, as for instance in the presence or absence of the auxiliary cell, etc., just as in all taxonomy the whole organization of the plants must be considered and not only single characters". Red algal taxonomy is slowly evolving out of a classification in which it had stayed for more than two to three decades and many an eminent phycologist had expressed the need for a change, or a better classification. This need has been more so felt in the Nemalionales, Gigartinales, and Cryptonemiales. The recognition of the Acrochaetiales, Bonnemaisoniales and the Chaetangiales would probably be right and

this step would leave the Gigartinales and the Cryptonemiales for future attention.

Latin Diagnosis

Order Chaetangiales (*Rhodophyta*)

Plantae cladomatibus multiaxialibus et distincto cortice multistrato ornatae; formae haplobionticae vel diplobionticae; ramuli carpogoniales praediti cellulis hypogynis, quae operantur ut cellulae auxiliares; gonimoblasta emergentia principaliter e cellula hypogyna consociata *cum* carpogonio fecundato vel secus.

Familia typica unica Chaetangiaceae.

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SOME OBSERVATIONS ON THE MORPHOLOGY AND EMBRYOLOGY OF *EUCOMMIA ULMOIDES* OLIV.

BY TH. ECKARDT

Institut für Systematische Botanik und Pflanzengeographie der Freien Universität Berlin, Berlin-Dahlem, Germany

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INTRODUCTION

IN a previous article we have communicated some new facts on the floral morphology of *Eucommia ulmoides* Oliv. and discussed the problem of the systematic position of this monotypic family (Eckardt, 1957, *see also* for further literature). As we have stated there, embryological data of *Eucommia* were hitherto unknown. Since then we have collected and studied some new material, but it is a very difficult and time-consuming task to get a complete series of stages because the few trees in our Botanical Garden, Berlin-Dahlem, do not flower regularly and often only scantily. We, therefore, cannot give here the whole life-history of *Eucommia*, the following contribution presents only some observations selected from a more detailed study on histogenesis, floral morphology and embryology in progress. A publication even of partial results seems to be justified, as we have independently found and can confirm some of the statements in a recent investigation of Tang (1962), with which we were acquainted only just now after having finished this contribution. On the other hand, a comparison of our results with those of Tang in each case is of interest.

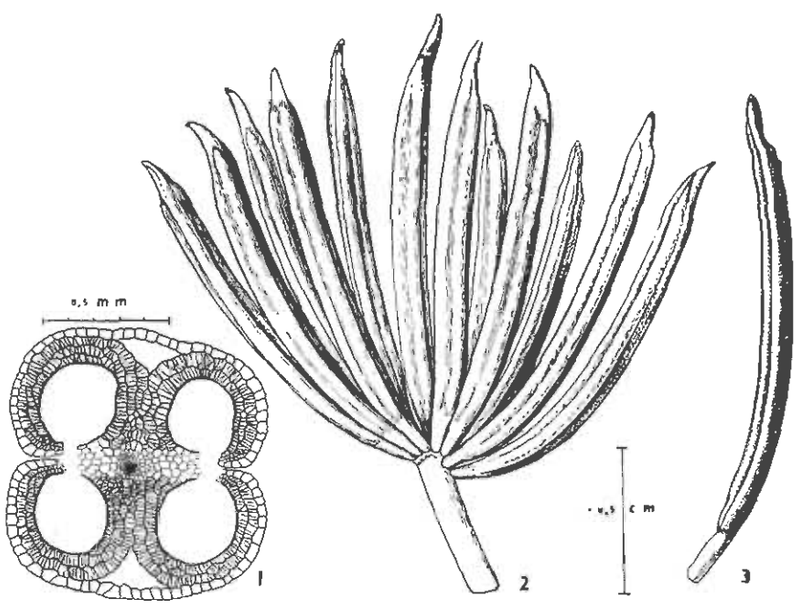
MATERIAL AND METHODS

Periodic collections of buds, flowers and ripening fruits were made from one male tree and two female trees growing in the Botanical Garden, Berlin-Dahlem. A part of the collected material has still to be examined. After partial dissection materials were fixed in either CRAF V (chromic acid - acetic acid - formalin) or FPA (formalin-propionic acid - alcohol) and then aspirated (for formulae see Sass, 1951, p. 15-18). The specimens as usual were embedded in paraffin. Sections were cut at 6–8–10 microns and stained in either Safranin O and Fast Green FCF, or in Heidenhain's iron-alum haematoxylin, or in tannic acid-iron chloride-Safranin as suggested by Foster (1943).

FLOWERING SHOOTS

Vegetative as well as flower-bearing shoots of *Eucommia* are sympodial, the uppermost functioning bud formed at the end of the growing

season being pseudoterminal. The flower buds begin with some 6–8 sterile scales, which are succeeded on incipient shoots by a varying number (from 1–6–8) of floriferous bracts of transitional nature and these are followed by a varying number of leaves. In such a way the flower-bearing shoots, the male as well as the female, produce from 1 to 6–8 and even more flowers according to their position within the twig system and the conditions of their nourishment. *Eucommia* is a dioecious plant, the flowers of both the male and the female trees are born singly in the axils of the bracts. Bracteoles are lacking, only in one case a small leaflet was observed on the pedicel. Fertile shoots of both sexes show a flower-bearing part only at the base, which is succeeded by an extensive leaf-bearing prolongation. These intercalary inflorescences are to be compared with an open raceme. This is in contrast to Varossieau (1942, p. 83), who writes: "The male inflorescence is most probably a cyme. However, this can only be conclusively stated by an ontogenetic study." Indeed ontogeny shows clearly the racemose arrangement of male flowers, which is just the same as of female flowers. The fertile part of a flowering shoot of *Eucommia* in a high degree resembles the intercalary inflorescences of *Euptelea* (Nast and Bailey, 1946).



TEXT-FIGS. 1–3. *Eucommia ulmoides*, male, May 10, 1962. Fig. 1. T.s. of anther at the time of shedding. Note large extensions of fibrous thickenings. ig. 2. Mature male flower with 12 stamens, seen from abaxial side. Fig. 3. A ngle stamen in lateral view, showing protrusion of connective above the thecae.

MALE FLOWERS

The male flowers of *Eucommia* have pedicels about 6–7 mm long, the apex of which is a somewhat flattened receptacle devoid of perianth (Text-Fig. 2). By compression within the bud the single flower is remarkably flattened, so that the stamens come to lie in two rows face to face. Originally the stamens seem to be arranged in one cycle, but for spatial relations some irregularities often occur (*see* Text-Fig. 2). The pedicel in transverse section shows an elliptic outline, indicating a slight dorsiventrality of the whole male flower. The vascular system of the flower is very simple. Throughout the pedicel extends a siphonostele, which resolves at the base of the receptacle into the whorl of the staminal traces.

The stamens vary in number from 5–12 (and even more) and are differentiated into a very short filament, an extensive anther of 10–14 mm length, and a prolongation of the connective above the thecae (Text-Figs. 2, 3). The four elongated and protruding microsporangia are laterally oriented in pairs. Their lateral position in mature anthers is preceded by a slightly introrse outline of staminal primordia. Prior to anthesis the septa between two adjoining microsporangia of each theca disappear. In mature anthers the thecae dehisce by a longitudinal split (stomium) in the grooves between the two microsporangia (Text-Fig. 1). The staminal vascular bundle runs unbranched almost to the short conical apex beyond the sporangial region. Remarkable is the large extension of endothecial fibrous thickenings in anther wall, almost completely enclosing the sporangia and uniting in the connective, surrounding the staminal bundle from the abaxial and adaxial sides (Text-Fig. 1). Note also the detaching of epidermis in the region between the thecae, which takes place in very young anthers.

The early stages in the development of the anther will be described elsewhere. The primary archesporium is hypodermal, by periclinal division giving rise to an outer parietal layer and an inner sporogenous layer. Finally we have, besides the epidermis, a wall of usually 4–5 layers, of which the outer two persist in mature anther (Text-Fig. 1). The tapetum, probably of parietal origin, is of the secretory type. The tapetal nuclei undergo some divisions, so that full-grown tapetal cells are 2 to 6-nucleate (Plate I, Figs. 1, 7).

MICROSPOROGENESIS AND MALE GAMETOPHYTE

The microspore mother cells with conspicuous nuclei (Plate I, Fig. 1) undergo meiotic divisions in the normal way to give rise to tetrahedral tetrads of microspores (Plate I, Figs. 2–5). These divisions are of the simultaneous type. After meiosis I (Plate I, Fig. 2) no wall is laid down between the two interphase nuclei (Plate I, Fig. 3) and only after meiosis II (Plate I, Fig. 4) the pollen mother cells become separated at once by furrowing (Plate I, Fig. 5). Soon the uninucleate microspores begin to separate from one another (Plate I, Fig. 6). The stages, figured in Plate I, Figs. 1–4, were observed in one and the same flower, and even in

the same anther one may see different stages of meiosis in the upper and lower parts. The first division of the microspores has not been observed, but the two cells thus formed are clearly visible in Plate I, Fig. 8, the vegetative nucleus with conspicuous nucleolus being by far larger than the small nucleus of the generative cell. The generative cell possesses a definite membrane and at first is situated towards the wall of the microspore, the vegetative nucleus being oriented towards the distal pole of the microspore. The pollen grains at the time of shedding (Plate I, Fig. 9) have a very dense cytoplasm and are of the tricolporoidate form (for details *see* Erdtman, 1952). Whereas the vegetative nucleus with its large nucleolus is prominent, the generative cell can be seen only in one pollen grain (marked by an arrow).

Female Flower

The female flowers of *Eucommia* are composed of two carpels, one of which is fully developed and fertile, whereas the other is solid and sterile (for further description *see* Eckardt, 1957, p. 489). There are neither perianth leaves nor rudiments of the male sex. The pistils resemble those of *Ulmus* and likewise are pseudomonomerous. They are shortly stalked, at the base being separated from the short (3–5 mm) pedicel by a constriction. The short style is crowned by two sessile stigmas of somewhat different size, according to the preponderance of the fertile carpel over the solid one.

Ovule, Megasporogenesis and Female Gametophyte

The ovary is unilocular and biovulate. The two ovules of the fertile carpel originate at the top of the locule on a submarginal placenta. They are pendulous with a dorsal raphe, that is they are apotropous (Plate II, Fig. 10). A stout bundle runs to the chalazal end of the ovule to reach the base of the nucellus. The shape of the ovule is very remarkable: it is anatropous and unitegmic with a relatively thick integument whose micropylar canal is as long (and longer) as the thin, long-stretched nucellus. The nucellus is of a peculiar type (*see also* Dahlgren, 1928), intermediate between the well-known crassinucellate and tenuinucellate types, and described by Mauritzon (1939, p. 20). Regrettably this third type with weakly developed nucellus is hitherto unnamed, so that we must classify the megasporangium as weakly crassinucellate, in correction of our former characterization (Eckardt, 1957, p. 494) as tenuinucellate.

The ovular primordia are at first directed downwards to the bottom of the locule, but together with the early differentiation of the integument they assume a hemianatropous direction (Plate II, Fig. 11). In this stage the integument at the outer side is almost as thick as the nucellus, which already reaches the definite number of 5–6 cell layers in transverse direction.

The primary archesporial cell, differentiated in the hypodermal layer of the nucellus, divides by a periclinal wall into primary parietal

and sporogenous cells. The primary parietal cell undergoes only a few divisions to form a small number of wall layers (Plate II, Figs. 11, 13, 14). The epidermal cells capping the parietal tissue also undergo periclinal and oblique divisions to produce a nucellar calotte of long-stretched cells (*e.g.*, Plate II, Figs. 13, 14, 16). The sporogenous cell functions as the megaspore mother cell (Plate II, Fig. 10). Almost regularly there are found more than one megaspore mother cell either in a linear arrangement (Plate II, Fig. 13) or side by side (Plate II, Fig. 14). These supernumerary cells of a multicellular archesporium at first remain resting, but may undergo meiosis and develop rudimentary embryo sacs, as clearly seen in Plate II, Fig. 15, which may reach the 4 and 8-nucleate stages, but never develop further. Only the innermost of several archesporial cells forms the functional megaspore mother cell. The first meiotic division of the megaspore mother cell was not observed, but the second division in the two dyad cells. Only the chalazal megaspore of the linear tetrad of megaspores develops into the 8-nucleate embryo sac of the Normal or Polygonum type. The remnants of the degenerating three megaspores are striking by their red colour in safranin-stained preparations (Plate II, Fig. 13, marked by arrows). The cells of the egg apparatus are not so prominent as those of the antipodals (Plate II, Fig. 12), the secondary nucleus being very conspicuous before fertilization. The most outstanding feature is the pushing away of the nucellar cells by the fully organized embryo sac, thus coming in direct contact with the inner layer of the integument which, however, is not differentiated as an endothelium.

Fertilization and Endosperm

Normally the pollen tube of *Eucommia* after arriving at the top of the ovary enters the ovule through the micropyle (porogamy). The course of the tube is clearly observable, details of fertilization are given elsewhere. *Eucommia* is also characterized by having branched pollen tubes and occasional chalazogamy. Twice we have seen a case of chalazogamy. The pollen tube travels outside the embryo sac, that is to be fertilized, in the thick integument and enters the nucellus through the chalaza. Then near the antipodals it enters the embryo sac and reaches the egg by a direct penetration: intravesicular chalazogamy (*see* Swamy, 1948, p. 22) (Plate II, Fig. 18).

After fertilization the embryo sac expands in a direction parallel to the long axis of the ovule (Plate II, Fig. 17). The zygote remains undivided for a long time after fertilization. In the meantime the development of the endosperm has begun, which is of the Cellular type. The first deposited and somewhat oblique wall after the division of the primary endosperm nucleus is visible in Plate II, Fig. 12, an older stage of endosperm development is shown in Plate II, Fig. 15. Subsequent divisions in the endosperm cells take place in various oblique planes (Plate II, Figs. 16, 17), and finally a compact mass of nutritive tissue is produced, a large proportion of which, however, is used up by the

growing embryo. Of the two ovules only one produces the seed, the other degenerates and shrivels in about 4–6 weeks after pollination (in June).

As to the systematic position of *Eucommia* a much broader outlook is needed, involving evidence from all organs of the plant (*see also* Swamy and Bailey, 1949).

Summary

Eucommia ulmoides Oliv. has been studied especially from the point of view of embryology. A complete account of life-history can be given only after further investigations, which may throw new light on the systematic position of *Eucommia*.

Microsporangium and pollen.—The tapetum is of the secretory type. The tapetal cells are multinucleate. The endothecial thickenings are largely extended to the anther wall and the connective. The anther dehisces by a longitudinal split. The division of the microspore mother cells is of the simultaneous type. Mature pollen grains are 2-celled.

Ovule.—The ovules are pendulous with dorsal raphe (apotropous), anatropous, unitegmic with a long micropylar canal, and weakly crassinucellate. By divisions of the epidermal cells a nucellar calotte is formed. Parietal cells are cut off.

Female archesporium and embryo sac.—Usually some megaspore mother cells are produced either in linear or lateral arrangement. Only the innermost megaspore mother cell produces the functional embryo sac of the monosporic, 8-nucleate, Polygonum type, the supernumerary ones may also undergo meiosis and develop embryo sacs of the 4-nucleate or 8-nucleate stages, which soon shrivel.

Fertilization.—Normally the pollen tube enters through the micropyle (porogamy). In two cases intravesicular chalazogamy has been observed. The pollen tubes may branch. The zygote divides only after a conspicuous amount of endosperm is produced.

Endosperm.—The endosperm is of the Cellular type.

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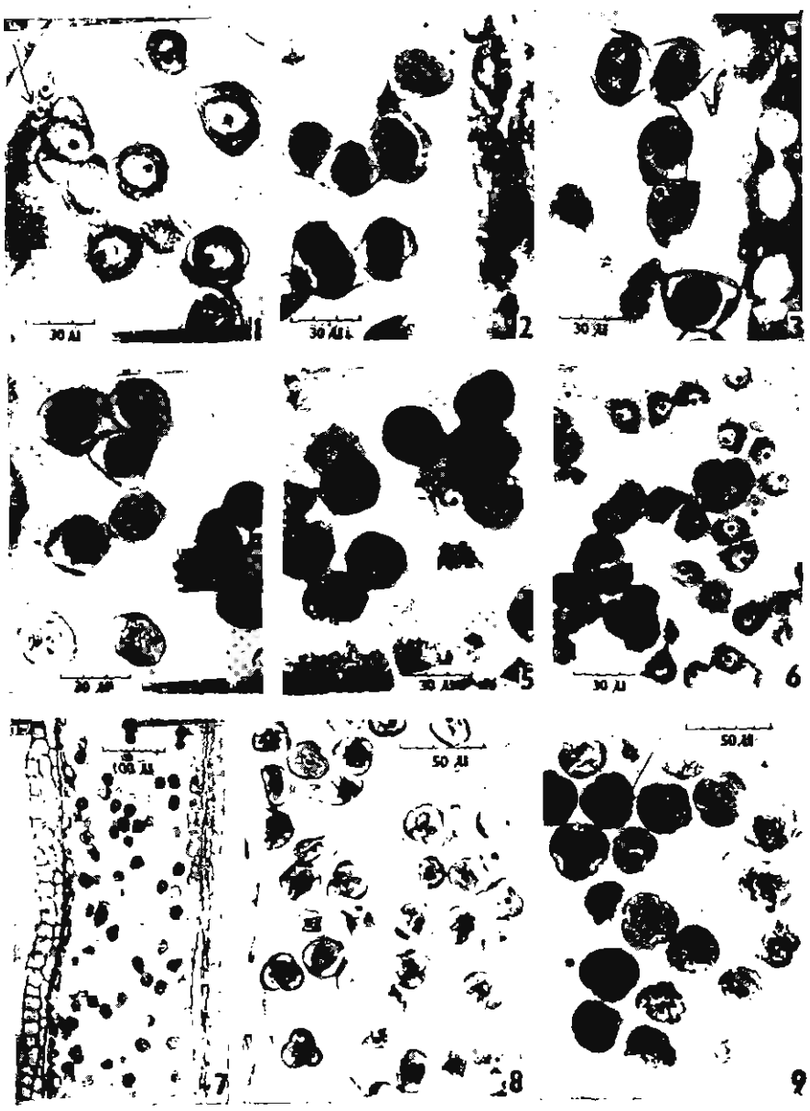

FIG. 1-9

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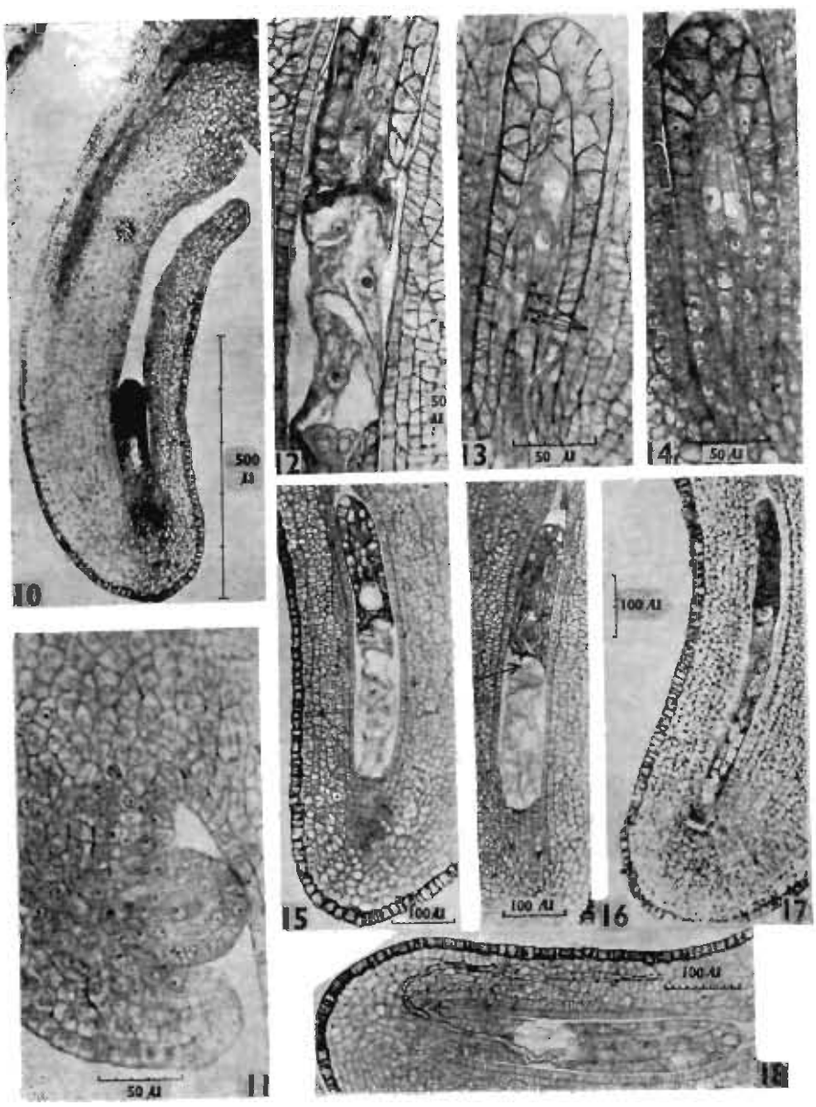

FIGS. 10–21

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EXPLANATION OF PLATES I & II

PLATE I

FIGS. 1–9. *Eucommia ulmoides*, microphotographs illustrating stages in the development of pollen grains. Material of Figs. 1–7 fixed April 29, 1954, of Fig. 8 April 26, 1962 and of Fig. 9, May 10, 1954.

FIG. 1. Microspore mother cells in early prophase. Note a 3-nucleate tapetal cell (arrow).

FIG. 2. Metaphase I.

FIG. 3. Interkinesis.

FIG. 4. Metaphase II.

FIG. 5. After meiosis II furrowing by special walls has begun.

FIG. 6. Separation of 1-celled microspores.

FIG. 7. L.s. of anther lobe with stages of meiosis II, below anaphase II, above telophase II. Note the tapetal layer with multinucleate cells.

FIG. 8. Two-nucleate pollen grains with a large vegetative nucleus and small generative one.

FIG. 9. Pollen grains at the time of shedding. Note the predominant tube nucleus and tricolporoidate pollen grains. A generative cell in the dense plasma was only once visible (arrow).

PLATE II

FIGS. 10–18. *Eucommia ulmoides*, microphotographs illustrating some stages in the development of ovule and embryo sac.

FIG. 10. L.s. through ovule about two weeks after pollination. In the lower part the section is not quite median. Note the bundle in the dorsal raphe, the thick integument, the long micropylar canal, the relatively thin nucellus, and the large megaspore mother cell.

FIG. 11. L.s. of young ovule with primary sporogenous and parietal cells.

FIG. 12. L.s. of embryo sac with cellular endosperm. Note two antipodal cells.

FIG. 13. L.s. of nucellus showing in the upper part a row of two sporogenous cells and in the lower part a tetrad of megaspores with the chalazal cell functioning. Note three dark dots (arrow) on the place of the three degenerated megaspores.

Fig. 14. Two sporogenous cells lying side by side.

FIG. 15. L.s. of nucellus showing a supernumerary 4-nucleate embryo sac (two upper nuclei visible !) and the cellular endosperm of functional embryo sac.

FIG. 16. L.s. of nucellus and embryo sac with zygote (arrow).

FIG. 17. L.s. of embryo sac six weeks after pollination. Note the shrivelling cap of nucellus and the resting zygote.

FIG. 18. L.s. of ovule showing a case of chalazogamy. The course of the pollen tube in the ovule is accentuated by India ink.

Dates of fixing of the materials: Fig. 10, May 19, 1954. Fig. 11, May 10, 1954. Figs. 12 and 18, May 29, 1954. Figs. 13 and 14, May 19, 1954. Figs. 15 and 16, June 5, 1954. Fig. 17, June 22, 1954.

SPOROMORPHOLOGY AND PHYTOMORPHOLOGY

By G. Erdtman

Palynological Laboratory, Nybodagatan 5, Solna, Sweden

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Pollen and spore morphology is, owing to its special character, to a great extent dealt with in special journals devoted to palynology and adjacent branches of science. Two aspects of modern pollen morphology are, however, of more general interest and may thus properly be discussed also in connection with ordinary phytomorphology. One of them is the ultrastructure (including also the fine relief) and morphogenesis of the sporoderm. The other is the classification of pollen and spore characters and the establishment of a terminology that may be instrumental in making the microfeatures of the wall of the first male gametophyte cells (as well as those of the megaspore walls) better known and more extensively considered in various contexts, particularly taxonomy.

With regard to morphogenesis and fine structure attention may be drawn, among other things, to papers by Larson *et al.* (1962), Larson and Lewis (1962), Heslop-Harrison (1963), Rowley (1962, 1963), de Sloover (1963), Sitte (1963), and by Stix and Takeoka (1963).

Information concerning certain details of the intine has recently been given by Bailey (1960), Baker (1955), Hyde and Adams (1958), Martens and Waterkeyn (1962), Saad (1961) and others. In Plate I in the present paper photomicropgrahs of minute, chemically not very resistant, densely spaced rod-like intine elements directed at right angles to the pollen surface or to the surface of an emerging pollen tube have been collated. They can be demonstrated in *Dracaena hookeriana* (Pl. I, Fig. 1) after the almost instantaneous swelling that takes place if fresh pollen grains are immersed in water. Similar elements are also encountered in the related genus *Sansevieria* (Pl. I, Fig. 2). The rods in *Morina longifolia* (Pl. I, Fig. 3) are very delicate (diameter about 0·1 μ), those in *Hedychium coronarium* (Pl. I, Fig. 4) and *Heliconia aurantiaca* longer, more substantial. Small intinous, rod-like elements are also found in pine pollen (Martens and Waterkeyn, 1962; for the chemistry of the intine, *see also* Bouveng, 1963; a combined study of the chemistry, morphology and physiology of the intine would be welcome in order, among other things, to shed some light on the function of the rods).

The intricate details of the outer part of the sporoderm, the exine have, as a rule, attracted much more attention than have the details of the intine. Special terms (about a dozen or less) are necessary in writing

ordinary, not particularly detailed pollen or spore diagnoses (for example, *see* Erdtman *et al.*, 1961):

Montia fontana ssp. *lamprosperma*—Pollen grains polycolpate (pantocolpate; number of colpi 12), spheroidal (mean diameter 37 μ), colpi narrow.

Exine 2–2·5 μ thick. Sexine thicker than nexine, gradually thinner towards colpi, tectate. Tegillum supported by bacula (rather coarse about halfway between adjacent colpi, gradually smaller towards colpi).

Anagallis arvensis—Pollen grains 3-colporate, subprolate (26 × 20 μ). Apocolpium diameter about 5 μ. Colpi narrow, crassimarginate. Ora elongate (about 4 × 6 μ), rectangular, sometimes united to an oral zone.

Exine about 2 μ thick. Sexine thicker than nexine, finely and regularly reticulate.

In order to avoid confusion all terms must be used in a consistent way. "Endexine", "nexine", "oblate", etc., etc., must not be used 'sensu A", "sensu B", etc. Usefulness must take precedence (although always *cum grano salis*) over priority and over ontogenetic, physical and chemical evidence. Thus the original morphological definition of ectexine and endexine ("sexine" and "nexine") cannot be "amended" by redefining "ectexine" and "endexine" on a purely physico-chemical basis (stainability, etc.). From an ontogenetic point of view definitions of the latter categories may perhaps seem to be acceptable or even necessary. Yet they can hardly be admitted since, in many cases, it is difficult or impossible—from lack of adequate instruments, material and time for investigation—to make the special studies that would be required: with a good microscope ectexine and endexine as originally defined can be easily traced also in very small pollen grains (*Myosotis*, etc.). It is virtually impossible, however, to decide—without very detailed investigations (electron microscopy, etc.)—whether the outer part of the "endexine" has the same fine structure and stainability, etc., as the (morphological) ectexine or if it shows the same characteristics as the rest of the (morphological) endexine.

In a world-wide classification of pollen grains and spores by their morphological features that can be observed with an ordinary microscope a more elaborate terminology must be used. In order to make pollen descriptions shorter and more easily comparable *inter se* terms can, however, be replaced by figures. Thus a "code" for classification of pollen grains and spores by means of the number, position and character of their apertures has recently been suggested (*cf. e.g.*, Erdtman, 1963, p. 158, 159, 162–167). Further "codes" concerning exine patterns, shape, size, and polarity, etc., of the pollen grains, may increasingly be established in attempts to make palynological characters more used in taxonomical contexts.

But numerous characters are still difficult to describe. There are many enigmas. Ever present transitions between certain characters

will remain a manifest source of annoyance (*cf.* Plate II). For the time being intensive investigations of well-defined morphological problems seem, therefore, to be more justified than many of the scattered contributions to "the pollen flora" of various parts of the world now being published—for phytogeographical, geological, or other reasons—at a rapidly increasing rate. Intensive, concentrated and well-planned investigations are indeed necessary in order to approach the scientific standard established in general phytomorphology by Professor P. Maheshwari.

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EXPLANATION OF PLATES I & II

PLATE I

FIGS. 1–4. "Radial striation" in intine

FIG. 1. *Dracaena hookeriana*, chemically untreated pollen grain; immersed in distilled water, × 1,000.

FIG. 2. *Sansevieria* sp., untreated pollen grain in distilled water, × 1,500.

FIG. 3. *Morina longifolia*, radial section through the outer part of one of the funnel-shaped apertures; UV micrograph (2536 Å), × 2,000.

FIG. 4. *Hedychium coronarium*, section through part of a pollen grain fixed in glacial acetic acid; UV micrograph (2536 Å), × 3,000.

PLATE II

FIGS. 1–8. Microtome sections (1, 2, 4–7) and optical sections (3, 8) through various pollen grains

FIGS. 1–2. Parts of pollen grain of *Bouchea fluminensis* (Verbenaceae); outer part of exine (sexine ?, ectosexine ?) more solid than most of the inner exine (nexine ?, endosexine ?); the innermost part of this inner part of the exine seems to be solid near the aperture, × 1,000.

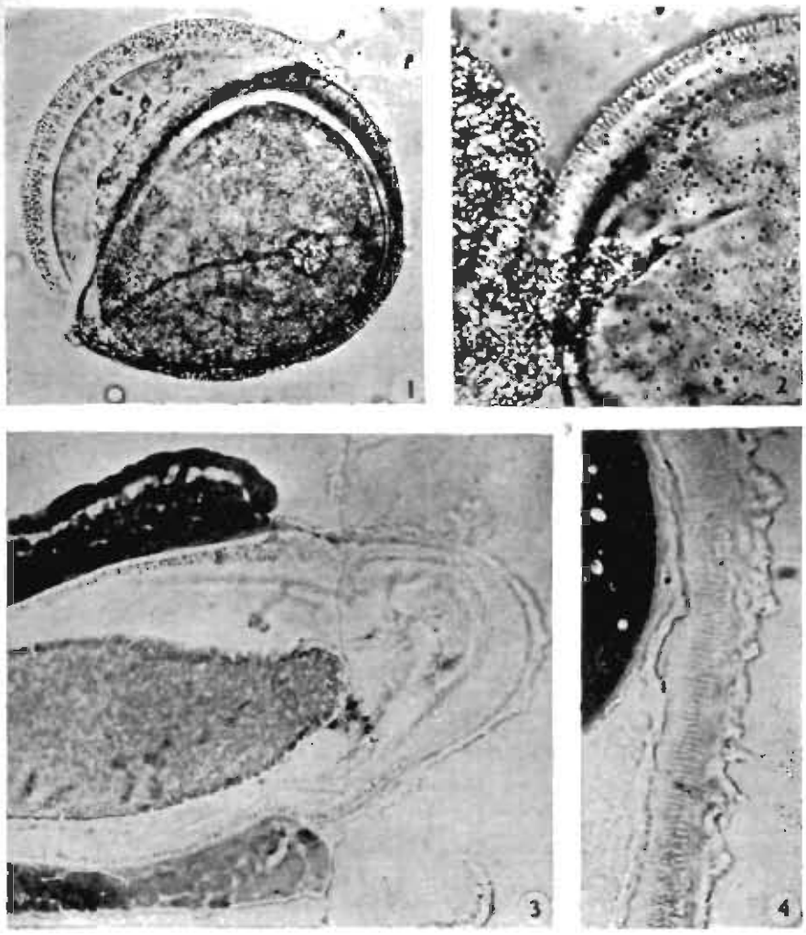
FIG. 3. Part of a Cretaceous (Maestrichtian) pollen grain from Oebisfelde, Germany (slide provided by Dr. W. Krutzsch); exine more or less homogeneous; the dark streak immediately above the lumen of the grain may correspond to the nexine in recent pollen grains, × 1,500.

FIG. 4. *Plumbago capensis*, pollen from a brevistylous flower; interpretation baculoid coarse suprategillar processes arise from a thin tegillum supported by slender, infrategillar bacula standing on a thin nexine. If it were impossible to see any infrategillar bacula under the microscope (without access to thin sections), the interpretation would be as follows: sexine consisting of baculoid coarse processes standing on a nexine with slightly undulating outer surface. Length of processes about equal to thickness of nexine.

FIGS. 5*a*, 5*b*. *Ceratostigma willmottianum*, exine details similar to those in *Plumbago capensis*; thin sections, however, show that the pollen grains are not "tectate", as in *Plumbago* (according to the first interpretation given above) but intectate; a thin nexine is densely beset with processes consisting of slender rods; the distal parts of the latter are united into coarse baculoid, flat-topped elements beset with a number of very minute spinules, × 2,000.

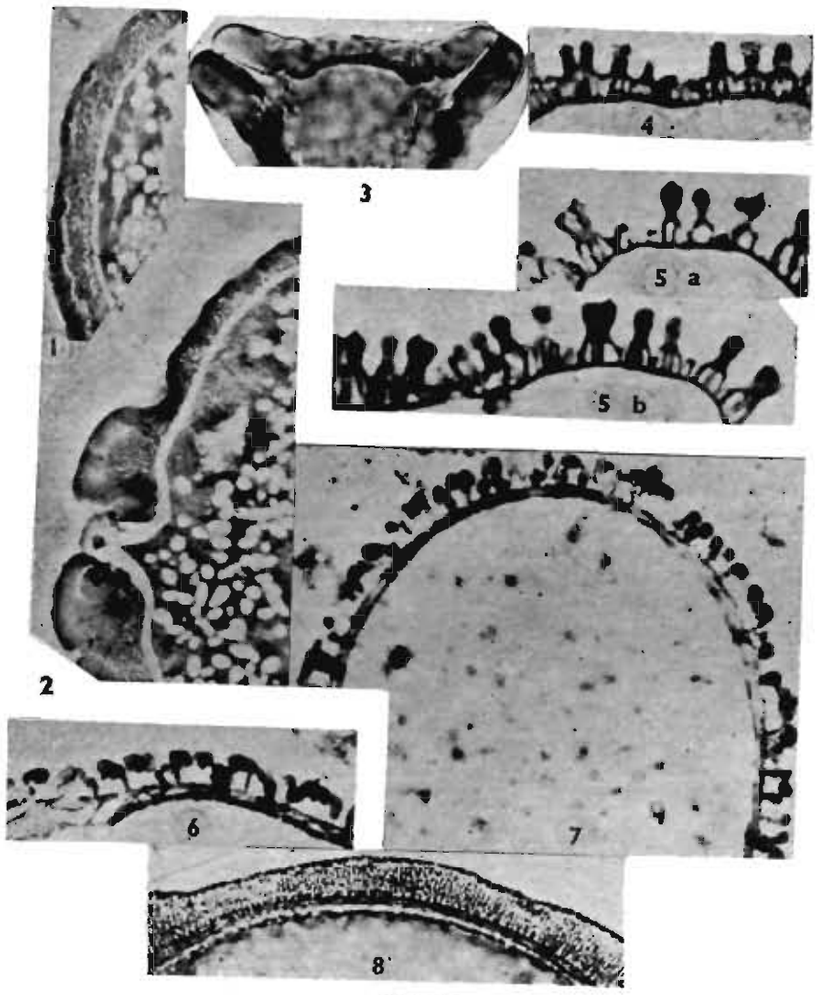
FIGS. 6, 7. *Nelumbo nucifera*, interpretation of the inner "nexinoid" exine layer doubtful; it seems to consist of two layers of which at least the outer seems to be lamellated, × 1,500.

FIG. 8. *Boisduvalia densiflora* (Oenotheraceae), optical cross-section through the exine of an acetolysed pollen grain; nexine compact, thinner than the less compact exine; the latter consists of a thick tegillum supported by a very thin layer of baculoid rods, × 1,000.



FIGS. 1–4

G. Erdtman



FIGS. 1-8

G. Erdtman

THE FLESHY CORTEX OF ARTICULATED CHENOPODIACEAE

BY A. FAHN

Department of Botany, The Hebrew University, Jerusalem, Israel

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MANY Chenopodiaceae growing in salt marshes and desert areas show reduction of leaf size and the young branches are built of succulent green internodes which give the young shoots an articulated appearance (Text-Figs. 1, 2; Plate I, Figs. 1–3).

Bentham (1858), de Bary (1877), Hooker (1884), Ganong (1903), Cross (1909) and Schischkin (1936) simply describe these plants as being aphyllous, or almost so, and as having stems with a green fleshy cortex. However, the peculiar structure of the articulated stems of these plants, whose leaves are variously reduced, caused many plant morphologists to interpret the cortex as being a tissue which has been derived from the leaves. So, for instance, according to Dangeard (1887–1888), Monteil (1906), Leysle (1949), Backer (1949) and Keller (1951), the fleshy cortex developed by fusion of the opposite leaves (or only their sheaths) and their adnation to the stem.

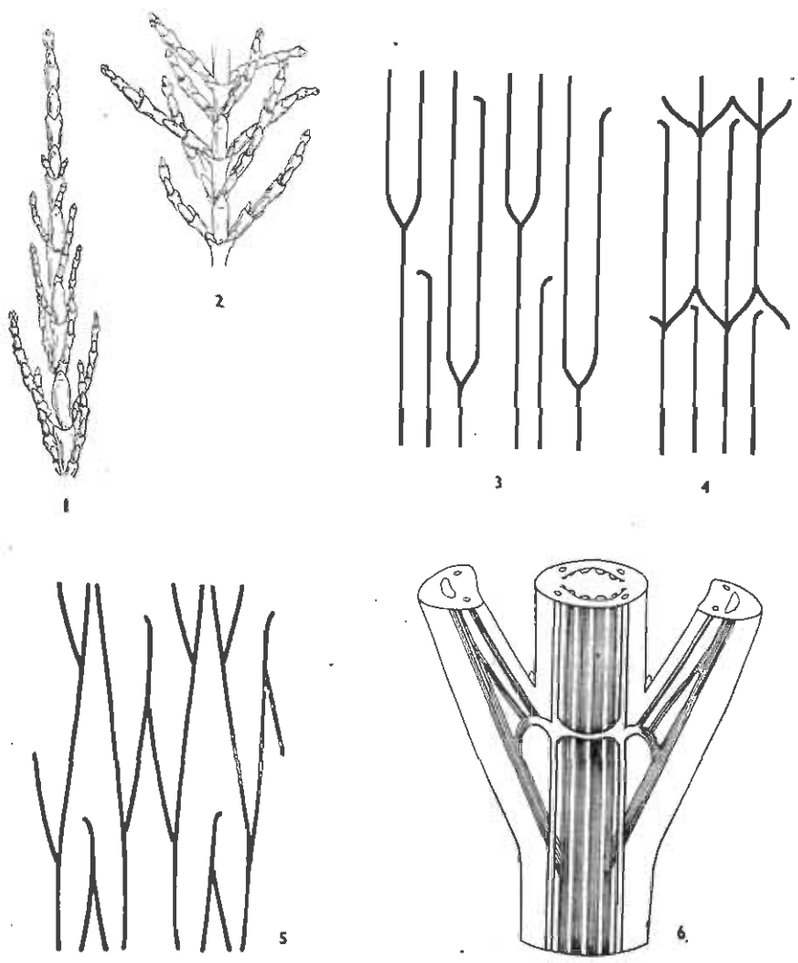
Other attempts to interpret the origin of the cortex as being foliar were made by Duval-Jouve (1868), Cooke (1911), de Fraine (1912) and Halket (1928). According to these authors the cortex originates through the decurrent growth of the leaf-bases.

The above two views of foliar origin of the cortex are completely different and one cannot be used as support for the other.

THE VIEW OF LEAF FUSION AND ADHESION TO THE STEM

Leysle (1949), who worked on *Anabasis aphylla*, supports his view of adnation of the opposite leaves to the internodes with the following: In the seedling the lowest nodes adjacent to the cotyledons have quite prominent leaves, while in the later developing nodes the leaves become gradually reduced. From the scaly leaves to the base of the internodal cortex similar palisade and water storage tissue can be seen. Leysle also regards the different leaf lengths in various species of *Anabasis* (*i.e.*, *A. micradena*, *A. brevifolia* and *A. salsa*) as a proof for his theory.

Keller (1951) reached the same conclusion by studying some anomalies in *Salicornia* plants. He found seedlings with three cotyledons and unifoliate nodes and asymmetric arrangement of the internodal fleshy cortex.



TEXT-FIGS. 1–6. Fig. 1. Branch of *Arthrocnemum glaucum* (Del.) Ung.-Sternb. Fig. 2. Branch of *Anabasis articulata* (Forsk.) Moq. Figs. 3–5. Diagrammatic reconstruction of the longitudinal course of vascular strands of the stele. Fig. 3. *Arthrocnemum glaucum*. Fig. 4. *Anabasis articulata*. Fig. 5. *Salsola longifolia* Forsk. Fig. 6. Diagrammatic representation of the primary vascularization of a young branch of *Calycanthus* in which the stelar and foliar strands are hatched and the cortical bundles merely outlined.

THE VIEW OF DECURRENT GROWTH OF THE LEAF-BASES

Duval-Jouve (1868) states that the young fleshy cortex originated through downward growth of the bases of two opposite leaves which

fused along the adjacent edges. Duval-Jouve supports his view by anatomical observations, additional to the similarity of the tissue in the cortex and reduced leaves. He found that there are two networks of vascular bundles in the fleshy cortical tissue, each of which derived from the two descending lateral branches of each opposite leaf-trace, which enters the leaf of the node above. These two networks (Plate I, Figs. 5, 6) anastomose (Fahn and Arzee, 1959). The median branch of each leaf-trace ascends into the reduced leaf. Duval-Jouve considers his view confirmed by the following behaviour of the fleshy cortex during the maturation of each internode. These cortex segments become yellow and then wither and so behaving, according to him, as deciduous leaves.

Cooke (1911) and de Fraine (1912) who investigated the various tissues and venation of the reduced leaves and fleshy cortical segments, as well as the structure of the flowering shoots of various species of *Salicornia*, came to the same conclusion as Duval-Jouve. De Fraine also brings evidence from the morphology of seedlings. Halket (1928) uses the form and structure of some abnormal nodes of *Salicornia herbacea* to support the above theory. He noticed plants in which two deep lateral indentations appeared along the internodes.

Recent Debate on the Nature of the Fleshy Cortex

Van Royen (1956), from his anatomical studies on *Tecticornia*, states that the theory of leaf-fusion and adnation to the stem cannot be accepted. He bases his view on the departure of the leaf-traces and branch traces, from the stele at the top of the articulated internode and not at its base, as we should expect if cortex represents fused leaves.

In order to clarify the problem whether fusion of leaves occurred along the internodes, Fahn and Arzee (1959) studied the vascularization pattern of the stele of some articulated genera of the Chenopodiaceae. They found two types of steles: *Salicornia-Arthrocnemum* type (Text-Fig. 3) including *Halocnemum* and *Anabasis* type (Text-Fig. 4) including *Haloxylon*. Comparison of the stele of the above plants with the stele of other opposite-leaved Chenopodiaceae genera, which have a similar stelar pattern but which are not articulated and do not exhibit a fleshy cortex (Text-Fig. 5) shows no sign of addition of foliar strands to the stele. If such a process would have taken place, a greater number of strands would be expected to occur in the stele and the leaf-traces in the stelar region would be expected to be longer than in species in which leaf-fusion and adnation to the stem is not suggested. From this investigation the contrary situation can be seen. The number of vascular strands in *Anabasis* for instance is smaller or the same as in the different *Salsola* species which also have opposite leaves and which lack a fleshy cortex. Therefore, the view of foliar fusing and adnation to the stem seems to me as an unsatisfactory one.

The theory of decurrent growth sounds more logical, but as will be shown later is not convincing, in my opinion.

The vascular network of the cortical segments which is derived from the lateral branches of the leaf-trace at the top of the internode could suggest a hypothesis of a downward prolongation of the leaf-bases. This, however, does not seem to me to be the only possible explanation of the existence of a vascular network in the cortex. Cortical vascular strands are known to appear not only in the articulated Chenopodiaceae but also in many families (Metcalfe and Chalk, 1950), which do not exhibit a fleshy cortex as, for instance, in the Calycanthaceae (Fahn and Bailey, 1957). The cortical strands here run throughout the stem but are not interrupted at each node, as the stem here is not constricted and no intercalary growth takes place. However, vascular connections between the cortical bundles and the leaf-traces at the nodal level exist also in the Calycanthaceae (Text-Fig. 6). The position of the phloem of the cortical bundles of the Calycanthaceae is interior to the xylem as is also in the cortical bundles of the articulated Chenopodiaceae. A fleshy cortex, such as that of the articulated Chenopodiaceae, in which the processes of photosynthesis take place, requires a more well-developed vascular system than the Calycanthaceae. The view of purely functional development of the vascular network of the cortex can be strengthened by the fact that in some articulated Chenopodiaceae, such as *Anabasis* species, the large part of this network is derived from branches given off from stelar strands at various levels along the internode (Plate I, Fig. 4). Additional support for the functional interpretation of the appearance of vascular bundles in the cortex can be found in the following statement from Eames and MacDaniels (1947, p. 314): "In plants with fleshy cortex, such as many of the Cactaceae, where the leaves are reduced and photosynthesis is carried on largely by the cortex, branches from the base of the leaf-traces penetrate the cortical tissues."

The shedding of the cortex, which occurs in each internode separately, has been interpreted by some authors as a proof of their foliar nature.

James and Kyhos (1961) doubt the view of Fahn and Arzee (1959), that the phellogen separates the vascular cylinder from the cortex and that the cortex is constricted at the base of the node, which is the cause of the characteristic way in which the cortex is shed. James and Kyhos write further that "If this were true, the cortex would have to be discontinuous, since in the constricted area the phellogen develops directly underneath the outer layer of cells. That is, we would have the rather anomalous situation of the stele being surrounded only by an epidermis".

If these authors had examined better a longitudinal section of two adjacent internodes, they would have seen in the constricted area of the internodes, several layers of compressed parenchyma cells not only between the phellogen and the epidermis, but also between the cells outside the periderm, which become suberized before phellogen activity starts. Exterior to the vascular strands, phloem or pericycle fibres can also be seen (Plate I, Figs. 7, 8). The constriction may be either the

outcome of the suppression of the activity of the intercalary meristem or of the pressure of the rudimentary leaves or of both.

The fact that the cortex of *Allenrolfea occidentalis* is thinner on the side of the internode opposite to that below the reduced leaf (James and Kyhos, 1961), or the fact that in some abnormal plants of *Salicornia* two deep lateral longitudinal indentations appeared in the cortex (Halket, 1928) does not necessarily prove the cortex to be of foliar origin. In a great many of dicotyledons there can be observed cortical thickenings running decurrently from the leaf-base down along the substanding internode. This is the cause of the oval outline as seen in cross-sections of, at least, the upper part of the internodes of most of the species with opposite leaves and the asymmetry of young stems with alternate leaves.

It is important, here, to bear in mind the difficulty in drawing an exact borderline between stem and leaf. Both organs develop from a common meristem, *i.e.*, from the apical meristem. The connection and the mutual interdependence of leaf and stem exist during the whole period of the plant growth (Wardlaw, 1960). Moreover, according to Arber (1950), the leaf represents basically a stem organ which became secondarily flattened. It has been shown in some ferns that it is possible to change by surgical methods, the developmental trend of primordia from future leaves to buds (Cutter, 1959).

The main reason which caused the earlier authors to regard the cortex of the articulated Chenopodiaceae to be of foliar origin was that photosynthesis is carried out in its tissues. However, numerous plants are known in which the leaves are reduced and in which stems then become concerned with photosynthesis, sometimes even resulting in the formation of phylloclades. This proves that the interchange of function between leaf and stem is possible.

Therefore, it seems to me that no convincing evidence exists as to the foliar origin of the fleshy cortex of the articulated Chenopodiaceae and so the simple view according to which the fleshy cortex is of axial origin and has acquired photosynthetic function as a result of the reduction of the leaves, has to be retained.

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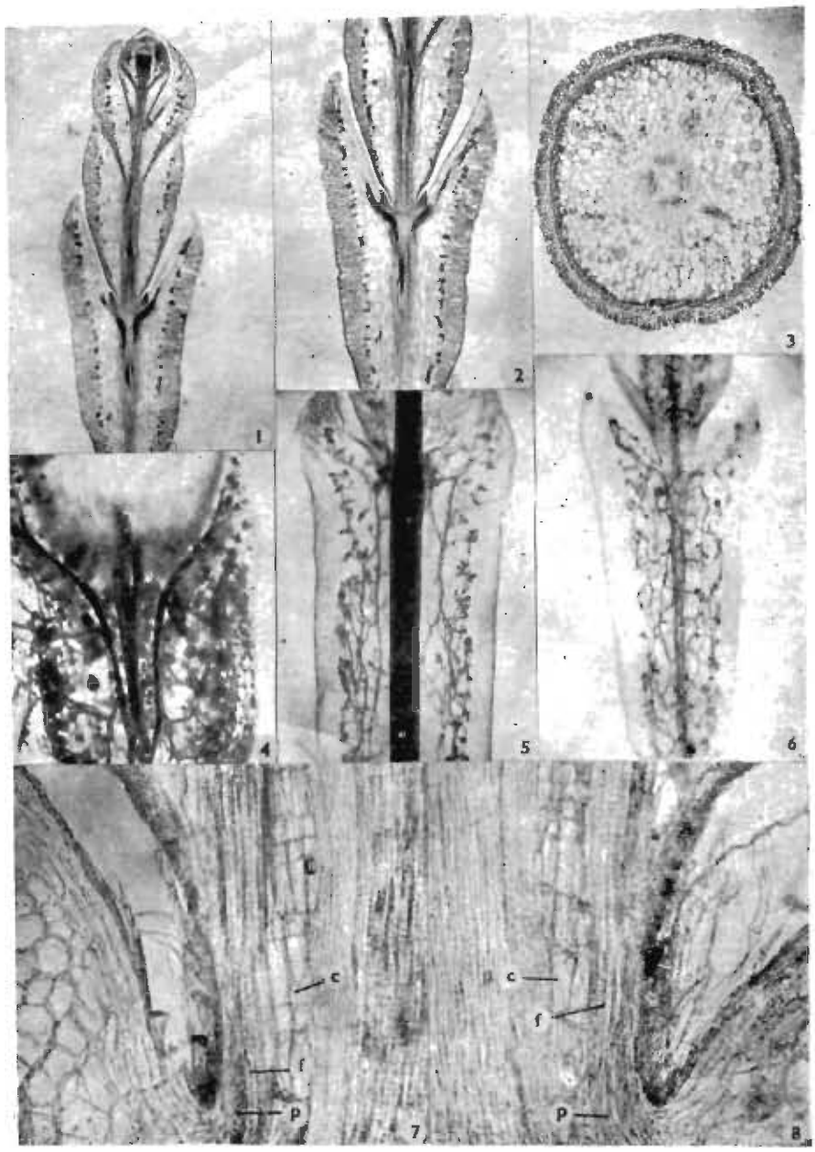
EXPLANATION OF PLATE I

FIGS. 1–8

FIGS. 1–2. Longitudinal sections of a terminal portion of a shoot of ***Salicornia fruticosa*** L. Fig. 1, × 10; Fig. 2, × 12.

FIG. 3. Cross-section of a young internode of *Anabasis articulata*, × 15.



FIGS. 1–8

A. Fahn

FIG. 4. A portion of a cleared shoot of *Anabasis articulata* in which branches given off along the internode from the vascular strands of the central cylinder can be seen entering directly into the cortical tissue. The strands of the central cylinder are seen to be somewhat spread as a result of pressure, × 13.

FIG. 5. A portion of a cleared shoot of *Arthrocnemum glaucum* showing the network of the cortex which is connected with the lateral branches of the leaf strands, × 11.

FIG. 6. As in Fig. 5 but showing the anastomosing of the network in cortex and reduced leaves. This photograph was made with the focus nearer the upper surface of the shoot, × 13.

FIGS. 7–8. Longitudinal hand-cut sections of a portion of a shoot of *Anabasis articulata* in which several cell layers consisting of fibres and compressed parenchyma cells, can be seen between the multiseriate epidermis and the cork in the constricted zone of the cortex, 68.

(*c*, cork; *p*, parenchyma cells; *f*, phloem or pericycle fibres.)

A PERSPECTIVE ON INOCULUM POTENTIAL

BY JAMES G. HORSFALL AND A. E. DIMOND

The Connecticut Agricultural Experiment Station, New Haven 4, Connecticut, U.S.A.

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WE submit here our perspective of inoculum potential as a contribution to theoretical plant pathology.

A BRIEF HISTORY

Since the term, "inoculum potential" was born 30 years ago (Horsfall, 1932), a few months before Professor Maheshwari's thirtieth birthday, it seems fitting to discuss it in this Commemoration Volume before his sixtieth birthday.

Inoculum potential was born with no fanfare whatsoever in a footnote to a very practical paper on seed treatment for damping-off. The effectiveness of seed treatments varied from soil to soil. We concluded that the amount of inoculum must vary proportionately.

The footnote said that the ".... concept carries with it the idea of mass action—the greater the mass of the organism present .... and the more virulent the organism, the more severe will be the disease." Curiously enough, the effect of environment was excluded and the host was ignored.

Of course, others were working in the field. Heald (1921) had published his classical paper on wheat bunt 11 years before our paper, and a few years after it Leach and Davey (1938) related the number of sclerotia in soils to a sugar beet-disease.

Holmes (1929) three years earlier had published his classical paper on the local lesion method for assaying inoculum potential of viruses.

We have continued to tinker with the concept in a sporadic way ever since. Environment could not be ignored very long. In 1938 Horsfall redefined inoculum potential in the special case of damping-off as "the disease-producing power of a soil". In 1941 Dimond generalized the term as "disease-producing power of parasite and environment".

Horsfall (1945) defined inoculum potential as the equilibrium between number of hosts, number of spores, distance between hosts, randomness of hosts and weather factors. Still host susceptibility is missing, but in 1960 we (Dimond and Horsfall, 1960) included it.

In the meantime the term slowly invaded the literature. Lin (1939) used inoculum potential for the number of spores in a pycnidium and Wilhelm (1951) was the first outside of our laboratory to use the term in a title.

What we call inoculum potential has been called by other names by others, as for example "infection index" (Wilhelm, 1950).

Most of the terms in the literature really refer only to the quantity component of inoculum potential, spore load (Heald, 1921), spore dosage (Zentmyer *et al.*, 1943), inoculum density (Hood and Stewart, 1957), Sporendichte (Kuhfuss, 1956).

On the other hand, the term inoculum potential has been clouded by using it for somewhat different concepts as well. Garrett (1960, p. 28) defines it as "the energy of growth of a pathogen available for infection of a host at the surface of the host organ to be infected".

We consider that this definition is too restrictive and not easily quantifiable. His concept is sound enough. It needs a term. Inoculum potential is not the term.

Weihing and O'Keefe (1962) seem to consider that inoculum yet to be produced is inoculum potential. This usage introduces an unknowable factor into a concept that must be a measurable factor to be useful.

Present Concept of Inoculum Potential

We now consider that inoculum potential is an abstract concept that involves the interaction between the amount of inoculum and its virulence, the amount of available host tissue and its susceptibility, *plus* the effect of environment on each. Environment affects the pathogen and its ability to produce disease as well as the host and its ability to contract disease.

Inoculum potential is the integrated likelihood that a given amount of disease will result from a given amount of inoculum. It comprises two components—a quantitative component and a qualitative component.

We shall analyze current knowledge as it can be used to appraise and measure these two components of inoculum potential. This will be based on the properties of the dosage-response curve, the dosage of pathogen and the response of host. We shall suggest that the quantity component is measured by the position of dosage-response curve, the quality component by the slope.

Significance of the Dosage-Response Curve

The use of the dosage-response curve has vastly improved our knowledge of the quantity and quality factors in fungicidal action. We

suggest that this curve will also tell much about the quantity and quality factors in inoculum potential.

The derivation of the dosage-response curve from raw data is discussed extensively by Horsfall (1956). The mathematics of it is discussed by Finney (1952). Suffice it to say here that if the number of propagules is expressed as logarithms and the percentage of disease is expressed as probability units (probits) a straight line is generated. In practice one plots his data on a log-probability grid.

The generality of this principle is illustrated in Text-Fig. 1 showing inoculum potential curves replotted from data on curly top, a viral disease of sugar-beet (Giddings, 1946), a bacterial disease of potato (Starr, 1947), wheat bunt (Heald, 1921) and sclerotial root rot of sugar-beet (Leach and Davey, 1938).

The straight line as illustrated in Text-Fig. 1 has two properties—position and slope. These are not mutually exclusive, of course. Position can vary without a change in slope. However, when the slope is altered, the position is ordinarily also changed. Position is usually defined by, say the dose to produce a 50% response. For many reasons this point can be defined with the best precision. It is normally called ED 50—that is, effective dose for 50% response.

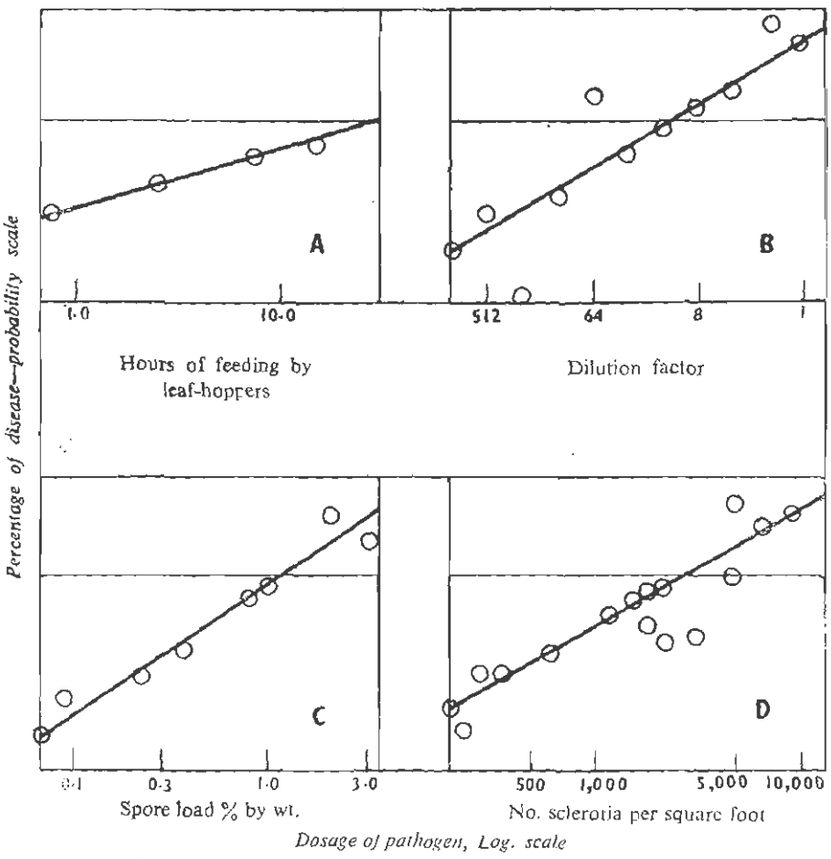
By analogy with fungicidal dosage-response curves, the slope of the inoculum potential curve gives information on the mechanism of disease induction. If slope differs between treatments, this evidences a change in the mechanism of disease induction. If a treatment changes the position of the inoculum potential curve but not the slope, this evidences a change in the effectiveness of inoculum without a change in the mechanism of disease induction.

We must not forget a basic assumption in the dosage-response curve. It assumes that each dose increment acts qualitatively like the one next smaller. It assumes on the one hand that cuprous oxide is the same chemical at 1 μ/cm^2 as at 100 μ/cm^2, and on the other hand that spores act the same at 10 per cc. as at 10,000 per cc.

Some spores may react on each other, however, as for example when they compete with each other for nutrient in the infection court. When this happens, non-linear curves are generated as we show below.

Environment may affect the pathogen and the host separately, either quantitatively or qualitatively. If environment changes the inoculum quantitatively, the position of the curve will shift to the right when the effect is deleterious to the fungus, to the left when beneficial to the fungus.

It is conceivable that environment could change a pathogen qualitatively. Garber (1954) says that a well-nourished pathogen is more aggressive than a starved one. If the well-nourished pathogen merely



TEXT-FIG. 1. Inoculum potential curves for four types of pathogens. The line through the middle is the 50% respo. se. A. Virus for curly top of sugar-beets; data of Giddings (1946) B. Bacterial ring rot of potato, data of Starr (1947). C. Bunt of wheat; data of Heald and Boyle (1923). D. Sclerotial disease on sugar-beet; data of Leach and Davey (1938).

breaches the host barriers more easily, it should shift ED 50 to the left, *i.e.*, fewer propagules will be required to produce 50% of disease. If, however, the well-nourished organism is in effect a qualitatively different strain, then it could affect different biochemical pathways in the host, and thus alter the slope of the inoculum potential curve.

To summarize this part of the discussion then, we consider that slope of the inoculum potential curve indicates the biochemical pathways of the disease process. Position of the inoculum potential curve gives a measure of the quantitative physical aspects of producing infection that are not an essential part of the mechanism of pathogenesis.

The force to breach the barriers of the host is such a physical factor in inoculum potential and is measured by the ED 50. The nature of the disease process after the barrier is breached is the quality factor in inoculum potential, and it can be indicated by the slope of the curve.

The Quantity Component

We now proceed to explore cases in which the quantity component has been varied experimentally in the attack on problems.

Distance

Where an organism must move outward from a source, the amount of inoculum should diminish progressively.

Several years ago we showed (Zentmyer *et al.*, 1944) for Dutch elm disease a linear relation between the log of the distance and the probability of disease. From the disease picture on elms, we inferred that the number of infective propagules falls off as the logarithm of the distance. We said that the position of the curve should be governed by: (1) the amount of fungus present, (2) the number of carrier beetles, and (3) the number of elm trees, and we suggested that with an increase in the amount of fungus or number of beetles at the source, or a decrease in the number of elms, the .... curve should shift to the right.

We find two other excellent sets of data where distance also plots out as a straight line on a logarithmic-probability grid. These are the old data of Posey and Ford (1924) on white pine blister rust and Bonde and Schultz (1943) on the phytophthoral disease on potato.

Time

As the season advances the density of propagules rises logarithmically and, of course, the amount of disease also rises. Barratt and Richards (1944) showed that amount of alternarial blight of tomato expressed as probits increases linearly with time during the season. Later Large (1945) showed that phytophthoral blight of potatoes follows the same pattern.

It is interesting that time is an arithmetic not a logarithmic function in both these studies. Dimond and Horsfall (1963) suggest that this is because the number of propagules increases logarithmically with time so that time is equivalent to the logarithm of the number of propagules present.

Density of Hosts

Density of hosts is a factor in the expression of inoculum potential. If uniform amounts of inoculum are spread at random over the field, the proportion of plants affected should decline as the number of plants is increased. If increasing the number of propagules increases the likelihood of disease, then increasing the number of hosts should reduce the likelihood of disease.

In 1941 Shapovalov *et al.* showed that the percentage of curly top in sugar-beets is inversely proportional to the density of planting. This stimulated Linford (1943) to offer better data, as did van der Plank and Anderson (1945).

Protective Fungicides

Protective fungicides are designed with the sole purpose of reducing the amount of inoculum, the higher the dosage, the lower the inoculum. Thus, as expected one derives straight lines from field-work on protective fungicides as Dimond *et al.* (1941) have shown.

Despite the obvious relation of fungicide dosage to inoculum potential, one is astonished to note that virtually no research has been done to vary inoculum independently of the fungicide dosage.

Such a procedure is difficult for foliage diseases, but it is easy for seed-borne fungi. We find three papers where the interaction has been studied for seed-borne wheat bunt. The data of Mackie and Briggs (1923) plot out as beautiful linear curves over wide ranges of fungicide and spore dosages. The curves are all parallel thus showing dramatically that fungicides affect inoculum potential quantitatively, not qualitatively.

Nutritional Status of the Host

The nutritional status of the plant affects its susceptibility. Studies of the interaction of inoculum potential and nutrition are difficult to find. Macfarlane (1952) has superimposed different nutritional levels on various inoculum densities in the case of club root of cabbage. His *inoculum potential curves differ in ED 50 but not in slope. ED 50* varies inversely with nutritional level which means that fewer spores are required to give 50% infection on the well-nourished plants. Presumably a changed nutritional status of the plant alters the amount of work necessary for invasion by a pathogen, but not the fundamental mechanism of invasion and pathogenesis.

Deverall and Wood (1961) investigated the interaction of calcium nutrition and inoculum density for *Botrytis fabae* on the broad bean. As calcium was increased, the inoculum potential curve was parallel but shifted to the right which means that more spores were required to produce ED 50. This suggests that calcium makes the establishment of the fungus more difficult but does not change the type of reaction. Presumably the extra calcium increases resistance quantitatively, but does not change the biochemistry of the disease process.

In our work here (Corden and Edgington, 1960; Edgington *et al.*, 1961) calcium confers some resistance on the tomato to fusaria wilt. We think that the calcium reduces the availability of pectic substrates as food for the fungus. Our interpretation is that this affects the rate of process of pathogenesis but not its nature. If so, high calcium

nutrition should increase the ED 50 but not the slope, as shown by Deverall and Wood.

Light and Temperature

Colhoun (1961) has studied the effect of light intensity on the inoculum potential of club root. High light intensity displaces the curve to the right, *i.e.*, it increases the ED 50, and the number of spores must be increased to give equal disease. Light does not change the slope, however. Presumably light increases the level of resistance but not the nature of the resistance. It affects the quantitative component, not the qualitative component of inoculum potential.

Presumably light increases some constituent or constituents in the resistance process, but does not introduce a different type.

Temperature also affects plant disease. Starr's (1947) data are elegant. He used 11 doses of ring-rot bacteria on potato and four dates of inoculation beginning in mid-August. Two August dates of inoculation gave parallel curves. Possibly the September inoculation may have given steeper curves, but the September data are so variable that this is far from certain. Hence we must conclude at least for the present that cooler temperatures do not change the slope. Heald (1921) has a few data on temperature and wheat bunt. Although his data on this point are scarce and variable, they hardly seem to suggest any change of slope due to temperature.

The Quality Component

We now proceed to a measurement of the quality component in inoculum potential.

Host Variety

If the biochemistry of the host reaction or the mechanism of disease induction differs among varieties, *i.e.*, if the quality of the disease process differs among varieties, the slope of the inoculum potential curve should differ. Heald (1926) says that the bunt disease makes the heads of *Triticum compactum* (wheat) noticeably more slender than those of *T. vulgare* and the heads of *T. vulgare* more loose and open and the leaves a darker green than those of *T. compactum*. This suggests that the biochemical processes in the disease differ between the two species.

We were delighted to find, then, in the data of Heald (1921) and Heald and Boyle (1923) that the slope of the inoculum potential curve for the *compactum* varieties like Jenkins Club and Little Club is steeper than for the *vulgare* varieties like Winter Fife. Please note that no difference in resistance is claimed to exist between these varieties. Their disease responses are qualitatively different and the slopes are also different.

On the other hand, Marquis is ranked distinctly more resistant than say Winter Fife, both *vulgare* types. Although Heald's data for Marquis are pretty variable there seems no valid evidence that the slope

is different for Marquis. From this we must infer that the nature of resistance of Marquis is qualitatively the same as that of Winter Fife, but that these varieties differ quantitatively in degree of resistance as a result of physical differences such as thicker cuticles or heavier cell walls.

The result for the resistant Marquis is duplicated for other diseases. The curves for resistant and susceptible varieties are parallel in the data of Giddings (1946) for the viral disease, curly top of sugar-beets, in the data of Crosse (1959) for bacterial disease of stone fruits, and in the data of Kuhfuss (1956) for the anthracnose disease of flax.

On the other hand, Beard (1943) of this Station shows that the inoculum potential for the corn-borer insect is steeper for the susceptible Marcross than for the somewhat more resistant Lexington variety of corn (maize). Presumably the mechanisms differ in these two cases.

We would like to see more work on inoculum potential for resistant and susceptible varieties. We would hope that the slope of the curve would indicate whether a new variety differs qualitatively from the old.

Age of Tissue

The effect of age of tissue on disease has received some attention in plant pathology. Several years ago, we (Dimond *et al.*, 1941) published an inoculum potential curve for apple scab in which we regulated spore load with sulfur. The curve was steeper in July than in June. We suggested that environmental effects could account for the action. We offer here an alternative suggestion that the slope is steeper for old apple leaves (late July) than for young apple leaves (June).

Turner (1944) also of this Station has looked into the inoculum potential curve for Mexican beetles on beans. He, too, found that the slope is steeper for old foliage than for young. Deverall and Wood (1961) showed that the inoculum potential curve for *Botrytis fabae* is steeper for old bean leaves than young.

The cited data all indicate that the slope of the inoculum potential curve is steeper on old leaves than on young ones, and, thus, the slope of the inoculum potential curve may provide valuable clues to youth and old age disease.

Soil pH

Club root of cabbage has long been known to be favored by low *p*H in the soil. What does change of soil *p*H do to the slope of the inoculum potential curve? Samuel and Garrett's data (1945) provide the answer. Their data show that as the *p*H is increased, the slope of the curve becomes steeper. These results suggest that *p*H somehow changes the mechanism of disease induction. One might at first think that this is because *p*H changes calcium nutrition, but data above show that calcium changes position and not slope of the curve.

The Case of Non-Linear Curves

As in the case of fungicides there are cases of non-linear inoculum potential curves. We (Dimond *et al.*, 1941) published a bimodal curve for dimethyldithiocarbamate fungicides. Barratt and Horsfall (1947) say that such curves are "generated by the interaction of two factors acting at different rates or in opposite directions". Petersen (1959) has published the best available data for a bimodal curve for inoculum potential. His organism was *Puccinia graminis* on wheat. Spores of this organism secrete an inhibitor. Thus as dose rises the amount of inhibitor rises and one has two oppositely acting factors. We would expect that a bimodal curve would be generated. It was.

Although we have seen only an abstract of Domsch's original paper (1954), we suspect that he too has a bimodal curve on *Erysiphe* on barley. He says that percentage of spore germination and percentage of infection declined as the spore density was increased. This looks like a fragment of a polymodal curve.

Now spores of *Botrytis fabae* also hinder their own germination at higher spore loads. Deverall and Wood (1961) show some spore load studies. Unfortunately, they used only three doses of spores, but only a few of their curves show the expected straight line. We suspect that they too had fragments of bimodal curves. If *Botrytis* were to be tested with a narrow dosage interval but over a wide dose range, we think that the bimodal curve would appear.

In any case, we suggest that if the inoculum potential curve is not linear on the standard log-probability grid, more needs to be learned about the infection process. Such a study could lead to productive results.

Where Next?

We hope we have supported the thesis, (*a*) that inoculum potential represents the amount of inoculum and its virulence, the amount of available host tissue and its susceptibility, *plus* the effect of environment on all, (*b*) that it has a quantity component measured by ED 50 of the curve, and a quality component indicated by the slope of the curve, (*c*) that ED 50 shows the forces in establishing infection, (*d*) that different slopes indicate different biochemical processes of infection.

We hope that the concept of inoculum potential and its measurement with the dosage-response curve will serve as an improved tool for studying many aspects of the process of disease induction. For example the slope of the inoculum potential curve should serve to show whether the resistance of a new variety is quantitative or qualitative. Is it due simply to more of what is in the susceptible variety, or is it due to a new type of resistance?

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THE TECHNIQUE OF *IN VITRO* CULTURE IN THE STUDY OF PHYSIOLOGY OF REPRODUCTION

By B. M. Johri and Sipra Guha

Department of Botany, University of Delhi, Delhi, India

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Reproduction is one of the chief attributes of life, and an understanding of its mode and mechanism is of great interest. In flowering plants, investigations concerned with reproduction have been primarily devoted to the physiology of flowering and control of sex-expression. Much less is known about the factors responsible for the development of anther, ovary, ovule, endosperm and embryo, and the biochemical pathways operating within these tissues.

The advent of the aseptic culture technique in recent years has made it possible to approach these problems from an entirely new angle. The investigations employing this technique are briefly reviewed here.

Control of Flowering and Expression of Sex

Although much attention has been paid to the photoperiod and temperature control of floral induction, the chemical bases of these phenomena are yet unknown. In recent years the chelation of metals by compounds such as ethylenediaminetetraacetic acid (EDTA) has come to play a significant role in the development of flowers.

Lemna perpusilla, which behaves as a day-neutral plant in Hoagland's medium, becomes photoperiodically sensitive by the addition of chelating compounds. With EDTA flowering occurs only under short-day conditions when the daily photoperiods are of 10 hours or less (Hillman, 1961). In *L. gibba*, which is a long-day plant, EDTA merely enhances the effect of long photoperiods (Kandeler, 1955). In *Wolffia microscopica* flowering cannot be induced *in vitro* unless EDTA is present in the medium. While the addition of 10^{-4} M EDTA alone will induce flowering in some media, in others both EDTA and short photoperiods are necessary (S. C. Maheshwari and Chauhan, 1963). The exact role of EDTA remains to be elucidated, but in *L. perpusilla* there is some evidence to show that it chelates metal ions, perhaps Cu^{++} (Hillman, 1962).

Raghavan and Jacobs (1961) reported the induction of floral primordia in shoot apices of *Perilla* cultured *in vitro*. From this work it appears that the calyx and corolla can be formed regardless of photoperiod but short days are necessary for the formation of the androecium and gynoecium.

Cuscuta reflexa is a parasitic twiner which lacks foliage. When the cultures of its shoot apices are subjected to normal-day conditions, there is a profuse growth of the vegetative shoot. Flowering occurs if the cultures are kept in 72 hours of continuous darkness and it is much more when this period is extended to 132 hours. In most plants, so far investigated, leaf is the organ that perceives the photoinduction but in *Cuscuta* it is the shoot apex which is photoresponsive (Baldev, 1959, 1962).

Chouard and Aghion (1961) obtained interesting results with tobacco explants. The segments from the lower internodal regions or young vegetative plants regenerated calli which produced vegetative buds. Those from the upper internodes of flowering stems also formed calli which differentiated into rudimentary floral buds. The callus derived from the internodes from intermediary regions produced floral buds with a few bracts or leaves.

To determine if the exogenous supply of IAA or gibberellic acid would bring about a reversal in sex-expression, Galun, Jung and Lang (1962) cultured the floral buds of a cucumber (*Cucumis sativus*). The potential male buds were excised from the lower nodes of monoecious plants and cultured on White's medium containing IAA, or gibberellic acid, or both. With IAA (0·1 mg./l.) only the ovaries developed; with gibberellic acid the stamens grew better than the stamens of female buds formed on media containing IAA. When IAA and gibberellic acid were used together, the effect of IAA was reduced. These authors concluded that IAA seems to affect the sex-expression when applied to young floral buds.

Physiology of Meiosis in Excised Anthers

Anther culture offers a promising approach to a better understanding of the physiological and biochemical factors controlling meiosis. There is a long-standing question whether or not the biosynthetic requirements of chromosome segregation are fulfilled prior to the onset of prophase, and that the unfolding of the division-cycle is a natural consequence of the properties of preformed aggregates. The comparatively long interval covering the pairing and movements of meiotic chromosomes provides an obvious advantage for a study of this problem.

Gregory (1940), working with the excised anthers of *Lilium* in culture solutions, reported that meiosis was dependent upon substances produced by the vegetative parts of the plant. Sparrow, Pond and Kojan (1955) stated that the anthers of *Trillium* excised at different meiotic stages and grown in culture media supplemented with some growth factors were able to complete meiosis and reach the mitotic microspore division. Only 22% of the anthers excised at pachytene developed up to the microspore mitosis, but 67% of the anthers removed at the diplotene completed the meiotic and post-meiotic development. Vasil (1957, 1959) reported that when the anthers of *Allium cepa* excised as early as leptotene-zygotene were grown on a medium containing

gibberellic acid (1 mg./l.) and kinetin (0·05 mg./l.), or RNA, they passed through both meiotic divisions producing tetrads of microspores.

Hotta and Stern (1963) studied the incorporation of labelled compounds into RNA and proteins in the excised anthers of *Trillium* during meiosis. They produced evidence to show that the synthesis of RNA and proteins occurs well after the duplication of chromosomes.

These studies indicate that once meiosis is initiated, it can continue till its completion by receiving the stimuli from the nutrient media, but they do not suggest as to what induces meiosis. A further step in this direction would be to culture anthers excised at premeiotic stages and induce meiosis by specific chemicals.

Control of Fertilization

Many crosses do not result in the production of viable seeds due to (*a*) failure of pollen to germinate on a foreign stigma, (*b*) short length and bursting of pollen tubes, or (*c*) slow growth of tubes so that they fail to reach the ovary before the abscission of the style. The recent success in intra-ovarian pollinations in *Papaver rhoeas* by Kanta (1960), and in *Argemone mexicana, A. ochroleuca* and *Eschscholtzia californica* by P. Maheshwari and Kanta (1961) opens up the possibility of overcoming such barriers to crossability. A pollen suspension in 0·02% boric acid was injected into the intact ovaries, and fertile seeds were produced.

A far more effective device is *in vitro* fertilization (Kanta, Ranga Swamy and P. Maheshwari, 1962). The ripe pollen and ovules of *Papaver somniferum* at the mature embryo sac stage were sown together on Nitsch's nutrient medium which is suitable for the growth of both. The ovules showed normal fertilization and a satisfactory development of the endosperm and embryo. The seeds were viable and gave rise to healthy seedlings.

Dulieu (1963) reared the unpollinated ovaries of *Nicotiana tabacum* with the style and stigma intact, on a medium containing mineral salts + vitamins of B-group + 4% sucrose + 20% coconut milk. The ovaries were pollinated by means of a needle on the same or the following day. While fertilization occurred in 25% of the ovules, only 10–15 seeds were produced per ovary and some of them germinated *in situ*. By transferring them to fresh media, normal seedlings with root, shoot and leaves were obtained.

In many plants the requirements for the germination of pollen, growth of pollen tube, and development of ovule are so different that *in vitro* fertilization is not always successful. An intensive study of these problems is desirable.

An understanding of the factors which enhance the growth of pollen tubes is also of great importance in dealing with the problems of incompatibility (*see* Johri and Vasil, 1961). Smith (1942) and Addicott (1943) obtained stimulation of both pollen germination and

elongation of tubes by the addition of hormones and vitamins to the culture media. Kato (1955), Chandler (1957) and Lockhart and Bonner (1957) also reported the promotive effect of gibberellic acid on the growth of tubes. Bose (1959) observed that in *Pisum sativum* the addition of gibberellin (0·05 p.p.m.) to the culture medium (sucrose IM + boric acid 0·01%) produced tubes which were more than seven times as long as in the controls.

Thus, a knowledge of the techniques of intra-ovarian pollination and test-tube fertilization are of distinct advantage in overcoming some types of incompatibilities leading to the success of hybridization programmes.

Development of Fruit and Seed

The culture of reproductive organs of angiosperms was initiated by LaRue (1942) who obtained limited growth of ovaries and rooting of their pedicels in several plants. Using the ovaries of tomato and gherkin, Nitsch (1951) extended this technique to the study of fruit physiology. On a medium containing sucrose the pollinated ovaries of tomato could be grown into mature fruits while the non-pollinated ovaries abscised or dried up unless a growth-promoting substance was added to the medium. He stated that in cultures the main characteristics of fruit growth remained unaltered and the growth curve and ripening process followed similar patterns *in vitro* and *in vivo*. Comparable results have been obtained in *Althaea* (Chopra, 1958), *Anethum* (Johri and Sehgal, 1963), *Linaria* (Sachar and Baldev, 1958), *Ranunculus* (Sachar and Guha, 1962), *Tropaeolum* (Sachar and Kanta, 1958) and *Zephyranthes* (Sachar and Kapoor, 1959).

The pollinated ovaries of *Allium cepa* (Johri and Guha, 1963), *Althaea*, *Iberis* (N. Maheshwari and Lal, 1961 *a*), *Ranunculus* and *Zephyranthes* produce fruits which are comparable to controls. In *Allium* and *Iberis* (Fig. 1 A) the fruits were normal and sometimes even larger than the field-grown fruits. At the time of inoculation the ovules contained the zygote and primary endosperm nucleus in *Allium*, *Althaea* and *Zephyranthes*; and a 2 to 3-celled pro-embryo and a few endosperm nuclei in *Iberis* and *Ranunculus*.

For the best growth the fruits of *Allium*, *Althaea* and *Iberis* required an auxin such as IAA or IBA. Gibberellic acid was also stimulative in these plants but was much more effective for the growth of ovaries of *Ranunculus* and *Zephyranthes* which do not require exogenous auxin. However, when used together, IAA and gibberellic acid showed a synergistic effect on the fruit growth of *Allium*.

Whenever the calyx is persistent it has a favourable effect on fruit growth. In *Althaea* (Chopra, 1958) the retention of the calyx resulted in the formation of larger fruits than those grown without it. The fruits of *Allium*, *Iberis* and *Linaria* failed to mature if the perianth or calyx was removed at the time of inoculation. In a self-sterile strain of tomato, Leopold and Scott (1952) noted that leaves were necessary

for fruit-set—a requirement which could be fulfilled *in vitro* by adding sugars, organic acids, various amino acids, purines and vitamins to the medium. Nitsch (1963) proposed that in tomato the ovaries grow at the expense of nitrogenous compounds present in the sepals and in the pedicel at the time of excision. From these studies it is obvious that the calyx or perianth supplies some growth-promoting stimulus to the ovary.

Although the ovary often develops normally *in vitro*, the seeds are not always fertile. Therefore, it is important to determine how far the test-tube fruits support the normal growth of seeds. The fruits of *Althaea rosea* contained seeds with normal endosperm and embryo when pollinated ovaries were planted with the calyx intact. In contrast, when the calyx was removed the seeds showed only heart-shaped embryos. In *Iberis* and *Linaria* the seeds had normal endosperm and embryo when the medium contained IAA (5 p.p.m.) and yeast extract (0·25%), respectively.

The ovaries of *Nicotiana tabacum* (Dulieu, 1963) reared four days after pollination did not grow in size but some of the ovules developed into seeds and contained normal embryos which were larger than those produced in Nature.

The auxin content of the flower and fruit is regarded as a critical factor for preventing abscission. Since the response to rooting can be taken as an index of the concentration of auxin, it would be interesting to compare the rates of rooting in different flowers grown *in vitro*. There is a striking difference in the rooting of pedicels of pollinated and unpollinated flowers. In tomato, on a medium containing only sugar and minerals, 25% of the unpollinated and 75% of the pollinated flowers developed roots in three weeks after planting. Subsequently, roots were produced in both sets of flowers (Nitsch, 1951). It may be concluded that regardless of whether pollination has or has not occurred, the flowers of tomato either contain or synthesise enough auxin to initiate rooting. On the addition of IAA to the nutrient medium there was profuse rooting in *Linaria* (Sachar and Baldev, 1958), and somewhat sporadic rooting in *Althaea* (Chopra, 1958), *Iberis* (N. Maheshwari and Lal, 1958) and *Ranunculus* (Sachar and Guha, 1962). These results indicate that the developing fruits and seeds of tomato produced abundant metabolite required for the initiation of roots, but the pollinated ovaries of *Althaea*, *Iberis*, *Linaria* and *Ranunculus* were deficient in this particular metabolite and an external supply of auxin was essential.

In cultured ovaries of *Allium* and *Zephyranthes* the seeds germinated *in situ* without the incorporation of any special adjuvant in the nutrient medium, whereas in *Ranunculus* casamino acids (100, 500, 1,000 p.p.m.) were indispensable (Fig. 1 B). The addition of IAA (0·5, 1 p.p.m.) to the nutrient medium resulted in the inverted orientation (negative geotropic response) of the seedlings of *Allium* (Fig. 1 C).

As compared to the studies on ovaries, investigations on the ovules are relatively few. The earliest reports are those of White (1932) and LaRue (1942) on *Antirrhinum* and *Erythronium*, respectively. LaRue observed a 4-fold increase in the size of ovules and development of callus. Withner (1943) reared the ovules of *Epidendrum cochleatum* into seedlings.

N. Maheshwari and Lal (1961 *b*) reported that in Nature the pollinated ovules of *Papaver somniferum* mature in about 18 days. When the ovules containing a 2-celled proembryo and a few endosperm nuclei were planted on Nitsch's medium, they matured into seeds in 23 days and some of them germinated *in situ* (Fig. 1 D). When the ovules of *Zephyranthes* (Sachar and Kapoor, 1959) collected two days after pollination and containing the zygote and primary endosperm nucleus were reared on Nitsch's medium, the embryo developed up to the globular stage but the formation of the endosperm was inhibited. In the cultured ovules the globular proembryo failed to grow further even if growth substances like kinetin (0·5 p.p.m.), IAA (2 p.p.m.), 2, 4-D (2 p.p.m.) and gibberellic acid (3·5, 5·0 p.p.m.) were added to the medium. However, if seitz-filtered coconut milk (25%) was added, the endosperm and embryo developed normally and the seeds germinated *in situ* (Kapoor, 1959).

To study the morphogenetic effect of the placenta and some growth substances, Chopra and Sabharwal (1963) cultured the pollinated ovules of *Gynandropsis gynandra*. The ovules sown with the placenta matured into seeds in 22 days. In Nature the seeds mature in 9 days after pollination and have a long period of dormancy. In cultures, however, they germinated within 4.0 days after maturation.

Besides their usefulness in investigating nutritional requirements of fruits and seeds, the *in vitro* techniques have proved very effective in breaking the dormancy of seeds. This is illustrated by *Ranunculus sceleratus* (Sachar and Guha, 1962). In Nature the achenes remain dormant for almost a year but when reared 3 to 6 days after pollination, they matured and germinated *in situ* within six weeks. The *in vitro* seeds of *Gynandropsis* and *Papaver* also showed a similar behaviour.

Growth of Endosperm *In Vitro*

While several continuously growing cultures of diverse reproductive structures such as pollen, placenta, nucellus and embryo have been established, reports of successful endosperm cultures are rather few LaRue (1942), for the first time, raised a continuous culture of endosperm of *Zea mays*, and Lampton (1952) did the same in *Asimina*. Straus and LaRue (1954) reported that seitz-filtered 'Difco' yeast extract (0·5%) supported a good growth of *Zea* endosperm. The growth-promoting activity of yeast extract could be attributed to its high organic nitrogen content. Thus, casein hydrolysate or a less complex mixture of only five amino acids could replace only one-third of the activity of yeast extract. Of the carbon sources tested, arabinose, galactose,

lactose, rhamnose and glycerol did not permit any growth and the tissues suffered a loss in weight. Sucrose, fructose and glucose were all satisfactory, but sucrose was by far the best. Even the utilization of starch has been reported in *Asimina* and *Zea* endosperm cultures. Sternheimer (1954 and Tamaoki and Ullstrup (1 58) demonstrated that for *Zea* endosperm yeast extract could be replaced by tomato juice but the growth was often erratic. There was no response of this tissue to IAA, 2,4-D, kinetin, gibberellic acid, casein hydrolysate and coconut milk.

By adding asparagine only, Straus (1960) succeeded in eliminating the requirement for organic nitrogen complexes like yeast extract and tomato juice. Glutamine and glutamic acid also supported appreciable growth but were not as satisfactory as asparagine. Norstog (1956) observed that yeast extract or seitz-filtered coconut milk supported optimal growth of *Lolium perenne* endosperm. Nakajima (1962) cultured the endosperm of *Cucumis* on Tukey or White's medium containing yeast extract, and he emphasized that IAA, 1, 3-diphenyl urea and casein hydrolysate could be used as substitutes for yeast extract.

There are a few unconfirmed reports of the differentiation of shoots from the endosperm tissue of *Zea* (LaRue, 1947). In most cases, however, the cultured endosperm tissue fails to differentiate.

Embryo Culture

The technique of embryo culture has been employed mainly to study the nutritional requirements, differentiation, raising of hybrid seedlings, and overcoming dormancy in seeds.

Hanning (1904), Stingl (1907) and Dieterich (1924) were the pioneers in this field. Dieterich used Knop's solution for growing the immature embryos of several species, and observed that the embryos tended to skip the stages of embryonic development and directly grew into seedlings.

Experiments with the culture of immature embryos revealed that they failed to grow even if many vitamins and growth substances were added to the nutrient medium. Therefore, it was realized that the younger embryos required certain substances which were supplied by the adjoining tissues in the seed but could not be replaced by any known chemical. To overcome this deficiency van Overbeek, Conklin and Blakeslee (1942) added coconut milk to the nutrient media for cultivating the embryos of *Datura*. They observed that with the autoclaved milk the embryos produced an unorganized mass, whereas with the unheated milk the growth and development was normal.

Sanders and Burkholder (1948) used casein hydrolysate or equal parts each of cystein and tryptophan as present in casein hydrolysate and obtained appreciable growth of the embryos of *Datura*. Rijven (1952) observed that the growth-promoting influence of glutamine on the embryos of *Capsella* surpassed that of a complete mixture of amino

acids. Asparagine did not prove stimulative. Further, he reported considerable starch formation in the young embryos grown on a medium containing asparagine, while with glutamine there was scarcely any starch. Norstog (1961) grew the 60 μ long (smallest embryo cultured so far) embryos of barley to maturity on White's medium containing coconut milk.

The possibility of overcoming sterility in hybrids was first demonstrated by Laibach (1925, 1929). He cultivated the young embryos of a hybrid *Linum perenne* × *L. austriacum* and obtained healthy seedlings. Brink, Cooper and Ausherman (1944) crossed *Hordeum* with *Secale* and produced intergeneric hybrids; and Blakeslee and Satina (1944), McLean (1946) and Sanders (1948) reported several new hybrids of *Datura*. Emsweller and Uhring (1962) stated that in a hybrid of *Lilium speciosum* × *L. amatum* the seeds showed embryos partially digested by the endosperm. However, after excision the embryos could be successfully grown in cultures. By resorting to embryo culture, Randolph (1945) was able to shorten the period of dormancy of *Iris* seeds from several years (2 to 15) to less than one year.

The essentiality of endosperm for the growth of embryos was demonstrated by Ziebur and Brink (1951) for *Hordeum*, and Pieczur (1952) for *Zea*. Nakajima (1962) reported the existence of an auxin, a cell-stimulating substance, and amino acids in the endosperm of several cucurbits which influenced the growth of the embryos.

According to Corcoran and Phinney (1962) there is a positive correlation between the growth of the seed or embryo and occurrence of gibberellin-like substances in the endosperm-nucellus of the *in vivo* seeds of *Echinocystis macrocarpa*. The concentration increased during the elongation of the cotyledons and dropped when the cotyledons had reached about 60% of their final length. These authors suggest that native gibberellin-like substances present in the embryo or endosperm may be one of the controlling factors in the growth of the seed. Dure and Jensen (1957) found that gibberellic acid accelerated the normal development of the embryos of *Gossypium hirsutum* grown in culture, whereas IAA caused abnormal growth.

Induction of Polyembryony

Haccius (1957) obtained regenerants on the basal end of the embryo by treating freshly harvested seeds of *Eranthis hiemalis* with isopropyl-N-phenyl-carbamate (0·2%) and maleic hydrazide (0·1%). She (Haccius, 1959) also observed cotyledonary abnormalities in young seeds by subjecting them to 600–1,200 p.p.m. phenylboric acid. Treatment with 2,4-D also resulted in syncotyly and monocotyly (Haccius, 1960; Haccius and Trompeter, 1960).

Adventive embryos originating from the nucellar cells produce seedlings which inherit the features of the mother plant. This is of great advantage to geneticists and plant breeders and is utilized on a large-scale propagation of desirable varieties.

Ranga Swamy (1961) cultured the nucellar tissue of *Citrus microcarpa* on White's medium containing 400 p.p.m. casein hydrolysate. It yielded a callus capable of unlimited proliferation and subculture. Further, the nucellar callus could be differentiated into 'pseudobulbils' which developed into new plantlets (Fig. 1 E). Only the upper half of the nucellus containing the young adventive embryos is capable of proliferation. P. Maheshwari and Ranga Swamy (1958) pointed out that by this method an indefinite number of plants, true to the maternal parent, can be obtained and this should be a great aid to the horticulturists.

Sabharwal (1963) obtained more or less similar results in *Citrus reticulata* but concluded that the proliferations arise not from the nucellus *per se*, but from nucellar embryos.

The embryos of *Cuscuta reflexa* (P. Maheshwari and Baldev, 1962) proliferate profusely when grown on White's medium containing 1 p.p.m. IAA and 400 p.p.m. cas in hydrolysate. When ovoidal embryos (0·5–1 mm long) were cultured, they swelled, became spherical, and developed superficial outgrowths (Figs. 2 A, B). The transections showed these outgrowths to be developmental stages of adventive embryos (Figs. 2 C, D). In six weeks numerous greenish proliferations were formed. These simulated young (adventive) embryos some of which germinated *in situ* (Fig. 2 B). The older embryos responded differently. Four weeks after inoculation the radicular end callused profusely and adventitious outgrowths, similar to those of ovoidal embryos, differentiated from the callus. When subcultured, the callus showed an unlimited capacity for proliferation and every subculture produced a fresh crop of adventive embryos. Many of these gave rise to normal shoots.

Johri and Bajaj (1963) cultured the mature embryos (length 4–8 mm) of *Dendrophthoe falcata* on modified White's medium supplemented with IAA (0·5 p.p.m.) and casein hydrolysate (500 p.p.m.). The radicular end of the embryo developed a marginal meristem and produced a discoid holdfast which later gave rise to as many as eight similar holdfasts in succession. In 25 weeks after inoculation well-developed seedlings were formed. If the embryo was sown while still enclosed in the endosperm, the radicular end again produced discoid holdfasts in succession but, in addition, it occasionally proliferated forming a mass of callus. Subsequently, accessory papillate leaves (Fig. 2 E) and foliar buds differentiated on the callus and the appearance of plumular leaves was either delayed or altogether inhibited. The globular proembryos (length 0·8–1·5 mm) cultivated on White's medium + IAA (1 p.p.m.) + cas in hydrolysate (1,000 p.p.m.) also produced a callus which differentiated into numerous adventive embryos (Fig. 2 F) in 3 or 4 weeks.

Johri and Sehgal (1963) reported induction of polyembryony in *Anethum graveolens*. The ovaries, excised three days after pollination with the ovules at the zygote and free nuclear endosperm stage, were inoculated on a modified White's medium supplemented with casein

hydrolysate (250, 1,000 p.p.m.) or yeast extract (100, 500, 1,000 p.p.m.). Four weeks after inoculation 16% of the cultures showed a polyembryonic and 20% a monoembryonic condition, while the embryo failed to develop in the rest of the cultures. A histological examination revealed that the embryonal mass undergoes cleavage and/or budding resulting in 15–52 accessory embryos. The polyembryonate mass grew enormously, ruptured the pericarp, and projected outside (Fig. 1 F). The adventive embryos usually had 2 to 6 cotyledons. The polyembryonal mass left *in situ* gave rise to seedlings with multiple shoots (Fig. 1 G). On the other hand, isolated mature embryos produced normal seedlings. During their growth the supernumerary embryos consumed the entire endosperm. In contrast, in fruits formed in Nature the seeds contain copious endosperm and only a small embryo.

From the callus of carrot root, Steward *et al.* (1958 *a*, *b*) obtained free cells and cell masses in suspension on White's medium containing 10–20% coconut milk. The earlier stages in the development of the cultured cells were reminiscent of the embryogeny of carrot in Nature. The most important achievement is the formation, from the cell masses, of complete plants which flowered and fruited and developed storage roots. Reinert (1963) also obtained embryo-like regenerants from the callus of carrot root.

Conclusions

The technique of plant tissue and organ culture has opened up several new frontiers but we are mainly concerned with the physiology of reproduction. Besides numerous other problems, it includes the induction of flowering; removing the barriers to crossability; test-tube fertilization; rearing fruits, seeds and embryos; induction of polyembryony; and the influence of intact calyx, corolla or perianth, and androecium on the development of ovary, ovules, endosperm, and embryo. These topics have been discussed in the foregoing pages.

The response to growth and development of different tissues and organs varies with the concentrations of chemicals added to the nutrient media. In combination the chemicals have a synergistic or an antagonistic effect. The influence of organic substances (like sugars, starch, organic acids, casein hydrolysate, yeast extract, malt extract, coconut milk and tomato juice) has been studied chiefly to determine the effective sources of carbon, nitrogen and growth-promoters. Sucrose is universally recognized as the best carbon source; other carbohydrates which are effective are fructose and glucose. The endosperm cultures of *Zea* can thrive even when starch is added to the medium (Straus and LaRue, 1954).

Sanders and Burkholder (1948) observed that for the young embryos of *Datura* a single amino acid or even a mixture of amino acids did not promote embryonic growth at a rate equal to that by casein hydrolysate. Therefore, they suggested that the "growth stimulus of the combination of twenty acids results from physiological interaction rather than from

the summation of effects of individual acids". Casein hydrolysate is now one of the important adjuvants used as growth-promoters. It has also proved effective in the induction of polyembryony, *e.g.*, in *Citrus*, *Cuscuta* and *Dendrophthoe*.

Another important growth-promoter is coconut milk which is also very extensively used in tissue cultures. Since the discovery of its role by van Overbeek *et al.* (1941), it has been subjected to detailed analysis by Pollard, Shantz and Steward (1961), and Tulecke, Weinstein, Rutner and Laurencot (1961). Which of the particular fraction/s of coconut milk promote/s growth of various tissues and organs is not yet fully understood, and further advances in this direction would be very useful.

Except when the level of endogenous auxin is adequate, an exogenous supply is essential for satisfactory growth of the excised tissues and organs. The promotive effect of auxin is considerably more when kinetin and/or gibberellic acid is/are also added.

This is the beginning of a new era and whereas the growth of excised tissues and organs on synthetic nutrient media has yielded some results, much more remains to be achieved. For example, very few attempts have as yet been made to use a defined medium which can be considered to contain the minimal nutritional requirements in respect of particular tissues and organs. Moreover, we know very little about the utilization and metabolic fate of the various chemicals present in the complex media. This should not be very difficult to follow with the use of labelled chemicals.

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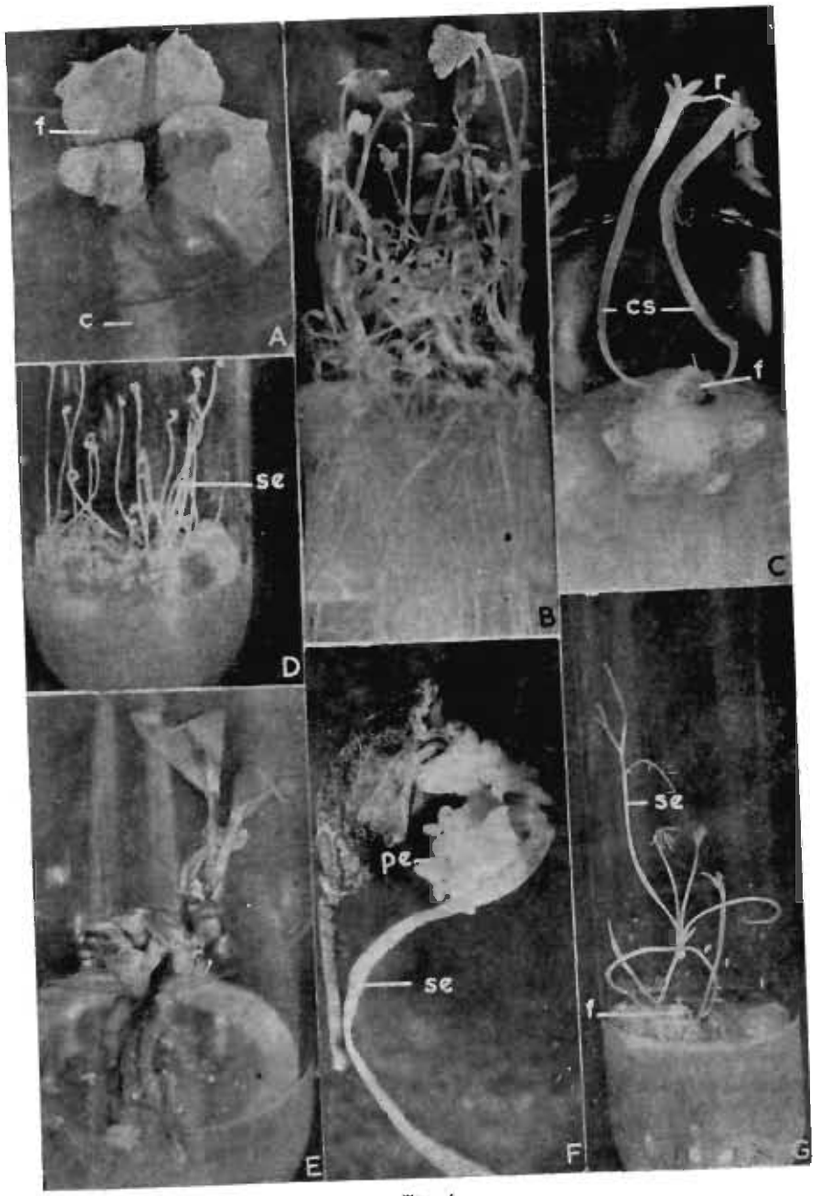

FIG. 1

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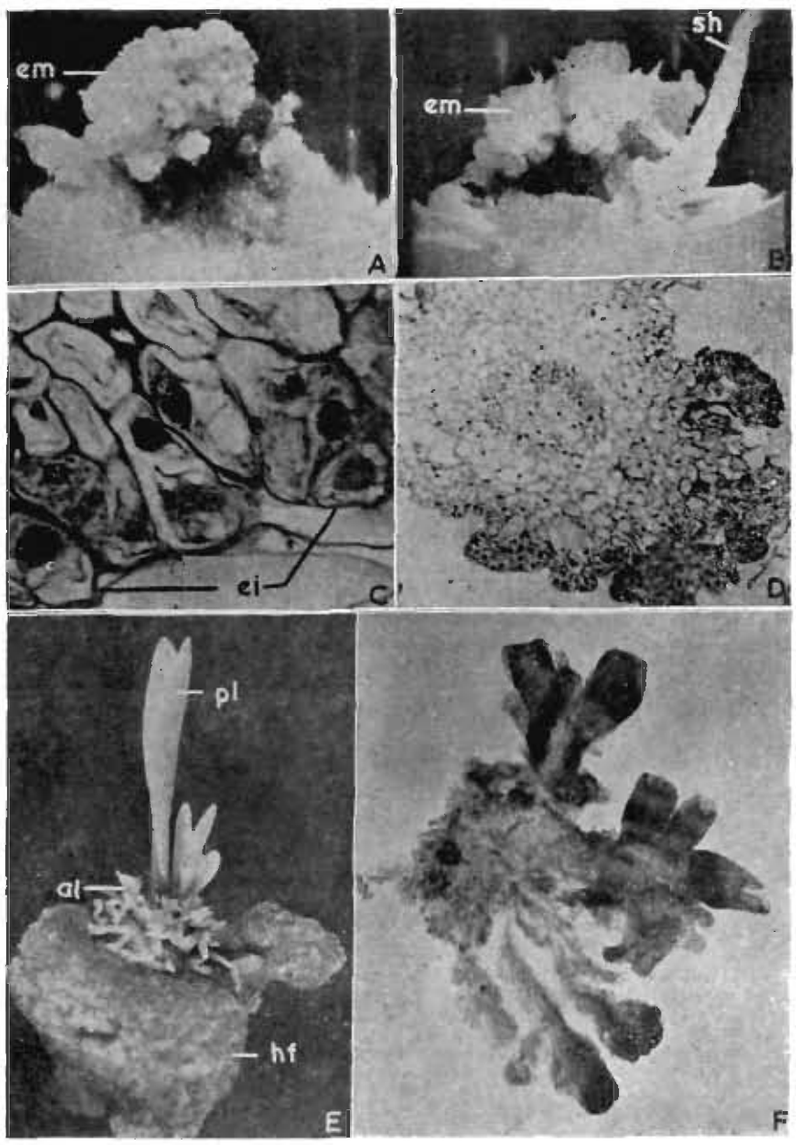

FIG. 2

B. M. Johri & Sipra Guha

EXPLANATION OF PLATES I & II

PLATE I

FIGS. 1 A–G. (*c*, callus; *cs*, cotyledonary sheath; *f*, fruit; *pe*, polyembryonal mass; *r*, root; *se*, seedling.) A. *Iberis amara* L.—Normal fruit from a pollinated flower, grown on Nitsch's medium + IAA (*5 p.p.m.*) + *kinetin (5 p.p.m.). (After N. Maheshwari and Lal*, 1961 *a*). B. *Ranunculus sceleratus* L.—*In vitro* germination of achenes in a 12-week-old culture on Nitsch's medium + casein hydrolysate (500 p.p.m.); ovaries were inoculated 6 days after pollination. (*After* Sachar and Guha, 1962.). C. *Allium cepa* L.—6-week-old mature fruit from pollinated flower grown on Nitsch's medium + IAA (0·5 p.p.m.); note the two seedlings with inverted orientation. (*After* Johri and Guha, 1963.) D. *Papaver somniferum* L.—3-week-old seedlings obtained from ovules inoculated 6 days after pollination on Nitsch's medium. (*After* N. Maheshwari, 1958.) E. *Citrus microcarpa* Bunge.—6-month-old seedling from a pseudobulbil on White's medium + casein hydrolysate (400 p.p.m.). (*After* Ranga Swamy, 1958.) F–G. *Anethum graveolens* L.—F. Ruptured fruit showing a polyembryonal mass, on White's medium + casein hydrolysate (100 p.p.m.) + yeast extract (100 p.p.m.); 18 weeks after incoluation. G. 10-week-old seedlings with multiple shoots, on White's medium + yeast extract (500 p.p.m.). (*After* Johri and Sehgal, 1963.)

PLATE II

FIGS. 2 A–F. (*al*, accessory leaves; *ei*, embryo initials; *em*, embryonal mass; *hf, holdfast; pl, plumular leaf; sh, shoot*). A–D. *Cuscuta reflexa* Roxb. — A, B. Embryonal callus from the subculture of callus of an ovoidal proembryo grown on White's medium+IAA (1 p.p.m.) + casein hydrolysate (400 p.p.m.); note the formation of a shoot in B. C. Some of the peripheral cells of the callus simulate embryo initials; note the 2-celled proembryo-like structures. D. A number of embryonal masses formed peripherally on the callus. (*After* P. Maheshwari and Baldev, 1962.) E, F. *Dendrophthoe falcata* (L.f.) Ettings. E. 20-week-old seedling produced from a mature embryo inoculated with the intact endosperm on White's medium + IAA (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.); note the prominent holdfast and numerous accessory leaves. F. 6-week-old culture on White's medium + IAA (0·5 p.p.m.) + casein hydrolysate (500 p.p.m.) showing differentiation of supernumerary embryos from callus derived from a globular proembryo. (*After* Johri and Bajaj, 1963.)

CYTOGENETICAL EVOLUTION WITHIN THE *SISYMBRIUM IRIO* COMPLEX

BY T. N. KHOSHOO

Post-graduate Department of Botany, Jammu and Kashmir University Naseembagh, Srinagar, India

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SIGNIFICANT results have been obtained in the field of cytogenetical evolution in the angiosperms in the United States of America, Europe, Japan, Canada and lately also in Australia and New Zealand. Out of these, the work of the Californian group is synthetic in approach and is the most illuminating. It has become apparent that chromosomes are a valuable guide to the understanding of the evolutionary processes, and it is natural that their study has received the maximum attention in recent years. Few integrated studies of this type have been made in India, particularly on the wild angiosperms. Considering the richness of the flora and the diversity in eco-geography and geology of the Indian subcontinent, such studies on our plants should prove to be particularly rewarding. We are using such an approach for the study of the angiospermic flora of our region, hoping ultimately to monograph it with an evolutionary bias. We are at present concentrating on some polymorphic taxa which are taxonomically "problem-species". A short account of the cytogenetical mechanism underlying the evolution within one such species, *Sisymbrium irio* Linn. (Cruciferae), is summarized here (*cf.* Khoshoo, 1959 *e*, 1960, 1963 *b* and unpublished).

A combined genecological and cytogenetical attack on a large number of selections gathered from diverse sources and covering almost all variations seen in Nature in the Punjab, revealed the occurrence of diploid, tetraploid, hexaploid and octoploid races, and stray triploid hybrid individuals. Furthermore, the tetraploid itself contains three morphologically and ecologically distinct types. These are *caulis*, *subcaulis*, and *acaulis*, suited respectively to wet, dry and moist-grazed habitats. Their morphological and physiological characters appear to have a definite adaptive value, because these are in tune with the requirement of the three types of habitats which they occupy (*cf.* Khoshoo, 1957 *a*, 1958 *c*). The cytological characteristics of each of the races (*cf.* Khoshoo, 1955, 1957 *b*, 1959 *a*) may be summarized as below.

CYTOLOGY OF THE RACES

Diploid.—It possesses 14 chromosomes in the leaf cells and corresponding with this number, 7 bivalent were noted at diakinesis. Meiosis is normal, resulting in perfect pollen and seed fertility.

Autotetraploid.—These plants were raised by the colchicine treatment of the diploid and possess 28 chromosomes in leaf cells. During diakinesis 7 quadrivalents are most commonly formed. The meiosis is on the whole normal. Pollen fertility is about 98% but percentage of good seeds is lower being 83–94.

Tetraploid.—All the three types were investigated and showed uniformly 28 chromosomes in the leaf cells. Fourteen bivalents were persistently seen during diakinesis. The pollen and seed fertility is ordinarily normal.

Polyhaploid.—One plant of this type was discovered which resembled the tetraploid *subcaulis* qualitatively. The 14 chromosomes found in its somatic tissue generally formed 14 univalents during diakinesis. Average number of bivalents per cell was 0·4. The plant was totally pollen and seed sterile.

Triploid.—These hybrids possess $2n = 21$ and the most predominant association during meiosis is 7 bivalents and 7 nnivalents. The bivalents are of rod type and often loosely attached, and during anaphase I they often form bridges with or without fragments. Anaphase II is also not normal. Trivalents are conspicuous by their virtual absence (mean number 0·02 per cell). Pollen and seed fertilities are 0·C05% and 0·0001–0·002%, respectively. The progeny of triploids, if viable, is always hexaploid.

Hexaploid.—Always 42 chromosomes were counted in leaf cells with 21 bivalents as the most preponderant association during meiosis. However, in some cells 1 or at the most 2 quadrivalents were observed during diakinesis. The average number of quadrivalents was only 0·5 per cell. Fertility of pollen is 75–93% and that of seeds 68-89%.

Octoploid.—Somatic chromosome number is 56 and during diakinesis there are 13–5 quadrivalents + 2–18 bivalents. However, mean number of quadrivalents is only 6·70 per cell. Anaphase I and II are generally clean. The percentages of pollen and seed fertilities are 92–100 and 70–96, respectively.

Synthesis of Higher Polyploids

'It has been demonstrated that the triploid individuals arise every year by the crossing between diploid and tetraploid *caulis* (Khoshoo, 1959 *b*). Both parents occupy the same habitat and grow indiscriminately mixed together. The hexaploid race has been raised as the progeny of the triploids, and has also been synthesized by doubling the chromosome number of the triploids by colchicine.

The octoploid individuals arise from the "off-season" plants of the tetraploid race. Due to "modificative asynapsis" in the "off-season" plants there are produced octoploid individuals in their progeny, in all probability as the result of fertilization between unreduced spores. The colchicine-induced octoploids from tetraploid confirm

such an origin. The artificial hexa- and octoploids show higher multivalent frequency than their natural counterparts (Khoshoo, 1959 *b*).

Cytology of the Hybrids

The various races including the colchi-autotetraploid from the diploid race and synthesized hexa- and octoploid types were crossed amongst one another in all directions. From such crossing only the following types of hybrids have been secured whose meiotic details are given below (*cf.* Khoshoo and Sharma, 1959; Khoshoo, 1959 *d*).

Hybrids within the tetraploid race.—The six reciprocal hybrids involving the three types of tetraploid always show 14 bivalents as in their parents. Hybrids are fully fertile and segregate in F_2. The genetics of the morphological and physiological differences that enable them to have distinct habitat preferences is under study.

Hybrids within the octoploid race.—The two types of octoploid have also been reciprocally crossed. The meiosis and fertility of the hybrids resemble their parents.

Diploid × tetraploid and reciprocal.—The progeny in both the cases was triploid and meiosis resembles the natural triploid plants.

Autotetraploid × tetraploid (Hybrid-tetraploid).—The diakinesis in this complex is conspicuous by the presence of 7 bivalents + 14 univalents. The mean number of trivalents per cell is only 0·2. Anaphase is characterized by being highly abnormal because of the presence of univalents. Though the hybrid was tetraploid in constitution, yet it esembled neither of its parents in the nature of its meiosis.

Natural × synthesized hexaploids and octoploids.—These hybrids did not reveal any aberrant behaviour up to metaphase I, but at anaphase I and II bridges were noted with and without fragments, thereby indicating that structural differences exist between the chromosomes of the raw and natural counterparts.

Genome Relations

The following conclusions emerge after taking together the foregoing cytological analysis of the various races, polyhaploid plant and natural and artificial hybrids (Khoshoo, 1960):

1. The tetraploid can be regarded as a genomic allotetraploid for two reasons. Firstly, it shows only bivalents, while autotetraploid (from the diploid) shows quadrivalents, even though both have the same chromosome number and size. Secondly, the polyhaploid plant from the tetraploid has 14 univalents which is further strengthened by the presence of 14 univalents even in the hybrid-tetraploid. In the latter the 7 bivalents are formed by the 14 chromosomes of the auto-tetraploid while the 14 univalents by the chromosomes of the natural tetraploid. In short, the latter has arisen after an intercenospecific cross and is a perfect allotetraploid.

2. The relationship between the diploid and the tetraploid is clarified by the meiosis in triploid and hexaploid. In triploid there are 7 rod bivalents and 7 univalents, while in hexaploid there are 21 bivalents. Therefore, the 7 bivalents of triploid are due to the pairing between homeologous chromosomes which is further supported by the presence of bridges with and without fragments formed by the bivalents of the triploid. Evidently, in triploid the chromosomes form bivalents because of the differential affinity between the homologous chromosomes, but on doubling (*i.e.*, in hexaploid) chromosomes again form bivalents because of the preferential pairing between the homologous chromosomes. Therefore, if the genomic constitution of the diploid is AA, then that of tetraploid is 2 (A_1B), of triploid AA_1B and of hexaploid 2 (AA_1B). The constitution of the hexaploid is auto-allohexaploid in which the autoploid portion [2 (AA_1)] is segmental-alloploid in nature.

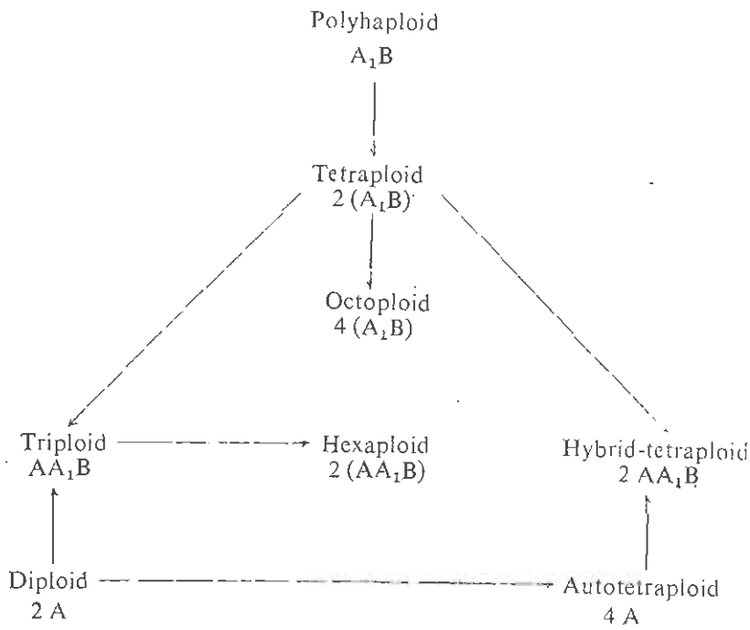
3. The constitution of the octoploid is simple to infer since it is only a duplication of the tetraploid. It is, therefore, 4 (A_1B) and is auto-allo-octoploid.

4. The presence of higher quadrivalent frequency in the raw hexa- and octoploids than their natural counterparts is due to their being raw. As such, they contain more duplicated genic material. Furthermore, the raw types have not undergone selection for the reduction of quadrivalents. The hybrids between the natural and synthesized types show evidence of rearrangement of chromosomes. The reason for this is the possible difference in the chromosomes of the strains involved for the origin of these races in Nature and those utilized by the writer. Alternatively, it may be looked for in the difference in modes of their origin. The natural types arise through fertilization between unreduced spores, while the synthetic types were prepared by the somatic doubling by colchicine.

5. The three genomes, A, A_1 and B, are not entirely non-homologous. Of course, the degree of homology between A and A_1 is considerable; however, the intergenomic pairing seen in polyhaploid and also in triploid and hybrid tetraploid, though low, nevertheless, shows that the A and A_1 genomes on one hand, and B on the other, do contain some segments in common.

6. According to the present analysis the A genome in tetraploid is differentiated in comparison to the A genome of the diploid. It does not necessarily mean that the diploid is not one of the parents of the tetraploid. The latter is very widely distributed and appears to be an old taxon (*cf.* Khoshoo, 1961 *a*). It is possible that ever since the A genome of the diploid entered in association with the B genome, to form the present tetraploid, the two genomes have undergone a measure of structural rearrangement and genetical divergence in comparison to the original condition, one of which is still preserved in the present diploid (AA).

The foregoing analysis has been summarized in the following diagram.



Extent and Nature of Barriers to Gene Exchange

Isolating mechanisms, though negative factors, are nevertheless almost an integral part of the evolutionary processes. There are four types of barriers to crossability in this complex, namely, ecological preferences, autogamy, seed incompatibility resulting from the matings between parents with different ploidy levels and, lastly, chromosomal sterility (Khoshoo, 1959 *e*. 1963 *b*).

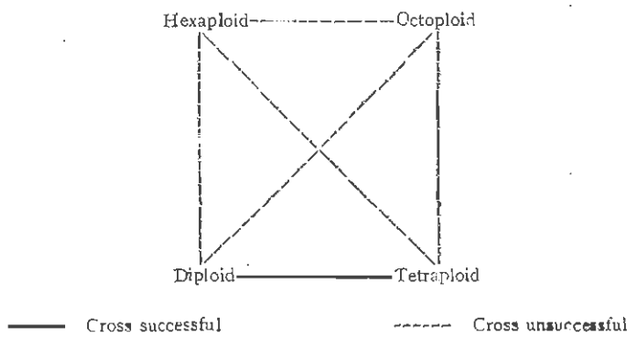
On the basis of the habitat preferences the complex can be classified into three categories, accordingly as the races occupy dry (tetraploid *subcaulis* and octoploid), moist-sun or shade (diploid, tetraploid *caulis* and hexaploid), and moist-grazed (tetraploid *acaulis*) situations. Ordinarily there is no chance of hybridization between members of the three different categories. On the other hand, one can expect hybrids to arise between races occupying one and the same type of habitat. However, such crossing is prevented due to more or less obligate autogamy. Autogamy is augmented by the fact that there is decrease in length of the ovary in the higher ploids (Khoshoo, 1959 *c*). The only hybrids possible are with the protogynous flowers of the diploid as the female parent. Due to seed failure, not all the expected types

of hybrids arise. An embryological examination has shown that a serious endosperm malfunction results in crosses involving parents of different ploidy levels (Kboshoo and Sharma, 1959; Khoshoo and Raj, 1960). These three types of isolation barriers are often superimposed upon one another. In Nature, due to their interplay, there results only one type of hybrid, *i.e.*, triploid.

It may be emphasized that even this hybrid does not break the isolation between its parents, *i.e.*, diploid and tetraploid. This is because of the chromosomal sterility (*cf.* Stebbins, 1950) resulting in total sterility. No seed is produced except for the rare occurrence (0·0001–0·002%) of hexaploid seeds resulting from the chance fusion between unreduced spores. Such seeds, as indicated earlier, result in hexaploid individuals which again are isolated. The net result is that the diploid and the tetraploid though bridged by the triploid and hexaploid, remain, nevertheless, isolated and there is no gene exchange between the parents and their amphiploid, *i.e.*, hexaploid.

There is, however, no seed-incompatibility between races with the same chromosome number, whether they are genetically akin or not. This is apparent from the vigorous hybrids that result from the matings among the three tetraploid types, two octoploid types and, lastly, what is more significant, between autotetraploid (4 A) and tetraploid ($2A_1$ 2B). Furthermore, it is apparent that the extent of natural hybridization is very limited primarily because of the autogamy. So far the only natural hybrids seen were the triploid individuals; however, recently I have discovered, what may be looked as inter-ecotypic (between *caulis* and *subcaulis* types of tetraploid) hybrid swarms in the intermediate habitats. These are being analysed at present.

The foregoing discussion has been summarized in the following diagram.



General Remarks and Conclusions

Sisymbrium irio is not only very widely distributed, but also occupies a geologically, edaphically and climatically diversified area (*cf.*

Khoshoo, 1963 *a*, *b*). It is, therefore, understandable that the species is polymorphic. The polymorphicity owes its origin to the phenotypic modifications induced by the varying environments on one hand, and the genetic diversity on the other (Khoshoo, 1958 *b*, 1963 *b*). The former is not polymorphicity in the real sense, because it vanishes as soon as the plants are cultivated under uniform environmental conditions. It is completely reversible. However, capacity of the various genotypes to modify in relation to the environments is a distinct advantage, because it enables them to grow in a variety of habitats, from adverse to favourable.

The genetic diversity is primarily due to the auto- and/or allopolyploidy, and secondarily to the gene mutations and structural alterations in chromosomes. These phenomena constitute the cytogenetic mechanisms that underly evolution in this complex.

At least two (A and B) and at the most three (A, A_1 and B) genomes have been interwoven by auto- and allopolyploidy to give rise to one phylogenetic unit. In this unit there occur three distinct polyploid races (4*x*, 6*x* and 8*x*) and stray triploid hybrid individuals. Furthermore, at the tetraploid level there have resulted three distinct ecotypes due to genic differentiation. These types seem to have arisen due to the selective action of the edaphic and biotic factors. The hybrids between the raw and the synthesized polyploids have revealed the role played by the structural alterations in chromosomes.

One of the important features of evolution in the complex is the simultaneous and abrupt origin of the barriers to crossability of the various races, since polyploidy *per se* acts as an effective barrier. Its action is supplemented by habitat preferences, autogamy and chromosomal sterility. For their perpetuation and further spread these taxa have to be effectively isolated from one another, particularly when some of them occupy the same habitat. The various races do not show very strong morphological differences, but with the strong isolating mechanisms in operation, these are likely to evolve independently and may, in course of time, attain the requisite morphological distinctness. Here, therefore, we are faced with a situation where the various races have a taxonomic status of not more than varieties, but a cytogenetic status of distinct and mature species.

In conclusion, it may be said that the present results reveal the combined role of polyploidy, hybridization, gene mutations and structural changes in the evolution and variation of a single species.

It is of interest to point out that there appears to be a correlation between the annual habit, self-pollination and polyploidy in this complex (*cf.* Grant, 1956; Khoshoo, 1958 *a*; Baker, 1959). This is because of the fact that all the polyploid races of this complex have arisen as the result of fertilization between unreduced spores, and chances of production of polyploids in this manner are greater with

self-pollination than with the cross-pollination. Furthermore, such a mode of origin assumes importance because the species has an annual habit, which, so to say, acts as the limiting factor.

The species complex is able to maintain its far-flung boundaries (*cf.* Khoshoo, 1963 *a*) because of its excellent genetic system which promotes weedy tendencies. Some of its traits are: extreme aggressiveness, capacity for extreme phenotypic modifications (that arise in response to the diverse environmental conditions in which it can grow), obligate autogamy, high reproductive potential and auto- and allopolyploidy accompanied by efficient isolating mechanisms. The autogamy is a distinct advantage under the habitat conditions which this complex exploits, because it causes these races and ecotypes to be ordinarily inbred and, therefore, these are true-breeding. Furthermore, it also ensures high seed production and high viability.

Similar type of data are being obtained for the various taxa of the angiosperms of this region and some interesting results have been already obtained. It is hoped that in course of time it will be possible to present an integrated picture of evolutionary corspectus of the angiospermic element of the flora of this region. This in turn would throw light on the composition, evolutionary status, affinity and migration of the flora of this region in relation to past geographical and geological events.

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COMPARATIVE GENETICS IN WHEAT*

By Hitoshi Kihara and Koichiro Tsunewaki

National Institute of Genetics, Misima, Japan

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Introduction

Continuous search for genetic information and incessantly deeper and deeper delving cytogenetic analysis of diverse materials have opened the ground to a special branch of investigations, the comparative genetics.

Nachtsheim (1959) divides comparative genetics in animals into two categories, namely, comparative genetics within species and that between species. The former consists of studies of genocopy, a term coined by him for mimic genes in contrast to phenocopy. The task of the latter is to find out by morphological, physiological, embryological and phenogenetic investigations if homologous genes are actually involved; how far the parallelism of various types goes; if eventual differences could be shown to be type-specific; if in sufficiently analyzed types the compared traits belong to the same linkage-group, etc. (Nachtsheim, 1959, p. 192).

On the basis of assembled results, the pursuit of comparative genetics of the second category seems to be highly promising in plants. Nachtscheim did not mention any special methods for this kind of studies.

In wheat, however, excellent methods for such studies are at our disposal. They are:

(1) Nullisomic and monosomic analysis,

(2) Chromosome addition and substitution, and

(3) Use of amphidiploids.

The first method was used by many authors in locating a gene or genes in the corresponding chromosome. At present this method is used only for 6*x* wheat, where 21 monosomics are available.

The genome formula of 6*x* wheat is AABBDD. A normal plant has 21 bivalents. Nullisomics, with 20 bivalents instead of 21, can be obtained in many ways, namely, by

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* Contributions from the National Institute of Genetics, Misima, Japan, No. 469.

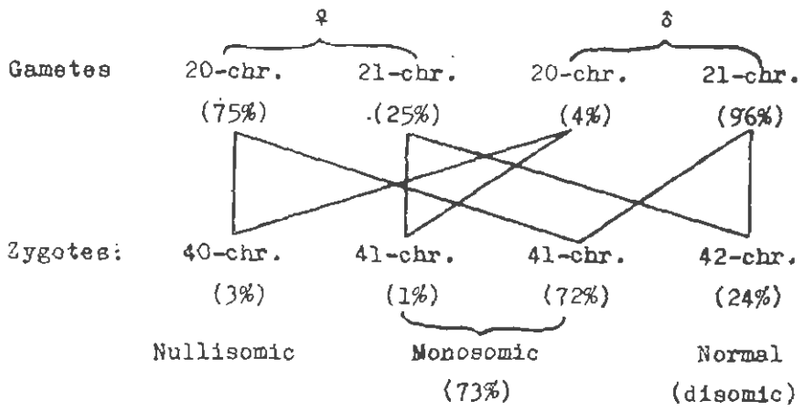
(*a*) crossing the 5*x* hybrid by 6*x* wheat,

(*b*) crossing a haploid by 6*x* wheat, and

(*c*) using partially asynaptic 6*x* wheat.

Among the offspring of a pentaploid hybrid or those obtained from the backcross, 5*x* × 6*x*, we find 41-chromosome plants, which give rise in the next generation to 40-, 41- and 42-chromosome plants (nulli-, mono- and disomics, respectively).

From selfing of monosomic plants, we obtain offspring of three chromosome types, namely, with 40-, 41- and 42-chromosomes. Their ratio is shown in Text-Fig. 1.



TEXT-FIG. 1. Frequencies of nulli-, mono- and disomics in the selfed progeny of wheat monosomics (after Sears, 1959).

A 75 : 25 ratio for the 20- and 21-chromosome female gametes is expected when univalent elimination occurs in 50% of EMCs. (embryo sac mother cells). Frequency of elimination differs more or less among the 21 chromosomes. On the average, however, a ratio very close to 75 : 25 is obtained.

There occurs certation between 20- and 21-chromosome male gametes in fertilizing the eggs; the 21-chromosome male nuclei fertilize the eggs in much higher frequency (96%), notwithstanding that only one-fourth of male gametes have 21 chromosomes. From the first method, we get 7 nullisomics of D-genome only, while from the second and third ones we get all possible nullisomics, namely, 21 lines. They differ in their morphology to a certain extent. We can easily distinguish any two nullisomics by crossing them with each other. The hybrids show $19_{II} + 2_{I}$. Such plants have normal height and almost normal fertility. In the following generation we get among others normal plants with 21_{II}.

Since the first nullisomic (g-dwarf) was obtained from the offspring of a pentaploid hybrid between *T. polonicum* and *T. spelta* in 1924, it took almost 30 years to establish a complete set of different nullisomics (Kihara, 1924; Sears, 1944, 1954). In the beginning, nullisomics were designated by the missing chromosomes such as *a*-dwarf, *b*-dwarf, etc. (Kihara and Matsumura, 1942), or nulli-I, nulli-II, etc. (Sears, 1954). Usually the 7 nullisomics from 5*x* wheat hybrid breed true. Sometimes, or fairly frequently, in the offspring of some of the selfed nullisomic dwarfs, we obtained tall plants. The origin of these so-called gigas-plants, possessing one chromosome more than their parents and showing $1_{III} + 19_{II}$ (or $20_{II} + 1_{I}$), is probably due to the union of 20-chromosome- with 21-chromosome-gametes. The formation of the latter may be attributed to non-disjunction. In the subsequent generation of the gigas-plants we found 42-chromosome plants, whose PMCs had $1_{IV} + 19_{II}$. They were almost normal regarding height as well as fertility. We have assumed that the missing chromosome of a nullisomic plant was substituted by a corresponding chromosome, *i.e.*, semi-homologous (Kihara's old designation) chromosome, belonging to the A- or the B-genome which was, for instance, designated by γ_1 or γ_2 (Kihara and Wakakuwa, 1930). Later the three corresponding chromosomes of A, B and D genomes were designated by Sears as homoeologous.

After elaborate studies undertaken by Sears and his co-workers (Sears, 1959; Okamoto, 1962), it became clear that the 21 wheat chromosomes can be divided into 7 groups of 3 homoeologous chromosomes. Each of 3 chromosomes within a group was ascribed either to A-, B- or D-genome. Accordingly, symbols for the 21 wheat chromosomes were completely revised. For instance, 3 homoeologous chromosomes of group 1 were designated as 1A, 1B and 1D — A, B and D denoting affiliation to the respective genomes. The revised system is given in Table I.

According to Huskins' terminology (1941), two chromosomes are homoeologous when they are homologous in part (Sears, 1954). Now we found that a missing chromosome of any homoeologous group can be complemented by one of the two other members. So the function of the three chromosomes of a given group must be similar. Therefore, we may assume that the homoeologous chromosomes have originated from one common ancestor-chromosome in the course of evolution. The finding of the 7 homoeologous groups is the most outstanding achievement from the standpoint of the development of comparative genetics in wheat.

Methods and Results

1. *Nullisomic and Monosomic Analysis*

For locating a gene or genes in the corresponding chromosomes, 21 monosomic lines of Chinese Spring were used as female parents in crosses. The method will be described briefly as follows;

(*a*) *Dominant gene.*—We will assume that a recessive allele (*a*) is found in Chinese Spring and its dominant allele (*A*) in the variety we want to investigate. Then F_1 between Chinese Spring ($2n$) and that variety will show the dominant character and 3 : 1 simple segregation will be observed in F_2. However, if monosomics are used as female parents, 2 types of F_1 for each monosomic line will be produced. One has 42 chromosomes. This F_1 is a monogenic hybrid (*Aa*) and 3 : 1 segregation can be observed in F_2.

TABLE I

Assignment of the 21 *wheat chromosomes to* 7 *homoeologous groups*

Homoeologous group	Genome						
	A		B		D		
1	1A	XIV	1B	I	1D	XVII	*c*
2	2A	XIII	2B	II	2D	XX	*e*
3	3A	XII	3B	III	3D	XVI	*g*
4	4A	IV	4B	VIII	4D	XV	*d*
5	5A	IX	5B	V	5D	XVIII	*f*
6	6A	VI	6B	X	6D	XIX	*b*
7	7A	XI	7B	VII	7D	XXI	*a*

a~*g*: Used by Kihara and Matsumura (1942)

The other F_1 type has 41 chromosomes. This hybrid behaves just like a 42-chromosome hybrid, if the lacking chromosome is one of the 20 chromosomes which do not carry the *a* locus. However, if the chromosome carrying *a* is missing, then this F_1 has only one *A*, which will be expressed by the formula 0*A*. Such a hybrid can be obtained only once among the 21 hybrid combinations. The segregation of 0*A* will give rise to 00 (nullisomics), 0*A* (monosomics) and *AA* in the ratio 3 : 73 : 24 (*see* Text-Fig. 1). Thus, the *A* gene can be located on the chromosome, whose monosomic line shows such an aberrant F_2 ratio.

One example will be given as a model. Tsunewaki (1961) studied the location of a gene controlling ear density, compact ear (*C*) being dominant over lax (*c*).

Only in F_2 segregation with mono-XX we see a significant deviation from the usual 3 : 1 ratio. From this result, it is assumed that the gene (*C*) locates on the chromosome XX (2D).

TABLE II

Segregation of compact and lax ear types in F_2 lines of Chinese Spring monosomics × Elgin

F_2 lines		No. of plants		x^2 (3. 1)
		Compact ear	Lax ear	
Disomic		542	185	1.08
Mono.	I	84	20	1.85
	II	67	24	0.09
	III	50	18	0.08
	IV	48	22	1.54
	V	41	10	0.79
	VI	58	21	0.11
	VII	29	8	0.23
	VIII	51	20	0.38
	IX	46	18	0.33
	X	65	14	2.23
	XI	73	23	0.06
	XII	45	17	0.19
	XIII	36	12	0.00
	XIV	66	18	0.57
	XV	40	14	0.03
	XVI	80	18	2.30
	XVII	73	31	1.28
	XVIII	121	32	1.36
	XIX	52	19	0.12
	XX	205	1*	66.03†
	XXI	84	17	3.57

* Apparently nullisomic † Significant at the 1% level

(*b*) *Recessive genes.*—On the contrary, we will assume the dominant gene *A* in Chinese Spring and the recessive gene *a* in the variety used for examination. Among 21 kinds of monosomic F_1's from crosses between Chinese monosomics and the tested variety the one, whose lacking chromosome carries the *A* gene, should be genotypically 0*a* and should show the recessive character. In F_2 progeny from this plant, we get 00, 0*a*, and *aa* in a ratio 3 : 73 : 24, namely 3% of F_2 are nullisomic and the rest show the recessive character. The other 20

monosomics should have *Aa* and should express the dominant character. We see the ordinary 3 : 1 ratio in their F_2. Thus, we can locate the *a* gene as well, on a specific chromosome from both F_1 p henotype and F_2 segregation ratio.

(*c*) *Duplicate genes.*—In 6*x* wheat duplicate gene system is common. Therefore, we will consider now a monosomic analysis of duplicate genes. Assuming that the Chinese Spring has the genotype $A_1A_1A_2A_2a_3a_3$ and the variety for examination $a_1a_1a_2a_2a_3a_3$, then F_1 has the genotype $A_1a_1A_2a_2a_3a_3$, and 15 dominant (*A*) : 1 recessive (*a*) is obtained in F_2. Now this variety is crossed with all 21 monosomics. All 42-chromosome F_1 plants obtained from 21 cross-combinations will segregate 15 *A* and 1*a* in F_2. One of the monosomic (41-chromosome) F_1 from monosomic Chinese Spring would be $0a_1A_2a_2a_3a_3$. The F_2 segregation from this F_1 plant is calculated as follows:

$$(75A_2 + 25a_2) \times (3\text{Nulli} + 97a_1) = 3A. : 1a.$$

In this calculation the percentage of 00 (nullisomics) and that of the recessive segregants ($0a_1 + a_1a_1$) is 3 and 97 respectively. However, the 3 : 1 ratio of *A* and *a* in F_2 remains constant for any percentage of nullisomics, which is evident from the following derivation:

$(75\,A + 25a)\,(m\,\text{Nulli} + na) = 3\,A : 1\,a$, where m = per cent. frequency of nullisomics, n = per cent. frequency of mono- and disomics, and $m + n = 100$. This 3 : 1 ratio is not the same as in simple Mendelian F_2 segregation. It is the ratio of 20- and 21-chromosome gametes as already mentioned.

Another monosomic F_1 would be $A_1a_1\,0a_2a_3a_3$. The F_2 segregation from this F_1 is also $3A : 1a$. All the other 19 monosomic F_1's must have the same genotype, $A_1a_1A_2a_2a_3a_3$, as the normal F_1 and will segregate $15A$ and $1a$ in F_2. Thus, we can locate A_1 and A_2 loci each on one of the two chromosomes, whose monosomic F_1's give the 3 : 1 F_2 ratio.

(*d*) *Some results of monosomic analysis.*—Using the principles of monosomic analysis many workers have tried to assign various genes to their respective chromosomes. A part of the results hitherto obtained is given in Table III.

It may be seen from Table III that genes with similar effects were found within many homoeologous groups. For instance, 3 awn-promoter genes a_3, a_1 and a_2 are associated with chromosomes 2A, 2B and 2D, respectively, while 3 modifiers of awn forms occur in the third homoeologous group (3A, 3B and 3D).

It seems plausible to conclude that the homoeologous chromosomes of the second and third groups have retained their homoeologous genes* during the long period of evolution. As the three promoters and the

* Those genes must be homologous. However, as they are located on homoeologous chromosomes, we call them homoeologous genes for convenience's sake.

TABLE III

Location of genes with similar functions in wheat

Character	Chromosome	Locus
Awnedness (promotion)	2A, 2B, 2D	a_3, a_1, a_2
Waxy foliage	2A, 2B, 2D	I_1-W, I_3-W, I_2-W
Lethality	3A, 3B, 3D	(ls_1), v, (ls_2)
Awnedness (modification)	3A, 3B, 3D	a_3, a_6, a_4
Squarehead ear	5A, 5B, 5D	q_1, q_2, q_3
Growth habit	5A, 5D	Sg_2, Sg_1
Pigmentation	7A, 7B	Rc, Rs
Awnedness (inhibition)	5A, 4B, 6B	B_1, Hd, B_2
Progressive necrosis	2A, 5B, 3D	Ne_2, Ne_1, Ne_3

three modifiers belong to different homoeologous series, it might be better to designate them accordingly, for instance, the latter by symbol *mo-a* with subscripts 1–3 added to *mo*, *i.e.*, mo_1-a, mo_2-a and mo_3-a.

The inhibitors for waxy (I_1-W and I_2-W) were found on 2A and 2D respectively. On 2B, however, Tsunewaki (1962) believed to have found W (a dominant waxy gene). Recently, Tsunewaki was inclined to accept the view that I_3-W might be present on 2B. This gene inhibits weakly the waxy character restricting its effect to the haulm and foliage, leaving the ear without waxy bloom (half waxy). It is already known that waxy genes are independent from waxy inhibitors.

For winter habit, only 2 genes, Sg_1 and Sg_2, were found in 5A and 5D, respectively. No corresponding gene was detected in 5B by monosomic analysis (Tsunewaki and Jenkins, 1961; Tsunewaki, 1962).

We cannot explain why 5B monosomics did not show any sign of change in growth habit. About this problem we want to speak later in detail.

As to homoeological series for lethal genes and pigmentation, our knowledge is still meagre. We cannot tell whether they are homoeologous with each other.

Let us consider the last two gene series of similar effects of Table III, first the genes for necrosis. They are found in different homoeologous groups. The three genes, Ne_1, Ne_2 and Ne_3, are complementary. Their effects appear only when all three dominant alleles are present. It is interesting that two of them, Ne_1 and Ne_2, are included in the tetraploid species. Only when they are combined with Ne_3 from *Ae. squarrosa*, the F_1 will be necrotic. In the hexaploid varieties, this complementary effect of necrotic genes can be detected by a crossing experiment.

We presume that these genes are consistent with normal development and have different functions in their respective parents. But when they are brought all together into one system, the physiology of zygotes seems to be strongly disturbed. If so, it is no wonder that they are not found in the same homoeologous group.

Also, the awn inhibitors are non-homoeologous. They seem to inhibit the activity of other genes in different ways. Most probably they are different genes.

To this category might belong complementary dwarf genes (Tanaka, 1961 *b*). However, their location is not yet determined.

Major genes differentiating species-characters were investigated by Sears (1954). They are, *S* gene for *spelta*, *C* gene for *compactum* and *sp* gene for *sphaerococcum* characters. They are not found in homoeologous chromosomes as shown in Table IV. The corresponding homoeologous genes in Emmer ($4x$) and Einkorn wheats ($2x$) are also given in this table. These data are still tentative. However, the occurrence of *Sp* and *c* in *Ae. squarrosa* was ascertained by the production of *vulgare*-type amphidiploids from the hybrid, *T. persicum* × *Ae. squarrosa*.

TABLE IV

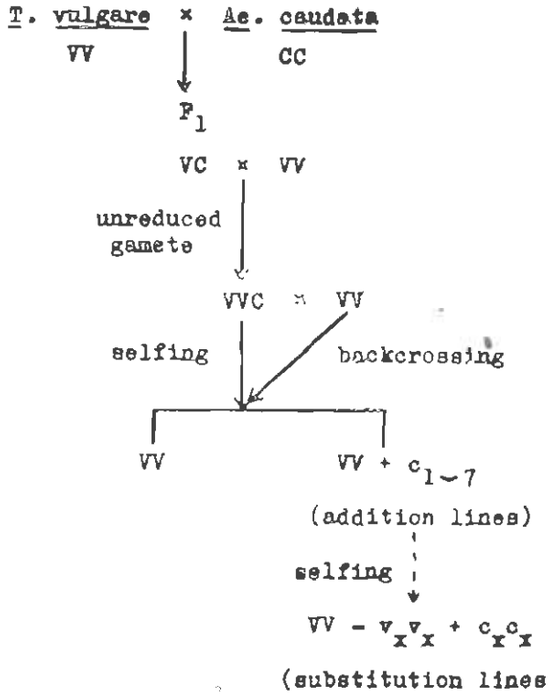
Location and distribution of 6x species-specific genes

6x species-specific genes	Location	Distribution			
		Common wheat	Emmer wheat	Einkorn wheat	*Aegilops squarrosa*
S, s	5A	*S, s*	*S, s*	*S*	
C, c	2D	*C, c*			
Sp, sp	3D	*Sp, sp*			*Sp*

2. *Addition and Substitution*

Comparative gene analysis may be carried out by addition of an alien chromosome to a normal chromosome complex. Such plants correspond to allotrisomics of Nishiyama (1934).

Addition lines are obtained by backcrossing an allotriploid hybrid (AAB) to the ancestral analyser (AA). Among the offspring of the backcross hybrid we should find allotrisomics having AA + b_1, AA + b_2, ... One of such examples is shown in Text-Fig. 2, where the chromosomes of *Ae. caudata* were added to the *vulgare* genome (V = ABD).



TEXT-FIG. 2. A method of alien chromosome substitution.

By selfing these addition lines we could obtain in some cases substitution lines, as the result of substitution of the additional chromosome for one of the homoeologous chromosomes of the recipient nucleus.

Among the substitution lines of this *vulgare-caudata* combination, three deserve further explanation.

One is the substitution line with C-sat-1, which is one of the two sat-chromosomes of *caudata*. This chromosome shows no visible effect on the phenotype. The second is the substitution line with C-sat-2, the second sat-chromosome of *caudata*. This chromosome manifests itself by black awn colour and by restoring male fertility (when the plasma is *caudata*).

It became known by monosomic analysis that C-sat-2 is homoeologous to XVII, or 1D (Muramatsu, 1959). The substitution of the third chromosome (J_1) is not yet karyologically clear. However, it is proved that non-waxy character of *caudata* is transmitted to *vulgare* genome. To explain this phenomenon, we have two explanations:

(*a*) Substitution of the whole chromosome (J_1);

(*b*) Transmission of a part of J_1 to the corresponding *vulgare*-chromosome by crossing over.

The second assumption was adopted in a previous paper (Kihara, 1959). However, the first one cannot be excluded as there are chromosomes in the 6*x* complement morphologically similar to J_1.

Not many substitution lines have been reported. However, it is known that hairy neck chromosome of rye can substitute for 5A of *vulgare* (O'Mara, 1947).

In cereals we have many beautiful examples of addition lines. They are given in Table V.

TABLE V

Examples of alien chromosome addition lines

Recipient	Common wheat		Emmer wheat	
Donor	*S. cereale*[1]	*Ha. villosa*[2]	*Ag. elongatum*[3]	D-genome of common wheat[4]
Addition line	AABBDD + r_1	AABBDD + v_1	AABB + e_1	AABB + d_1
	AABBDD + r_2	AABBDD + v_2	AABB + e_2	AABB + d_2
	AABBDD + r_3	AABBDD + v_3	AABB + e_3	AABB + d_3
	AABBDD + r_4	AABBDD + v_4	AABB + e_4	AABB + d_4
	AABBDD + r_5	AABBDD + v_5	AABB + e_5	AABB + d_5
	AABBDD + r_6	AABBDD + v_6	AABB + e_6	AABB + d_6
	AABBDD + r_7	AABBDD + v_7	AABB + e_7	AABB + d_7

[1] Evans and Jenkins, 1960; [2] Hyde, 1953; [3] Mochizuki, 1962; [4] Yamashita, 1947.

From Table V we see that whole series of addition lines were obtained when the recipient plants were tetraploid or hexaploid, but until now never with diploids.

Addition lines give helpful clues to the location of genes having dominant, epistatic or modifying effects on the phenotypes of the recipient species. Plate I, Fig. 1 shows spikes of 7 monosomic addition lines of *T. durum* by *Agropyron elongatum* ($n = 7$). Some of them are easily distinguished morphologically. For instance, e_4 of *Agropyron elongatum* has a gene for non-waxy, namely, an inhibitor of waxy. Therefore, AABB + e_4 is non-waxy and the other 6 addition lines are waxy. The coexistence of waxy genes with their inhibitors was clearly demonstrated in this experiment.

It would be worthwhile to study using a large material the possibility of introgression of *elongatum* genes or chromosome segments to *durum* genome.

3. *Use of Amphidiploids*

Amphidiploids are very useful tools for comparative genetics. We can combine the genomes of two species into one system, creating a new species of higher polyploidy. When the synthesized form has the same genome type as a naturally occurring polyploid species, they can be easily crossed with each other and the F_1 will have normal or almost normal chromosome conjugation in meiosis and will be highly fertile. Synthesized 6*x*-wheats between Emmer wheats and *Ae. squarrosa* are good examples. In our laboratories, we have obtained 23 amphidiploids using 4*x* wheat species on one hand and *Ae. squarrosa* on the other. As the supplier of AABB genomes, wild Emmer (*T. dicoccoides*) and many cultivated species (*e.g.*, *T. durum*) were used as female parents. As the source of DD lines serving as male parents, many varieties of *Ae. squarrosa* were employed. They are given in Table VI.

The hybrid between ABD No. 4 and *T. vulgare erythrospermum* showed normal chromosome conjugation and high fertility.

The genic constitution of the amphidiploids should be the sum of those of the parental species. Therefore, if we know the genotypes of the parents, we can use the amphidiploids for the gene analysis of *T. vulgare* and *vice versa*.

Using spring and winter habit as an example, let us see how the genes concerned express the character in amphidiploids. To obtain the needed information, growth habits were determined in many diploids and tetraploids. In Table VII, the distribution is given for spring and winter habit genes among various species of *Triticum* and *Aegilops* which were used for the production of artificial polyploids.

Table VIII shows the growth habit of those artificial auto- and allopolyploids.

TABLE VI

List of synthetic hexaploid wheats

Stock No.		Cross-combination
ABD 1	*T. dicoccoides spontaneo-nigrum*	× *Ae. squarrosa* var. *typica* No. 2
ABD 2	*T. durum coerulescens*	× *Ae. squarrosa* var. *typica* No. 1 × No. 2 F_1
ABD 3	*T. turgidum nigro-barbatum*	× *Ae. squarrosa* var. *typica* No. 2
ABD 4	*T. persicum stramineum*	× *Ae. squarrosa* var. *typica* No. 2
ABD 5	*T. persicum fuligi-nosum*	× *Ae. squarrosa* var. *typica* No. 2
ABD 6	*T. turgidum nigro-barbatum*	× *Ae. squarrosa* var. *typica* No. 1
ABD 7	*T. dicoccum liguli-forme*	× *Ae. squarrosa* var. *typica* No. 1
ABD 8	*T. orientale*	× *Ae. squarrosa* var. *typica* No. 1 × No. 2 F_1
ABD 9	*T. dicoccoides spontaneo-nigrum*	× *Ae. squarrosa* var. *strangulata* KUSE 2124
ABD 10	*T. dicoccoides spontaneo-nigrum*	× *Ae. squarrosa* var. *typica* KUSE 2129
ABD 11	*T. persicum strami-neum*	× *Ae. squarrosa* var. *strangulata* KUSE 2135
ABD 12	*T. dicoccoides spontaneo-nigrum*	× *Ae. squarrosa* var. *strangulata* KUSE 2112
ABD 13	*T. dicoccum* (Vernal)	× *Ae. squarrosa* var. *strangulata* KUSE 2112
ABD 14	*T. durum* (Gulab)	× *Ae. squarrosa* var. *strangulata* KUSE 2118
ABD 15	*T. durum* (Gulab)	× *Ae. squarrosa* var. *typica* KUSE 2129
ABD 16	*T. durum* (Gulab)	× *Ae. squarrosa* var. *meyeri* KUSE 2144
ABD 17	*T. persicum strami-neum*	× *Ae. squarrosa* var. *typica* KUSE 2107
ABD 18	*T. persicum strami-neum*	× *Ae. squarrosa* var. *strangulata* KUSE 2112
ABD 19	*T. persicum strami-neum*	× *Ae. squarrosa* var. *strangulata* KUSE 2118
ABD 20	*T. persicum strami-neum*	× *Ae. squarrosa* var. *typica* KUSE 2129
ABD 21	*T. persicum strami-neum*	× *Ae. squarrosa* var. *typica* KUSE 2130
ABD 22	*T. persicum strami-neum*	× *Ae. squarrosa* var. *meyeri* KUSE 2144
ABD 23	*T. orientale insigne*	× *Ae. squarrosa* var. *strangulata* KUSE 2112

(Data after Kihara *et al.*, 1950; Tanaka$_2$ 1961 *a*.)

TABLE VII

Distribution of genes controlling growth habit in some species of Triticum *and* Aegilops

Genome	Species	Growth habit	Possible genotype (*n*)		
			5A	5B	5D
A	*T. aegilopoides*	W	sg_2		
A	*T. monococcum*	W, S	sg_2, Sg_2		
S	*Ae. speltoides*	S		Sg_3	
S^1	*Ae. longissima*	W		sg_3	
S^2	*Ae. bicornis*	I		Sg_3^e	
D	*Ae. squarrosa*	W, I, S			sg_1, Sg_1^e, Sg_1
AB	*T. dicoccoides*	W, S	sg_2, Sg_2		
AB	*T. dicoccum*	W, S	sg_2, Sg_2		
AB	*T. durum*	W, S	sg_2, Sg_2^e		
ABD	*T. aestivum*	W, S	sg_2, Sg_2^e, Sg_2		sg_1, Sg_1^e, Sg_1

(Data after Tsunewaki, 1962, revised.)

It is clear from Table IX that spring type is dominant or epistatic over winter type. One exception was found in the amphidiploid between *Ae. umbellulata* (winter) and *Ae. caudata* (winter). The amphidiploid was unexpectedly a spring type. Intermediate types (*bicornis*) behave very irregularly. They are sometimes dominant and sometimes recessive to winter type. Autopolyploids of intermediate species became spring, winter or intermediate types. Species of Sitopsis were variable in growth habit.

Next let us look at the artificial allohexaploids which correspond to 6*x*-wheat. The relationship between them and their parents regarding spring/winter habit is as follows:

Parents		Amphidiploids
Emmer	*Ae. squarrosa*	
4*x* (winter)	+ 2*x* (winter)	= winter (ABD-1)
4*x* (spring)	+ 2*x* (winter)	= spring (ABD-2, ABD-3, ABD-4)
4*x* (spring)	+ 2*x* (winter)	= intermediate (ABD-8)
4*x* (intermediate)	+ 2*x* (winter)	= spring (ABD-5)
4*x* (winter)	+ 2*x* (spring)	= no example.

TABLE VIII

Growth habit of synthetic auto- and allopolyploids in Triticum *and* Aegilops

Species, variety and cross-combination[1]	Chromosome number (n)	Genome type	Growth habit[1]
AUTOPOLYPLOID:			
T. durum reichenbachii (S) 8x	28	AAAABBBB	S
Ae. sharonensis typica No. 1 (I) 4x	14	$S^lS^lS^lS^l$	S
Ae. bicornis typica No. 1 (I) 4x	14	$S^bS^bS^bS^b$	I
Ae. ovata No. 1 (I) 8x	28	$C^uC^uC^uC^u$ $M^oM^oM^oM^o$	W
T. aegilopoides boeoticum No. 1 (W) 4x	14	AAAA	W
Ae. umbellulata typica No. 2 (W) 4x	14	$C^uC^uC^uC^u$	W
Ae. longissima No. 1 (W) 4x	14	$S^lS^lS^lS^l$	W
Ae. uniaristata No. 1 (W) 4x	14	$M^uM^uM^uM^u$	W
Ae. squarrosa typica No. 2 (W) 4x	14	DDDD	W
AMPHIDIPLOID:			
T. monococcum (I) × *T. Timopheevi* (S)	21	AAAAGG	S
Ae. sharonensis (I) × *T. durum* (S)	21	S^lS^lAABB	S
Ae. crassa (S) × *Ae. squarrosa* (W)	21	$DDM^{cr}M^{cr}DD$	S
T. Timopheevi (S) × *Ae. umbellulata* (W)	21	$AAGGC^uC^u$	S
T. Timopheevi (S) × *Ae. squarrosa* (W)	21	AAGGDD	S
T. Timopheevi (S) × *Ae. longissima* (W)	21	$AAGGS^lS^l$	S
T. durum (S) × *Ae. squarrosa* (W)	21	AABBDD	S
T. turgidum (S) × *Ae. squarrosa* (W)	21	AABBDD	S
T. persicum (S) × *Ae. squarrosa* (W)	21	AABBDD	S
T. orientale (S) × *Ae. squarrosa* (W)	21	AABBDD	I
Ae. sharonensis (I) × *Ae. umbellulata* (W)	14	$S^lS^lC^uC^u$	W
Ae. bicornis (I) × *Ae. squarrosa* (W)	14	S^bS^bDD	W
Ae. sharonensis (I) × *T. aegilopoides* (W)	14	S^lS^lAA	W
T. persicum (I) × *Ae. squarrosa* (W)	21	AABBDD	S
Ae. umbellulata (W) × *Ae. bicornis* (I)	14	$C^uC^uS^bS^b$	I
Ae. comosa (W) × *Ae. bicornis* (I)	14	MMS^bS^b	I
T. aegilopoides (W) × *Ae. bicornis* (I)	14	AAS^bS^b	W
T. dicoccoides (W) × *Ae. squarrosa* (W)	21	AABBDD	W
Ae. longissima (W) × *T. aegilopoides* (W)	14	S^lS^lAA	W
Ae. uniaristata (W) × *Ae. umbellulata* (W)	14	$M^uM^uC^uC^u$	W
Ae. uniaristata (W) × *Ae. squarrosa* (W)	14	M^uM^u DD	W
Ae. umbellulata (W) × *Ae. squarrosa* (W)	14	C^uC^uDD	I
Ae. caudata (W) × *Ae. umbellulata* (W)	14	CCC^uC^u	S

[1] S=spring type; I=intermediate type; W=winter type (Data after Tanaka, 1960).

TABLE IX

Growth habit in autotetraploids and allotetraploids (summarized)

	Parental combinations		Tetraploids
5 species	spring + spring	= spring	auto-tetraploids
1 species	winter + winter	= winter	
1 species	intermediate + intermediate	= intermediate	
1 species	intermediate + intermediate	= spring	
5 combinations	winter + winter	= winter	allo-tetraploids
9 combinations	spring + winter	= spring	
2 combinations	winter + intermediate	= winter	
2 combinations	winter + intermediate	= intermediate	

From these results we see again that spring habit is usually dominant over winter habit. Here too it is quite unexpected to find that in the case intermediate + winter the amphidiploid had spring character.

CONSIDERATIONS ON THE ORIGIN OF 6x WHEAT

From the results so far obtained by the three above methods, some considerations may be given concerning the origin of 6x wheat.

According to Tanaka (1960) diploid wild species of *Triticum* and *Aegilops* have mostly winter growth habit. Only in the Sitopsis section there is a wide variation in this respect. Among Sitopsis species, *Ae. speltoides* is the only one having winter type. This species is found in the northern parts of Sitopsis distribution (Iraq, Turkey, Syria, Palestine, etc.). *Ae. bicornis* is an intermediate type and is distributed in the southern parts (Palestine, Egypt, Tripoli and Cyrenaica). The third species, *Ae. longissima*, consists of two, winter and intermediate, types. The distribution of this species covers almost the whole area except Tripoli and Cyrenaica.

Spring and intermediate types are more adapted to warmer climates than the winter type. When the intermediate type was combined in polyploids with other types, it behaved sometimes as a dominant, sometimes as a recessive character, and sometimes a type different from those of the parents was obtained.

This complicated situation in the growth habit of polyploids might be one of the reasons that have made the monosomic analysis very difficult.

It might also be interesting to note that the cultivated Emmer wheats are usually of spring type. They are cultivated not only in moderate climatic regions but also in the tropics (South Egypt, India, etc.).

By addition of D-genome to AABB (Emmer), common wheat has acquired strong winter habit (Tsunewaki, 1962), a favourable character for cultivation in the northern parts of wheat distribution. The growth of winter forms is not hastened during the vegetative growth period and the plants reach to full development. So we are inclined to think that *squarrosa* is not a poor material for building up common wheat, as it was once assumed by Shebeski (1959). The winter habit produced by D-genome is very strong and is dominant over the intermediate *Ae. bicornis* habit. Also, other species possessing D-genome have strong winter habit, *e.g.*, *Ae cylindrica.*

Among *squarrosa* varieties however there are intermediate and spring types. The spring type is found only in the southern part of *squarrosa* distribution (Quetta) (Kihara and Tanaka, 1958).

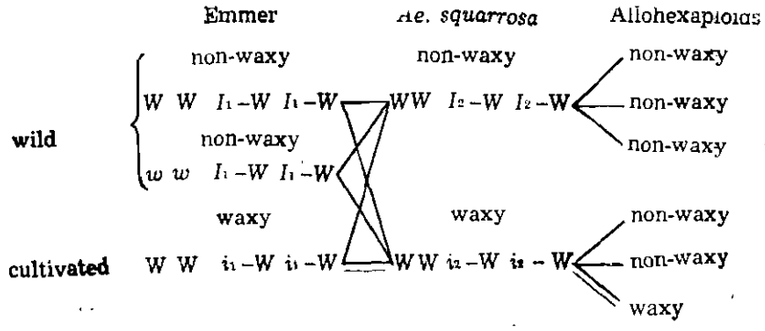
In this connection an experiment made by Yasuda (1961) with barley deserves mentioning. He crossed spring and winter-type barleys and raised segregating generations in several localities of Japan. In the north the winter type became predominant (80%) in the population already in F_6, while it has decreased in the south (less than 10%).

Waxy is another character, whose distribution in different wheat species should be taken into consideration in discussions on the origin of 6*x*-wheat.

So far as we know at present, all wild Emmers (*T. dicoccoides*) have non-waxy foliage. From genic analysis we have known that two genes are responsible for this character. *I-W* is an inhibi or of waxy, and *W* is a dominant gene for waxy. So there are three genotypes for non-waxy, namely, *W W I-W I-W*, *w w I-W I-W* and *w w i-W i-W*. For waxy character there is only one genotype, *W W i-W i-W*.

Cultivated 4*x* wheats are usually waxy. So they must have the genotype *W W i-W i-W*.

If we combine all possible variations of Emmer with those of *Ae. squarrosa*, we get the following 6 combinations.



Only the combination connected with double lines gives rise to waxy offspring in the amphidiploid generation. Existing 6*x*-wheat varieties are mostly waxy and non-waxy varieties are seldom found. But the non-waxy character of 6*x*-wheat is recessive. Thus, no wild species having all *I-W* could be the progenitor of 6*x*-wheat. From cultivated Emmer only the AB genomes could have been derived.

Complementary dwarf genes are also important from the standpoint of the origin of 6*x*-wheat. This character does not give conclusive evidence by means of hybridization between cultivated 4*x*-wheat and *Ae. squarrosa*. Still these genes reduce further the possibility of 6*x* formation from a hybrid between wild Emmer and *Ae. squarrosa*. Tanaka (1961 *b*) has found that Dw_1 and Dw_2 are complementary for the production of dwarfness. If these 2 genes are brought together by crossing, the hybrids are dwarf and very weak. Most probably they could not survive in Nature. Dw_2 is always present in *Ae. squarrosa* while Dw_1 occurs in most wild Emmers except *T. dicoccoides* var. *spontaneo-nigrum* (dw_1). So *T. dicoccoides* having Dw_1 can be excluded as a possible progenitor of 6*x*-wheat. But *T. dicoccoides spontaneo-nigrum* cannot be discarded. However, it cannot be the progenitor owing to its non-waxy gene, as mentioned above.

There are other series of gene systems for necrosis and similar lethal genes which are found in wild Emmer species and *Ae. squarrosa*. One of such complementary necrotic lethals is *Ne* series mentioned above. Three dominant genes Ne_1, Ne_2 and Ne_3 are responsible for the formation of necrotic plants. Wild 4*x* species have in many cases dominant alleles of necrotic genes (Ne_1 Ne_2), while cultivated species have usually recessive alleles. All *squarrosa* varieties so far have Ne_3. Also, therefore, the chance of formation of 6*x*-wheat from wild Emmer and *Ae. squarrosa* is very limited. It should be emphasized here that we have never found a wild 6*x*-wheat in Nature.

Our considerations lead us to the conclusion that common wheat originated from a cultivated, not *wild*, Emmer crossed by *Ae. squarrosa*.

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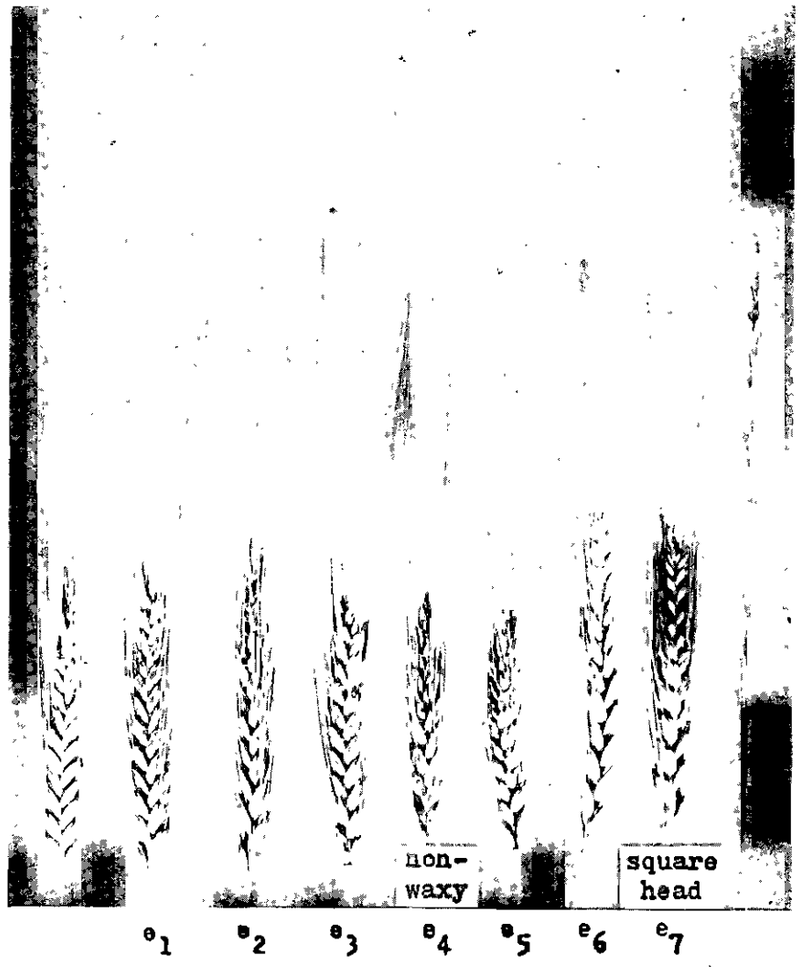

FIG. 1

Hitoshi Kihara & Koichiro Tsunewaki

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EXPLANATION OF PLATE I

FIG. 1. Spikes of seven monosomic addition lines and the parental species. From left to right: Stewart *durum*, monosomic addition lines (e_1~e_7) and *Ag. elongatum* (Mochizuki, 1962).

EUPHORBIOXYLON SAGARENSE SPEC. NOV., A FOSSIL WOOD FROM SAGAR (M.P.) BELONGING TO THE FAMILY EUPHORBIACEAE

BY T. S. MAHABALE AND S. R. DESHPANDE

Botany Department, University of Poona, Poona, India

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INTRODUCTION

SAGAR (Madhya Pradesh) has been one of the earliest known localities in the Deccan Trap area from which silicified fossil plants have been known for over a century. One Mr. Nicolls had obtained pieces of palm from it as early as 1833, and the well-known fossil palm described by Sahni (1946) under the name *Palmoxylon sundarum* had come from this locality.

This locality is about a mile away from the town of Sagar (latitude 23° 45″ E., longitude 74° 45″ N.) having an altitude of 1906′. A lacustrine bed hardly 2-3 feet deep extends around a hillock, not very far from the Sagar University. It contains pieces of fossil palm tree trunks, shales of *Bullinus* and other water molluscs, and some other plant remains, all petrified. However, to the best of our knowledge no dicot wood from this locality has been so far described and hence this paper.

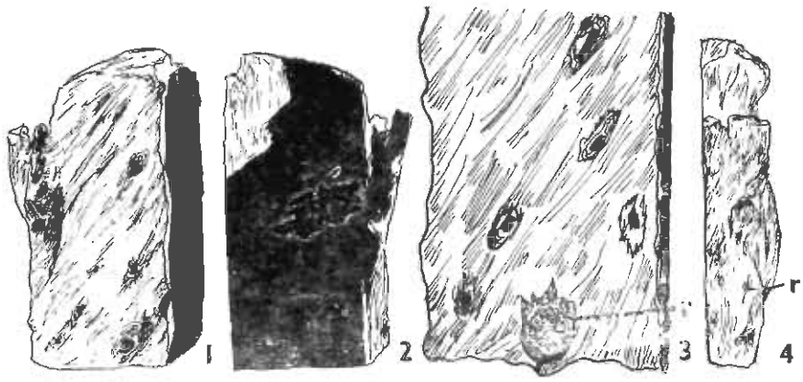
DESCRIPTION

A piece of dicot stem was collected by one of us (T. S. M.) in 1960 which forms the material on which this paper is based.

It is a piece of chert, $6 \cdot 8 \times 3 \cdot 52$ cm., not round, but flat on one side (Text-Figs. 1, 2 and 4; Plate I, Figs. 1 and 2). It includes a node also from where a small axis of a branch must have been going out. It is ash-grey in colour outside and shows longitudinal and oblique lines of weathering on outer surface. There are at least four prominent round scars embedded in vertical lines (Text-Fig. 3, *r*). In the centre of one of these scars, a basal stump of an axis was visible. This is deep brown in colour contrasted with the dark matrix of the rest of the chert. Possibly, therefore, it was endogenous in origin. Growth rings are indistinct, preservation being not very good. On cutting and examining the microscopic structure in transverse, radial and tangential longitudinal sections, the following points were made out:—

In t.s. the wood seems to be of the diffuse porous type, vessels being typically solitary, sometimes in the radial multiples of 2, 3 or

even 4 cells (Text-Fig. 5; Plate I, Figs. 3 and 5). When more than one vessel lies in a row, size of the xylem cells progressively becomes smaller. The distribution of vessels is 5–10 per mm.^{2} Comparatively their walls are thin suggesting poor development of wood tissue. They are oval to round in outline, their long and short diameters being 60–100 μ and 44–60 μ respectively. The vessels, therefore, are small to medium-sized.

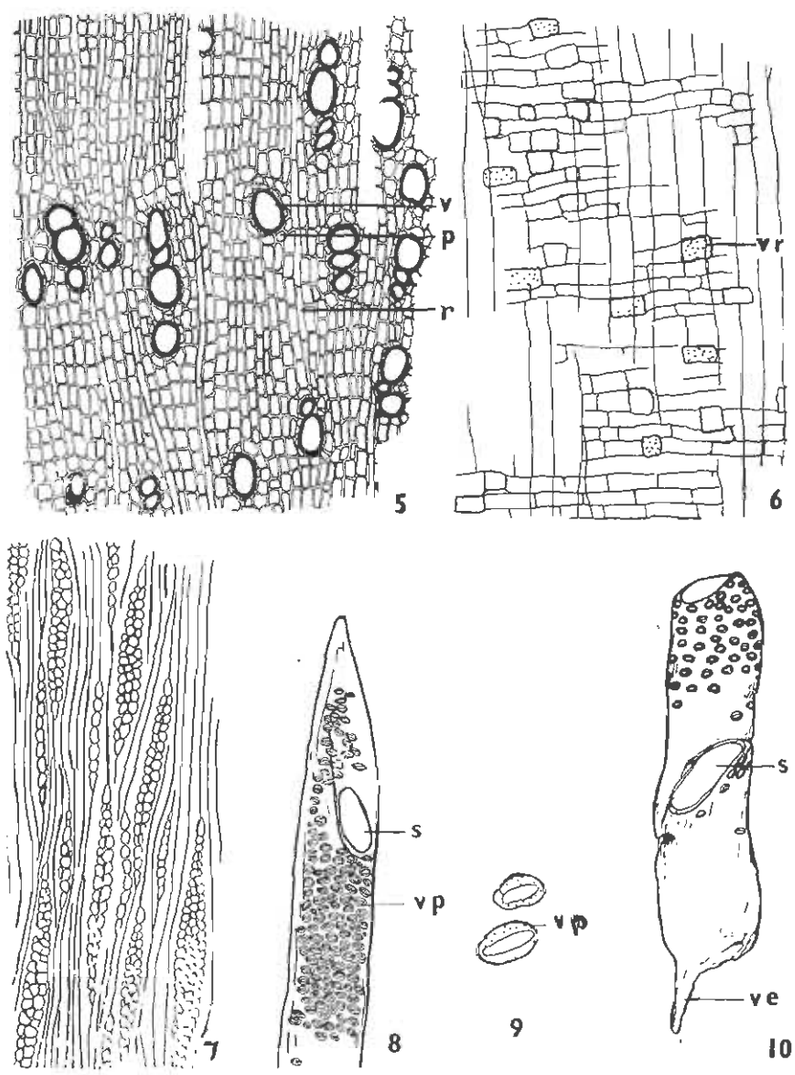


TEXT-FIGS. 1–4. *Euphorbioxylon sagarense* spec. nov. Figs. 1, 2 and 4. Entire specimen in different views, × 2/3 nat. size. Fig. 3. A part of the specimen magnified to show round scars of intra-cortical roots, × nat. size.

In l.s. they appear to be dark brown in colour due to pigment. The vessel ending is oblique and shows simple perforation (Text-Figs. 8 and 10; Plate I, Figs. 4 and 7). The intervessel pits are in 5-6 rows, transversely arranged, with oval to lenticular apertures. The intervessel pits are bordered and vestured (Text-Fig. 9). The vessel ray cell pits are different from the intervessel pittings, being simple, round, small and numerous per cell (Text-Fig. 6; Plate I, Figs. 8 and 9). Fibres are moderately thick-walled, libriform and aseptate. They are arranged in regular radial rows with characteristic zigzag lines as seen in T.L.S. (Text-Fig. 7; Plate I, Fig. 6). Parenchyma is paratracheal and scanty, there being only a few cells around the vessels (Text-Fig. 5; Plate I, Fig. 3). The parenchyma cells are oval, 20–30 μ in diameter. Medullary rays are evenly distributed and are uniseriate to multiseriate (Text-Fig. 7; Plate I, Fig. 6). They are distributed 8–10 per mm.^{2} slightly heterogeneous. In some cells of the rays, crystals are seen (Plate I, Fig. 10). In transverse section they are very fine, being 12 μ in breadth. The height of a ray varies from 5-20 cells. Ray cells are 30 μ in diameter.

AFFINITIES

The fossil wood seems to belong to some member of the Euphorbiaceae with which it compares well in many respects. It shows some resemblance with the woods of the Guttiferae and Simarubaceae on



TEXT-FIGS. 5–10. *Euphorbioxylon sagarense* spec. nov. Fig. 5. T.s. of stem, × 40 (*p*, parenchyma; *r*, ray; *v*, vessel). Fig. 6. R.l.s. of stem, × 50, (*vr*) vessel ray pits. Fig. 7. T.l.s. showing medullary rays, × 40. Fig. 8. Vessel in l.s. × 160. (L.s. simple perforation). Fig. 9. Magnified view of the vestured pits, (× 600. *v*, vestured pits). Fig. 10. A vessel in l.s. showing attenuated vessel ending, × 160. *s*, simple perforation; *ve*, vessel ending).

account of the presence of gum canals and light paratracheal parenchyma, but the combination of characters it possesses shows its affinities with the wood of the Euphorbiaceae rather than with members of any other family.

The important diagnostic characters of the family Euphorbiaceae having systematic value are:—

Vessels solitary or sometimes in radial rows of 2-3 cells, vessels moderately small to medium, about 100 μ in diameter; the distribution of vessels 5-10 per mm.^{2}, intervessel pittings bordered and vestured with oval to lenticular apertures; vestured pits showing characteristic markings; parenchyma paratracheal and scanty; fibres libriform and aseptate; rays one- to many-seriate, and slightly heterogeneous; vessel ray cells having different types of pits than the pits on the vessels.

According to Metcalfe and Chalk (1950) all these characters are typical of the Euphorbiaceae. The family Euphorbiaceae has been divided into two groups anatomically: (1) Phyllanthoideae and (2) Crotonoidae. The present specimen belongs to the group (1) Phyllanthoideae, as it has the following anatomical characters:—

1. Vessels with simple perforations, small to medium-sized, intervessel pits transversely arranged with apertures oval to lenticular.

2. Parenchyma paratracheal, scanty, with only a few cells around the vessels.

3. Rays slightly heterogeneous, uniseriate to multiseriate.

The group Phyllanthoideae is further divided into three types: Aporosa type, *Glochidion* type, and the rest. The slides of this material were, therefore, compared with the slides of living genera of the *Glochidion* type available at the Forest Research Institute, Dehra Dun, such as *Bischoffia*, *Glochidion*, *Cleistanthus* and *Bridelia*. Our findings were further scrutinized and confirmed by Dr. K. Ramesh Rao, Plant Anatomist at the F.R.I., Dehra Dun, who agreed with the view expressed by us that this fossil wood belongs to the family Euphorbiaceae. Of the different genera belonging to this type which were examined, this wood showed resemblance with the wood of *Bridelia*, a genus with about 11 species not uncommon in the deciduous forests of India. But the slides of *B. retusa* could only be compared, as slides of other species were not available. However, in the fossil wood there are no bands of parenchyma supposed to be characteristic of this genus so far known; and, therefore, notwithstanding its considerable resemblance with that genus, the specimen has not been named after that genus. It may be possible to settle this question later on knowing the range of variation of this character in other species of *Bridelia* such as *B. stipularis*, *B. hamiltoniana*, etc., available in India and elsewhere.

In this connection, it may be mentioned here that besides anatomical resemblances with the genus *Bridelia*, the fossil also shows some

external features of that genus. For example, the genus *Bridelia* is distinguished by the presence of intra-cortical root-thorns described by Parija and Misra (1933). These root thorns are very characteristic of *B. retusa* and the fossil also shows them. In our specimen there are four clear scars with endogenous plugs, encircled by elevated edges. This adds weight to its resemblance with the genus *Bridelia*. But one should certainly wait to examine this feature anatomically in the other living species of the genus and see if it occurs in all species of that genus or not, rather than rush into calling it *Bridelioxylon*. About its family identity, however, there is no doubt.

The family Euphorbiaceae has been represented by some fossil woods reported recently from various Tertiary horizons of India. Ramanujam (1956) has described three woods of this family under the names, *Putranjivoxylon puratanum*, *Bridelioxylon cuddalorense* and *Glochidioxylon tertiarianum*, all from the Tertiary rocks of South Arcot District, Madras. Prakash (1959) has described *Euphorbioxylon krauseli* from the Intertrappean beds of Madhya Pradesh, from a specimen obtained at Keria, and another member *Glochiodioxylon sahnii* from Bharatwada near Nagpur.

Outside India, only a few fossil woods belonging to the Euphorbiaceae have been described from the Cretaceous of Arizona by Bailey (1933), from the Tertiary of Columbia by Felix (1887), from East Africa by Bancroft (1932), from Kinshi and Tobata in Japan by Ogura (1932) and Watari (1943), from Algeria by Boureau (1951) and from the Eocene beds of Eden Valley of Wyoming by Krause (1954). Recently Grambast (1961) has also described a fossil wood of *Piranoxylon stockmansi*, belonging to the Euphorbiaceae, from the Eocene of Belgium and Salard (1961) has described a species of *Euphorbioxylon brideliodes* from Perou. The ornamentation of the vestured pits in our material is of the same type as in *E. brideliodes* described by her. The present material, therefore, adds one more wood to the list of this family which seems to be essentially a Tertiary one. We consider it better to place it under the family name rather than under a generic appellation to avoid confusion till it is firmly established as belonging to a particular genus and hence the name *Euphorbioxylon sagarense*. Its diagnosis is given under:—

Diagnosis

Euphorbioxylon sagarense **sp. nov.** A piece of diffuse porous wood, $6 \cdot 8 \times 3 \cdot 5 \times 2$ cm., with indistinct growth rings.

Vessels typically solitary, sometimes in multiples of 2 or 3 cells, rarely four, arranged radially; distribution of vessels 5–10 per mm.^{2}, small to medium, 50–100 μ in diameter, oval to round; vessel segments attenuately tailed at one or both ends; perforation simple, intervessel pits round or oval medium-sized, about 10 μ in diameter; bordered and vestured; their aperture lenticular; vessel ray pits different from those on the vessels in r.l.s., simple, round and many per cell.

Fibres moderately thick-walled, libriform and aseptate; radial arrangement of fibres regular, and highly characteristic of the family Euphorbiaceae.

Parenchyma cells oval, 20–30 μ in diameter, paratracheal, scanty around the vessels.

Rays 8–10 per mm.^{2}, evenly distributed 1–3-seriate, slightly heterogeneous, height of the ray 5–20 cells; ray cells 30 μ in diameter.

Holotype No. 67, and slides 671, 672, 673 and 674 deposited in the Palaeobotany Museum, Department of Botany, University of Poona, Poona, India.

Locality.—Sagar (M.P.).

Horizon.—Deccan Intertrappean Series (Tertiary).

Age.—Eocene.

Latin Diagnosis

Euphorbioxylon sagarense **spec. nov.** Fragmentum ligneum poris diffusis, 6·8.... cm., incrementiannui annulis indistinctis.

Vascula typice solitaria, nonnumquam constantia e cellulis binis vel ternis, raro quaternis, radialiter dispositis. Vascula distributa 5–10 in mm.^{2}, minuta vel mediocira, 50–100 μ diam., ovalia vel rotundata; vasculorum segmenta attenuate caudata ad unum vel ad utrumque apicem; perforationes simplices; foveoli intervasculares rotundi vel ovales, mediocres, ca. 10 μ diam., margineti et vestiti, poro lenticulari; foveoli radiales vasculares different ab eis in vasculo in r.l.s., simplices, rotundi, plures in singulis cellulis. Bibrea parietibus sat crassis, libriformes et aseptatae; dispositio radialis fibrarum regularis, alte typica familiae Euphorbiacearum. Cellulae parenchymaticae ovales. 20–30 μ diam., paratracheales, rarae circum vascula. Radii 8–10 in mm.^{2} equaliter distributi, 1–4 seriati, paulum heterogenei, 5–20 cellulis alti; radiorum cellulae 30 μ diam.

Holotypus No. 67 et preparationes microscopicae 671–674, positae in Musaeo Palaeobotanico, Universitatis poonensis, ad Poona in India; locus collectionis; Sagar, M.P.; in serie Deccanensi intertrapeana, aet. Eocaena.

Summary

This paper gives an account of a new species of *Euphorbioxylon* under the specific name *Euphorbioxylon sagarense* Mahabale and Deshpande, found at Sagar (M.P.) in the Deccan Intertrappean Series of India. This is the first dicotyledonous wood being described from this locality. It is of the diffuse porous type, with solitary vessels, scanty paratracheal parenchyma, slightly heterogeneous, uniseriate to multiseriate medullary rays and libriform aseptate fibres. The vessels show

bordered vestured pits. and simple perforation. The vessel ray cell pittings are different, being small, simple and numerous. The anatomical characters show its definite affinities with the family Euphorbiaceae; and some of its morphological characters suggest relationship with the modern genus *Bridelia*, *e.g.*, the intra-cortical roots. But as the identity with that genus is not complete it has been named after the family to avoid the rejection of generic name later.

ACKNOWLEDGEMENTS

In the end, the authors wish to thank Dr. K. Ramesh Rao, Plant Anatomist, Forest Research Institute, Dehra Dun, for the help in examining wood slides of Euphorbiaceae, and the Rev. Father Dr. H. Santapau, Chief Botanist, Botanical Survey of India, for his help in rendering Latin diagnosis.

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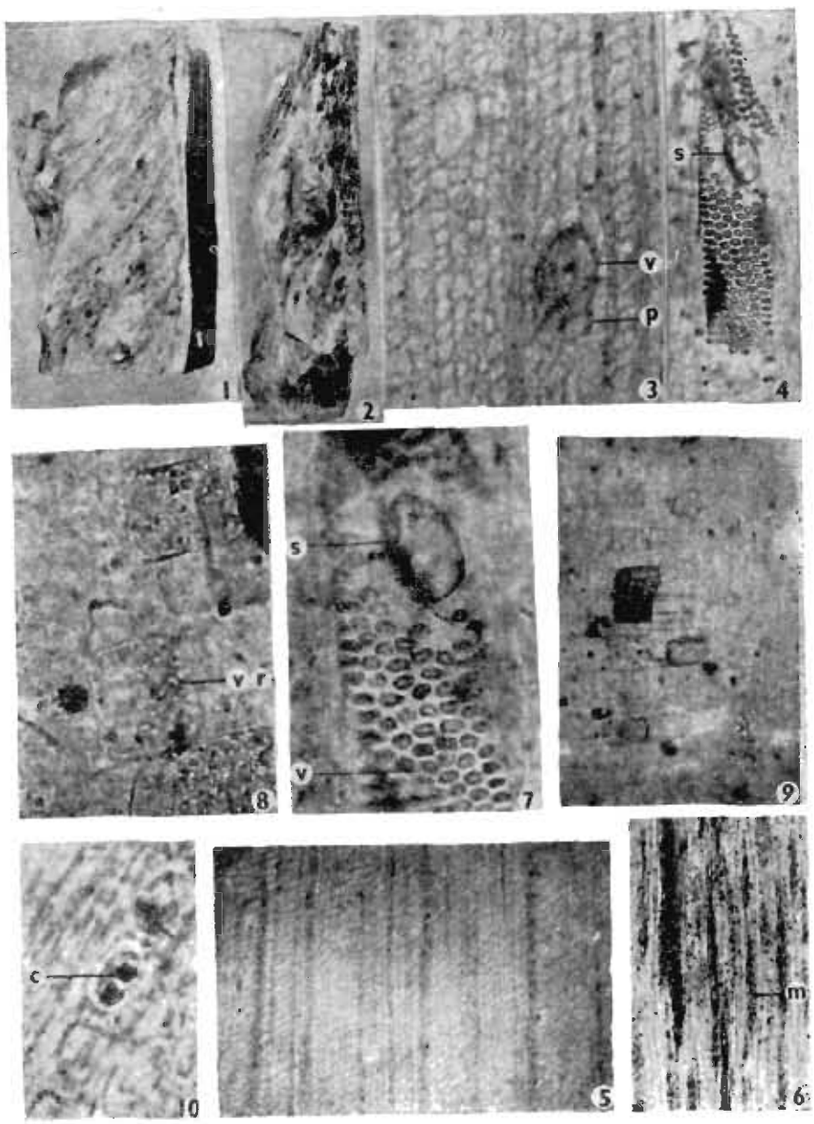


FIGS. 1–10

T. S. Mahabale & S. R. Deshpande

EXPLANATION OF PLATE I

FIGS. 1–10. *Euphorbioxylon sagarense* spec. nov.

FIG. 1. Entire specimen, × nat. size

FIG. 2. Side view of the same, × 1½ nat. size.

FIG. 3. T.s. of stem, × 120. (*p*, parenchyma; *s*., simple perforation; *v*, vessel.)

FIG. 4. A vessel in l.s., × 240. (*s*, simple perforation.)

FIG. 5. T. s. of stem, × 40.

FIG. 6. T.l.s. of stem, × 80. (*m*, medullary ray.)

FIG. 7. A vessel in l.s., × 440. (*r*, vessel ray pits.)

FIGS. 8, 9. R.l.s. of stem, × 80.

FIG. 10. T.l.s. of stem, × 100. (c, crystals.)

POLYMORPHICITY AND CYTOGENETICS OF *ADIANTUM LUNULATUM* COMPLEX

By P. N. Mehra and S. C. Verma

Department of Botany, Panjab University, Chandigarh, India

(Received for publication on May 27, 1963)

Introduction

The Malabar maidenhair fern, *Adiantum lunulatum* Burmann f. (*Fl. Ind.* 235, **1768**) is one of the prettiest and most delicate species of Indian Adiantums. The species derives its name from lunate (half moon-shaped) subdimidiate, glabrous pinnae. It is better known in literature as *A. philippense* Linn. (Sp. Pl. 2, 1094, **1753**). However, recently Verma (1961) has advocated valid reasons for the preference of specific epithet *lunulatum* over *philippense* from a cytotaxonomic view-point.

Mehra (1938, 1944) was the first to report apogamy in the species from Sikkim and Dehra Dun. Since then it has been investigated from various parts of its range and so far reported, the species occurs largely as a triploid apomict, from S. Africa, Ceylon to Himalayas (*cf.* Manton, 1959; Manton and Sledge, 1954; Mehra and Verma, 1960; Verma and Loyal, 1960; Roy and Sinha, 1961, 1962). Extensive collections made in the Himalayas during four consecutive years (1955–58) have revealed a high degree of polymorphicity in the species. A cytological analysis of the different populations has shown that besides the triploid apomict three additional cytotypes, diploid sexual, diploid apogamous, and tetraploid sexual also exist in the Eastern Himalayas. A brief reference to this effect was made earlier in a paper listing the chromosome numbers of Himalayan Ferns (*cf.* Mehra, 1961).* In the present contribution a detailed study of this complex as it occurs in the Himalayas is presented.

Material and Methods

The species occurs plentifully on forest margins and moist rock crevices throughout the Himalayas at lower elevations, usually up to 1,050 m. It prefers gravelly and acidic soil or moist limestone. The present investigations are largely based upon plants growing all along the pony path from Badamtam (600 m.) to Teesta (150 m.) near Darjeeling in the E. Himalayas.

The material was collected during the months of July–September, the peak season of growth for the ferns in the Himalayas. For cytological preparations, fixations of unripe sporangia was done both in 1 : 3 acetic-alcohol and modified Carnoy's (1 part acetic acid + 3 parts

* More recently diploid and tetraploid sexuals have also been reported from South India (Abraham *et al.*, 1962).

absolute alcohol + 2 parts chloroform). Acetocarmine squashes of S.M.C. were made in the usual fashion.

Morphological Variation

The characters which best exhibit variation in the different populations are: frond size, pinna shape, size and extent of lobing, soral length and the basal angle formed by the acroscopic and basiscopic edges of pinnae at the junction of the stalk. In general, the entire range of variation agrees broadly to *A. lunulatum* plan in the presence of a short ascending rhizome, clothed with brown narrowly lanceolate scales, and bearing naked polished deep brown-black stipes supporting nearly as long or rarely far larger simply pinnate lamina, either terminating in an apical pinna or the rachis apex is prolonged bearing gradually reduced pinnae and terminating in a vegetative bud. Pinnae are more or less typically lunate, a character specific of *A. lunulatum*, and absolutely glabrous. The spores are similar in structure, being trilete with smooth-slightly rough exine. Some of the important variable characters which need be mentioned are given below.

Frond size.—The size variation is by no means continuous in this complex. The data on the size of the individuals and their relative frequencies (*cf.* Table I) representing a random sample of 775 indi-

Table I

Frequency of individuals in various size classes (in cm.)

Cl.	Fr.	Cl.	Fr.	Cl.	Fr.	Cl.	Fr.	Cl.	Fr.
4	2	17	4	30	23	43	41	56	6
5	5	18	3	31	25	44	29	57	5
6	7	19	2	32	24	45	37	58	..
7	15	20	5	33	..	46	27	59	9
8	23	21	5	34	27	47	..	60	..
9	24	22	7	35	..	48	21	61	3
10	30	23	6	36	..	49	20	62	3
11	37	24	15	37	26	50	15	63	..
12	36	25	10	38	32	51	9	64	2
13	21	26	..	39	..	52	..	65	..
14	13	27	10	40	38	53	7	66	1
15	8	28	..	41	36	54	7	67	2
16	5	29	6	42	..	55	..	68	1

Cl.=Class, Fr.=Frequency.

viduals from the populations growing by the side of Badamtam-Teesta pony path is very significant. Fractional measurements have purposely been avoided and these have been grouped in one class up or below depending upon the fraction. The entire *lunulatum* element seems to contain at least two widely different modes. If this data, without selecting an appropriate class interval, be plotted as such in a frequency polygon (Text-Fig. 1), the above conclusion becomes quite apparent, *i.e.*, the size factor sharply delimits the entire element into two major categories. One of them ranges between 4 and 18 cm. (8–13 cm. in general, $\bar{m} = 10{\cdot}71$ cm), while the other category ranges between 20 and 58 cm. (largest number falling between 30 and 49 cm., $m = 40{\cdot}34$ cm). This data is highly significant since the former class represents in large majority the diploid sexuals while the second comprises mainly of triploid apogamous types.

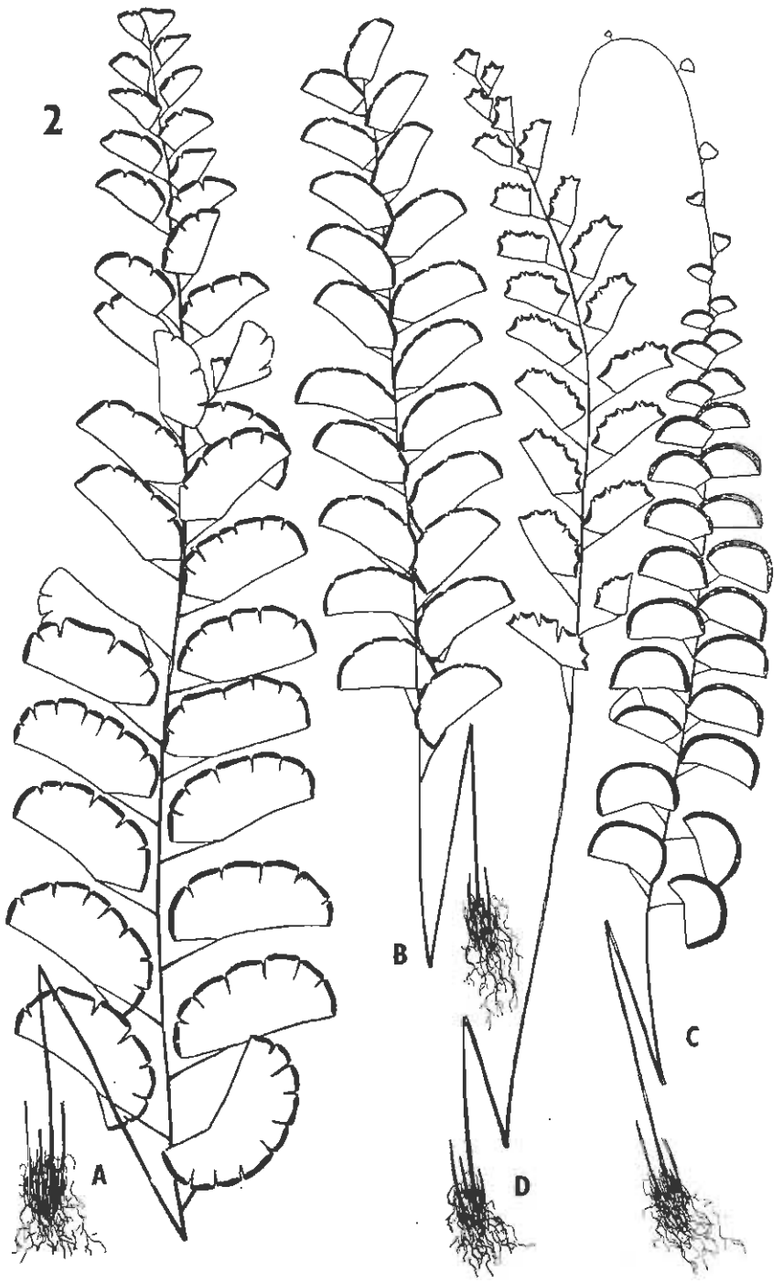
On the whole the fronds in the small-sized category are delicate and occupy moist, shady and well-protected rock crevices (limestone in general), whereas the large-fronded individuals inhabit shaded aspects of open forest margins or roadsides and the fronds are comparatively much tougher. It should be pointed out that so far the lower size limits given in various authentic works for *A. lunulatum* does not decrease beyond 20 cm and the general size range given is 30–45 cm (*cf.* Ching, 1957). In fact, the sharp discontinuity in the size-range was the first indication that led us to suspect some intrinsic cause rather than sheer environmental modification to be responsible for this variability.

Pinnae.—Frond size in general is directly proportional to the pinna size and what is more important some of the size-overlap individuals (extremes of the two categories) from the two size-groups do not generally have the overlapping of the pinna size. The large size category possesses thickly herbaceous larger pinnae borne on longer stalks (total range of pinna size 2–5·0×0·8–2 cm.), which at least in the lower few pairs are fully lunate or more often with the basal angle (where acroscopic and basiscopic edges of pinnae meet at stalk) more than 180° (*cf.* Text-Fig. 2). The basal angle for the pinnae placed higher up gradually decreases to roundabout 120° and in the distal region it becomes nearly 90°.

Apart from general shape and size, the extent of lobing varies considerably. The largest forms usually possess lobed pinnae, lobes extending to $\frac{1}{2}$–$\frac{1}{3}$ the breadth (Text-Fig. 2 A). In another set of individuals the pinnae have almost a continuous outer edge being only interrupted by a few narrow notches (Text-Fig. 2 B). Still others have a completely continuous outer edge with a continuous sorus (Text-Fig. 2 C).

Distinctly small-sized populations (Pl. I, Fig. 1; Pl. II, Figs. 7, 8), on the other hand, bear correspondingly small-sized pinnae (average dimensions 0·4–1·0×0·2–0·6 cm) borne on relatively much smaller stalks. There is lesser variability in the extent of lobing and shape of pinnae. The lower pair seldom becomes fully lunate and the outer

TEXT-FIG. 1.—Frequency polygon on size of individuals from a sample population of 775 individuals from Badamtam-Teesta pony path (Darjeeling). Note two widely different modes.



TEXT-FIG. 2

TEXT-FIG. 2. Line drawings of triploid apogamous *Adiantum lunulatum*, showing architecture of pinnae, lobing, soral construction and basal angle, ×½ nat. size. (A) Pinnae lobed, soral lines straight (from P.U. Coll. No. 212). (B) Pinnae simply notched (P.U. Herb. No. 4531). (C) Pinnae entire, soral line continuous (P.U. Herb. No. 1540). (D) Pinnae lobed or notched, sori concave (P.U. Herb. No. 4530).

edge is shallowly lobed or simply notched. The soral line never becomes continuous. The basal angle in the large majority falls between 90° and 150° in the lower ⅓ of the frond, while in the upper ⅔ region it approaches 90°.

Sori.—The range of variability in soral construction among the large-sized forms is comparatively far more than in the second category, where the situation is more or less constant. In the deeply-lobed or shallow-lobed forms, one lobe possesses in general a single elongate soral line, although more than one sorus may occur occasionally. In extremely shallow-lobed forms, the lobing is often obscured by the overlapping of adjacent indusia (Pl. II, Figs. 3, 4). Forms with entire pinnae have a continuous line of sorus (Pl. II, Fig. 5; Text-Fig. 2 C), and in some cases with lobed pinnae each sorus becomes protruded outwardly at the edges and curves centripetally, so that the concavity faces outwards (Pl. II, Fig. 6; Text-Fig. 2 D).

The category of small-sized fronds has nearly uniform type of sori, each lobe possessing a smooth-elongate sorus or a slightly concave sorus (Pl. II, Fig. 7).

CYTOLOGY OF MORPHOVARIANTS

As stated above cytological studies of these variants have revealed the existence in the E. Himalayas of four cytotypes, namely, triploid apogamous, diploid sexual, tetraploid sexual, and diploid apogamous, all based on $x = 30$.

Triploid apogamous.—This is by far the commonest cytotype in which 8-celled sporangia far outnumber the 16-celled ones and show '*n*' = 90 at diakinesis (Text-Fig. 3). The frond size ranges between 20 and 68 cm. Morphologically three categories of individuals are recognizable here on the basis of pinnae and soral construction:

1. Pinnae variously lobed with discrete smooth sori (Text-Figs. 2 A, B).
2. Pinnae simple with continuous soral line (Text-Fig. 2 C).
3. Pinnae shallow lobed, sori prominently concave (Text-Fig. 2 D).

All the three categories broadly adhere to the description of *Adiantum lunulatum* (*see* Holttum, 1954). The illustrations by Beddome (F.S.I., t.1., **1863**), Ching (*Icones Fl. Sinicarum*, Fas. 5, pl. 215, **1958**), Pichi-Sermolli (Fig. 4, p. 667, **1957**) and Nayar (1962) only conform to the category 1, although most authors do mention about the variability in the size and lobing of pinnae. The forms in this category are the

TEXT-FIGS. 3–6. Fig. 3. Diakinesis in a spore mother cell of triploid apogamous *A. lunaatum*, ' '= 90, ×2,000. Fig. 4. Silhoutte of tetraploid sexual cytotype. (P.U. Herb. No. 3812), ×½ nat. size. Fig. 5. A S.M.C. of tetraploid, showing *n* = 0, 6H×2,000. Fig. 6. Silhoutte of diploid apogamous cytotype (P.U. Herb. No. 3811), ×½ nat. size.

commonest occurring most often in open and shaded localities preferably on soil. These forms were collected under P.U. Herb. Coll. No. 212, a specimen out of which was sent to Dr. R. E. Holttum (Kew) for comparison with the Burmann's type. He expressed (letter dated March 24, 1959) "the specimen (Burmann's type) comes nearest your No. 212 but is rather less deeply incised". To be very exact, it should therefore be proper to relate strictly the shallow lobed forms as nearer to the type of *A. lunulatum*. From the spore dimensions and the occurrence of abortive spores, it has been inferred that Burmann's type represents a triploid apomict (*cf.* Verma, 1961).

Diploid sexual.—It is characterized by its much smaller delicately herbaceous fronds, 4–18 cm, the basal pinnae seldom assume a lunar

shape (Pl. I, Fig. 1). Generally, the pinnae are small, 0·4–1·0×0·2–0·6 cm, and the basal angle lies between 90° and 150° but never more than 180°. In the upper $\frac{2}{3}$ region it is often 90°. Sixty-four normal and viable tetrahedral spores result in a sporangium and at diakinesis the spore mother cell shows 30 bivalents (Pl. I, Fig. 2).

As compared to triploids, this cytotype is plentiful only nearabout Teesta. As expected, the spore and stomatal dimensions are smaller when compared to the triploids.

Tetraploid sexual.—In addition to the above stated common cytotypes met with in Darjeeling (Teesta), tetraploid sexuals (Text-Fig. 4) have also been discovered based on the presence of 60 bivalents at M_1 (Text-Fig. 5). Meiosis in these is perfectly regular resulting in a normal output of 64 spores per sporangium. This form is midway between the triploid apogamous and diploid sexuals in frond size, but the texture and the basal angle resembles more or less the diploids in a wider sense.

Diploid apogamous.—Very few individuals (Text-Fig. 6) which were at first taken to be the larger forms of the diploid taxon proved to be cytologically different. These were diploid apogamous showing 'n' = 60 and released 32 spores per sporangium. From morphological appearance it is hard to isolate these from the tetraploid sexual cytotype.

Discussion

The Himalayan *A. lunulatum* Burm. is in fact an "aggregate species" and so far known is comprised by four cytotypes, namely, triploid apogamous, diploid sexual, tetraploid sexual, and diploid apogamous. Out of these, triploids are widely distributed having been reported from S. Africa, W. Trop. Africa, Ceylon, Bombay, Mt. Abu, and Western and E. Himalayas. Diploid and tetraploid sexual cytotypes have been recently reported also in S. India (Abraham *et al.*, 1962). There is no report yet of the occurrence of diploid apogamous elsewhere.

Cytogenetic Considerations

It has been indicated all along that all the cytotypes broadly agree to the *lunulatum* plan and there is probably no other species which may be considered to be involved in this complex. The obvious assumption, thus, which one is likely to make on gross morphology is that the entire range represents an "autoploid series". This assumption, however, is not supported by the pairing behaviour of chromosomes in the spore mother cells.

At M_1 the tetraploids show complete bivalent formation, a condition prevalent in genomic alloploids. The remaining two cytotypes are apogamous and in apogamous ferns the chromosomal association analysis in the 16-celled sporangia are taken to be of paramount importance, since they supposedly reflect the genetic constitution of the species. This information is not known for the diploid apomict. Ghatak (1960), however, has reported approximately $30_{II} + 30_{I}$ in the

16-celled sporangia of triploids from W. Bengal. Same is corroborated by the investigations of Roy and Sinha (1961). This would again suggest that two diploid species are involved in it, one represented twice. In other words the tetraploid sexual or the diploid apomict which could serve as the other parent in the triploid are of hybrid origin or genomic alloploids. The situation requires experimental evidence but on the face of it and in cognizance with morphological data, it appears that there are to be suspected at least two distinct genetical lines in the diploids which although morphologically similar would create on hybridization a cytological situation akin to alloploids.

In conclusion, it shall suffice to state that *lunulatum* complex promises an excellent material for further experimental investigation. Morphologically it appears as a range of "autoploid" series but available cytological evidence disproves it. The situation seems to be rather parallel to N. American *Pityrogramma triangularis* (*cf.* Alt and Grant, 1960) where also three cytological races occur, all morphologically similar and two genetical types are suspected to occur at the diploid level.

Incidentally, a reference should be made here to the karyotypic studies in the genus *Adiantum* by Roy and Sinha (1960, 1962), which includes triploid *A. philippense* (= *A. lunulatum*). The authors state "In *A. philippense* one pair again has both primary and secondary constrictions. Nineteen pairs of chromosomes have median centromeres and 25 pairs have sub-median to sub-terminal centromeres". It is evident that Roy and Sinha (*loc. cit.*) detected 45 pairs in the triploid ($2n = 3x = 90$) and they did not take into account $x = 30$ in species of *Adiantum* of its affinity. The position thus cannot be taken as correct and requires reinvestigation. Presently looking from any angle it neither corroborates Ghatak's (1960) analysis nor approves of its being an "autoploid".

Taxonomic Evaluation of Cytotypes

It is rather curious to note that all the sheets of *A. lunulatum* present in the Central National Herbarium, Sibpur (Howrah, India) belong to the group of triploids. The small-sized individuals (diploids) are not at all represented and this fact is reflected in the range of size mentioned in earlier literature. The triploids in general and P.U. Coll. No. 212 (Group I here) in particular conform to Burmann's type. Three morphological forms were recognized in the triploids and cytotaxonomically speaking each form, since it can breed true effectively apogamously, could possibly be segregated as a variety, especially the one with a continuous line of sorus. This form seems to be widespread as evident from Dr. Holttum's letter (dated April 12, 1957), "*A. philippense* occurs in West Tropical Africa and in West Indies and in both areas is very variable with some forms having continuous sori". However, at present the varietal names already existent in *A. philippense* or *A. lunulatum* are not known to us. Hence the matter of nomenclature is postponed. It may be remarked that the morphovariants in the tri-

ploid apomict could have evolved through gene mutations or chromosomal rearrangement during a long history of its apogamous nature evident in its present-day wide distribution from S. Africa to Himalayas. Apogamous reproduction would preserve all such changes, if otherwise not detrimental to the proper functioning of growth processes and able to withstand the test of natural selection.

The diploid sexual cytotype is morphologically distinct from the triploids and on this basis it demands a separate specific status. It has been named *Adiantum teestae* sp. nov. (Pl. I, Fig. 1), based on its place of collection, Teesta.

The tetraploid sexual has more similarities in general aspect to the diploid sexual than the triploid apomicts but for the size of pinnae and frond. The diploid apomict similarly comes nearer to the diploid sexual cytotype (*A. teestae*) than the common triploids. Both the cytotypes can, for the present, be retained as additional elements in *A. teestae* (diploid). The specific name *Adiantum lunulatum* (s.s.) is thus limited only to the triploid apomicts.

SUMMARY

Adiantum lunulatum Burm. (= *A. philippense* L.) is widely distributed in subtropical and tropical regions of Asia, Australia and Africa. The extremely variable elements of the species from the E. Himalayas (about Teesta) have been analysed.

In all, four cytotypes have been found, namely, triploid apogamous (commonest), diploid sexual (quite common in the area), tetraploid sexual (occasional) and diploid apogamous (rare). Burmann's type specimen of *A. lunulatum* resembles the triploid cytotype as regards the size and general configuration of the fronds, as well as the spore size. *A. lunulatum* (s.s.) may, therefore, be regarded to represent triploid apogamous taxon. Because of the overall morphological distinctness of the diploid sexual cytotype (small-sized individuals), it has been discussed to segregate it into a new species, *A. teestae*. The two other cytotypes, namely, diploid apogamous and tetraploid sexual because of their general resemblance with *A. teestae* are also retained in it for taxonomic convenience although each one of these has a distinct biological status from evolutionary point of view.

It has been pointed out that all the cytotypes and morphovariants broadly agree to *lunulatum* plan and no other species is involved in the *lunulatum* complex. The cytogenetic considerations have been discussed on the available cytological and morphological data. Gross morphology suggests a case of autoploid series whereas cytology disproves it and indicates "alloploid" nature of cytotypes. In conclusion, it has been indicated that in the diploid sexual cytotype there probably exist at least two distinct genetical lines which on hybridization yield a situation like the genomic alloploids.

ACKNOWLEDGEMENTS

The writers feel it a great privilege to thank Dr. R. E. Holttum for taking great interest in the taxonomic issues. They are thankful to the University Grants Commission for making possible a visit of the Junior author to Central National Herbarium, Sibpur (Howrah), for comparing the specimens. Grateful thanks are also due to Dr. J. K. Bhatnagar for taking the photographs and to Mr. B. Khanna for inking the illustrations.

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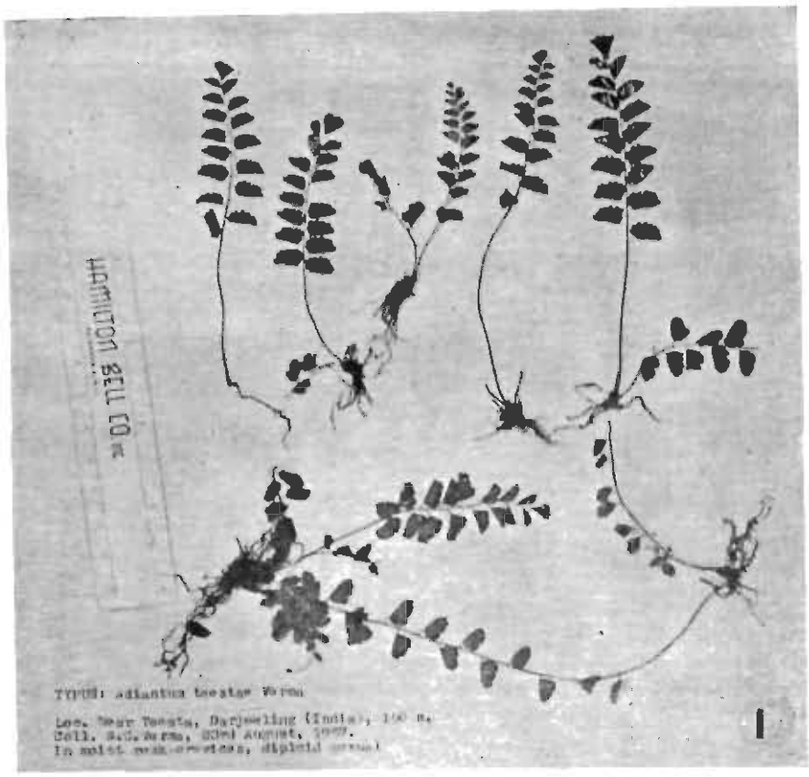
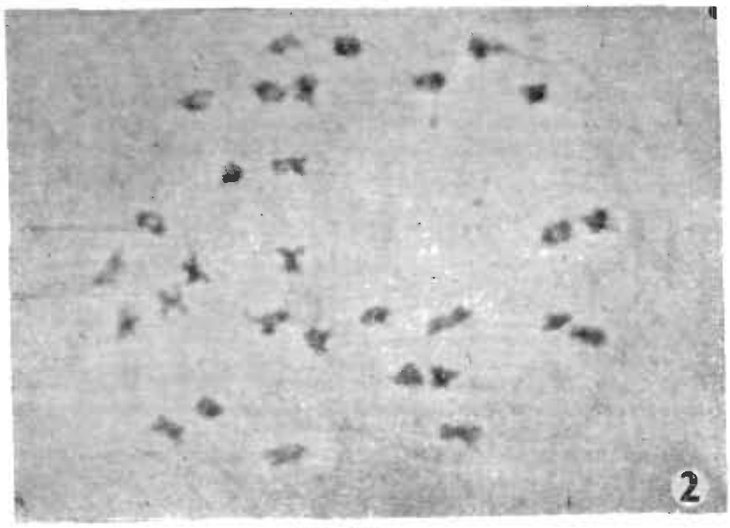
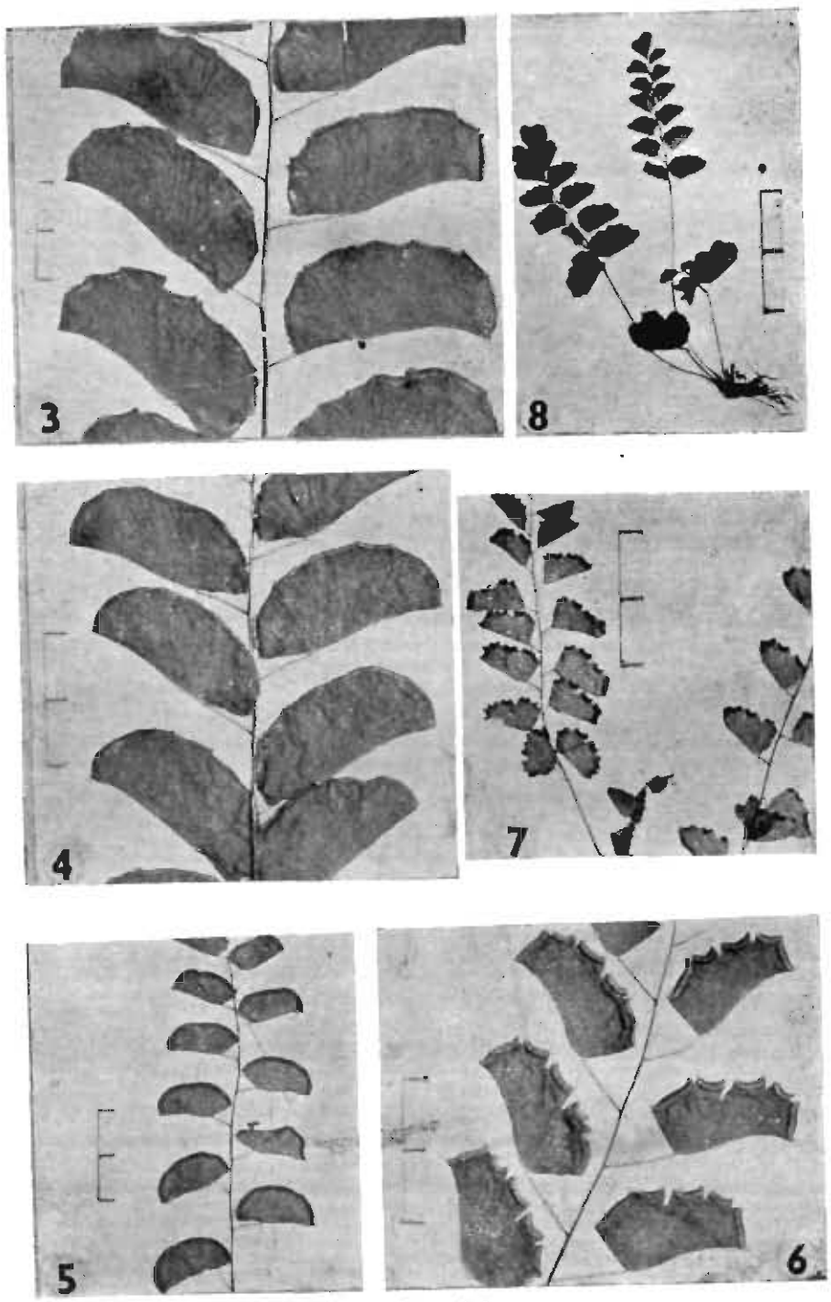




FIGS. 1–2

P. N. Mehra & S. C. Verma



FIGS. 3-8

P. N. Mehra & S. C. Verma

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EXPLANATION OF PLATES I & II

PLATE I

FIG. 1. Individuals of diploid sexual *A. teestae* Verma. This sheet represents the Typus deposited in the Panjab University Herbarium (No. 4534), × *approx.* ½.

FIG. 2. Diakinesis in a S.M.C. of *A. teestae*, showing $n = 30, \times 1{,}200$.

PLATE II

FIGS. 3–8. Close-up photographs of some of the major forms in *A. lunulatum*, both large and small-sized category, showing details of soral construction. A two-centimetre scale is attached along with each. Fig. 3. Pinnae lobed, soral lines straight, triploid apogamous (P.U. Herb. No. 3813). Fig. 4. Pinnae simply notched, sori restricted one to each lobe, triploid apogamous (P.U. Herb. No. 4531). Fig. 5. Pinnae entire, soral line continuous, triploid apogamous (P.U. Herb. No. 4532). Fig. 6. Pinnae lobed or notched, sori sharply concave, triploid apogamous (P.U. Herb. No. 4530). Fig. 7. Pinnae shallow lobed, sori straight-concave, diploid sexual (*A. teestae*), note the small size and basal angle (P.U. Herb. No. 4534). Fig. 8. A small individual of diploid sexual *A. teestae* to show the range of size (P.U. Herb. No. 4533).

ANOMALIES OF THE LIFE CYCLE OF *CHLORELLA* CAUSED BY SOME ANTIMETABOLITES

By Yuji Morimura and Hiroshi Tamiya

Institute of Applied Microbiology, University of Tokyo, and The Tokugawa Institute for Biological Research, Tokyo, Japan

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Introduction

It has been shown in previous work (Tamiya *et al.*, 1962) that the life cycle of *Chlorella*, as studied by the technique of synchronous culture, is modified in multifarious ways by various antimetabolites. Some substances, such as hexachlorocyclohexane and 8-azaguanine, applied at certain concentrations, inhibited the process of cellular division without, or only slightly, affecting the growth (mass increase) of individual cells. In striking contrast, chloramphenicol suppressed markedly the growth process, but allowed the algal cells to perform cellular division.

The manner in which the nuclear patterns of cells and the formation of essential cellular substances such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein may be modified in the algal life cycle in the presence of these substances is of interest. This paper reports the results of observations made with synchronized cultures of *Chlorella* exposed to hexachlorocyclohexane, 8-azaguanine, dihydrostreptomycin and chloramphenicol.

Materials and Methods

The experimental organism used was *Chlorella ellipsoidea* grown synchronously by the D_sLD'-method of Tamiya *et al.* (1961). The starting material of the synchronous culture was a homogeneous population of young and small cells ("D_s-cells") which was prepared according to Morimura (1959) with a modification that the pre-culture lasted only 4–5 days at 25° C under light of 10 kilolux before the cell population was subjected to differential centrifugation. The synchronous culture was run at 21° C with 9 kilolux light, and the other experimental conditions were those described previously (Tamiya *et al.*, 1962). The culture vessels used were large oblong flat flasks (1 lit. in capacity; *see* Kanazawa *et al.*, 1959) or, when large quantities of algal cells were needed for analyses, a flat culture chamber made of acrylate resin (15 lit. in capacity). The Feulgen staining of nuclei was performed according

to Tamiya *et al.* (1961), and the quantities of DNA, RNA and protein-nitrogen in algal cells were analysed by the method described by Iwamura *et al.* (1955; Iwamura, 1955; *see also* Tamiya *et al.*, 1961).

RESULTS

In Tables I and II are summarized the results of analyses of DNA and RNA in the cells grown synchronously in the presence and absence of hexachlorocyclohexane, 8-azaguanine, dihydrostreptomycin and chloramphenicol. Before we consider the effect of each antimetabolite tested, a few words seem to be relevant concerning the results obtained with the control culture which was run by the D_sLD'-method (*see* Tamiya *et al*, 1961). In this method, observation is made of one cycle (starting from D_s-cells) of the growth and division occurring under continuous illumination, so that the cells receive light not only in their light-requiring phases of development (phases of "growth" and "ripening", or from the start of culture to the stage of incipient cell division), but also in their light-independent developmental stages (phases of "post-ripening" and "division"; *see* Tamiya *et al.*, 1961). In the latter stages some daughter cells newly liberated from mother cells perform further growth and ripening until all the mother cells existing in the culture complete their division. The daughter cells produced under such a condition (called D'-cells) are distinguished from the "dark-born" D-cells by the fact that the former are somewhat larger than the latter, and that while the latter are all uninucleate, some of the D'-cells are bi- or even tetranucleate (*see* Tamiya *et al.*, 1961). Correspondingly, as may be seen in the tables, the D'-cells contained more DNA and RNA than did the initial D_s-cells.

Although not mentioned in the table, the cycle in the control culture was completed with the "division number"* of 4. It may be noted that the DNA-content per cell at the stage of incipient cell division was almost exactly 4 times that of the initial D-cells.

Hexachlorocyclohexane.—This substance, which is an analogue of meso-inositol, has been found to cause an anomaly in the algal life cycle at the concentration of $10^{-4}M$ (Tamiya *et al.*, 1962). As shown in Text-Fig. 1, the growth of D-cells was partially suppressed, but the cells continued to grow to an enormous size without undergoing cellular division.† At the concentration of $10^{-3}M$, growth was completely halted, while at $10^{-5}M$ no effect at all was observed.

Cells grown in the presence of $10^{-4}M$ concentration contained large amounts of DNA and RNA (*see* Tables I and II). The Feulgen

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* The number of daughter cells produced from one mother cell.

† In repeated experiments it sometimes happened that the large cells divided in a later stage of culture with the division number of 4 (*see* the data previously reported, Tamiya *et al.*, 1962).

TABLE I

Effects of chloramphenicol, hexachlorocyclohexane, 8-azaguanine and dihydrostreptomycin on the formation of DNA in synchronized Chlorella *cells*

(D_sLD'—cycle; temperature: 21° C.; light intensity: 9 kilolux.)

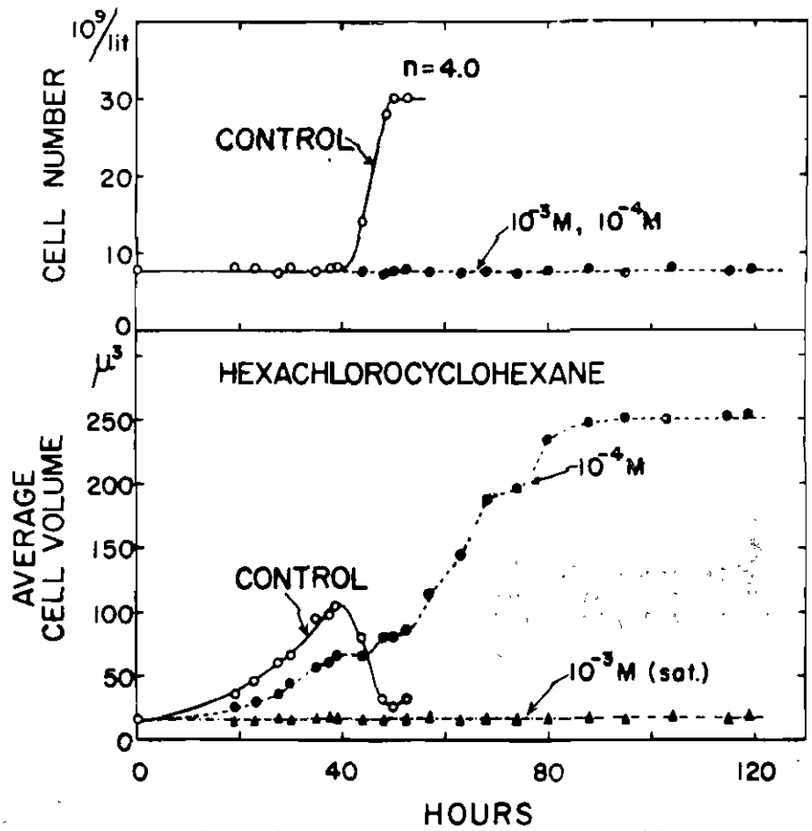
	DNA-content: μg./cell $\times 10^{-8}$					
	Control	Chloramphenicol		Hexachlorocyclohexane	8-Azaguanine	Dihydrostreptomycin
		10^{-3} *M*	10^{-2} *M*	10^{-4} *M*	10^{-3} *M*	$10^{-2\cdot5}$ *M*
Initial D-cells	2·8	2·8	2·8	3·2	2·8	2·8
At incipient cell division	11·9	9·1	5·9	↓	↓	↓
After completion of cell division	4·2 (D'-cells)	5·0	2·2 (Dead)	↓	↓	↓
Giant cells incapable of cell division				27·5 (Dead)	35·6	9·1

TABLE II

Effects of chloramphenicol, hexachlorocyclohexane, 8-azaguanine and dihydrostreptomycin on the formation of RNA in synchronized Chlorella *cells*

(D_sLD'—cycle; temperature: 21° C.; light intensity: 9 kilolux.)

	RNA-content: μg./cell $\times 10^{-8}$					
	Control	Chloramphenicol		Hexachlorocyclohexane	8-Azaguanine	Dihydrostreptomycin
		10^{-3} *M*	10^{-2} *M*	10^{-4} *M*	10^{-3} *M*	$10^{-2\cdot5}$ *M*
Initial D-cells	11	11	11	12	11	1
At incipient cell division	113	123	18	↓	↓	↓
After completion of cell division	52 (D'-cells)	45	5·5 (Dead)	↓	↓	↓
Giant cells incapable of cell division				295 (Dead)	167	99



TEXT-FIG 1. Effect of hexachlorocyclohexane (10^{-3} and 10^{-4} *M*) upon the life cycle of *Chlorella ellipsoidea*. (Temperature: 21°C; light intensity: 9 kilolux.)

staining of these cells revealed that a part of the cells contained two large nuclei per cell, while the rest were uniformly stained permitting no counting of the number of nuclei. The large cells were nicely green in color and looked apparently normal containing as much as 345×10^{-8} μg. per cell of protein-N compared with 22×10^{-8} μg. in the initial D-cells. They were, however, quite unable to divide (both in the light and dark) when transferred to the normal medium. No division occurred even when meso-inositol (10^{-4} *M*) was added to the medium, indicating that the capacity for the cellular division in the large cells had been irreversibly damaged by the action of the antimetabolite.

8-*Azaguanine*.—As has been reported in a previous paper (Tamiya *et al.*, 1962) the effect of 8-azaguanine at the concentration of

10^{-3} *M* is similar to that of hexachlorocyclohexane (10^{-4} *M*) shown in Text-Fig. 1; namely, it allows the cells to grow to an enormous size with inhibition of cellular division. Feulgen staining showed that the abnormally large cells in this case contained, at first, a multiple number of irregularly-shaped nuclei, which later disappeared giving apparently uniformly stained cells. Analyses of the cell material showed (Tables I and II) that the contents of DNA and RNA in the large cells were about 13 and 15 times, respectively, those of the initial D-cells. When these cells were thoroughly washed with water and resuspended in the normal medium, cellular division took place in the light—but not in the dark—with a division number of 6·2. The requirement for light for the division of the large cells in normal medium indicates that the process which had been directly or indirectly affected by 8-azaguanine involved a photochemical process.

Dihydrostreptomycin.—Experiments reported earlier (Tamiya *et al.*, 1962) have shown that in the presence of dihydrostreptomycin in concentrations of 10^{-2}–10^{-3} *M*, the growth of algal cells is halted at a certain stage and the cells remain unable either to grow further or to perform cellular division. Results of analysis of DNA and RNA performed with the cells whose division was halted by $10^{-2.5}$ *M* dihydrostreptomycin are shown in Tables I and II. These cells were able to resume growth and division when transferred (after washing) to the normal medium and kept under illumination.

Chloramphenicol.—This antibiotic, which is a well-known specific inhibitor of protein synthesis (Gale and Folkes, 1953), has been shown to have a strong inhibiting action upon the growth of synchronized *Chlorella* (Tamiya *et al.*, 1962). The anomalies of the life cycle caused by this substance were markedly different according to the concentration used.

When added at a concentration of 10^{-2} *M*, the antibiotic caused a 100% inhibition of growth, but strangely enough, the cells divided almost at the same time as the normal cells (about 20–35 hours after the start of synchronous culture). The division number was 2 (compared with 4 in the control culture), and the extremely small daughter cells produced were unable to grow when transferred (after washing) to the normal culture medium. Feulgen staining showed that the nucleus, which had been clearly visible in the initial D-cells, became difficult to discern at the stage of incipient cell division, with some cells being uniformly stained, and some others appearing to be uni- or binucleate. After cell division, the small daughter cells were mostly ambiguously stained and only some were distinctly uninucleate. As may be seen from Table I, cells at the stage of incipient cell division contained about twice as much DNA as the initial D-cells. The DNA-content per cell decreased to less than half following cell division. Analysis of RNA (Table II) showed that there occurred only a slight increase of RNA during the transformation of initial D-cells into the cells at the stage of incipient division. In a separate experiment, it was found that the

synthesis of protein was completely halted by 10^{-2} *M* chloramphenicol: thus, the content per cell of protein-N in the cells after cell division was only a half ($11{\cdot}8 \times 10^{-8}$ μg.) of that in the initial D-cells ($22{\cdot}3 \times 10^{-8}$ μg.). Another characteristic phenomenon observed with 10^{-2}*M* chloramphenicol was the loss of chlorophyll. Whereas the initial D-cells contained 11×10^{-8} μg chlorophyll per cell, the cells after the division in chloramphenicol contained no detectable amount of the pigment.

The inhibitive effects of the antibiotic were, in general, much milder when it was applied at the concentration of 10^{-3} *M*. The growth of initial D-cells was only partially suppressed, and the cell division also took place with the division number of 4. The cells remained green during the whole course of the growth cycle and the daughter cells produced were able to grow normally when transferred to the normal medium. As may be seen from Tables I and II, the contents of DNA and RNA in cells at the stage of incipient cell division and after the completion of division were almost comparable with those found in the control culture.

A remarkable effect observed with 10^{-3}*M* chloramphenicol was, however, that the onset of cellular division was conspicuously retarded compared with that observed with control culture or with the culture affected by 10^{-2}*M* of the same antibiotic. Thus, in an experiment reported in a previous paper (Tamiya *et al.*, 1962), the cells, whose growth was partially suppressed by 10^{-3} M chloramphenicol, started to divide at approximately the 160th hour of the experiment, while the incipient division in the control culture occurred at the 33rd hour. The apparently paradoxical fact that the onset of cellular division was retarded by 10^{-3}*M* but not by 10^{-2} *M* chloramphenicol may be explained on the basis of an important finding made by Hase *et al.* (1961), who discovered that the cellular division of algal cells is evoked by an endogenous substance(s), and that the formation of this substance(s)—and, therefore, the occurrence of cellular division—is in competition with the process of protein synthesis. (For further details on this problem, *see* Tamiya, 1963 *a*, *b*.).

Summary

Effects of hexachlorocyclohexane, 8-azaguanine, dihydrostreptomycin and chloramphenicol upon the life cycle of *Chlorella* were investigated using the technique of synchronous culture. In the presence of 10^{-4}*M* hexachlorocyclohexane, the growth of young algal cells was slightly suppressed, but the cells continued to grow to an enormous size synthesizing corresponding amounts of DNA, RNA, protein and chlorophyll, without, however, being able to perform cellular division. The resulting giant cells were unable to divide when transferred subsequently to normal medium with or without the addition of meso-inositol (10^{-4} *M*). Giant cells having no capacity for cellular division —but with uninhibited formation of DNA and RNA—were also produced in the presence of 10^{-3} *M* 8-azaguanine. These cells were capable

of division—with the "division number" of 6·2—when transferred to the normal medium and kept illuminated.

In the presence of $10^{-2.5}$ *M* dihydrostreptomycin, the growth of algal cells and the formation of DNA and RNA were halted at a certain stage and the cells remained unable either to grow further or to perform cellular division. These cells were also able to resume growth and division when transferred to the normal medium and kept under illumination. Interesting phenomena were observed when chloramphenicol was added at concentrations of 10^{-2} and 10^{-3}*M*. At the concentration of 10^{-2} *M*, the algal cells could not grow or synthesize protein, but they divided almost at the same time as the normal cells and each cell gave rise to two daughter cells. These cells were devoid of chlorophyll and incapable of growth when transferred to the normal medium. When added at a concentration of 10^{-3} *M*, the antibiotic caused a partial suppression of growth, but the cells continued to grow, synthesizing DNA and RNA, and eventually each cell divided into 4 daughter cells that could grow normally when transferred to the normal medium.

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ON LATEX

By K. Mothes and L. Meißner

German Academy of Sciences at Berlin. Institute of Biochemistry of Plants Halle/Saale, Germany, G.D.R.

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Latex and latex vessels are often investigated in respect of phytochemical, histological as well as cytological studies. We know that chemically there can be great variations in latex of different species. We also know that this medium with its high water content is capable of chemical reactions, and that hydrolytic, oxidative and other enzymes with a predominantly degrading activity are present in high concentrations. But recently on account of certain synthetic reactions, the question was raised again whether latex is cell-sap, a sort of protoplasm, a mixture of protoplasm and cell-sap or a degenerated protoplasm. Is the sap-space of the latex vessels an excretion-space? Are the substances excreted there biochemically active? If they are, is this activity of a physiological importance for the plant?

Recently it was shown that isolated latex has the capacity to form rubber from acetate (Bandurski and Teas, 1957; Park and Bonner, 1958; Kerwick *et al.*, 1959; Teas and Bonner, 1960; Lynen and Hennig, 1960). This process requires several enzymes and co-factors, some of which could be demonstrated (McMullen, 1959; 1960). After the first demonstration of presence of nuclei in some latices modern biochemistry (McMullen, 1962) and researches on structure and particulate inclusions of cells (Moir, 1959; Kleinschmidt and Mothes, 1959) made it probable that at least the best studied latex of *Hevea* cannot be compared either with common cell-saps or to the "full" vacuole saps, but that it is rather more a cytoplasmatic liquid. During these investigations we found that isolated latex of *Papaver somniferum* is able to convert ^{14}C-labelled tyrosine to a complicate spectrum of radio-active alkaloids (Kleinschmidt and Mothes, 1959, 1960; Mothes and Schütte, 1963; Schenk and Schütte, 1961), some of which can be classified among the normal opium-alkaloids. Though the biosynthesis of these alkaloids with their quite complicated structure is likely to be simpler than assumed as yet, certainly a reaction chain of several parts and with the participation of several enzymes is involved. There was some hope that latex represents a sort of natural homogenate which perhaps makes the investigation of certain syntheses easier than plant homogenates resulting from tissue destruction.

In order to find out whether *Hevea* and *Papaver* represent exceptions which do not allow a generalization, we made some preliminary experiments on the chemistry and biochemistry of latex. In these first

experiments done with L. Engelbrecht, I. Liß and W. Schenk we noticed the remarkable fact that the latex of about fifty different species of the genus *Euphorbia* is distinguished in most cases (about 30) by specific ninhydrin positive water-soluble compounds. Of these compounds first the group of amino acids was investigated, and it was found that most of latices contain a certain amino acid in such a high concentration as to surpass the others. Often this concentration is higher than the sum of all the other amino-acids, and often this amino acid does not occur in the tissues of these species or it is found there only in traces. Besides these proteinogenous amino acids (as tryptophan in the latex of *Euphorbia polychroma*) there are frequently non-proteinogenous or rare amino acids in the latex or amino-acids which have not yet been observed elsewhere or which appear to be species-specific (Schenk and Schütte, 1961; Liß, 1961, *a*, *b*, 1962; Schenk *et al.*, 1962; Schütte and Schütz, 1963).

Here a wide field opens for phytochemical work, for the latex of other plant families is equally interesting and presents a wide variety.

After it was shown that latex is markedly different from a tissue, the question arose whether these characteristic latex substances are also formed in the latex. If this is so, an excellent possibility is given to study the synthesis of these substances in an undisturbed system.

This problem was investigated more thoroughly with two materials. The latex of *Euphorbia lathyris* contains 3, 4-dioxyphenylalanine (DOPA) in such a high amount (1–1·7% of fresh weight) that the solution is by far oversaturated. Probably the crystals observed in the latex are DOPA crystals (Liß, 1961). ^{14}C-labelled tyrosine fed to these plants is not found as such in the latex, but only in the form of DOPA. Isolated latex, however, does not form DOPA from tyrosine. Doubtlessly, DOPA is formed in the parenchymatous cells of the tissue accompanying the latex vessels or in the non-effluent plasma covering the latex vessels themselves and secreted into the latex. It is also interesting that from the latex a phenoloxydase of the laccase type can be prepared which oxidises DOPA, staining it dark. This enzyme is not active in the native latex. The cause of this inactivity is not clear. Perhaps the redoxpotential is not favourable or the enzyme is specifically inhibited. It is more probable, however, that the enzyme exists in a stereostructure which does not allow its activity.

Very similar conditions are found with *Strophanthus scandens*. Besides considerable amounts of 4-hydroxypipecolic acid we observed 4-aminopipecolic acid which was unknown as yet (Schenk and Schütte, 1961). Whereas shoots are able to convert pipecolic acid to the two above mentioned derivatives, the isolated latex cannot do so. Here again these substances must be considered to be secretion products of the neighbouring cells or of the protoplasm covering the cell walls (Schütte and Schütz, 1963). Other latices containing special amino acids are being investigated.

We are far from generalizing these observations but we would like to emphasize the contrast between the observations made with *Hevea* and *Papaver* on the one side, and with *Euphorbia* and *Strophanthus* on the other.

In parallel investigations we tested for processes requiring oxygen. In all *Euphorbia* species examined the freshly isolated latex shows no or only a very small oxygen consumption. *Strophanthus scandens* has a peculiar oxygen consumption curve which rises almost logarithmically from very low values after the isolated latex has been kept for two or three hours at 30° C. Similar conditions are described with *Hevea* (Hsia, 1958). We do not think that bacteria might be the cause of the considerable increase of O_2-consumption, because this increase is as well obtained in the presence of antibiotics and because with the common methods of bacteriology no noticeable amounts of bacteria could be observed.

The CO_2-production is also increased to a certain degree. But after 4 hours it remains almost constant and far below the oxygen curve, thus excluding the possibility of it being a plain respiratory process.

This O_2-consumption, however, is probably based on an enzymatic reaction, as it is completely inhibited if the latex is heated to 100° C under nitrogen.

The most interesting example is *Papaver somniferum*, the latex of which has a considerable O_2-consumption with an R_o 3–4.

We may conclude, therefore, though with some precaution, that a process comparable to respiration exists perhaps only in *Papaver*. As to *Strophanthus* and *Euphorbia lathyris* we possibly may think of an oxidase reaction induced by the destruction of latex particles and acting upon a substrate existing in latex.

This special capacity of *Papaver* latex is also encountered in investigations of protein metabolism. From the preliminary experiments of Dr. Böttger (unpublished) an activation of amino acids in the latex appears to be probable. Incorporation experiments with radioactively labelled amino acids into the protein of freshly isolated latex showed a noticeably positive result only in the case of *Papaver* as yet; they make us consider, however, the possibility of a protein synthesis. This problem needs further investigations with fractionated particles and enzymes. Anyway the results of McMullen with *Hevea* (McMullen, 1959, 1962), who could demonstrate nucleotides of various sorts in latex indicate postulates for a protein synthesis.

The biochemical investigation comparing isolated latex and intact plant parts point out that latex of different plant species behaves very differently. A true metabolism can perhaps only be demonstrated in the isolated latex of *Papaver somniferum* and only for a certain time. This latex, certainly, shows the highest degree of biochemical organization. But, of course, electron microscopical investigation must give

here information on the structural fundamentals. In contrast to the *Papaver* latex, the latices of *Euphorbia* species examined are chemically rather inactive and did not show a reaction involving a high grade of co-ordination. Nevertheless, it is possible that all latices can be classified into a graduated system of complicated mixtures of protoplasmic particles and cell-sap which is marked by an increasing disarrangement of the vital structure of the organellae by cell-sap and which results in a gradual inhibition of the processes going on in the protoplasm.

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ON THE PRIMITIVE TYPES OF STELAR SYSTEM IN THE PTERIDOPHYTES*

BY Y. OGURA

University of Tokyo, Tokyo, Japan

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INTRODUCTION

SINCE the stelar theory has been proposed by van Tieghem and Duliot in 1886, numerous attempts concerned for the relationship of these types have been undertaken. The stelar theory is considered to be very important, especially in the Pteridophytes, because in this group there are numerous stelar types, which are considered to show a phylogenetic relationship. The nomenclature of the stelar types and the consideration for their relationship are, however, not unique in different authors, and there are some prominent discrepancies in their classification. The outline of the stelar theory has been demonstrated by some authors, such as Tansley (1907–08), Chauveaud (1911), Meyer (1916), Thompson (1920), Schoute (1938), etc., and the present author has given a detailed contribution in his handbook on the anatomy of Pteridophytes (1938).

This paper is dealt with a compensatory attempt concerning the primitive types of the stelar system.

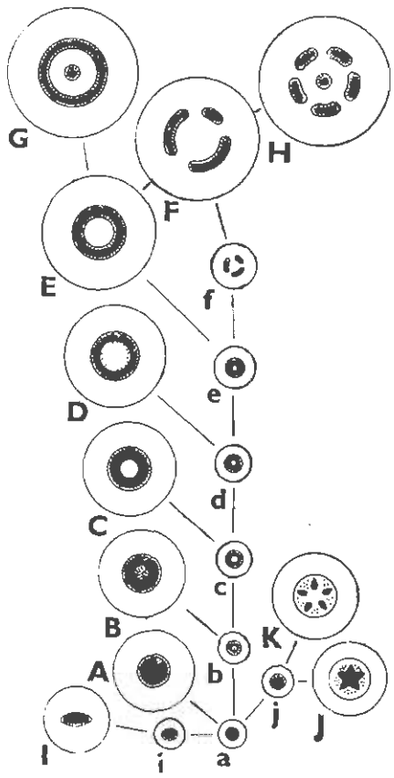
THE PRIMITIVE PROTOSTELE

The protostele, one of the fundamental types among the stelar system in the Pteridophytes, is a single stele constituting of a central xylem, surrounded by a ring of phloem. It was Jeffrey (1897, 1899), who proposed this name for the above noted stele, and he considered it to be a simple and primitive type among the steles. Brebner (1902) used the name haplostele for such a stelar type, and considered the protostele to be a stelar type supposed to be primitive, including his haplostele and actinostele. Many authors hereafter considered his haplostele as identical with the protostele of Jeffrey, and at present, the name protostele is generally used for the stele described above.

Besides the typical form of the protostele, there are some types, which are considered to be modified from the typical one, and the author classified the protostelic type into 11 subtypes, such as typical protostele, protostele with mixed pith, medullated protostele, *Lindsaya*

* Contribution from the Division of Plant Morphology, Department of Botany, Faculty of Science, University of Tokyo, N.S. No. 86.

type, stellate protostele, radial protostele, dorsiventral protostele, etc. (Text-Fig. 1).



TEXT-FIG. 1. Diagrammatic illustration of the development of the stelar types (A–K) from the primitive stelar types (a–j). a, primitive protostele; A, typical protostele; b, primitive protostele with mixed pith; B, protostele with mixed pith; c, primitive medullated protostele; C, medullated protostele; d, primitive protostele with internal phloem; D, protostele with internal phloem; e, primitive solenostele; E, typical solenostele; *f*, primitive dictyostele; F, typical dictyostele; G, H, dicyclic solenostele; H, i, primitive dorsiventral protostele; I, dorsiventral protostele; j, primitive stellate protostele; J, stellate protostele; K, radial protostele. Black, xylem; dotted, phloem; lined, endodermis; outer circle, epidermis; lines between steles show the ontogenetic or phylogenetic relationship of stelar types.

In the typical protostele the xylem consists of uniformly distributed tracheids and parenchyma (Text-Fig. 1 A), while in the medullated protostele the central part of xylem is occupied by the group of parenchyma constituting the pith (Text-Fig. 1 C), and if the central part is occupied mainly by parenchyma, a few tracheids intermingled within

it, it is called the protostele with mixed pith (Text-Fig. 1 B). The stems or rhizomes of some species of *Gleichenia*, *Lygodium*, *Botryopteris*, *Lepidodendron*, etc., show the typical protostele. In these typical protostelic forms the stele is generally large[1] and distinctly differentiated, and protoxylem and metaxylem are clearly distinguished in the xylem, and in the phloem also protophloem and metaphloem are distinguished, though not so sharply as in the xylem. The protoxylem may be either endarch, mesarch or exarch.

It is well known that some species of Psilophytes, such as *Rhynia* and *Horneophyton*,[2] and of other primitive Pteridophytes possess the stems showing the protostelic system. But the protostele of these species is generally small[3] and consists of a few elements, in which the differentiation of protoxylem and metaxylem is hardly distinguishable or is not sharp. Moreover, the stele occupies only a few parts of the stem, just like the conducting bundle in some mosses, whose membrane is, however, very thin. Though the author included the protostele in *Rhynia* and *Horneophyton* in the typical protostele, he considers, now, that it is rather reasonable to distinguish it from the typical protostele, and wants to call it as "primitive protostele" (Text-Fig. 1 a), one of the subtypes of the protostelic type, as it seems to be more primitive than that of the typical protostele.

The primitive protostele may be found not only in the stem of primitive Pteridophytes mentioned above, but also in the sporelings or young stems of higher Pteridophytes, as will be described below.

Primitive Solenostele and Dictyostele

It is well known that in the species in Filicales, which show the solenostelic or dictyostelic system in the adult stems, the sporelings show a protostelic system. Such a protostele is small, consisting of a few elements, and if we trace the ontogeny of the stele we will see the formation of parenchyma group, or the pith, within the xylem, so that the protostelic stage is seen in a very short distance. The protostele in this stage is similar to the primitive protostele mentioned above, and may be called by the same name (Text-Fig. 1 a). Tracing the stele toward the young stem, successive stages of the formation of pith (Text-Fig. b, c), internal phloem (Text-Fig. 1d), and internal endodermis (Text-Fig. 1 e) may be recognizable, and at a certain part we will meet with the stele of a solenostelic type (Text-Fig. 1 E), consisting of a xylem ring provided with external and internal phloem and endodermis. But such an ontogenetical development is not always identical in different species, as have been investigated by many authors, such

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[1] Diameter of the stele in *Gleichenia glauca* is 1·6 mm, in *Lygodium japonicum* 1·2 mm., in *Botrychium cylindrica* 1·0 mm., and in *Lepidodendron esnotense* 4·5 mm.

[2] *Horneophyton* Barghoorn and Darrah, 1938, is an emended name for *Hornea* Kidston and Lang, 1920.

[3] Diameter of the stele in *Rhynia gwynne-vaughani* is 0·1 mm.

as Chandler (1905), Bower (1911), Thompson (1920), Gottlieb (1959), and others.

In some species of *Lepidodendron*, the stele shows a typical protostele, in the xylem of which parenchyma is uniformly distributed between the tracheids (*e.g. L. esnotense, L. saalfeldense*), and in other species, in the centre of protostelic xylem is found a group of parenchyma (*e.g., L. harcourtii, L. brevifolium*), while in others, among such a parenchyma group are found tracheids intermingled (*e.g., L. selaginoides, L. intermedium*). In this second case the group of parenchyma constitutes a pith, and the last case is the intermediate suggesting the process of the pith formation, or the medullation. The last case is called the protostele with mixed pith (Text-Fig. 1 B), and the second the medullated protostele (Text-Fig. 1 C). Thus, in *Lepidodendron*, 3 types of protostele may be distinguished: the typical protostele, the protostele with mixed pith, and the medullated protostele. In the last type, the size of the pith is variable, and if it is very large, tracheids of xylem constitutes a thin ring, which sometimes looks like a xylem ring of the solenostele. Such a medullated protostele containing a large pith, is found also in most species of *Sigillaria* and *Bothrodendron*, in which the tracheid ring is surrounded usually by thick secondary tracheids.

In the sporelings and young plants we will meet with the simple protostelic and transitional stelar forms, just described in *Lepidodendron*, but as they are small, differentiation of elements is not always distinct, consisting of small elements, and sometimes certain features of irregular or peculiar structure will be seen.

Tracing the young plant further upwards, there is a part, where the internal phloem, and then the internal endodermis appear, finally reaching the part provided with a small solenostelic or dictyostelic construction (Text-Fig. 1 a–f). In this part, the stele becomes a little larger and consists of a little differentiated element, but still in the primitive condition. When the stem enlarges further, the stele also enlarges gradually until it constitutes a completely differentiated solenostelic or dictyostelic system (Text-Fig. 1 E. F). In accordance with it, the leaves become larger, and as the leaf-trace also becomes larger, its departure leaves a large gap on the stelar ring, and gives a great influence to the stelar construction. Generally, the dictyostele results from the solenostele by the overlapping of leaf gaps (Text-Fig. 1 E, F).

The transition of the stelar construction in the sporeling and young plant occurs within a short distance, and successive stages described above are not always regularly shown, and at certain stages may be omitted, or peculiar construction will be seen. It is one of the reasons why formation of pith is considered to be either intrastelar or extrastelar.

In short, it has been described by many authors that the solenostele is derived from the protostele through the formation of pith.

internal phloem and internal endodermis (Text-Fig. 1A-E). This concept is not a mistake in general sense, though it is not in accordance with that of Zimmermann based on his telome theory (1954, 1956, 1959). Nevertheless, it may be more reasonable to explain that the solenostele is derived from the primitive protostele, passing through the primitive forms of intermediate types and primitive solenostelic condition (Text-Fig. 1 a-e-E). In other words, the solenostele is not derived directly from the typical or completely differentiated protostele, but is derived from the forms in a primitive protostelic condition, passing through the primitive condition of medullated protostelic and other intermediate forms.

The dictyostele may be derived either from the primitive solenostele through primitive dictyostelic condition (Text-Fig. 1 e-f-F) or from the solenostele directly by means of the overlapping of leaf gaps (Text-Fig. 1 E, F).

In the stelar types with more complicated construction, such as dicyclic, tricyclic or acyclic stelar system, the successive process of ontogeny from the primitive protostelic type to the primitive solenostelic or dictyostelic type, passing through intermediate stages, will be traced in the same way with that described above. After the formation of solenostele or dictyostele, one part of the stele separates into the pith and becomes a medullary meristele. This is the first step of dicyclic stelar system (Text-Fig. 1 G, H).

The Actinostelic System

The actinostele, a kind of the protostelic type, consisting of a radial vascular bundle, which is characteristic in the root, is found also in the stem of a few species of Pteridophytes.

In the stele in *Asteroxylon*, *Psilotum*, *Sphenophyllum*, etc., its xylem is stellate, the phloem being situated between the arms of xylem rays. The author called such a stele as the stellate protostele. The actinostele is generally considered to be derived from the protostele, and the stellate form may be the first step toward the actinostele. Strictly speaking, however, such a transition from the circular xylem toward the stellate one occurs in a small protostelic stage, so that it is reasonable to consider that the stellate protostele may be derived from the primitive protostelic type passing through a "primitive stellate protostele" (Text-Fig. 1 a, j, J). The xylem in *Sphenophyllum* is very remarkable in its regular triangular shape, but the xylem in most species is more or less irregularly shaped.

Further reduction of the xylem derives the radial vascular bundle, in which the xylem is separated in some groups, arranging side by side with the phloem on a ring. This form, the radial protostele of the author (Text-Fig. 1 K), which is most familiar in the root, is found, accompanying a certain irregular arrangement, in small branches in a few species of *Lycopodium* (*e.g.*, *L. selago*, *L. inundatum*, *L. holstii*). The

stele consisting of some plate-shaped xylems, which is found in most species of *Lycopodium*, the *Lycopodium* type of the author, may be derived from this radial form secondarily.

The stems of *Selaginella* show some peculiar stelar type (Harvey-Gibson, 1894), among which a dorsiventrally flattened stele, the dorsiventral protostele of the author, is most familiar (Text-Fig. 1 I). This type may be derived from the primitive protostele by means of the flattening process (Text-Fig. 1 a, i, I), and the other types may be considered to be derived from this type due to further modifications, though there are other types, such as a protostele (*e.g.*, *S. selaginoides*) or a solenostele (*e.g.*, *S. lyallii*), developed in other directions from the primitive protostele.

Summary

1. The stele of the stem in some primitive Pteridophytes, such as *Rhynia* or *Horneophyton*, is protostelic, consisting of a few, not fully differentiated elements. It is distinguished from the large, differentiated typical protostele, and may be called the " primitive protostele".

2. The primitive protostele is observed also in the stem in the sporelings in most kinds of Pteridophytes. Tracing the ontogeny of the stele in sporeling toward the young stem, this protostele enlarges gradually to construct a solenostelic or dictyostelic type, passing through the transitional types showing the formation of pith, internal phloem and internal endodermis.

3. These stelar types in successive stages may be denoted as the " primitive " type of protostele, protostele with mixed pith, medullated protostele, protostele with internal phloem, and with internal endodermis, and solenostele or dictyostele, respectively.

4. The primitive stelar type denoted above may be primitive phylogenetically and ontogenetically, and the adult stelar types may be derived from the corresponding primitive types.

5. The deviation from the primitive protostele in other directions originates the stellate and radial protostele and also the dorsiventral protostele, passing through their primitive forms.

6. The polycyclic or acyclic stelar types may be derived from the solenostelic or dictyostelic type in the young stem, by means of the appearance of accessory meristele originating from the main stele.

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EMBRYOLOGY AND PLANT BREEDING

By B. P. Pal

Indian Agricultural Research Institute, New Delhi, India

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A plant breeder of today needs no apology for attempting a discussion on embryology, especially if we recall two important discoveries, namely, that of the pollen tube by Amici who was a mathematician and astronomer of Italy, and of the laws of inheritance by Mendel, a theologian of Austria who also taught mathematics. Apart from these facts of history, the advances in our knowledge of genetics and embryology have reduced to oblivion the gulf between these sciences and both have evolved from the narrow classical discipline of the past into the wider experimental philosophy of today. It is proposed here to discuss briefly the application of embryological studies in an important aspect of plant breeding research, namely, distant hybridization, a field which is the meeting place not only for embryologists and geneticists but, as will be seen later, also for the physiologists and biochemists.

The increase in our knowledge concerning the isolation mechanisms (Clausen, 1951) in plant species and the evolution of techniques to overcome the barriers to species crossing, have further widened the spectrum of variability available for recombination breeding. It will be proper, first, to consider the barriers to crossability between species and the techniques available for overcoming these. The application of embryological data concerning seed development in distant crosses will then be discussed briefly.

Barriers to Crossability—Problems and Techniques

Broadly these barriers can be divided into two kinds, (*a*) those appearing before fertilization, and (*b*) those occurring after fertilization. The pre-fertilization barriers include, non-synchronization of flowering time, failure of pollen of one species to germinate upon the stigma of another, lack of pollen tube growth in crosses between species having different lengths of style, and finally the failure of the fertilization process itself. As regards techniques, flowering time can be synchronized by adjusting the photoperiod and temperature, but with present-day facilities of air transport, it is even possible to send pollen from one part of the world to another or even store the pollen from one season to another, with the help of pollen-banks which now operate on a commercial scale (Antles, 1951). Our knowledge of the physiology of pollen (Johri and Vasil, 1961) and the techniques for extending the storage life of pollen are now fairly advanced and form an important

adjunct to plant breeding. Failure of pollen germination and bursting of pollen tubes has also been tackled successfully in some crosses. Blakeslee (1945) could overcome the incompatibility between certain $2n \times 4n$ *Datura* crosses by the use of periclinal chimeras where the style layers were of $4n$ and $2n$ constitution so that the $4n$ epidermis prevented bursting of the pollen tubes of the $4n$ parent. Stylar amputation was successfully employed by Mangelsdorf and Reeves (1931) to effect the cross between *Zea* and *Tripsacum*. Since the cut surface of the style may not be always as good as the stigma, style-grafting techniques have been evolved by Buchholz *et al.* (1932) in *Datura*, by Yasuda (1931) in *Petunia* and by Hecht (1960) in *Oenothera*. However, such delicate operations are feasible only in plants having stout styles. A simpler method of replacing the natural stigma by an artificial medium containing sucrose-agar-gelatin was employed by Swaminathan (1955) in securing crosses between *Solanum pinnatisectum* and *S. bulbocastanum* and between the former and *S. lanciforme*, and by Swaminathan and Murty (1957) in the crosses, *N. tabacum* × *N. rustica* and *N. tabacum* × *N. debneyi*.

Stylar incompatibility may also be overcome in the future by what is known as intraovarian pollination, a technique standardized in the poppy by Kanta (1960). An offshoot of this technique is "test-tube fertilization" brought about by culturing ovules and then landing pollen upon them or near them in the artificial medium (Kanta *et al.*, 1962). It remains to be seen how far this fascinating technique will work in distant crosses.

Coming next to the post-fertilization barriers, these may appear in the form of zygotic lethality, premature cessation of growth in the embryo and/or endosperm tissue, or sterility in the F_1 plant and so on. The precise location of the post-fertilization abnormality necessarily calls for an investigation of the process of seed development in crosses. We owe much of our existing knowledge on the mechanisms of seed failure in crosses to the excellent work of Brink and Cooper (1947) and of Blakeslee's school of *Datura* workers (*see* Avery *et al.*, 1959) and of several others. The various investigations of diplontic sterility may be considered under four categories for the sake of convenience.

(i) *Abnormal endosperm.*—Embryologists consider the endosperm tissue as an evolutionary device in angiosperms that probably compensates for the highly reduced female gametophyte as compared to a massive one in the gymnosperms, and also confers upon the endosperm the physiological superiority of hybridity. This cytogenetic equipment presumably enables the endosperm to maintain a certain aggressiveness over the maternal tissues surrounding it, so that it can serve as an efficient intermediary for the nutrition of the embryo. From this it will be evident that any malfunctioning of the endosperm would lead to the starvation and death of the embryo. Different kinds of abnormalities have been reported in distant crosses, such as dumbel-shaped and giant-

sized nuclei in the crosses *Avena strigosa*×*A. fatua* (Kihara and Nishiyama, 1932), *Hordeum jubatum*×*Secale cereale* (Cooper and Brink, 1944; *see* review by Brink and Cooper, 1947) and in *Gossypium arboreum* ×*G. hirsutum* (Weaver, 1958). Reusch (1959) in his study of the cross *Lolium perenne*×*Festuca pratensis* noticed precocious wall formation but further divisions were suppressed due to pycnosis of nuclei and stickiness of chromosomes, a situation considered parallel to that reported by Koller (1943) in tumorous cells. Other cases of abnormal endosperm reported include those by Sachet (1948) in *Datura*, Johansen and Smith (1956) in *Arachis*, and by Greenshields (1954) and Shastry (1958) in *Melitotus* crosses.

(ii) *Abnormalities in maternal tissues.*—Since the maternal envelope forms an integral part of the nutritional mechanism in the seed, histological changes in the cells of the nucellus and seed coat can often provide useful clues regarding the nature of the incompatibility reaction. Cooper and Brink (1940) have designated as "somatoplastic sterility" the phenomenon of over-growth or hyperplasia of the endothelium lining the endosperm, in the cross *Nicotiana rustica*×*N. glutinosa*. Similar cases of somatoplasia have been reported by Sachet (1948) in *Datura*, Beaudry (1951) in *Elymus virginicus* × *Agropyron repens* and Johansen and Smith (1956) in *Arachis hypogaea*×*A. diogoi*.

Since abnormalities in endosperm may also occur coincidentally with somatoplasia, it is often difficult to establish definite relationship between cause and effect. Brink and Cooper (1947) consider the endosperm as the primary cause of seed failure in crosses since it often precedes the somatoplasia of nucellus or endothelium.

As regards the rôle of antipodals in early post-fertilization development, opinions differ. Brink and Cooper (1944) attributed seed abortion in a *Hordeum jubatum* ×*Secale cereale* cross to the weak functioning of the antipodals which occupy a vital position in the nutrient stream. Maheshwari (1950) stated that antipodals enlarge and become glandular after fertilization and thus offer a close analogy to tapetum of the anthers. Beaudry (1951) also assumed that antipodals were secretory in function and their hypertrophy caused death of the endosperm. On the other hand, Thompson and Johnston (1945) claimed that antipodals were not different from normal to be of significance in the breakdown of the *Hordeum*×*Secale* cross. Brock (1955) has shown that breakdown in lily endosperm is not caused by antipodals but by chromosome abnormalities. Morrison (1955) has also shown that antipodals are not responsible for abnormalities in endosperm in the cross between *Hordeum marinum*×*H. vulgare*, and in wheat.

(iii) *Abnormal embryo.*—Greenshields (1954) found different kinds of abnormal embryogeny in *Melilotus* crosses ranging from a complete inhibition of division in the zygote to abortion at later stages. Buell (1953) also attributed the failure of the cross *Dianthus plumaris* ×*D. chinensis* to the abortion of the embryo. Weaver (1957) also held the embryo responsible for seed failure in the cross *Gossypium hirsutum*

× *G. arboreum*, where the absence of zygotic division resulted in "embryoless" ovules The imbalance between the endosperm and embryo has been ascribed to the production of an inhibitory substance by the embryo. Moav and Cameron (1961) postulate that the embryo is the site of hormonal upset leading to abnormality in maternal and endosperm tissues in the cross *Nicotiana rustica* × *N. tabacum*, and suggest that the changing of chromosome number level in favour of the embryo partially restored viability.

(iv) *Abnormalities due to different chromosome numbers in the parents of a cross.*—Several cases of seed failure have been attributed to an alteration of the 2 : 3 : 2 ratio of the embryo, endosperm and maternal tissues. In general the abnormalities resulting from such matings have been more prominent when the seed-parent had a lower chromosome number, as reported by Kihara and Nishiyama (1932) in *Avena*, Wakakuwa (1934) in *Triticum* crosses, Boyes and Thompson (1937) in a wheat × rye cross and Weaver (1957, 1958) in *Gossypium*.

Theories Concerning Mechanism of Seed Failure

Stephens (1942) used the concept of "genetic strength" of a genome to explain differences in seed-setting in crosses between autotetraploid *Gossypium arboreum* and diploid *G. arboreum* and several wild species of *Gossypium*. He concluded that differences in compatibility are associated with quantitative but not qualitative differences in cytological balance between endosperm and zygote, and that the extent of differences in "strengths" between different genomes would account for the success or failure of the cross. Howard (1947) employed a similar hypothesis to explain his results on seed size in crosses between diploid and autotetraploid *Nasturtium officinale* and allotetraploid *N. uniseriatum*. Valentine (1956) has assigned genetic values to genomes of different *Primula* species, and the ratio (R) between the endosperm and maternal tissue which in normal intraspecific combinations is 3/2. The success or failure of a cross depends on the extent of deviation from the normal. R. Woodell (1960 *a*, *b*) suggests that differences in genetic values may be analogous to differences in chromosome number and the rate of growth prompted by pollen at fertilization may be dependent on genetic value. and may condition the course of seed development.

From a detailed study of fertilization and seed development in crosses between the two jute species Iyer *et al.* (1961) have suggested a nuclear-cytoplasmic disharmony as being responsible for seed abortion. The incompatibility in this cross was overcome by the method of hormone application by Islam and Rashid (1960) and by the use of reciprocally grafted parents and irradiated pollen by Swaminathan *et al.* (1961).

With regard to other techniques employed to overcome post-fertilization barriers mention must be made of embryo culture. which is now a well established field by itself and an important aid in plant breeding.

Several workers have used embryo culture in obtaining distant crosses which normally fail due to premature abortion of the hybrid embryo. Important among these are the work of Skirm (1942) who obtained several interspecific crosses in *Prunus* and *Lilium*, Keim (1953) in *Trifolium*, Davies (1960) in *Hordeum*, Brink *et al.* (1944) in the cross *Hordeum* × *Secale*, and Farquharson (1957) in *Tripsacum* × *Zea* cross.

Embryo transplantation is another interesting method tried successfully by Hall (1954, 1956) in crossing wheat with rye. The crossability of wheat with rye was enhanced by raising the wheat plants from embryos planted on rye endosperm.

Concluding Remarks

From the above review, which by no means is comprehensive, it is sufficiently clear that the detailed studies on the process of seed-development made by the embryologists and plant breeders in collaboration, have immensely enriched our knowledge concerning mechanisms of seed abortion. Success in this field of investigation largely depends on the standardization of techniques, and the skill of the worker. Though the customary methods in embryological studies are lengthy and time-taking, the availability of shorter and less cumbersome techniques such as the dissection squash technique (Morrison, 1955) and feulgen staining prior to embedding and sectioning (Newcomer, 1959), will prove extremely useful for the plant breeder for whom it would otherwise be hard to fit into his programme the lengthy processes of embryological methodology. Besides, there are more elegant techniques also available such as the radioactive tracer-method used by Linskens (1955) in *Petunia*, by Tupy' (1961 *a*, *b*) in studying changes in carbohydrate and amino acid levels in styles and ovaries of *Nicotiana* after compatible and incompatible pollinations and by Polyakov *et al.* (1957) also in *Nicotiana* crosses.

As regards the mechanism of seed abortion, no single theory can explain all the different facts observed at the histological level. With increasing work on the biochemistry of the seed and on the changes following distant crossing, it may be possible to obtain deeper insight into the observed processes in the seed. The fact that the plant breeder is now fully aware of the problems in distant hybridization in all its various aspects has served to alter the earlier belief of the embryologist that plant breeders "merely put the pollen upon the stigma and pray for results in the ovary" (Maheshwari, 1962).

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ON *MAHESHWARIELLA BICORNUTA* GEN. *ET* SP. NOV., A COMPRESSED SEED FROM LOWER GONDWANAS OF KARHARBARI COALFIELD, INDIA

By Divya Darshan Pant and Devendra Datt Nautiyal

Department of Botany, University of Allahabad, Allahabad, India

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Introduction

Compressed seeds are found abundantly in the Lower Gondwana beds of the Southern Hemisphere and India. However, the vast majority of them are known only by their superficial characters although the cuticular membranes and other structural details of a few of them have been described lately by some authors (*see* Pant and Nautiyal, 1960). The structure of a new type of Lower Gondwana seed is described hereunder.

Material and Methods

Compressions of the seed are found in grey-coloured, fine-grained shales from the Karharbari coalfield, India. The shales are literally littered with these seeds which often lie in groups. As is usual with compressions of fossil seeds, their substance is either found as a compressed film of carbon or it is mud filled. They were extracted from the rock matrix by the usual transfer and maceration methods.

All type and figured specimens of this paper have been deposited in the Divya Darshan Pant Collection of Fossils at present located in the Botany Department, Allahabad University, Allahabad, India.

Description

Maheshwariella gen. nov.

Generic diagnosis.—Compressed seeds occurring detached, or attached to naked stalks, seeds bilaterally symmetrical, orthotropus, each flat face of seed showing a longitudinal ridge, margins of seed showing a narrow border, seed with two horns on either side of micropyle and a small beak between horns, outer cuticle of integument tough, nucellar and inner cuticles of integument extending up to base of seed, inner cuticle of integument delicate, nucellar cuticle tough, pollination possibly by monosaccate pollen grains.

Type species.—Maheshwariella bicornuta n. sp.

Discussion.—The new genus is erected for some unique Lower Gondwana compressions of small seeds showing four longitudinal ridges and two horns at the micropylar end. Their finer details too are distinctive. At present the genus includes only a single species *M. bicornuta.* A few of the seeds attributed to the genus are found attached to branched or unbranched stalks but other parts of the plants which bore these seeds are unknown.

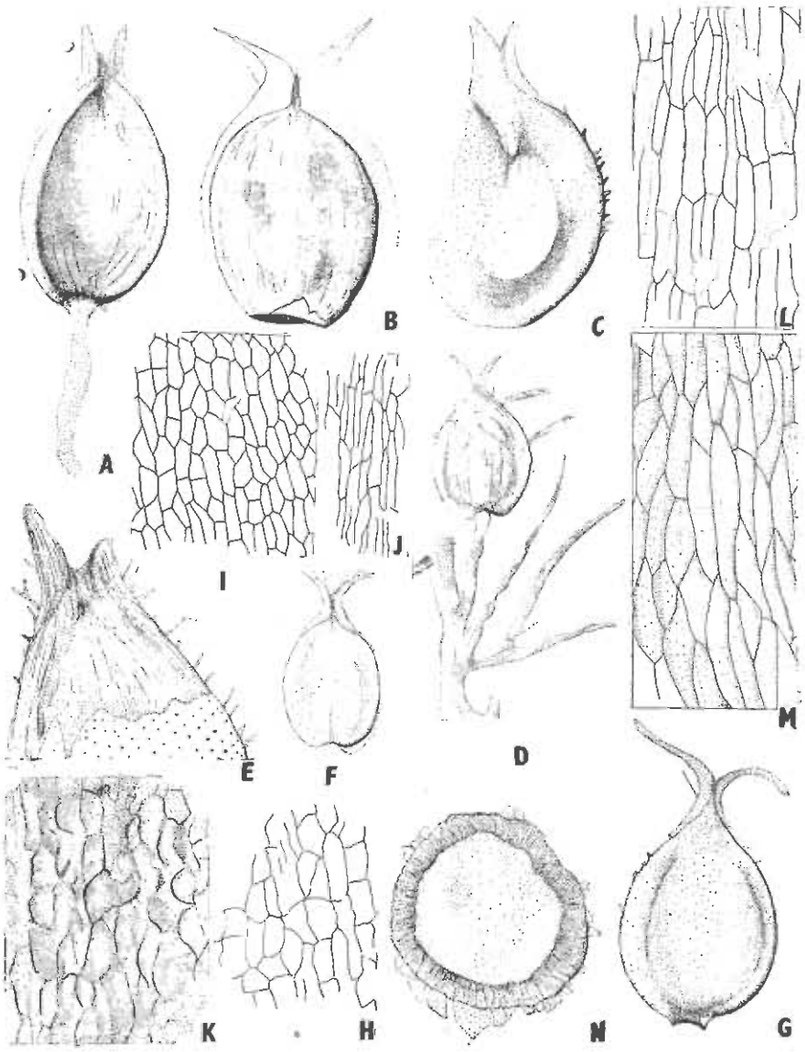
In token of our gratitude to Professor P. Maheshwari whose contributions towards the morphology of the ovule are well known we have great pleasure in naming this genus of Palaeozoic seeds in his honour on the occasion of his 60th birthday.

Maheshwariella bicornuta n. sp.

(Pls. I–III, Figs. 1–14; Text-Fig. 1, A–N)

Diagnosis.—Seed, flattened, orthotropus, averaging at 4·3 × 2·5 mm., broadly oval, micropylar end gradually tapering, chalazal end obtusely rounded, sometimes found attached terminally on slender branched or unbranched stalks, stalk up to 444 μ thick. Seed often showing a single median ridge, about 178 μ high, margins of seed usually showing remnants or marks of a narrow border up to 266 μ wide, border often becoming somewhat wider at chalazal end and usually extending into two prominent horns on either side of micropyle, horns straight or recurved, length of horns averaging at 755 μ, their width at base* being 244 μ; distance between apices of horns averaging at 781 μ, a small micropylar beak often present between bases of horns, beak 550 μ long × 115 μ wide, margin of seed and outer side of horns often showing small hair-like tapering processes, processes at margin of seed up to 222 μ long, processes on outer side of horn longer, up to 448 μ long, tips of processes simple and rounded or sometimes bifid. Surface of seed showing more or less longitudinally arranged short or elongated polygonal cells, averaging at 82 × 35 μ in size, epidermal cells tending to become narrow and sinuous walled over margins or border, average size of marginal cells 121 × 26 μ, elsewhere cell walls arched or almost straight, surface of seed-stalks showing polygonal cells like those on surface of seed but cells over stalk usually narrow and longitudinally elongated. Maceration of seed substance yielding three resistant membranes—outer cuticle of integument tough, up to 5 μ thick, granular in texture, cells of outer cuticle like those on seed surface, stone of integument fibrous, inner cuticle of integument smooth, delicate, about 2 μ thick, showing short and broad cells, 99 × 43 μ, cells towards margins relatively narrow and elongated. Nucellar cuticle tough up to 5 μ thick, showing longitudinally elongated polygonal cell outlines all over but cells towards tip of nucellus shorter, sides of cells arched or usually sinuous in the region of pollen chamber, cells 52 ×

* The base of a horn is measured at the level where the two horns become joined.



TEXT-FIG. 1, A–N. *Maheshwariella bicornuta* gen. *et* sp. nov. Figs. A–G. Compressed seeds. The seed in A shows almost straight micropylar horns and a long slender stalk at the chalazal end (*see* also Pl. I, Fig. 3), Seeds in B–E and G show processes at the margins. Note the fibre-like processes on the outer side of horns in Figs. B, G and on the sides of the body in C, E, G. The seed in Fig. G is attached terminally to a fork of a dichotomising axis. Fig. F. Seed showing a thin film of carbon between two horns (shaded by dots). A–F. Specimen Nos. 963 A, Selected holotype 964, 965, Syntype 963 B, 966 and 967 respectively, A–C, G, × 10; D, F, × 6; E, × 16. Fig. H. Surface cells of the seed. Specimen No. 964. Fig. I. Cells

of the outer cuticle of integument. Slide 968 B. Fig. J. Surface cells of horn-like structure of the seed in Fig. B. Fig. K. Cells of inner cuticle of integument (*see* also Pl. III, Fig. 12). Slide No. 968 A. Fig. L. Cells of the nucellar cuticle (*see* also Pl. III, Fig. 13). Slide No. 969. Fig. M. Surface cells of a seed whose integument is not preserved. Specimen No. 976. H–M, ×75. Fig. N. A monosaccate pollen grain recovered by the maceration of the seed substance from the micropylar end (*see* also Pl. II, Fig. 9). Slide No. 971 A, ×270.

14 μ in size, megaspore membrane not seen, chalazal hole up to 977 μ wide, pollination seemingly by one-winged pollen grains, size of pollen grains averaging at 166×118 μ.

Selected holotype.—Pant collection specimen No. 964.

Syntype.—Pant collection specimen No. 963 B.

Horizon and Locality.—Karharbari Stage, Karharbari coalfield, India.

Description and discussion.—Over two hundred seeds, all flattened along the principal plane have been assigned to the present species. Out of the total number, forty-four seeds show a partially or completely preserved border and horns. The size of seeds with intact horns and border varies between 4–5 mm (standard deviation or $\delta = \cdot 38$ mm.) in length and 2–3 mm. ($\delta = \cdot 25$ mm.) in width. The remaining seeds which have lost most of their border and horns have been assigned to this species because their body size and finer details are identical. The two horns may be straight or their tapering ends are recurved. They are usually free from the base but a single specimen shows a thin film of carbon between them (*see* Text-Fig. 1, F). This, however, may be extraneous but if it represents a tissue which originally joined the horns the seed may have had a funnel like that of *Stephanostoma crystallinum* at the micropylar end (Pant and Nautiyal, 1960). Sometimes a seed may show an obscure impression of the border and such seeds often show marginal processes at places covered by the border (*see* Text-Fig. 1, G) but at other points freely exposed (*see* Text-Fig. 1, C, E, G). We believe that the processes either represent strengthening fibres originally embedded in the tissue of the border and subsequently exposed by the decay of a soft outer tissue which is seen as a compression border or they may represent free marginal growths attached by the side of the border if it were of the nature of a narrow marginal ridge. Similar but finer processes are also sometimes seen along the outer margins of the horns (*see* Pl. I, Fig. 2; Text-Fig. 1, B, E, G). These too may represent stiff superficial hair-like growths or they also could be regarded as fibres originally embedded in a surrounding soft flesh but not a single specimen in our two hundred and odd seeds shows any trace of that tissue along the horns. The processes gradually taper towards their simple rounded ends or are occasionally lobed (*see* Text-Fig. 1, E). Seeds with well-preserved micropylar ends show a small slender beak between the two horns. This may represent a micropylar beak of the integument or a projecting nucellus. Attached seeds show a slender stalk up to 3 mm. long and 0·444 mm. thick. The stalk is somewhat broadened out at the base of the seed. A single specimen

shows a seed attached terminally to a fork of a dichotomising axis (*see* Pl. I, Fig. 4; Text-Fig. 1, D). Maceration of the carbonaceous substance of all completely preserved seeds yields fibres from the integument which come off and dissolve in the alkali and three resistant membranes. The presence of a tough outer cuticle in the seed indicates that it was freely exposed and the presence of a tough nucellar cuticle closely appressed to a delicate inner cuticle of integument and both extending up to the base of the seed, possibly suggest that the nucellus was free from the integument. The cells at the micropylar end of the nucellar membrane are relatively short and narrow and their anticlinal walls are arched or sinuous. Their structure and arrangement is in general similar to that of the pollen chamber walls of some other Lower Gondwana seeds, *e.g.*, *Semenites*, *Stephanostoma*, *Pterygospermum* and *Platycardia* (*see* Pant, 1958; Pant and Nautiyal, 1960) and it is possible that the nucellus of *M. bicornuta* too had an excavated pollen chamber of the same type. None of our preparations show the presence of a megaspore membrane.

Maceration of the seed substance from the micropylar end of a seed yielded about eight one-winged pollen grains, all of the same type. The spores are firmly attached to a delicate overlapping granular membrane which may represent the inner cuticle of the pollen chamber wall (*see* Pl. II, Fig. 9; Text-Fig. 1, N). The size of the pollen grains ranges between 130–223 $\mu \times 90$–143 μ while the central body shows a range of size between 100–116 $\mu \times 50$–103 μ. The width of the wing is about 31 μ. The wing is granular, it takes a darker stain and shows fine radiating striations from the sides of the central body. The central body is hyaline and often shows a few small thin-walled cells. The general form of the pollen grains is of *Perisaccus* Naum. type.

The surface characters of the seeds vary according to the quality of their preservation. Where the surface of the seed is intact it shows longitudinal rows of elongated polygonal cells like those seen in their outer cuticle. Other seeds which lack this superficial layer show a surface marked by numerous fine longitudinal lines representing fibres of the seed coat. The substance of these seeds yields no outer cuticle but only the inner cuticle of integument and the nucellar membrane. Still other seeds show a surface marked by cell outlines like those of the nucellar cuticle and these yield only a single membrane showing cell outlines like those of their surface. We regard that this third type of seeds have lost the integument completely.

Any fractured face of the shales usually shows a number of seeds assignable to *M. bicornuta* and numerous branched naked axes resembling their stalks. Their occurrence in groups and a single specimen showing an attached repeatedly forked stalk suggest that they were originally borne in bunches. The only other recognizable fossils occurring with the seeds are some short linear conifer-like leaves and rare specimens of another seed which will be described elsewhere. The association of leaves and seeds in the rock may be significant but at present there is nothing else to confirm this.

Comparison.—The most striking external features of *M. bicornuta* include a narrow border, two micropylar horns in the principal plane and a median ridge on either face of the seed. A comparison of this seed with other similar-looking and similar-sized previously described Lower Gondwana seeds reveals that it is different from all of them. In having a "V"-shaped sinus at the apex it resembles *Samaropsis goraiensis* (Surange and Lele, 1956) but there is only a narrow border in *M. bicornuta* and it shows two distinct micropylar horns. The seed of *S. goraiensis* is distinctly winged and shows no trace of the micropylar horns. *M. bicornuta* may also be compared with *Cordaicarpus furcata* (Surange and Lele, 1956) which apparently shows two similar but short blunt growths. However, *C. furcata* reportedly lacks a border (although at one place the authors admit that a faintly visible border may be present) and the horns of *M. bicornuta* are much longer and tapering. In possessing a marginal border *M. bicornuta* presumably resembles some seeds from the Punjab Salt Range attributed by Virkki (1938) to *Cordaicarpus* but the latter clearly differ from *M. bicornuta* in the absence of micropylar horns. Among all previously described Lower Gondwana seeds the form of *M. bicornuta* comes closest to that of an African seed called *Cornucarpus* sp. by Du Toit (1932). The narrow wing of *Cornucarpus* sp. is, however, described as "all but absent towards the rounded base" while the border of *M. bicornuta* widens out at the base. The margin of the African seed is smooth (in *M. bicornuta* it often shows fibrous processes). Moreover, a detailed comparison of *M. bicornuta* and these various Lower Gondwana seeds is not possible because the finer details of all these previously described seeds are unknown.

In possessing four surface ridges *M. bicornuta* resembles *Semenites* (*Spermatites*) *tetrapterus* (Pant) Pant and Nautiyal.

A single specimen (Pl. I, Fig. 4; Text-Fig. 1, D) showing a seed attached terminally to a dichotomizing axis suggests its comparison with some branched seed bearing fructifications described by White (1908) and Lundqvist (1919) as *Arberia minasica* and *Arberia* (?) *brasiliensis*, respectively. *Arberia* (?) *brasiliensis* even shows attached seeds of *Cordaicarpus* type but the seed stalks in the Brazilian fructifications are recurved and none of their seeds shows micropylar horns like those of *M. bicornuta*. A closer comparison is not possible because the finer details of the seeds in *Arberia* are unknown.

We have also compared *M. bicornuta* with some northern Palaeozoic seeds with converging or diverging micropylar horns, *e.g.*, *Comptosperma berniciense* Long, *Cardiocarpus* (*Ptilocarpus*) *bicornutus* Lesquereux, *Deltasperma fouldenense* Long, *Eccroustosperma langtonense* Long, *Lyrasperma scotica* Long and its possible compressed form *Samaropsis* (*Cardiocarpus*) *bicaudatus* Kidston (*see* Long, 1960). Many of them are within the same size range as *M. bicornuta* but they are all distinct from the latter in various structural details (*see* Long, 1960, 1961 *a*, 1961 *b*). Moreover, it is usually not possible to compare the structure of a compressed seed with the multifarious details available

in a petrified form. Apparently they also resemble some compressed seeds attributed to *Stephanospermum akenioides* Brongniart (*see* Langford, 1958, Fig. 631, p. 329) but any detailed comparison with this northern genus is out of question.

Seeds of *Nystroemia pectiniformis* Halle (1929) described from the Permian beds of China also seem to have two micropylar horns but the figures of its seeds available to us show no trace of a border. We have no access to the type material of *Nystroemia* for a first-hand comparison and a closer comparison is impossible because the finer details of the Chinese seeds are unknown.

In having bifid micropyles, seeds of *M. bicornuta* may as well be compared with some compressed Mesozoic seeds, *e.g.*, *Umkomasia*, *Pilophorosperma* (Thomas, 1933) and *Lepidopteris* (Harris, 1932) but their micropylar processes are recurved asymmetrically on one side and their finer details too are different. In addition it seems quite unlikely that *M. bicornuta* belongs to any plants which may be similar or related to such Mesozoic forms.

SUMMARY

The structure of some detached and attached seed compressions from Lower Gondwanas of the Karharbari Coalfield, India is described under the name *Maheshwariella bicornuta* gen. *et* sp. nov. The seed is characterized by showing a narrow marginal border, marginal processes and two micropylar horns. Maceration of the seed substance yields an outer and an inner cuticle of integument and a tough nucellar membrane all extending almost up to the chalazal hole. The pollination of the seed is seemingly by one-winged pollen grains.

ACKNOWLEDGEMENTS

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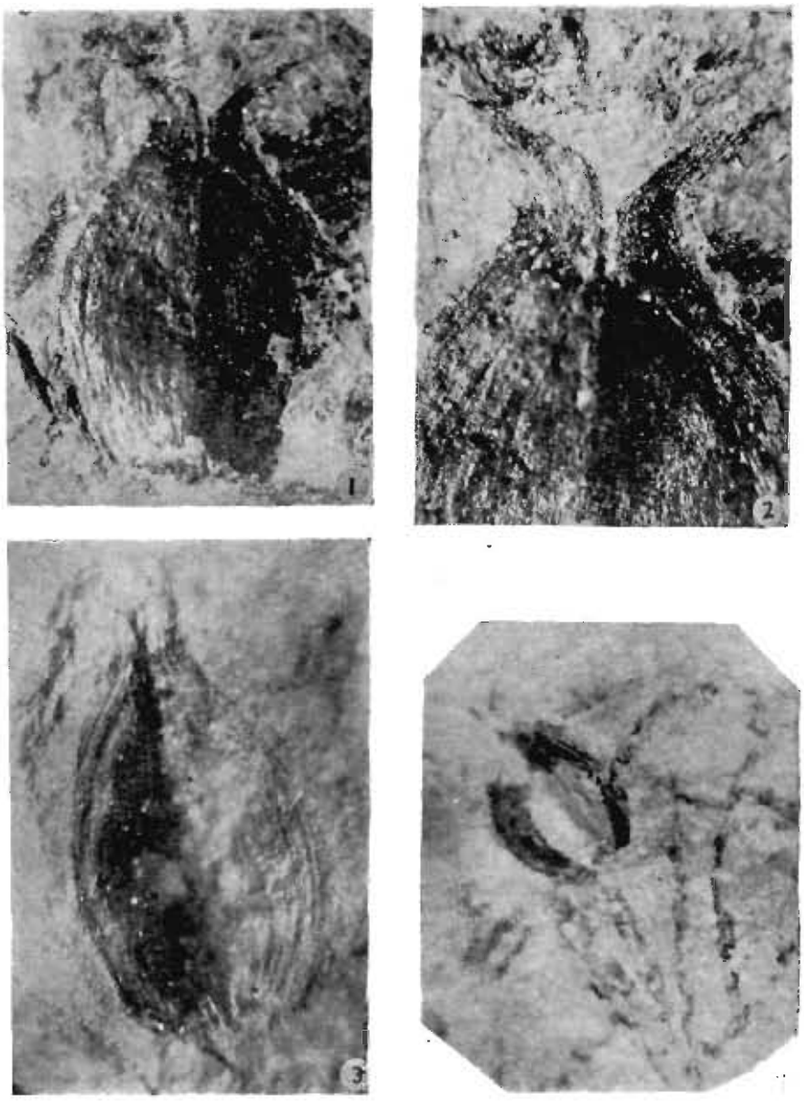
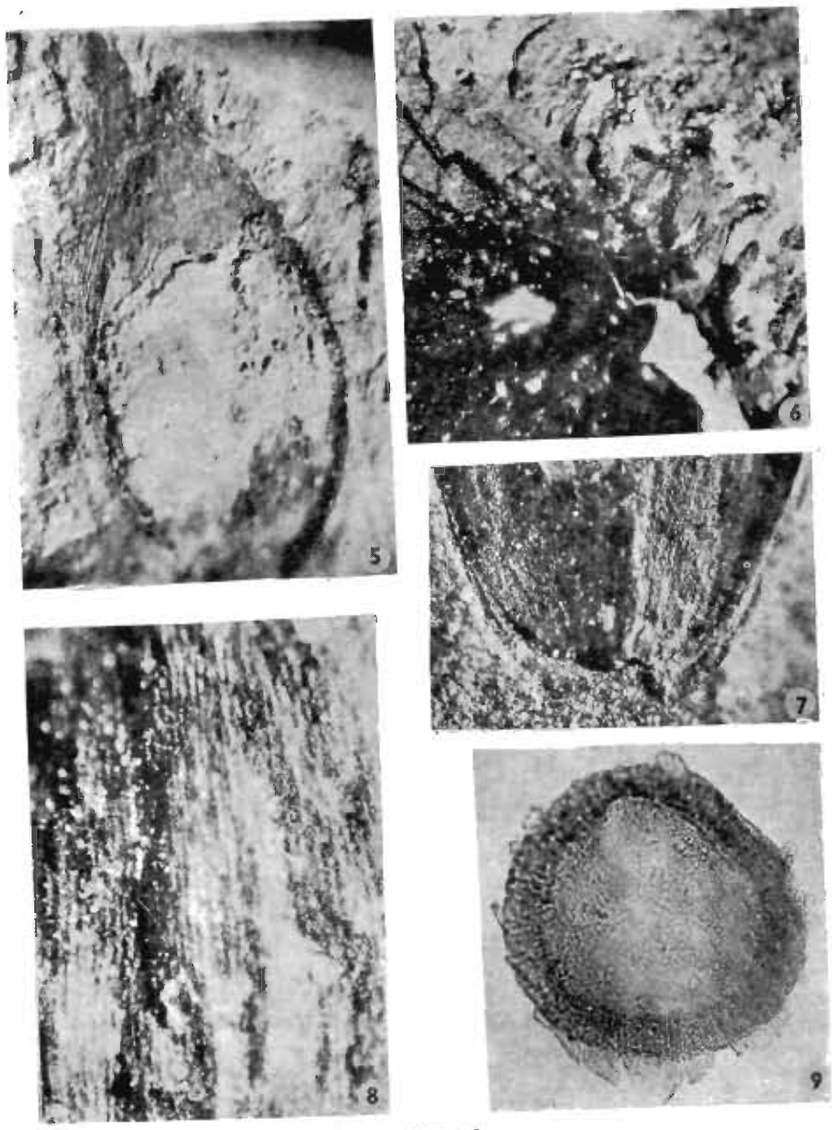
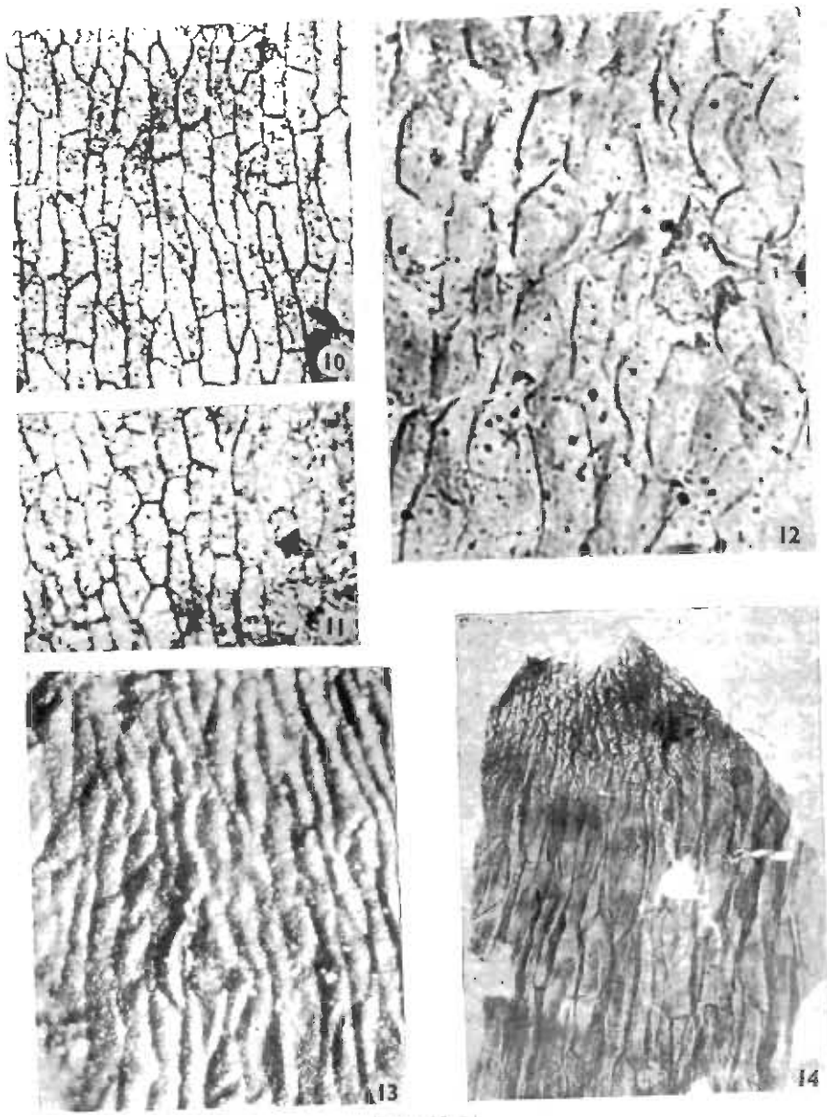


FIGS. 1-4



FIGS. 5-9



FIGS. 10–14

LONG, A. G. 1961 *a*. On the structure of "*Deltasperma fouldenense*" gen. *et* sp. nov. and "*Camptosperma berniciense*" gen. *et* sp. nov., petrified seeds from the Calciferous Sandstone Series of Berwickshire. *Trans. roy. Soc. Edinb.* **64**: 281–95.

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EXPLANATION OF PLATES I–III

PLATE I

Maheshwariella bicornuta gen. *et* sp. nov.

FIG. 1. Seed showing a narrow border and two diverging horn-like structures at the apex. Note the characteristic fine longitudinal marks representing longitudinal rows of surface cells. Selected Holotype-Specimen No. 964, ×18.

FIG. 2. Micropylar end of seed in Fig. 1. more magnified, ×31.

FIG. 3. Seed showing straight micropylar horns and chalazal hole. Specimen No. 963 A, ×18.

FIG. 4. A forking axis showing seed attached to a slender branch. Syntype specimen No. 963 B, ×9.

PLATE II

M. bicornuta gen. et sp. nov.

FIG. 5. Seed showing processes at margins. Specimen No. 966, ×18.

FIG. 6. Processes of a seed more magnified. Slide No. 972, ×51.

FIG. 7. Chalazal end of a seed showing a continued border. Slide No. 973 A, ×26.

FIG. 8. Longitudinal rows of surface cells of a seed. Specimen No. 974, ×80.

FIG. 9. A monosaccate pollen grain recovered by maceration of the seed substance from the micropylar end. Note the adhering membrane. Slide No. 971 A, ×500.

PLATE III

M. bicornuta gen. *et* sp. nov.

FIG. 10. Narrow elongated cells of outer cuticle of integument. Slide No. 975, ×200.

FIG. 11. Short and relatively broad cells of the outer cuticle of integument. Slide No. 968 B, ×200.

FIG. 12. Cells of inner cuticle of integument. Slide No. 968 A, ×200.

FIG. 13. Nucellar cells from the surface of a seed whose integument is not preserved. Specimen No. 976, ×135.

FIG. 14. Nucellar cuticle of a seed showing short cells towards the micropylar end. Slide No. 969, ×150.

THE DEVELOPMENT OF THE SEXUAL ORGANS AND THE CYSTOCARP IN *TAENIOMA PERPUSILLUM**

BY GEORGE F. PAPENFUSS

University of California, Berkeley, Calif., U.S.A.

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IN a previous paper (1944), I described the structure of the thallus and the development of the tetrasporangia in *Taenioma perpusillum*. Sexual plants, which have rarely been encountered, were not available to me at that time. Until recently spermatangia had been recorded for the genus only by Schmitz and Hauptfleisch (1896) and Thompson (1910), and cystocarps, with certainty, only by Thompson (1910).

In 1950 Dickinson and Foote recorded female plants of *Taenioma perpusillum* from Ghana. Lawson (1960) reported male plants of *T. nanum* from Sierra Leone and both male and female plants of *T. perpusillum* from Ghana. Thompson described the development of the spermatangia and also included observations on the gross structure of the cystocarp. Her account of the ontogeny of the spermatangia does not seem to be entirely correct, however, in the light of subsequent studies of the development of these structures in other Delesseriaceae. The development of the procarp and cystocarp has not been described.

Some of the gaps in our knowledge of this interesting genus can now be filled, thanks to the receipt of formalin-preserved sexual plants collected in Ghana by Miss Foote. Some of the material (*Foote 557*) was collected at Sekondi on 11 April 1949 and contained both male and female plants. (Material of this collection I received from both Miss Foote and Miss Dickinson.) Other specimens (*Foote 791*), including female, male and tetrasporangial plants, were collected on 3 April 1950 at Prampram. (Material of this collection was received only from Miss Foote.)

Taenioma as presently understood includes two species: *T. perpusillum* (J. Agardh) J. Agardh (1863), the type of the genus, which usually has three terminal hairs on the determinate branches but sometimes one or two (Desikachary and Balakrishnan, 1957; Lawson,

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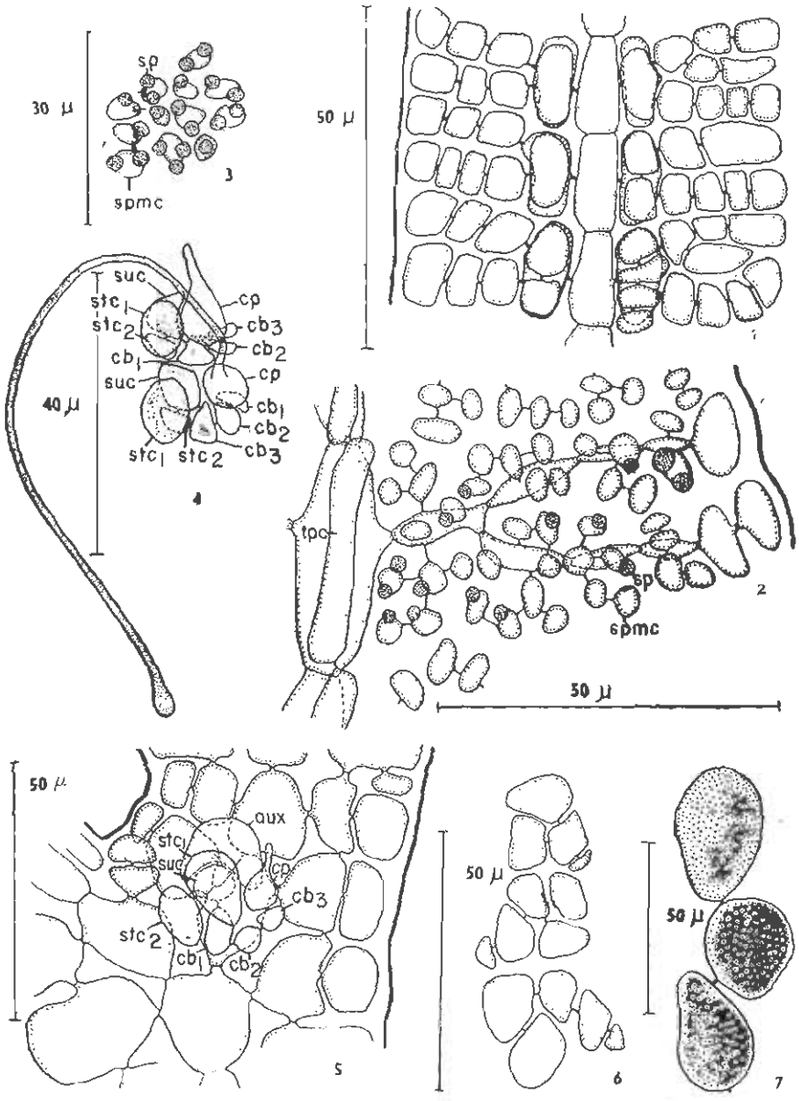


TEXT-FIGS. 1–7. *Taenioma perpusillum.* Figs. 1–3. Stages in the development of spermatangia. In Fig. 1 the transverse pericentral cells are not shown. Fig. 4. Two procarps before fertilization has occurred; the trichogyne in the upper (younger) carpogonium is in an early stage of development. The central cells of the two fertile segments are not shown. Fig. 5. Procarp with an auxiliary cell. Fig. 6. Division of pericentral cells on surface opposite the procarp, forming a cortical tissue; the middle group of cells represents a pericentral cell of the fertile

segment. Fig. 7. A short chain of carposporangia. (*tpc*, transverse pericentral cell; *corc*, cortical cell; *spmc*, spermatangial mother cell; *sp*, spermatangium; *suc*, supporting cell; stc_1, mother cell of sterile cell group one; stc_2, mother cell of sterile cell group two; cb_1, cb_2, cb_3, first, second and third cell of carpogonial branch, respectively; *cp*, carpogonium; *aux*, auxiliary cell.)

1960), and *T. nanum* (Kützing) Papenfuss (1952), which has two terminal hairs on the determinate branches (Tseng. 1944; Papenfuss, 1944; Desikachary and Balakrishnan, 1957; Lawson, 1960). The Ghanaian material used in the present study belongs to *T. perpusillum.*

Development of Spermatangia

The sexual plants are usually dioecious, but in rare instances predominantly female plants also produce spermatangia. I did not see spermatangia (or procarps) and sporangia on the same plant as Lawson (1960, p. 365, foot note) believed he saw in material of *Taenioma nanum* from Tangier.

It will be recalled that in *Taenioma* the erect parts of the thallus are composed of two kinds of branches: (1) terete indeterminate branches, in which each segment of the apical cell becomes divided into a central (axial) cell and four pericentral cells, and (2) flat determinate branches (ending in hairs), in which each segment becomes divided into a central cell, four pericentral cells, and four flanking cells, two on the marginal face of each of the two lateral pericentral cells. The axial (midrib) region of these branches is thus three cells thick, whereas the marginal wings, each two cells wide, are monostromatic.

The spermatangia are produced on both surfaces of the determinate branches, as is known from the observations of Schmitz and Hauptfleisch (1896), Thompson (1910, Fig. 15) and Lawson (1960, Pl. II, Fig. *d*). Thompson described the development of the blade in preparation for spermatangium formation, but her account is not precise and is partly incorrect.

A preliminary step in the formation of spermatangia is an increase in width of the determinate branches that are destined to become fertile. This is brought about by division of the flanking cells in typical delesseriaceous fashion to form cell rows of the second order and an occasional cell row of the third order (Text-Fig. 1, upper right). The blade thus becomes 9–12 cells wide (Text-Fig. 1). Next, starting with the lateral pericentral cells, the primary cells of the wings of the determinate branches (except the marginal cells and an occasional submarginal cell) divide to cut out cortical cells. These cells are cut out in one of two ways: (1) They may divide periclinally twice to cut out a cortical cell on each surface (Text-Fig. 1, upper end). The cortical cells then divide by anticlinal walls to produce spermatangial mother cells. (2) Or, they may cut out by concave walls two, three, or more cortical cells on each surface. The cortical cells themselves may function as spermatangial mother cells or they may divide by anticlinal

walls to form two or more spermatangial mother cells. The sterile marginal cells form a border on the sides of the soral area (Text-Fig. 2). Each spermatangial mother cell produces from one to three protuberances toward the surface; these are cut out by concave walls and develop into spermatangia (Text-Figs. 2, 3). The primary (innermost) cells of the fertile areas are greatly stretched during the maturation of the sorus (Text-Fig. 2).

Not infrequently the transverse pericentral cells on one surface or on both surfaces of a fertile blade also become subdivided into spermatangial mother cells.

Development of the Procarp and Cystocarp

Contrary to what I had expected to find the procarps in *Taenioma* are not produced on the flat determinate branches (blades), as are the spermatangia and tetrasporangia, but on the terete indeterminate branches. Thompson had noted that the cystocarps occur on the terete branches, but the material available to her did not allow study of the stages leading up to the cystocarp, and in my previous paper on *Taenioma* I had expressed the view that the cystocarps might have been initiated on young determinate branches and that such branches failed to complete their usual development into flat branches. This is not the case, however.

The carpogonial branches are produced on the younger parts of the erect indeterminate branches, but, to judge from the early stages observed, it seems that they are initiated some distance back of the tip of a branch: proximal to the place of insertion of at least one determinate branch. The statement by Kylin (1956) that the cystocarps are borne on the prostrate filaments is the result of incorrect interpretation of Thompson's illustration (Text-Fig. 20) of a cystocarp-bearing axis.

The procarp is initiated by a pericentral cell, but, as it was not possible to determine which of the pericentral cells of the terete branches are homologous to the transverse pericentral cells of the flat branches, it cannot be stated whether the carpogonial branches are produced by transverse pericentral cells, as they are in other Delesseriaceae. However, as in other Delesseriaceae, the fertile segments have only four pericentral cells. (It will be recalled that in the Rhodomelaceae, the only family outside the Delesseriaceae with whose genera *Taenioma* needs to be compared, the procarp-producing segments always form five pericentral cells, irrespective of the number of pericentral cells present in vegetative parts of the thallus, except in *Laurencia*, in which the procarp-bearing segments also form four pericentral cells.)

As in *Claudea* and *Vanvoorstia* (Papenfuss, 1937), *Platysiphonia* (Silva and Cleary, 1954; Womersley and Shepley, 1959), *Sarcomenia* and *Sarcotrichia* (Womersley and Shepley, 1959), and *Caloglossa*

(Papenfuss, 1961), only one pericentral cell in a segment initiates a procarp. Usually, the procarps are not produced in longitudinal series as they are in other Delesserioideae, although a few tips were observed in which two successive segments had each formed a procarp (Text-Fig. 4).

The earliest stages in the development of the procarp were not observed. To judge from the later stages seen, it would seem that the sequence of events corresponds to those in other Delesseriaceae. In Text-Fig. 4 are shown two young procarps, each consisting of a supporting cell (the original fertile pericentral cell), a 4-celled carpogonial branch, mother cell of sterile-cell group one, and mother cell of sterile-cell group two. This is the full complement of cells in the procarp prior to fertilization. In one of the young procarps observed, the mother cell of sterile group two had been cut out from the supporting cell while the carpogonial branch was still in the 3-cell stage. Whether this cell is always formed before the carpogonial branch has completed its development is not known.

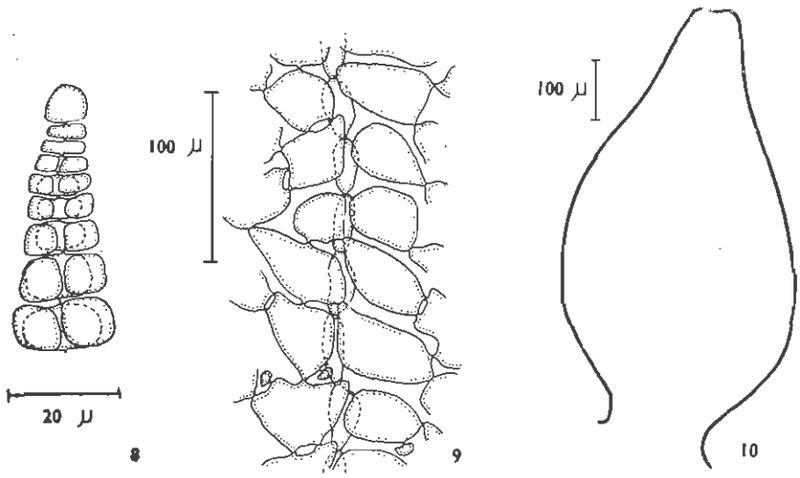
Following fertilization of the carpogonium, the supporting cell enlarges and cuts off an auxiliary cell from its distal end (Text-Fig. 5). The connection between the carpogonium and the auxiliary cell was not observed and it is unknown, therefore, whether the carpogonium forms a connecting cell, as it does in *Caloglossa* (Papenfuss, 1961).

Early in the development of the procarp, the pericentral cells to the left and right of the supporting cell and those in comparable positions in the segments above and below the fertile segment each divide once by a longitudinal wall (Text-Fig. 5). In procarpic areas the branches are thus five cells broad. The pericentral cells on the opposite surface undergo anticlinal division, producing a cortical tissue (Text-Fig. 6).

Young stages in the development of the gonimoblast were not observed. In a well-advanced young cystocarp four sterile cells, two in each group, were seen. It seems doubtful that more than four sterile cells are produced, and it is likely that at times fewer than four may be formed. In *Caloglossa leprieurii* four apparently is also the maximum number of sterile cells produced (Papenfuss, 1961).

During the development of the gonimoblast, a small fusion cell is produced at its base. This cell is formed by the fusion of the central cell of the fertile segment, the supporting cell, the auxiliary cell, and a few of the basal cells of the gonimoblast. The gonimoblast, in usual delesseriaceous fashion, consists of filaments of cells radiating from the fusion cell The carposporangia are produced in chains (Text-Fig. 7). The statement by Kylin (1956) that they occur singly is taken from Thompson, whose casual remark that the carposporangia are formed at the tips of the gonimoblast filaments was conducive to erroneous interpretation.

The wall of the cystocarp is produced by a series of filaments that issue from vegetative cells in the vicinity of the supporting cell. In a



TEXT-FIGS. 8–10. *Taenioma perpusillum*. Fig. 8. Terminal end of young pericarpic filament, showing division of segment cells to form two cortical (pericentral) cells on external face. Fig. 9. Central region of mature pericarpic filament, showing axial and cortical cells. Fig. 10. Outline of cystocarp.

manner similar to that described and illustrated by Silva and Cleary (1954) for *Platysiphonia parva*, these filaments grow by means of a transversely dividing apical cell, forming rib-like axes that over-arch developing gonimoblast. The segment cells thus produced soon cut out, by concave walls, two cortical (pericentral) cells on their external face (Text-Fig. 8). During further growth of the cystocarp, the segment (now axial) cells of the filaments are stretched while the cortical cells enlarge considerably and become joined by secondary pit-connections to neighbouring cortical cells, forming a coherent investment around the gonimoblast (Text-Fig. 9). In a mature cystocarp, the pericarp thus consists of a single layer of cells, except along the axial regions of the pericarpic filaments, where it is 2-layered (Text-Fig. 9). The ascending pericarpic filaments do not meet at the summit, leaving an opening which is the ostiole of the cystocarp. The mature cystocarp is urceolate in shape (Text-Fig. 10).

DISCUSSION

In a recent paper on *Caloglossa* (1961), I discussed the systematic position of *Taenioma* and related genera, concluding that they belonged in the Delesseriaceae rather than in the Rhodomelaceae. In that paper I also removed *Taenioma* from the *Sarcomenia* group of the Delesseriaceae to the *Caloglossa* group on the basis of three characters shared by *Caloglossa* and *Taenioma*: both genera show exogenous branching, alternate branching, and monopodial growth.

The present study did not bring to light features that would speak against allying these two genera. On the contrary, a seemingly important additional feature of agreement has come to light: in *Taenioma* as in *Caloglossa* the carposporangia are formed in chains. A perhaps unimportant feature of agreement also lies in the low number of sterile cells produced in the procarps of both genera.

An unexpected feature of *Taenioma* is the formation of procarps on the terete indeterminate axes instead of on the flat determinate branches, on which the spermatangia and tetrasporangia are produced. It is of interest to note in this connection that the pericentral cells lateral to the supporting cell and the comparable pericentral cells in the segments above and below the procarp-bearing segment divide by a longitudinal wall during the development of the procarp, causing the axis to become five cells broad in procarpic regions.

Summary

The development of the sexual organs and the cystocarp of *Taenioma perpusillum* from Ghana is described and illustrated. The plants are usually dioecious, but spermatangia are at times produced on predominantly female plants. The spermatangia are formed on the flat determinate branches. Their development in general agrees with that in related genera. The procarps are produced on the terete indeterminate branches. Only one of the four pericentral cells in a segment forms a procarp. The development of the procarp conforms to that in other Delesseriaceae. As in *Caloglossa* the carposporangia are formed in chains, a feature which lends further support to the belief that *Taenioma* and *Caloglossa* are somewhat closely related.

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HABIT IN RELATION TO AGE IN NEW ZEALAND TREES

BY W. R. PHILIPSON

Department of Botany, University of Canterbury, Christchurch New Zealand

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MORPHOLOGICAL differences between young and older trees are well known. These differences are usually slight, so that trees in general show a gradual development of the adult form from that of the sapling. However, the juvenile and adult stages of a tree may be so distinct that their specific identity cannot be recognized without developmental evidence. The term habit-heteroblastism is applied here to an abrupt break in the development of the habit of a plant.

The present brief account of habit-heteroblastism in New Zealand trees will be concerned mainly with one type of transformation. That is, from a much-branched shrub to a tree with single trunk (Pl. I, Figs. 1, 2). There are 11 trees, in New Zealand, with a marked juvenile divaricate-shrub stage in their life-cycles: these belong to nine genera in eight unrelated families, which fall within both the dicotyledons and the gymnosperms (Table I). Possibly the only examples of this type of development recorded outside New Zealand are two trees in Queensland belonging to a family, the Rutaceae, which strangely enough has no habit-heteroblastic representative in New Zealand (Rumball, 1961). Other types of habit-heteroblastism occur, both in New Zealand and elsewhere. The most familiar example is the lancewood, *Pseudopanax crassifolium*. These curious life-cycles have long been familiar to New Zealand botanists (Hooker, 1853), and the main facts are known to morphologists generally. Although much has been written on the subject, especially by Cockayne (1899–1928), most references are in old New Zealand journals. Even in this country no general review has appeared. It is hoped that the present account may prove of interest to overseas botanists even though it contains little new information.

Of the 26 species of *Pittosporum* included in the *Flora of New Zealand* (Allan, 1961) half are trees, none of large stature, and half are shrubs. Of these, about six have more or less densely interlaced twigs similar to the divaricate juvenile phase of *P. turneri*. In all the 11 trees listed in Table I leaf-shape as well as habit is very different in the juvenile and adult phases. Moreover, in *P. turneri* there are two distinctive leaf-shapes mingled together on the juvenile twigs. This feature is shared by some shrubby species of *Pittosporum* and also by

TABLE I

Trees with a shrubby juvenile phase and related species

Habit-heteroblastic trees	Divaricating shrubs	Non-heteroblastic trees
Pittosporum turneri	*P. divaricatum*, etc.	*P. eugenioides*, etc.
Elaeocarpus hookerianus	None	*E. dentatus*
Plagianthus betulinus	*P. divaricatus*	*P. betulinus* var. *chathamica*
Hoheria populnea, *Hoheria sexstylosa*, *Hoheria angustifolia*	None	*H. lyallii* and *H. glabrata*
Carpodetus serratus	None	None
Sophora microphylla	*S. prostrata*	*S. tetraptera*, *S. microphylla* var. *longicarinata*, *S. microphylla* var. *fulvida*
Paratrophis microphylla	None	*P. banksii*
Pennantia corymbosa	None	None
Podocarpus spicatus	None	*P. totara*, etc.

the superficially very similar juvenile stage of *Elaeocarpus hookerianus* (Pl. I, Fig. 3).

Fully mature specimens of *Elaeocarpus hookerianus* are tall round-topped canopy trees with a strong straight trunk (Pl. I, Fig. 2). Lateral shoots which arise from the trunk frequently exhibit the habit and leaf characters of the juvenile. These reversion shoots occur far above the height ever attained by the original juvenile shrub. The only other species of *Elaeocarpus* in New Zealand, *E. dentatus*, has no shrubby stage in its development.

The New Zealand woody members of the Malvaceae exhibit an interesting range of habit-forms. The two mountain species of *Hoheria*, *H. lyallii* and *H. glabrata*, are small deciduous trees without habit-heteroblastism but with a rather gradual transition in leaf-form from young to older individuals. *H. populnea*, *sensu lato*, comprises a number of forms some of which do not pass through a divaricate juvenile form, while in others metamorphosis is well defined. The remaining two species, *H. sexstylosa* and *H. angustifolia*, both have a well-marked

juvenile shrubby phase, with markedly heteroblastic leaf development. The juvenile shrub of *H. angustifolia* consists of a number of rather strong upright shoots bearing long whip-like lateral branches, horizontal or deflexed, which become closely interwoven. When the shrubs attain a height of about ten feet, the laterals become arched-erect and the leaf-shape shows transition towards the adult form. In this way a fastigiate small tree is formed with one, or sometimes a few, trunks still clothed with the persistent interlaced juvenile lateral branches. The fully mature tree loses the fastigiate habit, having a spreading crown with several strong branches.

The second Malvaceous genus, *Plagianthus*, has a wider range of habit forms. One species, *P. divaricatus*, is a low shrub bordering salt marshes. The Chatham Island form of *P. betulinus* is an erect tree with no shrubby juvenile phase. On the two main islands this species passes through a juvenile phase which varies from a shrub with densely interwoven branches very similar to that of *H. angustifolia*, to a sapling with a single strong leader whose side branches show more or less divarication and interlacing. It is not known to what extent this variation is phenotypic or genotypic. Progenies of natural populations showing different degrees of habit-heteroblastism are being reared in uniform conditions.

Corpodetus serratus (Escalloniaceae) and *Pennantia corymbosa* (Icacinaceae) are the only members of their genera represented in New Zealand. Both are medium-sized trees. The juvenile stage of *Carpodetus* consists of zig-zag branches rather loosely entangled. A more definite break occurs between the juvenile densely entangled shrub and the adult tree of *Pennantia*.

The large family Moraceae is represented in New Zealand by three species of *Paratrophis*. *P. smithii* is a small tree confined to the Three Kings Islands. *P. banksii* and *P. microphylla* both show leaf-heteroblastism but marked habit changes during development appear to be confined to *P. microphylla*. The juvenile phase has long wiry interlacing branches. In sheltered and shaded conditions the twigs are rather loosely woven together, but in exposed coastal situations the branches are locked together with extreme tightness.

Sophora (Leguminosae) resembles *Plagianthus* in its range of habit. *S. prostrata* is a low-growing densely divaricating shrub. It varies considerably in the height it attains, though some of this range of variation may be due to hybridization. *S. tetraptera* is a medium-sized tree which develops with a single leading shoot during its youngest stages. Successive internodes diverge only slightly. During the sapling stage the main branches tend to arch sideways, the identity of the leader may be obscured, and an open shrub-form be assumed. In *S. microphylla* the laterals of the young plants grow at a far faster rate than the main shoot whose identity soon becomes obscured unless tagged. This rapid growth in length without corresponding increase in thickness causes the

secondary branches and the main axis to droop. Tertiary branches repeat this process until a densely interwoven mass of wiry zig-zag stems is produced. Eventually several of the upper shoots become stronger, lose the divarication between successive internodes, and form the crown of the mature tree. The strong trunks of the mature trees soon lose the basal juvenile twigs. The form of this species which occurs on the Chatham Islands lacks habit-heteroblastism.

Marked leaf changes during development are frequent among gymnosperms. Associated with these are more or less well-defined changes in habit. *Podocarpus spicatus* is the only New Zealand gymnosperm, however, with a juvenile stage characterized by branches with successive internodes divaricating. As these branches do not always interlace to form a dense shrub, the juvenile of *P. spicatus* usually resembles the open rather tortuous juveniles of some forms of *Plagianthus betulinus* and *Hoheria populnea*, rather than the well-defined shrubs of most habit-heteroblastic trees.

This last example emphasises that the group of 11 species is arbitrarily defined. Less definite habit changes occur in juvenile trees, as for example in *Nothofagus fusca*, in which the saplings bear weakly divaricating lateral branches. The feature which links the chosen examples is the association of heteroblastic leaf development with striking habit changes. This combination results in such dissimilar plants that the transformation appears analogous to the metamorphosis of insects.

Variability in the degree of heteroblastic development is seen in different parts of the range of several New Zealand species. The leaflets of juveniles of *Schefflera digitata* are often deeply lobed in the northern part of the range of the species, while further south the plants are homoblastic. Similarly, *Cyathodes fasciculata* is heteroblastic for leaf-shape to the north but homoblastic further south. The differences between the mainland and Chatham Island *Plagianthus* and *Sophora* juveniles has been referred to. More detailed studies of these two genera in the South Island are showing that a wide range in the degree of habit-heteroblastism exists between populations. Current work in this department has established that the degree of divarication of seedlings from various populations of *Sophora microphylla* varies with environmental factors (Mrs. Denny, personal communication). The angle between successive internodes may be taken as a measure of the degree of divarication, and for a given seedling population this angle may be increased by increasing dryness. Day-length also affects degree of divarication. On the other hand, seedling populations from different sources display genetic variation. Similar findings are reported by Millener (1961, 1962) in relation to heteroblastism in *Ulex europeus*. The influence of temperature on the heteroblastic development of *Hedera canariensis* is reported by Stoutemyer and Britt (1961). Scattered early work reviewed by Schaffalitzki de Muckadell (1959) also shows that the inherent character of heteroblastic change is modifiable by the environment as in development generally. The interest

of the more recent work is the establishment of genetic variation on a geographical basis.

Very little information is available on anatomical differences between the shoots of juvenile and adult stages. A careful comparison of the anatomy of the wood of juvenile and adult specimens of the apple and pear was made by Fritsche (1948). Both vertical and ray parenchyma were more abundant in the adult wood. A greater proportion of the vessels of juvenile xylem occurred in the early wood, but the total area of vessels was less in juvenile branches, there being a high proportion of fibres. Juvenile and adult twigs may be so dissimilar that the apical meristems and the morphogenesis of the tissues behind them must be distinctive. Changes in apical organization during the life of a plant have been described (Philipson, 1949; Buvat, 1952) but rarely with reference to heteroblastic development. Hejnowicz (1957) compared apices of *Chamaecyparis pisifera* forma *squarrosa* with those of normal plants of the species at various ages. He concluded that this form should not be regarded as a persistent juvenile but as resembling an ancestral form. A comparison of the apical meristems of shoots from adult and juvenile specimens of *Plagianthus betulinus* is at present being undertaken by Miss Josephine Ward in this department. The greater part of the tissues of these shoots is derived from the cambium, and morphological changes in the cambium of *Hoheria angustifolia* during its transformation from the juvenile to the adult state are also being investigated here. At present no information is available on cambial modification in habit-heteroblastic trees, except indirectly through the studies of Rumball (1963). He worked with two habit-heteroblastic trees, *Elaeocarpus hookerianus* and *Podocarpus dacrydioides*, and two non-heteroblastic species belonging to the same genera, namely *E. dentatus* and *P. totara*. By examining wood-samples at intervals along a radius of a log he showed that there was a distinct check in the rate of increase in size with age of various characters of the secondary xylem of the heteroblastic species. This check occurred about the same time as the transformation in external form, and was absent from the two homoblastic trees whose xylem elements increased in size in a more uniform manner. It is well known that the wood laid down near the centre of a branch differs from that found further out on the same radius. This inner wood is frequently referred to as juvenile wood, but as it continues to be formed in shoots formed late in the life of mature trees, Zobel, Webb and Henson (1959) have suggested that the term 'core wood' is preferable. However, the wood formed in juvenile trees, which Rumball found to be distinctive, may justifiably be called juvenile wood. It should not be confused with the retention of juvenile characters in the secondary xylem of mature plants of woody herbs (Carlquist, 1962).

The frequent association together in the same genus of heteroblastic and non-heteroblastic trees and of divaricate shrubs is summarised in the second and third columns of Table I. A feature of New Zealand vegetation which strikes overseas botanists is the prevalence of the life-

form known as a divaricate shrub. The term is useful but difficult to define because the mode of branching of shrubs classed as divaricating is varied. General definitions will be found in Cockayne (1911, 1912, 1927, 1928) and in Bulmer (1958). The result of the various modes of branching is to produce a shrub with thin wiry, or sometimes excessively rigid, branches which closely interweave. In more typical shrubs the branches develop in relation to light so that the twigs rarely become entangled with one another. In divaricate shrubs, on the other hand, the direction of growth appears to be more definitely related to the parent axis than to the environment. Consequently some branches of second, third, and higher orders will grow into and through the densely shaded centre of the shrub.

Published photographs of the divaricate habit will be found in Laing and Goulay (1935), with figures of several species of *Pittosporum;* in Cockayne (1911, 1928), *Pittosporum divaricatum*; in Davies (1961), basal shoots on old *Pennantia corymbosa* and the transitional stage of *Pittosporum turneri*; and in Martin (1961), juvenile *Podocarpus spicatus*.

The divaricating life-form is not confined to New Zealand, but there is reason to believe that it is more important here than elsewhere. Bulmer (1958) states that the divaricate habit is also prevalent in Tasmania but not elsewhere. About 40 New Zealand shrubs clearly belong to this category and many of these are widely distributed and common plants. They belong to 15 genera in 13 families. Cockayne (1911) takes a wider view and records divarication in 15 families. The unusual frequency of interlacing shrubs in these islands, where trees with juveniles of this habit are virtually confined, can scarcely be a coincidence. After making extensive enquiries Rumball (1961) concluded that 2 rutaceous trees in Queensland might be the only examples of this type of habit-heteroblastism outside New Zealand. Balfour (1879) drew attention to the prevalence of dimorphic leaves in the flora of Rodriguez, and Cockayne (1911) suggested that one of these, *Fernelia buxifolia*, had a juvenile of divaricating shrub form. However, Balfour's figure hardly supports this interpretation, nor does his description of the habit of the plant as similar to that of the box tree. It may be assumed that some factors in New Zealand's present or past environment have favoured the intricately branched shrub habit. Whatever these factors may be, they might be expected also to account for the evolution of divaricate juvenile stages. Indeed, it is possible that in the genera *Sophora*, *Pittosporum* and *Plagianthus*, divaricate shrubs have evolved by paedogenesis from the juvenile stages of related trees. *Pennantia corymbosa* not infrequently flowers in the juvenile state and seeds from these juveniles have produced mature trees (Beddie, 1958). Flowering of *Plagianthus*, *Hoheria*, *Elaeocarpus* and *Sophora*, either in the juvenile state or on persistent juvenile branches at the base of mature trees, is reported (Cockayne, 1911, 1912, 1927, 1928). The leaf-heteroblastic liane, *Parsonsia capsularis*, includes a form which retains the juvenile-type leaf throughout its life, and therefore may be said to flower in the juvenile state (Cockayne, 1908).

Diels (1906) published an extensive account of the relationship between maturity to flower and vegetative development. The expression of vegetative characters is influenced by the environment, as also is the onset of flowering. However, these two aspects of the development of a plant are not influenced in the same way. Consequently, a plant will become ripe to flower at a different stage of vegetative development in different circumstances. In heteroblastic plants this is clearly seen when flowering occurs before vegetative development has attained the mature condition.

Diels proceeded to consider the implications of this on evolution. Plastic species with wide geographical range may flower in different phases of growth and appear so distinct as to be considered as separate species. If the separation of phase forms by the environment was maintained for long periods, Diels considered that they might become genetically fixed. Diels instances pairs of species which inhabit regions with different climates and which differ mainly by the plants in one region lacking the mature vegetative phase of those in the other.

The weaknesses in the belief that juvenile stages recapitulate the phylogeny of a plant are forcibly criticised by Diels (1906). If the relationship between flowering and vegetative attainment is so plastic as he believed, then the juvenile stage of one species may become the adult stage of a derived species. The reverse is equally possible, so that ontogeny alone can give little evidence of the past history of a plant.

Cockayne gave much thought to the evolution of heteroblastic plants in New Zealand. He first developed the theme in a series of papers on seedlings (1899, 1900, 1901). In the last of these he included his first full statement on the evolution of the divaricate juvenile stage of trees. He regarded the life-cycle of the Chatham Island *Plagianthus*, which lacks a juvenile shrubby phase, as primitive. The condition on the main islands is considered to reflect a former dry period there which favoured xerophytic forms, including the divaricate shrub habit. At this time Cockayne accepted recapitulation unreservedly and would explain the persistence of juvenile forms apparently out of harmony with their present environment as ancestral forms still persisting in development. In later publications (*e.g.*, 1927, 1928) Cockayne retains the essential features of this hypothesis, but expresses it more cautiously.

The clearest and fullest statement of his views appears in his important paper on evolution in relation to ecology (1911). On this occasion he uses *Sophora* as his main example. The original stock is envisaged as a mesophytic tree. This has remained virtually unaltered in the Chatham Islands. " A probable period of drought on the east of the Southern Alps at the time of the glacial period " induced the xerophytic divaricating shrub form. In *S. microphylla* (in which this form is confined to the juvenile) " the xerophytic stimulus never evoked an absolutely hereditary form, whereas in *S. prostrata* the effect of the stimulus is much more deep-seated". The juvenile stage of *S. microphylla* was suited to the dry period of the eastern South Island

"but it is certainly beneficial no longer, and the adult stage is more or less a return to the original form, but now called forth by the present mesophytic conditions". He considers that the potential of both forms is latent in the plant, and that whichever is expressed will depend on the stimulus of the environment. This view accords with his observations on phenotypic variation in *Pittosporum rigidum* (1901) and his experimental work on *Discaria* (1905). It fails to explain why the reaction of the individual plant should change without corresponding environmental change. Cockayne attempted to overcome this by supposing that the present mesophytic conditions are not sufficient to overcome completely the strongly inherited xerophytic form. But this does not explain its restriction to early phases of growth without recourse to recapitulation.

Prominence is given here to these early views of Cockayne to remind us that the findings of present-day experiments, presently to be described, must, like those of Cockayne, be related to evolutionary theory. We may explain transformations in terms of concentrations of plant hormones, but these physiological mechanisms must ultimately be explained in terms of the relation of the plant to the environment.

To Cockayne must be given the credit of having pointed out that the juvenile phase might be unsuited to its environment. Diels recognized the significance of the environment on the mature phase, which might be either more or less mesophytic than the juvenile. Passecker (1952) pointed out that the features of juveniles are adapted to the habitat of the young plants, and considered them to be adaptations resulting from natural selection. Cockayne drew attention to the fact that the divaricate juveniles of New Zealand mesophytic trees were in appearance xerophytic and therefore unsuited to the environment near the floor of the forest, a position even more mesophytic than the climate encountered by the mature crown of the tree. This supposition has since been substantiated for *Elaeocarpus hookerianus* by transpiration rates recorded by Rumball (1963). Juvenile foliage was found to lose water at a slower rate than the mature foliage.

It is this disharmony which singles out these divaricate juveniles from other examples of heteroblastic development. Divaricate shrubs are characteristic of open situations, and many lose this habit when growing in the shade of trees. Yet the shrubby stages of at least some of these 11 habit-heteroblastic trees grow in forest conditions where they are closely divaricating. It is true that the juvenile stages of some of these trees (*e.g.*, *Hoheria* and *Sophora*) frequently occur in open scrub, but they are also to be found in shade. How far their habit is influenced by the environment remains to be determined, but the fact remains that densely divaricating small-leaved juveniles of mesophytic trees characteristically occur in deep forest. No alternative has yet been offered to Cockayne's theory of the effect of a former dry period in New Zealand's history. It is to be hoped that the present interest being taken in the relation of structure to age will throw light on this problem.

In a recent paper Wardle (1963) has put forward views which are contrary to those of Cockayne. He contends that the divaricating habit occurs frequently in forest and mesic scrub and regards it as adapted to still existing though rather dry forest environments. Though divaricate juveniles of trees are relatively slow growing, he considers that their xeromorphy enables them to survive in these sites. The development of more mesomorphic foliage in the adult trees he presumes to be due to the development of larger, deeper and more efficient root systems.

The experimental work reported by Schaffalitzky de Muckadell (1959) goes far to substantiate his theory of meristematic ageing. Shoots arising from different parts of a tree are not only different morphologically but retain these differences as cuttings or reciprocal grafts. For example, shoots of young beech trees (*Fagus sylvatica*) characteristically retain their dead leaves in winter, whereas shoots of mature trees are deciduous. Leaf-retaining branches used as scions when grafted onto a branch on the crown of a mature tree continued to retain their leaves in winter. De Muckadell concludes that changes in shoot morphology of this kind are due to age changes in the apical meristem and that this is probably universal among woody plants. He has brought together much evidence in favour of this view, both as a result of his own experiments and from the literature particularly as it relates to forest trees and orchard fruits. To the many papers cited by him could be added the work on *Citrus* (Cameron and Soost, 1952; Frost, Cameron and Soost, 1957; Cameron, Soost and Frost, 1959). *Citrus* shoots of the same genotype differ greatly in characters such as direction of growth, thorniness and rate of elongation.

In spite of the great weight of evidence brought forward it is unlikely that the hypothesis of meristematic ageing will prove of general application in its original simple form. Habits of growth well known in trees do not comply with it. For example, this would apply to the restriction of flowers to the lower part of the trunk of a tree, or even to branches emerging at ground level, as in *Ficus* (Corner, 1961) or *Erythrochiton* (personal observation), leaving the whole crown of the tree vegetative. The normal occurrence of juvenile-type lateral branches high up the trunk of *Elaeocarpus* is also contrary to expectation. Nor does the theory readily apply to the condition described for *Michelia fuscata* (Tucker, 1962) in which the phyllotaxis of leading shoots is spiral and that of laterals is a modified decussate arrangement. Both types of shoot flower and the dorsiventral branches change spontaneously to the spiral condition. Differences due to relative position rather than to age are rightly distinguished by de Muckadell, following Büsgen and Münch (1929), as examples of topophysis.

A cytological mechanism whereby characters arising during development may be maintained during mitosis is postulated by Brink (1962). He assumes that, in addition to the genes, chromosomes also contain self-perpetuating accessory materials that undergo paramutation in somatic cells. Paramutations are considered to regulate the suc-

cessive processes of development. They are reversible. The hypothesis has the merit of being more generally applicable than that of meristematic ageing (which could indeed be an aspect of it) but lacks any direct supporting evidence. It is of great interest that the morphogenetic effects of *Agrobacterium tumefaciens*, which are cited by Brink as a counterpart of phase change in plants. have been shown to include, as a secondary effect, the induction of juvenile-type foliage in *Rubus* (Hudson and Williams, 1955).

In experiments with *Betula*, Wareing (1959) has shown that the juvenile phase of a tree's development can be shortened by factors which increase rate of growth. That is to say, attainment of maturity is dependent on reaching a certain size rather than having passed through a certain number of growth cycles. Wareing draws a distinction between characters associated with maturation and those with ageing. Characters associated with the attainment of maturity are commonly stable, being maintained in cuttings and grafts, whereas features characteristic of fully mature trees, such as lack of vigour and feeble apical dominance, are readily restored. Having briefly reviewed knowledge of the juvenile and adult conditions in plants and also the occurrence of morphological differences between shoots of different orders of branching, Wareing points out that all such phenomena seem to involve the existence of tissues in two stable alternative states and the transmission of a given state by cell-lineage through a large number of cell generations. Since the adult condition ultimately produces embryos which are once again in the juvenile state, he concludes that no nuclear genetic changes occur at the onset of maturity. He, therefore, postulates self-duplicating cytoplasmic factors.

It is well known that the mature state of the ivy (*Hedera*) retains its character if struck as cuttings. Recently a number of experiments have been reported in which this stability has been modified. Doorenbos (1954) caused adult shoots of *Hedera helix* to revert to the juvenile form by grafting them to seedlings or juvenile cuttings. Stoutmeyer and Britt (1961) using *Hedera canariensis* found that reversion of adult shoots grafted to juveniles occurred at 80° F. but not at 60° F. Frank and Renner (1956) grew rooted cuttings of adult and juvenile ivy together in a vessel containing Knop's solution. Adult cuttings were induced to produce juvenile growth. Robbins (1957, 1960) sprayed plants of *Hedera canariensis* var. *aborescens* with gibberellic acid. Growth increased substantially as compared with untreated controls, branches of some of the treated plants became completely juvenile in appearance. Similar experiments were carried out in New Zealand by Rumball (1961). Adult shoots of *Elaeocarpus hookerianus* were induced to produce juvenile-type foliage or intermediate foliage by spraying with gibberellic acid and also by incisions into the vascular tissue below the shoots. Juvenile shoots of *Podocarpus dacrydioides* were induced to form adult foliage by immersion in water at 42° C. at weekly intervals. It was presumed that the heat inactivated phytohormones in the shoots. It is to be hoped that this

promising pioneer work can be followed up. Only by the experimental approach can we hope to attain a further understanding of th.se transformations.

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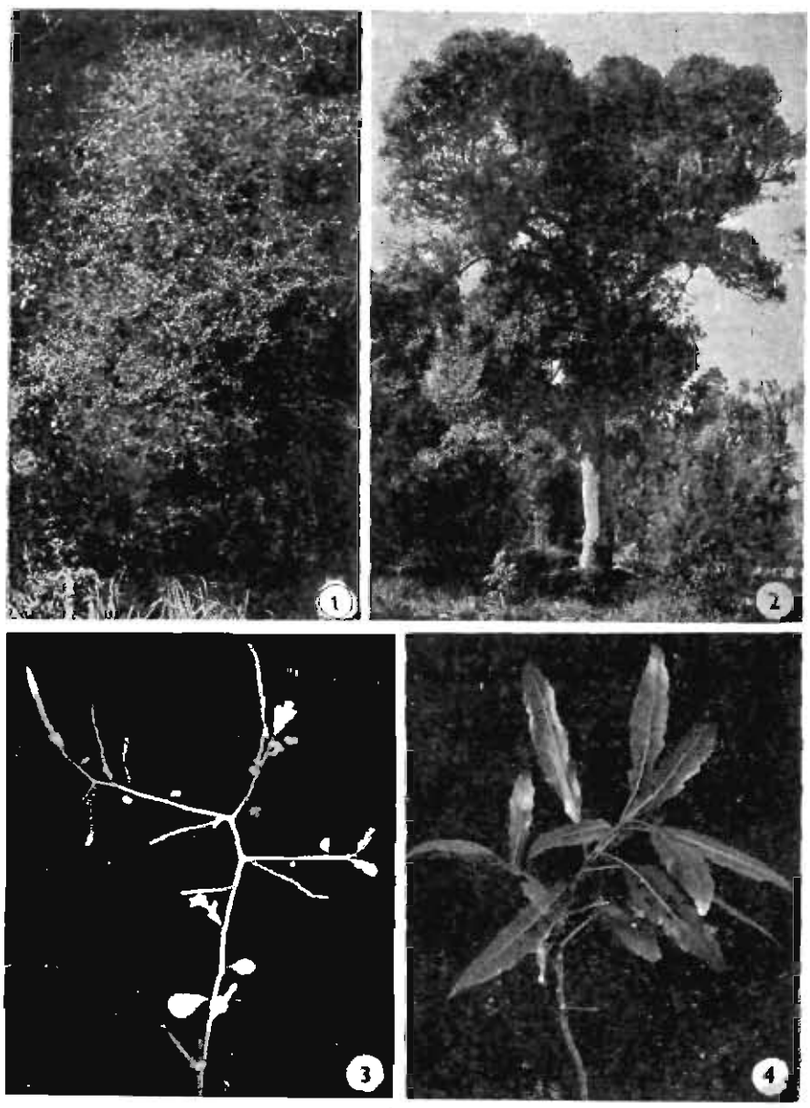

FIGS. 1–4

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EXPLANATION OF PLATE 1

FIGS. 1–4. *Elaeocarpus hookerianus* Raoul

FIG. 1. Juvenile divaricating shrub, about 15 dm high.

FIG. 2. Adult tree, about 17 m high.

FIG. 3. Twigs of juvenile, with leaves varying from linear to obovate, ×½.

FIG. 4. Twig of adult tree, ×½.

I am grateful to Mr. D. Hearn for taking the photographs for Figs. 1 and 2 and to Mr. M. Dukes for Figs. 3 and 4.

THE CULTURE OF SOME ORCHID OVULES ON AN ARTIFICIAL NUTRITIVE MEDIUM

By V. A. Poddubnaya-Arnoldi

The Main Botanical Garden, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.

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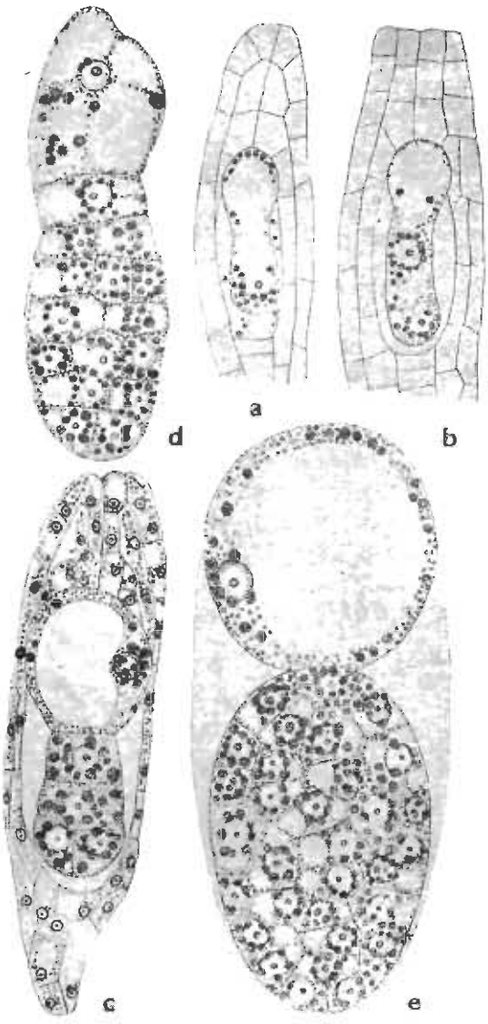
Ever more papers appear which are devoted to the culture of various generative organs and tissues of plants on an artificial nutritive medium under controlled conditions. This contributes to the development of physiological and experimental techniques in the investigation of generative processes. Several authors (Maheshwari, 1958; Ranga Swamy, 1958, 1961; Maheshwari *et al.*, 1959; Poddubnaya-Arnoldi, 1959; Sachar and Kapoor, 1959; Kapoor, 1959) have grown the ovules of *Papaver somniferum*, *Argemone mexicana*, *Zephyranthes*, of some *Citrus* species, and others on an artificial nutritive medium. In our experiments the ovules of *Dendrobium nobile*, *Calanthe veitchii*, *Phalaenopsis schilleriana* and *Cypripedium insigne* were grown in culture on Knudson's C artificial nutritive medium under sterile conditions. The ovules of all these orchids showed further development *in vitro*, provided that fertilization had taken place earlier.

The ovules taken at various stages of macrosporogenesis and at that of the development of the embryo sac did not undergo further development in our experiments, although they remained fresh and did not show any sign of degeneration for a long time.

The experiments on culturing the ovules of *D. nobile* on Knudson's C medium were especially successful, so that it is just these results which are described below.

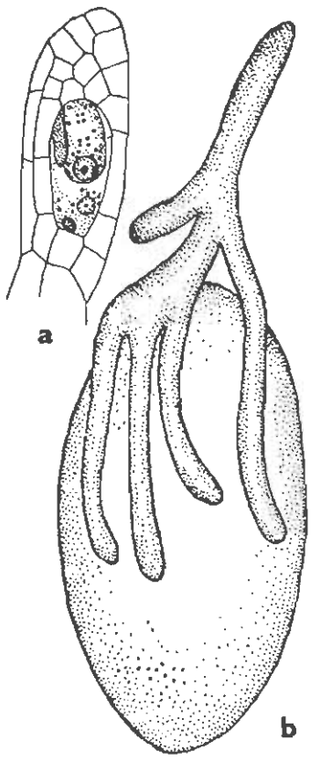
3–3·5 months after pollination the pieces of the placenta with the ovules were put into the test-tubes containing Knudson's C medium. These test-tubes were kept in an incubator at a temperature of 20–25° C. When the ovules were put on the nutritive medium they contained fertilized egg cells which had not undergone division. 1–1·5 months after the ovules were put on the nutritive medium, various phases of embryogenesis could be observed in the ovules of *D. nobile*, from the division of the zygote up to the formation of the multicellular embryo (Text-Fig. 1). After 4 months not only further development of the embryos but also the formation of seedlings was observed.

The development and structure of *D. nobile* embryo from the division of the zygote when culturing the ovules on an artificial nutritive medium were compared with those under natural conditions, upon



TEXT-FIG. 1. Embryogenesis in *Dendrobium nobile* ovules *in vitro*: (*a*) embryo sac with the zygote; (*b*) embryo sac with the 2-celled embryo; (*c*) ovule with the 5-celled embryo; (*d*, *e*) multicellular embryos. Drawn from total preparations of living material. All figures, × 700.

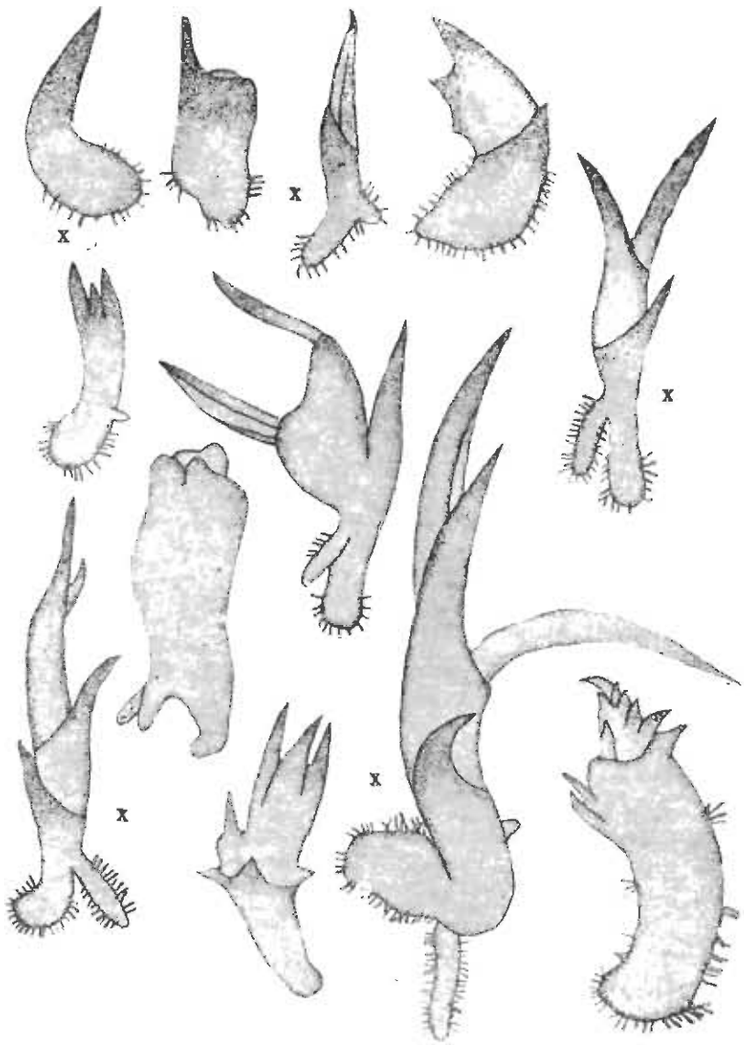
their development on the maternal plant. This comparison showed that all the cells and their constituents were considerably larger in the first case than in the second one and that the structure of the embryo cultivated on Knudson's C medium somewhat differed from that of



TEXT-FIG. 2. Embryogenesis in *D. nobile in vitro:* (*a*) embryo sac with the zygote; (*b*) mature embryo with a branched suspensor haustorium, × 700. Fig. 2 *a*. Drawn from total preparations of living material. Fig. 2 *b* is drawn from the preparation after its reaction to cellulose, since in this case the suspensor haustoria showing the positive response to this reaction could be clearly revealed, × 700.

the embryo grown on the maternal plant. This particularly concerned the plastids and the suspensors of the embryos (Text-Figs. 1, 2). The plastids in the embryonal cells of *D. nobile*, in the ovules with the fertilized egg cells cultivated *in vitro*, were larger and, as a rule, formed large aggregates.

While well-developed branched suspensor haustoria were usually formed in *D. nobile* embryos (Text-Fig. 2 *b*), in the culture of fertilized ovules *in vitro* suspensor haustoria did not branch and were vesicle-like (Text-Fig. 1 *c-e*).



TEXT-FIG. 3. *D. nobile* seedlings obtained from the culture of fertilized ovules, normally differentiated ones (X) and those abnormally differentiated. Figures are drawn from total preparations on living material, magnification, × 50.

D. nobile seedlings derived from zygote containing ovules upon their cultivation on Knudson's C medium were largely normally differentiated and did not show any considerable difference from *D. nobile*

seedlings from the embryos of ripe seeds that had developed on the maternal plant (Text-Fig. 3 *x*). However, the seedlings deviating from the normal development occurred more often when the ovules were cultivated on an artificial nutritive medium than in the culture of ripe seeds (Text-Fig. 3). In such seedlings the root was either feebly developed or lacking; the leaves were enlarged and developed abnormally.

Our experiments on the orchid ovules showed that orchid embryos could be grown under the conditions of the culture from the moment of the division of the zygote up to the formation of a multicellular embryo by putting the ovules with fertilized egg cells and with the embryos at various developmental phases on an artificial nutritive medium. This permits to experimentally investigate on the living material various stages of embryogenesis in orchids, including the very early ones, and to compare the course of their embryogenesis *in vivo* and *in vitro* which shall contribute to the study of the physiology and biochemistry of the embryos. The possibility to obtain normal orchid seedlings from unripe seeds considerably facilitates hybridization since it allows to shorten the time of the seed-ripening. This is of importance because in many orchids, like *D. nobile*, the ripening takes a very long time (a year and longer).

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RICCIA TUBEROSA TAYLOR = *ANOGRAMMA LEPTOPHYLLA* (L.) LINK, OR ON THE IMPORTANCE OF BEING BRYOPHYTIC

BY J. PROSKAUER

*Department of Botany, University of California, Berkeley, U.S.A.**

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IN 1846 (p. 415) Thomas Taylor described a plant from the Swan River (= Western Australia) gatherings made by James Drummond. Taylor was somewhat puzzled by what he had, and "temporarily placed" the material in the genus *Riccia*, under the name *R. tuberosa*. Reading his diagnosis, and remembering the provenance of the material, suggests as the most likely explanation that he was looking at a patch of sprouting *Petalophyllum preissii* Gottsche. Mitten (1855, p. 164) indeed tentatively relegated *R. tuberosa* to *Petalophyllum*. Stephani (1898, p. 53) assigned *R. tuberosa* to *Anthoceros* (and that is where I come into the picture). Later (1916, p. 1007) he perpetrated the error of basing the new combination *Anthoceros tuberosus* (Taylor) Steph. on it (illegitimate, by being a later homonym), applying it to the different taxon described from the same area as *Anthoceros tuberosus* Taylor, 1846, p. 412. ≡ *Phaeoceros tuberosus* (Taylor) comb. nov.

Superficial examination of isotype material of *Riccia tuberosa* in various herbaria (*e.g.*, BM), led me to the tentative sub-scientific interpretation, on the basis of cellular texture, of the specimens as being leptosporangiate fern gametophytes. I was kindly lent the type sheet in the Taylor herbarium (in hb. FH). It is labelled: "Riccia tuberosa *MSS. T. T*[aylor]. Swan River. Mr. James Drummond. 1843" (Taylor scr.), and annotated on a paper slip "Riccia tuberosa *Tayl.* Not a Riccia [lacuna] Fossombronia. CFA"= C. F. Austin. It bears a dense patch, *ca* 3 cm. in diameter, of the presumed fern gametophyte material, with a small pocket, annotated by Taylor exactly as the main sheet, containing Taylor's dissected specimens of it. The plants fit Taylor's description perfectly, orchid-like tubers and all. No sex organs are present on the thin erect lappets. Having the advantage over Taylor of a hunch on what to look for, I soon found, on some of the specimens, abundant leptosporangiate fern type antheridia among the rhizoids in the region of the tuber stalks, and some structures believed to be archegonial on a few of the larger but apparently still immature tubers. The material thus is a fern prothallus of the "archegoniophore" type as described repeatedly

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by Goebel (1877, 1889, 1930) for *Anogramma leptophylla* (L.) Link. (Involved is an extreme specialization of the "lateral meristem" type of gametophyte known in some other members of the gymnogrammoid alliance and probably ultimately of importance in evaluating the limits and systematic placement of that group; *cf.* Stokey, 1951, p. 54.) I was surprised to note the great similarity of most of the material in hand with Goebel's illustration of "young prothallia" (1889, pl. 1: 44–49). The occasional larger individuals demonstrate the funnel shape of the lobes of the bigger specimens figured by Goebel (1930). These facts in themselves are no reason for positive identification.

Appended to the type sheet in the Taylor herbarium is a further pocket with brown paper labels. One, written in a very large hand (? Drummond's), reads "Jungermannia-like plant Leaves plain"; the other is Taylor's label "Riccia tuberosa *MSS. T.T.*" The material appears to be the residue of stock left after Taylor's culling of the type. It contains assorted mosses, some anthocerote thalli presumed to belong to *Phaeoceros tuberosus*, a *Petalophyllum* thallus, and some more "*Riccia tuberosa*" specimens. By a stroke of fortune one small sporophyte was associated with them. Only one frond, immature, and but 1·5 cm. high, still had most of the blade part left. It was compared with corresponding fronds of *Anogramma leptophylla* from various collections in our herbarium. With these it agreed in basic architecture, venation, texture, marginal cells, etc. Indeed, minute though it is, it already shows developing sporangia in the correct soral position. Furthermore, it was possible to isolate numerous spores from the soil attached to the gametophytic material which also confirmed the identification. Goebel's (1877, pl. 12: 1, 2) figures of the spores are, incidentally, not very accurate.

Although this appears to have been overlooked by the pteridologists, *Anogramma leptophylla* has indeed previously been recorded as occurring also in Western Australia. Bentham and Mueller (1878) listed the Drummond collections 360 and 996 as belonging to it.

My interest in *Anogramma leptophylla* has been of long standing. It is rather appropriate that its gametophyte should have received description as a liverwort. For this species, with its perennial gametophyte and usually annual sporophyte which produces sori already on juvenile fronds, has adopted the main features of the bryophyte life-cycle. Already Goebel (1877, p. 709) remarked that *Anthoceros* on the one hand and *Anogramma leptophylla* on the other narrowed the gap between their respective groups. The key structure which permitted the bryophytic specialization of the latter is the gametophyte tuber which has become associated with the production of sex organs, as well as perennation and vegetative reproduction. It not only offers a ready food supply for the rapid growth of the embryo attached to it, but by virtue of usually becoming separated from the rest of the gametophyte when it is nursing a sporophyte (Goebel, 1877, p. 690), it defeats the vicious matricidal parasitism of the embryo to which other fern gametophytes normally fall prey (Proskauer, 1960, p. 16).

It is highly interesting to note that *Anogramma leptophylla*—just—managed to be included in Christ's (1910, p. 146) list of the dozen most successful ferns. (The specimens in our herbarium from Landour near Mussoorie in the Western Himalayas at over 2,000 m. elevation, from Mt. Kilimanjaro in East Africa, and those from Central Mexico, make an interesting addition to his list of localities.) It is a reasonable assumption that it owes this success precisely to its bryophytic way of life. Thus its distribution pattern should be judged in terms of the most successful cosmopolitan "native born" bryophytes, such as *Funaria hygrometrica* and *Phaeoceros laevis* subsp. *carolinianus*. We know that it shares with the latter two the basic attributes of some mechanism of gametophytic resistance, and monoecism to allow successful single spore colonization. An investigation would probably also show that its spores are resistant and that its development is relatively unimpeded by stringent photoperiodic requirements. Judging its distribution in terms of other ferns has evidently led to some puzzlement (*cf.* Copeland, 1947, p. 77).

Many a botany student has had it explained to him that the bryophytic way of life is rather a poor idea without a glorious future in the full exploitation of the land habitat. On the contrary, it is an excellent idea with so much future that the plants which adopted it have rigorously stayed with it, finding ample opportunity for themselves in a succession of geological epochs. The success story of *Anogramma leptophylla* is food to the theory that the bryophytes are derived from organisms with a pteridophytic life-cycle which realized a fine opportunity.

SUMMARY

The name *Riccia tuberosa* Taylor is based on fern gametophyte material from Western Australia shown to belong to *Anogramma leptophylla* (L.) Link. *Anthoceros tuberosus* Taylor is based on a different type, and the new combination *Phaeoceros tuberosus* is made for this taxon.

The remarkable success, in terms of its distribution, of the fern *Anogramma leptophylla* is explained on the basis of it having adopted a bryophytic mode of life.

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ON THE RELATION BETWEEN OVULE AND CARPEL*

BY V. PURI

School of Plant Morphology, Meerut, India

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ONE of the important biological generalizations of the twentieth century is the organismal hypothesis according to which an individual is essentially a mass of protoplasm endowed with the power of co-ordinating its different activities. In certain lower organisms, as for instance in *Caulerpa*, this mass grows much on all sides and becomes differentiated into 'roots', 'stems' and 'leaves'. In others it divides into cells which grow in different directions and in turn differentiate into various organs or parts. All this growth and differentiation is strictly controlled; but as pointed out by Sinnott (1960, p. 24), "A certain amount of self-differentiation undoubtedly exists, in which a given organ or structure, once its development has begun, proceeds more or less independently of the rest, but the parts are usually interdependent."

Conceived in this sense, these parts or organs are not *distinct* and consequently not homologous to those of a man-made machine; by virtue of their mode of formation they cannot be definitely demarcated from the rest of the organism. Yet quite often, for the sake of convenience of description and understanding, we have to speak of them as *distinct* organs or categories, to think in terms of them and sometimes, forgetting that they are of our own making, even quarrel about them (witness the controversy over structures being axial or appendicular!).

There is yet another sense in which we use these categories or terms. When we speak of their morphological nature we treat them as representing certain concepts rather than as distinct entities; we conceive them as abstract units rather than as material objects. It is immaterial for the purpose whether they have a distinct physical existence apart from the concept. Therefore, on isolated findings of structural differences, they (the concepts) cannot be supported or discarded; nor can they be materially altered by ontogenetic studies. They stand or fall on whether they offer an easy, satisfactory and consistent explanation of all known cases, without involving too much of speculation or assumption.

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It is in this conceptual sense that we look upon the controversy regarding the position of the ovule in angiosperms. In general there are three possible views on the subject:

1. Ovules are borne on floral axis.
2. Ovules are carpellary.
3. Ovules in some cases are axial and in others carpellary.

These views have been discussed sufficiently in the past and no useful purpose will be served by dilating upon them at this stage here (*see* Puri, 1952). However, there have been some recent attempts to interpret the ovules as cauline. It will be worthwhile to analyse the bases of these suggestions and see if any real advancement has been made in our understanding of this subject.

In the Primulaceae and certain Caryophyllaceae—where the placentation is free central—the question whether the ovules are carpellary or axial has ever assumed special significance. The supporters of the classical concept of the carpel believe that the central column has around it some carpellary tissue contributed by the carpellary margins which have broken off from the main body and that the ovules develop from, and are borne on, this carpellary tissue. This assumption has been very critically examined by Moeliono (1959) in a recent study of two species of *Stellaria*. He examined fairly close series of photomicrographs of longitudinal sections of young gynoecia. In sections believed to be passing through the septum he demarcates the limits between the septum (carpellary margins) and the central axial column. He thus concludes that the latter is entirely free from the carpellary tissue and that the ovules which arise from it are, therefore, axial rather than carpellary.

Moeliono has been cautious enough not to generalize his results. He has even loaded his final conclusion with a question mark. It may be pointed out here that Moeliono's entire study is based on longitudinal sections, which at best can show only vertical extent of separation; they cannot rule out the possibility of fusion of the septum tissue with that of the central column at another point. Only a transverse section could have shown, if it at all exists, a complete transverse separation of the column from the septum. I have had occasion to examine some series of transverse sections of *Stellaria media* but no such separation as envisaged by Moeliono could be seen. In view of this it appears that his Fig. 5 (Photomicrograph 5) may be based on a section passing through a side of the septum at a point where it is about to fuse with the column.

Again, assuming that Moeliono is able to establish the separation of the column from the placenta beyond any reasonable doubt, shall we be justified in concluding that the ovules are axial? I am somewhat sceptical about it for, it will be difficult to prove that this separation is *primary* and not *secondary*. This is especially significant

since Moeliono himself reports (p. 302) that "In later stages, the septum becomes delicate and fragmentary even disappears completely." What Moeliono has observed may be a case of precocious separation of the 'septum' from the 'central column'. Besides, what is the guarantee that the separation observed by Moeliono represents the limits between these two regions. To us, therefore, it appears that the problem is not so simple as it appears at first sight.

This leads us to a point where we may pause a little to consider if ontogenetic studies of organs are of any consequence in the elucidation of such problems. Fagerlind (1958) has discussed this aspect very ably and concisely. Referring to the excellent work of Eckardt (1957) he remarks that "In the young still undifferentiated flower of *Echinodorus* it is not possible to decide by ontogenetic analysis whether the ovule and the outstanding part of the pistil originate from a common primordium, soon to divide into two fractions, or it is the question of two different primordia lying in immediate mutual contact." This is indeed a very relevant point to take into account.

Further on, I associate myself wholeheartedly with the conclusion of Fagerlind that "we still do not have access to results that permit the adoption of any final attitude towards the actual problems" (of relationships between ovule and carpel, axillary bud and leaf, etc.). And may I add that we shall never be able to have those results, for the simple reason that our categories are artificial and not made by nature of distinct entities? We should not hope to find distinction where it is not implied.

Despite this clear statement, Fagerlind seems to be favourably inclined toward a stachyosporous interpretation of the angiosperm ovule. He wants us to believe that this ovule together with the carpel is homologous to the ovuliferous scale and its bract in Coniferales or to the axillary derivate and its subtending leaf in the strobilus of *Gnetum*. This means that a flower with several carpels will have to be considered as a *compound* strobilus like the megasporangiate strobilus of a pine and not as a *simple* strobilus. We are unable to accept this position for the present.

Recently, Melville (1961, 1962) has proposed a new interpretation, the *gonophyll* hypothesis, of the angiosperm flower, which is intended to replace the so-called carpellary view or the classical view. According to Melville the ovary is a compound structure consisting of sterile leaves forming the wall and a corresponding number of branches bearing ovules. Ordinarily these branches are epiphyllous to the leaves and the whole unit of leaf and ovuliferous branch is called a *gynophyll* (*gonophyll* being a general term for *androphyll* and *gynophyll*). Sometimes an ovuliferous branch may be ebracteate and may stand alternating with the sterile leaf as is believed to be the case in *Butomus*. In such a case the sterile leaf is called a *tegophyll*. The ovuliferous branches by repeated dichotomies or fusion in various combinations give rise to different conditions of placentation found in angio-

sperms. In the same way the style and stigma may also be compound structures formed partly by the subtending leaf and partly by the upward extension of the ovuliferous branch.

Melville (1962) has listed some nine objections to the classical concept of the angiosperm carpel. Some of these are determined by a faulty appreciation of this concept while others are due to assumed phylogenetic alliance of the angiosperms with the gymnosperms. His objections based on anatomy are:

1. The frequent overtwisting of the placental bundles is not accounted for.
2. No adequate reason has been advanced to account for fusion of ventral bundles of adjacent follicles.
3. The placental bundles frequently depart from the floral stele at some distance from the dorsal bundles and appear to have an independent origin.

In connection with the first point Melville emphasizes that the placental bundles in order to become inversely oriented twist more than do the margins of the carpel. This is made the basis for the suggestion that they do not belong to the carpellary margins but represent independent structures. Melville is perhaps putting too much faith in the word 'twisting'. Literally speaking the placental bundles do not undergo any twisting, rather they just differentiate in that position according to certain basic principles. The phloem of the bundle differentiates on the abaxial side and the xylem on the adaxial side (in leaves and carpels). In the classical concept the carpellary margins are believed to be involutely folded on the adaxial side, so that the abaxial surface of the carpellary margins comes to face the floral axis while the adaxial side is away from it. Exactly the same orientation is exhibited by the placental strands or their constituent ventral bundles. Therefore this objection does not have any relevance.

Regarding the second point, Melville (1962, p. 3) asserts that "When the placental bundles are provided with separate stelar gaps, distinct from those of the dorsal carpel trace, the appearance of separate organs is greater and when, as not infrequently happens, the adjacent placental bundles of adjacent follicles are fused at the base, the evidence is severely strained to make it fit the carpellary theory." This is a point that has been unnecessarily emphasized here. Students of floral anatomy know very well that in many families marginal bundles of adjacent sepals arise conjoint. Their nearness to one another is the only logical explanation of their fusion.

The third objection too is ineffective. It is common knowledge that in certain leaves the marginal traces may arise from all round the stele, even from the side opposite that of the dorsal bundle. But they are always interpreted as foliar traces. So the distance between the dorsal and the ventral bundles is of little consequence in determining

whether they belong to the same organ or to different ones. What is important is the subsequent behaviour of these bundles.

The other objections that relate to fertilization, distribution of transmitting tissue, ontogeny of the carpel and physiological requirements, are also of little consequence in the final analysis.

It is not necessary here to go into details of the gonophyll hypothesis. But its basic points have to be brought out and analysed at some length with a view to see if it can stand as a working hypothesis. In brief, Melville has attempted to apply the telome theory to the angiosperm flower in a manner analogous to that adopted by Florin (1951) for the conifer cone. The whole study is based on bleached and cleared flowers and transverse sections have been scrupulously kept out of consideration. Even where transverse sections had to be given (*cf.* Figs. 13 D and 16 E) they are only diagrammatic.

It may be pointed out that the underlying concept of this hypothesis is an old one, it has been proposed a number of times in one form or another from the days of Schleiden (1849) and Payer (1857). During recent years Hagerup (1938, 1939) has been a strong advocate of the independence of the placentae from the carpels. More recently Barnard (1957 *a*, *b*; 1958); Fagerlind (1958) and Moeliono (1959) have also lent themselves in favour of this view.

There are two salient features of the gonophyll hypothesis.

1. That the placentae are structures independent of the carpels which constitute just sterile envelopes.
2. That the placentae represent branch system axillant to sterile leaves.

None of these points seems to have been established beyond any reasonable doubt. Regarding the independence of the placentae it may be pointed out that there are a number of cases where the ovules receive their vascular supply from the dorsal bundles, *e.g.*, *Nymphaea* (Saunders, 1936), *Drimys* (Bailey and Nast, 1943); *Cananga* (Periasamy and Swamy, 1956), etc. Then Melville(1962) has himself reported cases where the dorsal bundles of the carpels are lacking and the placental bundles supply not only the ovules but also the wall of the carpel. If these dorsal bundles and the placental bundles belong to different organs, as Melville wants us to believe, then why should they behave in the manner they do? Vascular bundles as a rule are known to respect morphological limits.

Besides, the orientation of the ovules on either side of a placenta (*cf.* Gopal and Puri, 1962) and the orientation of the placental bundles always stand in a definite relationship with the dorsal suture of the carpel. This relationship cannot be explained by Melville in cases like *Butomus* where each placental branch is believed to ramify repeatedly. How can all the ovules, borne on the different ramifications that are believed to have become fused with the carpellary wall, have the same orientation? Melville ignores this feature completely.

The contention that the placentae represent dichotomous branch systems has just been taken for granted; there is little anatomical ground for this. One important distinction wh ch anatomists recognize between leaf and axis is that while the former has unifacial anatomy (dicotyledonous leaves) the latter has radial anatomy. In m joritv of cases that Melville has cited, the placentae have unifacial anatomy and yet they are interpreted as representing branch systems. What is more, the inverse orientation of their bundles has been considered as a point in favour of their axial nature. This is done by comparing the placentae having inversely oriented ventral bundles with coronal structures in *Ranunculus* (Arber, 1936), *Narcissus* (Arber, 1937) and *Passiflora* (Puri, 1948) which also have inversely oriented bundles. It may be emphasized here that these coronal structures are considered as enations from the inner faces of the floral leaves that have had bifacial anatomy. They have never been considered as branch systems.

In our understanding, therefore, Melville's hypothesis cannot explain satisfactorily the various structural features of angiosperm carpel. It is essentially a phylogenetic concept that does put angiosperms in line of gymnosperm and pteridophytes. It has to be emphasized here that Melville's hypothesis is not an alternative, nor even antagonistic, to the classical view of angiosperm carpel. The two have fundamentally different objects and can coexist if they have vitality enough for the purpose. Obviously, there is some confusion in the minds of certain morphologists which Melville has done well in bringing up to the surface.

The classical concept is essentially a morphological concept (as against phylogenetic) which attempts to explain angiosperm stamens and carpels as we see them here and now, in terms of vegetative leaves. It is just a means of understanding flower by comparing it with vegetative bud without implying anything as to its past history or evolution in course of time. In other words, it aims at achieving uniformity behind so much apparent diversity in nature, while at the same tim avoiding any speculation regarding the evolutionary history of thes organs. If some morphologists have read phylogeny or past histor into it, that is no fault of the concept. rather it is due to faulty under standing of the subject and lack of proper appreciation of distinguishin between pure morphological thought and phylogenetic speculation o the 'new morphology'.

According to the classical concept a carpel is visualized as equi valent to a leaf-bearing marginal (or sub-marginal) ovules, and enfold ing adaxially on its midrib (*see* Puri, 1952, 1961). For that matter leaf may also be visualized as a carpel that has lost its ovules and becom open. Such a comparison does not involve any phylogenetic implica tions. No initiated follower of the classical concept, for instance, wi ever suggest that a carpel at any stage of its development *was an ope leaf*.

Let us now refer to some structural features of the angiospern gynoecium and see how they stand, *vis-a-vis* this concept.

It is common knowledge that a placenta in majority of cases is a double structure consisting of two halves. Sometimes when this doubleness is externally lost it may be revealed by its vascular supply that may consist of two more or less distinct ventral bundles arising independently or conjointly from the parent stele. This is a very widespread feature exhibited by almost all major groups of angiosperms.

Intimately connected with this feature is the orientation of the ovules which on the two halves always face in opposite directions. In those cases where the two halves of a placenta lie far apart from one another, as in certain Gentianaceae (Gopal and Puri, 1962), Orobanchaceae, *Crataeva* (Puri, 1950), it is the orientation of all the ovules on this portion to one side that helps us in determining the true nature of the placenta.

Another important feature is that while in most of the open carpels the ventral bundles or their fusion products, the placental strands, are normally oriented, in closed carpels they are usually inversely oriented with reference to the floral axis.

While usually it is the ventral bundles or the placental strands alone that furnish ovular traces; in some cases the secondary marginals or even the dorsals may contribute ovular supply.

It will be seen that all these are important structural features of the carpellary region we are dealing with. These are very satisfactorily and easily explained on the basis of the classical concept according to which each placenta is formed by the fusion or appression of two margins of the same or adjacent carpels. Any view that envisages a placenta as a structure independent of carpels cannot account for these features so satisfactorily.

Having thus accepted the carpellary nature of ovules it is necessary to say something as to their actual location on the carpellary surface. Recently there has developed some difference of opinion as to whether the ovules are essentially marginal or superficial on the ventral surface of the carpel (*see* Puri, 1961). For a proper appraisal of this controversy it is essential to understand what constitutes the margin of a carpel. Ordinarily the extreme edge of the carpellary leaf that has the last traces of sub-marginal meristem is referred to as the carpel margin. But is that the total margin ? As emphasized earlier (Puri, 1962) the total margin must include the total thickness of the carpellary leaf at the margin as indicated by its lateral face. This lateral face, whose existence and extent have not been fully appreciated* in the past, is the surface that stands radially parallel to the ventral bundle of the margin. If we accept this we shall be able to explain all the recent cases of super-

* The statements that ovules in some cases, e.g., *Secamone* (Safwat 1962), and some other asclepiads (Baum, 1949), are borné on the dorsal surface of the carpellary margin, are based on lack of proper appreciation of this lateral face.

ficial placentation, *e.g.*, *Drimys*, *Degenaria*, *Cananga*, etc., in terms of marginal placentation.

Notwithstanding this, we have got to admit that in some cases, *e.g.*, *Butomus*, *Nymphaea*, etc., the ovules occur on most of the ventral surface and the placentation is superficial. Recently Gopal and Puri (1962) have drawn attention to some interesting variations in the placentation in certain Gentianaceae and they have indicated tentatively that the so-called superficial placentation "can be looked upon as a function of growth in which one part of the carpel (the marginal region) grows more than the rest". Whatever may be the fate of this suggestion, the fact remains that superficial placentation offers difficulties of explanation to the supporters of the classical concept as also to those who consider the placenta as an independent structure.

There is yet another point to be considered before we close. In some recent writings the placenta has been treated as a distinct structure arising probably as an outgrowth from carpellary margins. Heinig (1951), for instance, considers that in *Phaleria* (Thymelaeaceae) the two parietal placentae extend inward, carrying the ovules in the centre and dividing the ovarian cavity into two loculi. Evidence for such an interpretation is found in the occurrence of the carpellary ventrals in the ovary wall instead of their presence in the middle of the septum which should have been the case if the ovary were truly bilocular with axile placentation. There are several cases of this type on record (*see* Puri, 1952, p. 643).

The main point involved here is with regard to the nature of the structures that bear the ovules, whether they are *carpellary margins themselves* or just *outgrowths* from them. As pointed out earlier (Puri, 1954, p. 296) this issue cannot be settled in any logical manner; we can have either of the views without much difficulty and so no useful purpose will be served by discussing the point any further. It will, however, be more consistent with our classical concept to consider that the placentae in all such cases are the *swollen margins themselves* and that they are not borne on any *out growths from them*. Such a treatment has the advantage of enabling us to interpret the septum in all such cases as *true septum* and retain the expression *false septum* for sterile outgrowths from the placentae themselves (*cf.* condition in crucifers).

In the same way it is not advisable to draw a distinction between the *ventral bundles* and the *placental bundles*, as is sometimes done. It is true that in some cases where the placentae are large and massive as in the Cucurbitaceae (Puri, 1954), they have more or less distinct vascular supplies. But at some level or the other these latter may be connected with the ventral bundles and so it is not difficult to interpret them as branches of the ventral bundles given out to serve specific needs of large placentae.

In concluding this brief review it may be emphasized once again that the classical concept of the angiosperm carpel envisaging the ovules

as being borne on carpellary margins is still a valuable "instrument of description" in the hands of comparative morphologists. It is a pity that its aims and objects are not clearly understood in the present climate that appears to be too much vitiated by phylogenetic speculation. Even its ardent supporters, in their enthusiasm to elaborate it and strengthen it further, have sometimes introduced some weak links into it. However, its criticism usually comes from those who have not practised it long enough to enjoy its usefulness and advantages over the phylogenetic guess work. Those who have devoted their entire life to it have generally found it a "useful working hypothesis". No doubt there are some weaknesses in it but they will always be there in every concept of this nature.

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PECTIC ENZYMES AND PLANT DISEASE

By T. S. Sadasivan and D. Subramanian

University Botany Laboratory, Madras 5, India

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The importance of pectic enzymes of pathogenic micro-organisms has received special emphasis in studies on the physiology of parasitism in plants (De Bary, 1886; Smith, 1902; Brown, 1915, 1936, 1955; Davison and Willaman, 1927; Harter and Weimer, 1921, 1923; Wood, 1959, 1960). Decomposition or disintegration of tissues is one of the invariable components of disease syndromes in plants although the severity of this may be more or less marked. Indeed, rots, blights, cankers and anthracnoses showing obvious tissue breakdown were among the earliest recognized diseases. The mechanism by which microbes cause these diseases were largely unknown until de Bary (1886) studying the mode of invasion of *Sclerotinia libertiana* observed two types of action of the pathogen on host tissues, one leading to the partial solution of certain constituents of cell walls and consequent loss of coherence of tissues and the other, killing of the protoplast itself. The former effect was traced to an active principle produced by the pathogen, the activity of which was lost on heating and thereby proving its enzymatic nature. The latter effect could be due to the cell wall degrading 'enzyme' itself or it may be brought about by a second factor, possibly a toxin. However, de Bary left this question open. It was Brown (1915, 1917) who provided conclusive evidence that a pectic enzyme was involved in the maceration of plant tissue by *Botrytis cinerea*. Attempts to separate the factors responsible for these two effects have met with little success (Brown, 1915; Tribe, 1955; Fushtey, 1957) and the evidence so far available suggests only that both maceration of tissues and killing of the cells could be brought about by the same agent, *viz.*, the enzyme itself. Fushtey (1957), however, ruled out the possibility of the products of pectic degradation being responsible for killing of cells.

Pectin forms one of the basic components of cell walls in plants and occurs together with cellulose, hemicelluloses and lignin. The importance of pectic substances in tissue maceration rests on that they form the material of middle lamella cementing the component cells and a degradation of these materials results in the loss of coherence of the cells in a tissue. It is, therefore, fairly certain that wherever tissue maceration or rotting is seen, as in soft rots, damping off, anthracnoses, etc.,

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Abbreviations used: Pectin methyl esterase—PME; Pectin polygalacturonase—PG; Pectin depolymerase—DP; Protopectinase—PP.

the causal agents have been shown to be able to bring about enzymic degradation of pectic substances of the host tissues. In recent years, pectic enzymes have been implicated also in bacterial and fungal wilts of plants (Scheffer and Walker, 1953; Gothoskar *et al.*, 1953, 1955; Waggoner and Dimond, 1955; Beckman, 1956; Kamal and Wood, 1955; Scheffer *et al.*, 1956; Husain and Kelman, 1956, 1957, 1958; Husain and Dimond, 1958; Lakshminarayanan, 1957 *a*). Sufficient proof has been forthcoming as to their important role in many of these diseases either by their detection in the infected tissues or by other means such as histological or chemical study of these tissues. These observations indicate that the pectic enzymes are involved at some stage or the other in the host-parasite interaction.

In the establishment of parasitism, the parasite attacks the host and after breaching the barriers (if it does not enter through wounds) gains entry into it whereupon a series of interactions sets in. If this leads to the ill-health of the host with significant changes in its metabolism—disease—, then the parasite is described as *pathogen* and the events leading to this condition are termed *pathogenesis*. There has been a little amount of confusion with regard to the role of pectic enzymes as to their causal relationship with disease development in plants. Are these important in the primary parasitic phase when the host barriers are attacked and breached, or whether their role is only confined to the latter phases of pathogenesis or both ?

Role of Extra-Cellular Pectic Enzymes in Penetration of Host

There are three modes of entry of pathogen into the host: (i) direct penetration through the surface of the host, (ii) through the natural pores such as stomata and lenticels and (iii) through wounds caused by leaf fall, emerging rootlets, insects and other agencies. The point of interest would be to know if entry through intact surface of plants is achieved only by mechanical means or by dissolution of cell walls and surface layers by enzymes. In the case of shoots of young plants cuticle forms a continuous and relatively impermeable layer composed of cutin and waxy substances. At present the evidence indicates that this layer is penetrated by most pathogenic fungi mechanically and not by chemical action. This conclusion is based on the fact that the germ tubes of many fungi are able to penetrate intact wax film or collodion membrane or gold leaves provided they are neither too thick nor tough. Claims contrary to this general conclusion have been made as that of Chaudhuri (1935) who reported that cuticle of orange leaves was degraded by *Colletotrichum gloeosporioides*. The evidence in this case is indirect and not conclusive. The report of Roberts *et al.* (1949) that the cuticle of apple leaves shows channels containing pectic substances raises a suspicion if pectic enzymes produced by an invading pathogen could not be implicated in penetration. In this context, it is more than of passing interest to mention that the rust fungus, *Puccinia graminis* v. *tritici*, which hardly causes any extensive damage to the invaded tissues, has been shown to produce pectic enzymes (Sumere *et al.*, 1957).

The early phases of parasitism of root-infecting fungi are less clearly understood. In a few cases studied, the organisms seem to grow through the soil, and sometimes along the root surface before entry is made. Generally, the sites of entry are the wounds caused either by natural causes while growing in the soil and made by rootlets at the points of emergence or caused by nematodes. In the case of destructive pathogens like *Rhizoctonia* and *Pythium* causing pre-emergence wilt, damping off or seedling blights, it would appear that the tissues are attacked and the cells of the host tissue are killed in a zone in advance of the pathogen. In such instances there is clear evidence of cell wall degradation and the extra-cellular enzymes produced by the pathogen may act in advance of the growth of the pathogen through the damaged tissues. In foot-rot, take-all and vascular wilts, the destructive mechanisms are brought into action evidently at a later stage inasmuch as the roots and underground parts of infected host generally do not show any visible damage of rotting or browning on the surface. Perhaps, the entry and growth through roots are purely mechanical and the extra-cellular pectic enzymes produced by these pathogens apparently have no major role to play in the primary phases of pathogenesis.

Role of Pectic Enzymes in Disease Development

There is no correlation between the ability to produce extra-cellular pectic enzymes by micro-organisms and their ability to attack higher plants or their parasitic vigour. Indeed, many non-pathogenic organisms secrete powerful pectic enzymes. There was no correlation between the parasitic vigour of some species of *Rhizopus* and their capacity to produce pectinase on certain media (Harter and Wiemer, 1921) as also noticed in the case of a number of strains of *Botrytis cinerea* (Paul, 1929). There are, however, other instances where such a correlation was indicated (Damle, *1952*; Gregg, *1952*). These incongruities are largely a reflection of the varied role and extent to which pectic enzymes play in different host-parasite interactions. Comparison of virulence and pectic enzyme secretion between different organisms or isolates of the same organism against a particular host, has been generally on the basis of production of the disease itself and this is perhaps feasible in cases of diseases caused by the destructive pathogens producing rotting of tissues. Pathogens of a higher order in the scale of evolution depend more on the effective penetrative mechanisms and the 'action in advance' by secretion of enzymes are largely put down leading to a more balanced relationship with the host. Even in cases, where the production of enzymes have been established as in wilt diseases their action seems to be severely restricted or postponed to a later stage in pathogenesis. The correlation between pectic enzyme secretion by the pathogen and disease intensity, in such cases, becomes difficult since these enzymes may, after all, account only for a portion of the syndrome. Thus, McDonnell (1962) has shown that the abilities of several pathogenic strains of *Fusarium oxysporum* f. *lycopersici* to produce pectic enzymes on pectic substrates have no bearing on the final intensity of

tomato wilt; strain 80 which had no pectolytic activity was also shown to invade, establish and produce moderate disease symptoms in the host. Paquin and Coulombe (1962), however, were able to show that pectic enzymes were causally related to the virulence of the tomato wilt *Fusarium*. Recent work of Mann (1962) using mutants of *F. oxysporum* f. *lycopersici* is interesting. Mutants incapable of producing extra-cellular pectic enzymes induced typical wilting and vascular browning, although they were less virulent than the parent isolate. She concluded that evidently, pectic enzymes of the pathogen were not essential for development of wilt symptoms, but they seem important from the point of view of pathogenicity.

Whether or not pectic enzymes play an important role in disease development depends on satisfying at least two conditions: (1) the pathogen secretes these enzymes *in vivo* (2) the condition of host tissue is suitable for the action of these enzymes. Extensive studies on the physiology of pectic enzyme production by micro-organisms reveal a wide variation in their behaviour. Many of them produce the enzymes constitutively, while certain others like *Fusarium moniliforme* and *Sclerotium rolfsii* do so only in the presence of pectic substances. Partly adaptive enzyme secretion where pectin acts as an 'inducer substance' and stimulates greater enzyme secretion is known in several cases, *viz.*, *Penicillium chrysogenum* (Phaff, 1947), *Botrytis cinerea* and *Aspergillus niger* (Gäumann and Bühni, 1947) *Fusarium oxysporum* f. *lycopersici* (Waggoner and Dimond, 1955), and *F. vasinfectum* (Lakshminarayanan, 1957 *b*). Carbohydrate sources, sugars for example (Table I), exert a marked influence on pectic enzyme secretion by fungi and it is needless to emphasize that the pathogen confronts *in vivo* pectic substances not alone but together with other carbohydrates (Subramanian, 1959, 1960). The C/N ratio also exerts a marked influence on enzyme secretion.

The results indicate that both PME and PG secretion by *R. solani*, *F. vasinfectum* and *F. udum* were markedly increased as the concentration of sugar was reduced; presence of pectin in the medium stimulated enzyme synthesis at the higher sugar level (5%) in all cases. Indeed, a high sugar concentration appears to be less favourable for synthesis of PME and inhibitory for PG production by *R. solani*. The behaviour of *R. solani* presents a striking contrast to that of *F. vasinfectum*; with the decrease in sugar concentration, an enormous stimulation in enzyme synthesis was seen in the former, whereas in the latter the stimulation was slight. This appears to be of some significance in explaining the pathogenic behaviour of these two organisms: *F. vasinfectum* can apparently enter into a more balanced association with a host than *R. solani* which becomes very destructive with slight fluctuations in the availability of sugars. It would be relevant to mention here that sugars exert similar influence on cellulase production by *Verticillium albo-atrum* (Talboys, 1958).

The dynamics of pectic enzyme production by pathogens is yet another aspect that needs attention. The enzymes may be secreted

TABLE 1

The influence of sugar concentration on pectic enzyme secretion by some fungi

Organism		PME			
		1	2	3	4
1. *Rhizoctonia solani*	..	9·30	60·45	54·25	40·30
2. *Fusarium vasinfectum*	..	23·25	27·90	12·40	18·60
3. *F. culmorum*	..	1·55	1·55	0·00	13·95
4. *F. udum*	..	1·55	10·85	9·30	10·85
5. *F. solani*	..	1·55	0·00	1·55	1·55
		PG			
1. *Rhizoctonia solani*	..	0·000	3·098	2·655	2·124
2. *Fusarium vasinfectum*	..	0·708	5·133	4·248	1·859
3. *F. culmorum*	..	3·540	3·451	2·035	3·009
4. *F. udum*	..	3·009	5·478	5·044	3·098
5. *F. solani*	..	4·160	1·239	4·160	6·284

(1) Richard's solution. (2) Richard's solution with 2% sucrose only. (3) Richard's solution with 2% sucrose + 0·5% pectin. (4) Richard's solution with 5% sucrose + 0·5% pectin.

by the germinating spores and young and growing hyphal tips as noticed in *Botrytis cinerea* (Brown, 1936) and *Ceratostomella ulmi* (Beckman, 1956). We do not have clear-cut data if this is confined only to the younger portions of the hyphae or older portions also secrete the enzymes. If, only the hyphal tips produce the enzymes, the effects on the host tissue would be more or less localized and the quantity of the enzymes secreted would depend on the growth of the pathogen in the host tissues.

For instance, the conditions afforded by the host tissues must be suitable for secretion and activity of pectic enzymes of the pathogen. Water content is one such important factor as in the case of potato with an increase of about 2-4%, a definite increase in susceptibility to the enzyme was observed (Gregg, 1952). If the tissues are vacuum

infiltrated so as to replace air in the intercellular spaces with water, the tissue becomes susceptible to a much greater extent. The factor primarily concerned appears to be low oxygen tension as it is known that oxidising enzymes like polyphenol oxidases or the products of polyphenol oxidation exert an inhibitory action on pectic enzymes (Brown, 1955; J. S. Cole, 1956; M. Cole and Wood, 1961 *b*; Deese and Stahmann, 1962 *a*, *b*). Apple juice, if allowed to get oxidised, rapidly inactivates polygalacturonase of *Sclerotinia fructigena* and the substances responsible for this have been tentatively identified as leuco-anthocyanins which are oxidised by apple polyphenol oxidase (M. Cole and Wood, 1961 *b*). The action of the pathogen on the host itself would stimulate these deactivating mechanisms as an increased polyphenol oxidase activity has been recorded in plants consequent to infection by pathogens (Uritani and Maramatsu, 1953; Sanwal, 1956; Rubin and Aksenova, 1957; Lakshmanan and Subramanian, unpublished). Davis *et al.* (1953) reported that the hydrolytic enzymes produced by *Fusarium oxysporum* f. *lycopersici* libera e ortho- and poly phenols by causing their liberation from β-glucosides and the products of their oxidation polymerize into melanin-like pigments that cause vascular browning in tomato plants. This self-inhi itory action of the pathogens results in the rapid disappearance of pectic enzymes in the invaded tissue. In white rot produced by *Penicillium expansum* there is little of this inhibitory mechanism and continuous action of pectic enzymes results in greater loss of pectic substances from the tissues affected while in brown rots such as those caused by *Sclerotinia fructigena* this inhibition is greater. Obviously, in the former case, the pathogen does not itself secrete any polyphenol oxidase and, in fact, inhibits even the host oxidase. An interesting situation arises in the case of Fusar a which produce both pectic enzymes and fusaric acid, a potent inhibitor of polyphenol oxidase (Bossi, 1960; Sadasivan, 1961).

In wilt diseases of plants affecting young seedlings, the rapidity of lignification of vessel-walls appears to be an important factor in limiting the establishment of pathogen. Thus, in cotton, varieties resistant to *Fusarium* are characterised by a higher lignin and a lower pectin content of roots while in susceptible variety pectin rather than lignin occurs in higher quantities. Rapid lignification perhaps protects the substrates such as cellulose and pectin more vulnerable to the action of hydrolytic enzymes (Subramanian, unpublished).

Evidence for Production and Activity of Pectic Enzymes *in vivo*

The claims put forward by many workers that pectic enzymes operate *in vivo* and could be, at least, partly responsible for disease syndrome are based mainly on three lines of evidence: (i) the establishment of a higher activity of these enzymes in diseased tissues, (ii) histological and histochemical examination of infected tissues, and (iii) chemical changes in pectic substances or detection of the products of their breakdown. Each of these is severely limited by various sources of error and it would, therefore, be necessary to rely on more than one

or all of these evidences before assigning a role to pectic enzymes in plant disease.

The fact that a given enzyme is present in a tissue is no direct evidence that it is active *in vivo*. Clearly, the most that could be deduced is that it is potentially of significance; and the quantitative capability is subject to the reservation that the conditions *in vivo* must not be less suitable than those under which its activity is measured *in vitro*. Alternatively, if one could not extract an enzyme from a tissue, it may not always indicate its absence but that it may be in a non-extractable state, as it happens if it is adsorbed to some insoluble component of the tissue. Yet another possibility would be that it may have been inactivated by host reactions; polyphenol oxidase or the products of its action have been shown to be potent inhibitors of pectic enzymes (J. S. Cole, 1956; M. Cole, 1958; Deese and Stahmann, 1962 *a*). Uritani and Muramatsu (1953) found that when sweet potato was attacked by the black-rot organism, *Ceratostomella fimbriata*, melanin-like substances produced by the oxidation of polyphenols in injured cells adhered to the mycelium and inhibited further penetration. Studying the factors for resistance in tomato to *Fusarium*, Baumgardt (1953) was able to show a prompt and definite polyphenol accumulation in cut lateral roots exposed to preparations of the fungus. As hardly any fungal advance occurred in these areas which were characteristically developed in resistant plants, it was tentatively concluded that some fungistatic polyphenol may be present which accounted for inhibiting the pathogen. These observations on the restriction of the penetration and spread of pathogens by polyphenols or their oxidation products indicate that they either inhibit their growth or inactivate the enzymes they secrete for facilitating the advance. If the latter were true, then the condition of such tissues would neither permit extensive degradation of pectic substances nor permit accumulation of pectic enzymes. The estimates of pectic enzyme activity in tissues, invaded by pathogens, may further be vitiated by secondary invaders. It would, therefore, be necessary that the host-parasite combinations are grown under sterile conditions before pectic enzymes are studied in diseased plants so that we would be left only with host reactions to surmount.

Our studies on the pectic enzyme activities in tissue homogenates of susceptible cotton, pigeon-pea and rice plants infected by *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* respectively illustrate some of the points made out above. The PME and PG were determined when these plants showed typical wilt or foot-rot symptoms (*see* Table II).

The pectolytic activity (both PME and PG) in diseased cotton is much higher than in pigeon-pea. It is interesting to recall at this point that the development of wilt in cotton is more rapid (12–14 days) than in pigeon-pea where it takes about 3 weeks for the symptoms to appear. The symptoms of the latter disease are also less severe and the typical vascular browning is also absent. It would, thus, appear that a fairly good correlation exists between pectolytic properties of the pathogen and vigour of disease development. But the results on rice did not

TABLE II

In vivo *pectolytic enzyme activity of tissue homogenates from cotton pigeon-pea and rice*

Host	Variety	Roots		Stems		Leaves	
		PME	PG	PME	PG	PME	PG
Cotton	K2	1·24	3·12	6·82	2·32	1·86	1·55
		(17·98)	(9·29)	(22·63)	(3·08)	(22·63)	(1·59)
Pigeon-pea	NP24	1·55	1·77	12·30	1·95	55·80	7·35
		(3·10)	(4·78)	(7·75)	(2·30)	(27·90)	(3·54)
Rice	MTU 9	1·09	2·30	Foot and root region were taken together for enzyme assay			
		(1·09)	(0·89)				

Figures in parenthesis are values for the inoculated series.

conform to the trend noticed above despite the fact that this disease is characterised by a more obvious tissue degradation and rotting. Thus, the question arises as to how far our estimates of pectic enzymes indicate the correct picture.

The fact that foot-rot disease of rice, which shows the most obvious effects of the operation of pectic enzymes, records the lowest activity, probably indicates that it is exactly there that these enzymes excite the most violent host reactions leading to their rapid inactivation. That mere disorganisation of cells and tissues itself could lead to this effect is evident from the reports of inactivation of pectic enzymes *in vitro* by tissue homogenates (Byrde, 1957; Echandi and Walker, 1957; M. Cole and Wood, 1961 *b*).

HISTOLOGICAL METHODS

Histological examination of infected tissues would be helpful in indicating the action of pectic enzymes only after the middle lamella is dissolved and the tissue has lost coherence. Ruthenium red, often used as a specific dye to stain pectic substances, may not be reliable in all instances (*see* Kertesz, 1951). Although microscopic examinations reveal the degradation of pectin from cell walls, it does not indicate the type of enzyme that is active particularly, as pectic substances are acted upon by a number of enzymes. Sometimes, an examination of fresh free hand sections in water mount proves useful in revealing pectic degradation products rather than permanent

mounts (Ludwig, 1952). Such studies have revealed the dissolution of middle lamella before extensive cell wall degradation takes place as in soft rots, and this primary action of pectic enzymes would expose cellulose fibrils for enzymic degradation later. In tomato and tobacco plants infected with *Pseudomonas solanacea um* extensive cell wall breakdown and dissolution of middle lamella was noticed, and the effects produced on cut stems by the culture filtrates of the organism containing PME and PG were similar (Husain and Kelman, 1957). In tomato infected by *Fusarium oxysporum* f. *lycopersici* or treated to culture filtrates of the organism or commercial pectic enzyme preparation, when cross-sections of stem were stained with ruthenium red, showed a deep staining layer along the inner margin of the vessel-walls and also faintly staining large granular material inside the lumen of vessels. There were also some clear areas between the vessels and isolated areas of the vessel walls took the stain faintly, indicating that some of the pectic materials had been removed (Pierson *et al.*, 1955). In wilt diseases of herbaceous plants, the part played by pectic enzymes appears to be variable with the host-parasite combination. In those caused by Fusaria and Verticillia the disorganisation of xylem is less evident and extensive initially than in bacterial wilts where cells of the xylem, phloem and adjacent pith and cortex may be disorganised resulting in decay of localized areas (Husain and Kelman, 1958). On the other hand, in tree diseases, *e.g.*, Oak wilt and Dutch elm diseases, microscopic examination alone often fails to indicate any serious enzymatic digestion of vessel-walls. The xylem in these cases contains small quantities of pectic materials, perhaps the degradation of this fraction is less important in tissue disintegration.

Changes in Pectic Substances

When pectic enzymes play a part in the development of disease, their activity during this process will be reflected in qualitative as well as quantitative changes in pectic substances originally present in healthy tissues. In most cases there is a substantial reduction in the total quantity of pectins, they being converted to galacturonic acid residues. The appearance of galacturonic acid which was originally absent is taken as an evidence for pectolytic action in diseased tissues. But if pectic substances are acted upon by enzymes that merely de-esterify or reduce their viscosity by depolymerisation, they may not be degraded to the ultimate units. Only the action of PG-type of enzymes break down pectic materials to galacturonic acid. Pectic enzymes produced by *Pythium debaryanum* merely reduce the viscosity (Ashour, 1954) where no PME but only DP is produced and the enzyme acts on pectin molecules breaking it down to large fragments without the production of mono-, di- or tri-galacturonic acids (Gupta, 1956; Wood and Gupta, 1958). But di- and tri-galacturonic acids were present in tissues rotted by *Penicillium expansum* in addition to monomer and the organism was shown to produce PG-type of enzymes (M. Cole, 1958; M. Cole and Wood, 1961 *a*).

In general, we may sum up that PME would de-esterify methylated soluble pectin and convert it into pectinic acids of lower methoxyl content or completely de-esterify insoluble pectic acid which readily forms gels or salts with cations. The PG-type of enzymes act on pectinic or pectic acid and break them down to galacturonic acid residues, thereby having a solubilising action. The DP merely reduces the viscosity of pectic acid and the products are likely to have variable solubility as this is dependent on chain length. It would, therefore, appear that the nature of the changes wrought on pectic substances of the infected host plants or tissues would depend on the characteristics of the pectic enzymes produced during the host-parasite interaction. For instance, the firm-rot fungi (*e.g.*, *Sclerotinia fructigena*) reduced the water-insoluble fraction of pectic substances by only about 10–20% while soft-rot fungi (*Botrytis cinerea* and *Penicillium expansum*) caused much larger changes, up to 70%. The soft-rot fungi had little effect on pectic substances insoluble in dilute alkali or reduced their concentration, whereas the firm-rot organisms caused an increase in this fraction compared to healthy tissues (M. Cole and Wood, 1961*a*). In fruit rot of chillies caused by *Colletotrichum capsici* although the tissues are completely macerated, the total pectin is reduced from 24% to 20% on dry weight basis. This was somewhat surprising since the pathogen produces powerful PP, PME and PG in cultures. When pectic enzymes *in vivo* in healthy and infected fruits were studied, it was noticed that there was only a very high PME activity and low PG in infected tissues (Durairaj, 1959). Either the PG is rapidly inactivated in this case or more probably only solubilisation of pectic substances occurs by a reversal of PME action.

Interpretation of data on the qualitative and quantitative changes in pectic substances consequent to parasitic attack becomes difficult as the analytical methods employed are empirical and are based on the differential solubility characteristic of various fractions of pectic substances in the solvents usually employed in such studies. Solubility of pectic substances depends on the length of chain of the polymer, degree of esterification, the extent of cross-linking between adjacent chains and the types of cations associated with them. Admixture with other constituents such as cellulose, protein and lignin might also render them resistant to enzymic attack (Ginzburg, 1958; Wood, 1959). While considering transformations of native substances in plants, it may perhaps be necessary to revise our concepts that they are always brought about by the action of specific enzymes. This is indicated by our knowledge of the effects of ascorbic acid and peroxides on pectic constituents and other polysaccharides. Ascorbic acid when it gets oxidised causes degradation of both insoluble and soluble pectic substances; peroxides exert a similar action. A combination of both appears to be especially active (Robertson *et al.*, 1941; Kertesz, 1943; Deuel, 1944; Griffin and Kertesz, 1946). In light of the fact that both ascorbic acid and peroxides occur in plants and their oxidases increase in diseased hosts as in *Fusarium*-infected cotton (Lakshmanan, 1956; Lakshmanan and Subramanian, unpublished) it is perhaps difficult to overrule their

importance in bringing about changes in pectic constituents by themselves in infected plants.

CONCLUSIONS

It is now certain that in diseases such as soft rots, damping off, etc., where there is an extensive tissue disintegration, pectic enzymes have an important role to play. The disintegration of tissues facilitates free movement of materials into and out of cells and this is essential since the pathogen has to obtain its nutrition from the tissues thus attacked. In diseases such as wilts where there is no apparent tissue disintegration pectic enzymes are brought into action at a later stage in pathogenesis, perhaps when the pathogens enter into the vascular tissues. In diseases such as rusts and mildews produced by obligate pathogens it is doubtful whether these enzymes play any major role.

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A NEW SPECIES OF *OLDENLANDIA* FROM THE WESTERN GHATS OF INDIA

By H. Santapau and Y. Merchant

Botanical Survey of India, Calcutta, and St. Xavier's College, Bombay, India

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***Oldenlandia maheshwarii* Sant. & Merch. spec. nov.**

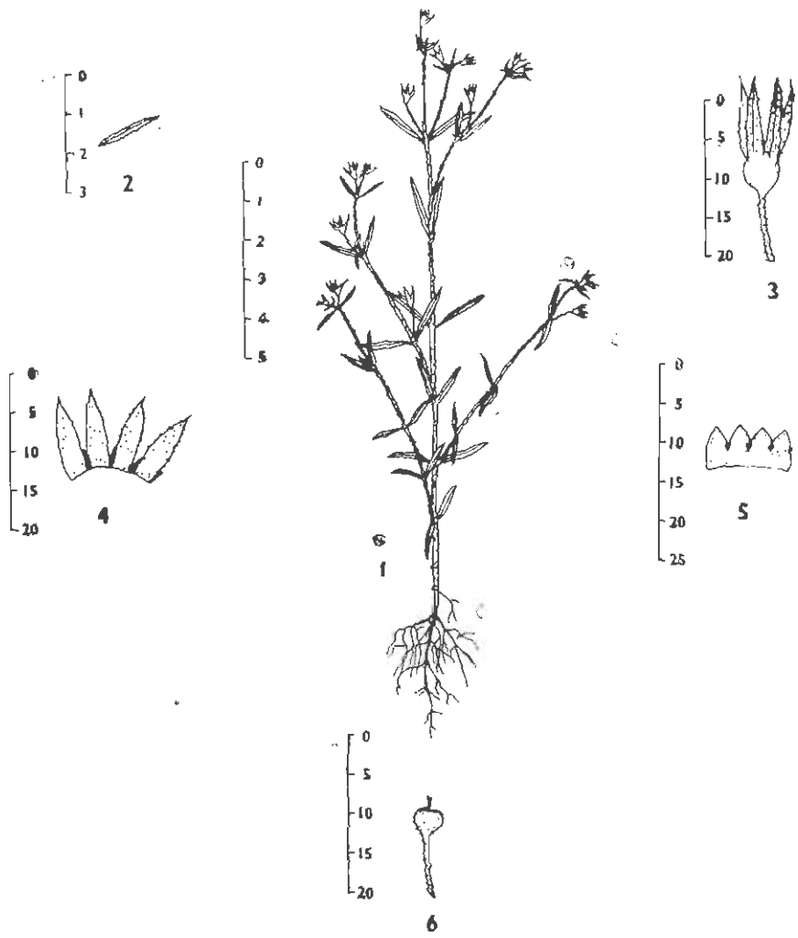
Accedit ad *Oldenlandiam stocksii* Hook f., a qua tamen differt caulsbus pubescentibus, floribus solitariis pedicello filiformi insidentibusi calycis dentibus corolla multo longioribus, florum colore albo vel roseo et magnitudine multo minore.

Herba erecta, 9–25 cm alta, ramosa vel simplex, caulibus et rami, maturioribus teretibus, pubescentibus, novellis vero quadrangularibus. *Folia* sessilia, 4–18×0·5–2 mm, linearia, integra, ad apicem acuta et seta tenui ornata, novella quidem scabra in utraque pagina, maturiora vero scabra supra et ad nervum medium infra; nervi laterales indistincti; stipulae 0·5–2·5 mm latae ad basin, membranaceae, setis 1–3 filiformibus ornatae, glabrae. *Flores* pedicellati, solitarii, axillares vel terminales; pedicelli 0·3–1·7 cm longi, graciles, pubescentes. *Calycis* laciniae 1·5–4×0·5–1 mm, ovato-oblongae vel oblongae, integrae, acutae, una seta capillari ad apicem ornatae, pubescentes in utraque pagina vel rarius glabrae, saepe dentibus vel setis interjectis separatae. *Corolla* alba vel rosea, tubularis, tubo 1 mm longo, glabro, laciniis ± 1 mm longis, ovatis, acutis, in utraque pagina pubescentibus. *Stamina* ori corollae inserta paululum infra basin laciniarum, exserta, filamentis brevibus, antheris dorsifixis. *Stylus* ± 0·5 mm longus; stigmata bina, minuta, vel nullo modo vel breviter tantum exserta. *Capsula* 1·5–3 mm lata, globosa, calycis dentibus pubescentibus coronata. *Semina* nigra vel brunnea elongata, elliptica, minutissime longitudinaliter verruculosa.

Typus lectus ab auctore juniore ad Mahableshwar, in ditione Maharashtra in India, die 13 septembris anni 1959 et positus in Herbario Blatteri (BLAT) in India sub numero *Merchant 1267*; paratypus, *Santapau 14963*, lectus ad Khandala in montibus Ghaticis occidentalibus die 20 septembris anni 1952 positus in eodem herbario.

***Oldenlandia maheshwarii* Sant. & Merch. spec. nov.**

Erect *herb*, 9–25 cm high, branched or unbranched; stem and branches terete, pubescent, the young branches 4-angled. *Leaves* sessile, 4–18×0·5–2 mm, linear, entire, acute at the apex and tipped



TEXT-FIGS. 1-6. Fig. 1. Entire plant. Fig. 2. Lower surface of leaf. Fig. 3. Calyx. Fig. 4 Calyx lobes opened out, with intermediate teeth. Fig. 5 Corolla. Fig. 6. Ovary with adnate calyx tube, style and stigmas. *Scale :* (1) in cm, (2) in mm, (3)—(6) in mm, but correct the numbers to read 0, ·5, 1·0, 1·5, 2·0, 2·5).

with a fine bristle, scabrid on both sides when young, scabrid above and on the midnerve beneath when old; lateral nerves indistinct; stipules 0·5–2·5 mm broad at the base, membranous, with 1–3 filiform bristles, glabrous. *Flowers* pedicellate, solitary, axillary or terminal; pedicles 0·3–1·7 cm long, slender, pubescent. *Calyx* lobes 1·5–4×0·5–1 mm, ovate-oblong or oblong, entire, pointed with a fine

capillary bristle at the apex, pubescent on both sides or sometimes glabrous, often with intermediate teeth in between the calyx lobes. *Corolla* white or pink, tubular; tube 1 mm long, glabrous; lobes ± 1 mm long, ovate, acute, pubescent on both sides. *Stamens* inserted on the mouth of the corolla tube a little below the base of the lobes, exserted; filaments short; anthers dorsifixed. *Style* ± 0·5 mm long; stigmas 2, small, shortly or not at all exserted. *Capsules* 1·5–3 mm, broad, globose, crowned with the calyx teeth, the crown protruding, pubescent. *Seeds* black or brown, elongate, elliptical, minutely longitudinally wrinkled (see Text-Figs. 1–6).

The type, *Merchant 1267*, was collected by the junior author at Mahableshwar in Maharashtra State, India, on September 13, 1959 and is kept in Blatter Herbarium, Bombay (BLAT); the paratype, *Santapau 14963*, collected at Khandala on the Western Ghats of India on September 20, 1952, has been placed in the same herbarium.

This new species has been repeatedly found in various parts of the Western Ghats during the last sixty years; its identification has remained problematic until the junior author took the materials from her collections to the Herbarium, Royal Botanic Gardens, Kew, and there proved that the material represents a new species. We have gathered this plant during the monsoon months in flower and fruit at Matheran, Khandala, Mahableshwar, Panchgani, Igatpuri, etc., usually growing on rocky plateaus, or in rock pools, or at times even on walls. It grows in rather dense patches, often in more or less pure stands.

For the sake of comparison, the following table is given showing the differences between the new species and *O. stocksii* Hook. f.

O. stocksii	*O. maheshwarii*
Stems glabrous	Stems pubescent
Flowers in di- or trichotomous cymes	Flowers solitary
Calyx teeth linear-lanceolate, about equalling the corolla tube	Calyx teeth ovate-oblong or oblong, longer than the corolla
Corolla blue, 4–6 mm long ('much the longest of the genus' Hook. f.), up to 6 mm diameter	Corolla white or pink, ± 2 mm long, about 1 mm diameter

SUMMARY

A new species of *Oldenlandia*, *O. maheshwarii* Sant. and Merch. spec. nov., is described from the Western Ghats of India, and dedicated to Professor P. Maheshwari.

A WORKING HYPOTHESIS CONCERNING THE MECHANISM OF CROWN GALL TUMOR FORMATION

By Harvey W. Spurr, Jr., A. C. Hildebrandt and A. J. Riker

Department of Plant Pathology, College of Agriculture, University of Wisconsin, Madison, Wis., U.S.A.

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The formation of tumors in the crown gall disease has attracted many scientists interested in growth, both normal and pathological. A large reservoir of data is available concerning many facets of this problem and involving many areas of botanical endeavour. Accordingly, many ideas about growth initiation and development have emerged. Crown gall tumor formation emphasizes the complexity of events or interactions as they occur during growth initiation and development. The crown gall problem has recently been reviewed by Braun and Wood (1961) and Braun (1962).

Our purpose is to relate a concept of nucleic acid interaction and selected observations from studies of crown gall. We then discuss a working hypothesis of special interest concerning the mechanism of crown gall tumor formation.

Studies of growth are concerned with cellular control mechanisms linked with the nucleic acids DNA (deoxyribosenucleic acid) and RNA (ribosenucleic acid). Recently, Bonner and Huang (1962) discussed chromosomal control in plants. The following sequence for cellular control comes mostly from this discussion: (*a*) DNA in the chromatin of the nucleus is necessary for the synthesis of RNA. (*b*) The function of the nucleolus is to manufacture ribosomal protein and further to assemble ribosomal protein and RNA to produce ribosomal subunits. (*c*) The ribosome under the direction of its RNA assembles enzymes from the 20 amino-acids present. (*d*) The enzymes assemble various molecules into the structures of the cells. With these ideas at hand, the following paragraphs attempt to bring together some of the anatomical, cytological, morphological, and physiological observations of crown gall tumor formation.

The bacterial origin of the crown gall tumor was established by Erwin F. Smith and C. O. Townsend (1907). Early researchers associated the necessity of wounding to tumor formation. For example, Riker (1923 *a*, *b*) established the requirement of a wound for tumor formation. He showed that tumor size was related to the size of the

wound, and that usually the bacteria must enter the wound within 4 days to produce tumors. The bacteria appeared intercellularly and exerted their tumorous influence from this position. The bacteria invaded injured cells and killed them. Large numbers of bacteria appeared after 2 days within the ruptured cells along the wound and in many intercellular spaces above and below the wound in all the tissues of the stem. Cell-walls bordering such spaces were yellow-tinged, stained more readily, were swollen, and had lost the property of double refraction of light.

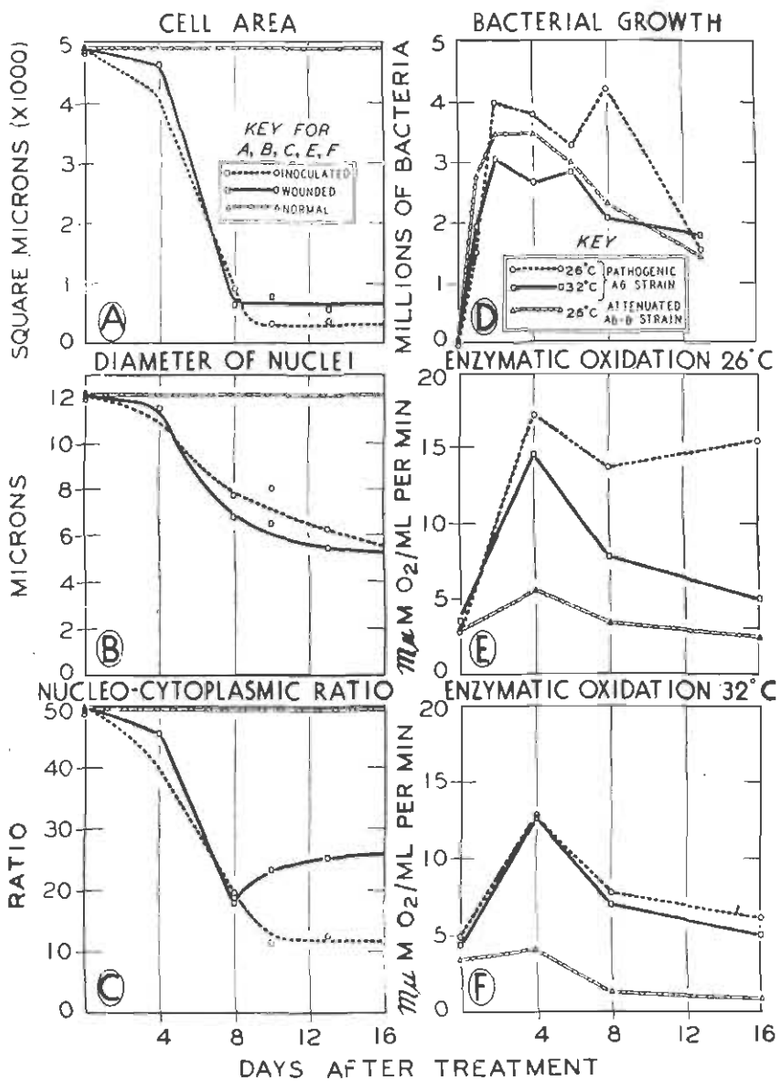
Within 4 days these changes were more extensive and a few cell divisions had occurred. With time, an increase in cell division was observed along with the development of hyperplasia and hypertrophy so characteristic of these tumors. Frequently, the nuclei of the plant cells were observed at cell-walls adjacent to intercellular spaces containing bacteria (Pl. I, Fig. 1). The early divisions often were unequal and new cells were formed within the older cells, resulting in the original cell becoming divided into a number of cells (Smith, 1922).

Studies of sterile wounds did not reveal the changes in cell-wall properties, however, yellowing was noticed in dead cell-walls adjacent to the wound in the endodermis; this persisted a few days and disappeared.

Cytologically, another sequence appeared (Riker, 1927). All living, unlignified tissues seemed able to respond to the bacterial stimulus. Regions in which cell division was most active were studied in merely wounded as well as wound-inoculated stems. The average size of cells, nuclei, and the nucleo-cytoplasmic ratio all decreased sharply during the 8 days after wounding and inoculating (Text-Fig. 1, A–C). Recovery toward normalcy began on the 13th day after wounding alone. However, a minimum cell size was not reached until 33 days after inoculating and was followed by a slight rise. Hypertrophied cells seemed to resist the stimulus to cell division.

Wounding alone influenced the plane of division and as many as 7 or 8 new cells formed in a row away from the source of the stimulus with the plane of each of the new walls parallel to the surface of the wound. The plane of division following inoculation reflected the position of the bacteria in addition to the wound influence. The stimulus for cell division disappeared after 5–10 cell divisions following wounding alone, whereas the stimulus persisted when tumors formed after wound inoculations. No definite evidence of amitosis or multinucleate cells was observed. Cell division followed karyokinesis.

An interesting temperature relationship, discovered by Riker (1926), has proved a very useful tool for differentiating changes caused by wounding from those caused by the bacteria. Inoculated tomato plants incubated in an air temperature of 26° produced large tumors and at 28° produced slower growing tumors, whereas those incubated at 30° C. and higher showed no tumors. Tomato plants grow well in the temperature range (26–32° C.) and the virulent A_6 strain of bacteria



TEXT-FIG. 1. A developmental sequence is emphasized by this composite illustration of research observations in relation to the factors critical for tumor formation in tomato. A-C, nucleo-cytoplasmic changes (Riker, 1927). D, growth of virulent and attenuated bacteria in the host following inoculation (Riker *et al.*, 1941). Tumors form at 26°, but do not form at 32° C. The attenuated strain produces a tumor with a slower growth rate. E-F, the enzymatic oxidation of catechol in terms of tissue dry weight ($m\mu$ M O_2/ml. per min. per mg. dry weight). Note the

reaction to wounding and the temperatures critical to tumor formation (Spurr *et al.*, 1962).

multiplies at a similar rate at both 26° and 32° C. (Text-Fig. 1 D) (Riker *et al.*, 1941). Thus, the following question may be asked: is the failure of tumor formation at 32° C. an effect on the action of the bacteria, the host, or their interaction? This question was answered by thermal manipulations which revealed that the effect was on the action of the bacteria and that the wound process in the host was conducive to tumor formation at both 26° and 32° C. (Braun, 1947).

Tumor growth rates also may be controlled by using different strains of bacteria. A virulent strain of single-cell origin (Wright *et al.*, 1930) was again single-celled and the virulence of these progeny examined; thus, a naturally attenuated strain was discovered (Hendrickson *et al.*, 1934).

The use of controlled growth rates by thermal manipulation was greatly expanded with periwinkle plants (Braun, 1947, 1952; Braun and Mandel, 1948). These studies revealed: the size or growth rate of tumors was dependent upon the time of exposure to a lower temperature (26°); the tumor formation took place in a delimited period of time, *i.e.*, between the 2nd and 4th day after inoculation; once tumors were formed during the 4 days following inoculation, the bacteria were no longer required for the continued growth of the tumors; the tumors grew well in the range 26–32° C. These studies led to the conclusion that the action of the bacteria could be thermally controlled to obtain tumors having various growth rates.

Investigations of the morphology and cytology of these tumors were conducted by Kupila (1958). One nucleolus generally appeared in the nuclei of normal tomato stem cells. In certain large nuclei in the cortex and pith cells, 2 or even 3 nucleoli appeared. However, in a growing tumor, even small nuclei often contained two nucleoli and the larger nuclei contained several nucleoli. In older tumors, the majority of nuclei had one nucleolus. A comparison of cytological structure in 3 hosts revealed a relationship between the normal stem and its respective tumor. If the normal stem contained cells of different degrees of ploidy, the tumors contained cells of highly variable size. Normal stems of tomato and pea had different degrees of ploidy. Tumors on these hosts contained dividing cells, mainly diploid and tetraploid, and large, continuously enlarging cells which in old tumors represented degrees of ploidy higher than ever observed in normal tissue. The stem of sunflower is wholly diploid and the tumor cells of sunflower were diploid and uniform in size. It was suggested that the type of mitosis prevailing in each nucleus at the time of tumor formation becomes fixed and the cell continues with that type of mitosis.

The interaction of the wound and the pathogen within the host were further elucidated through biochemical studies of DNA and enzymatic oxidation. The DNA content of broad bean internodet

increased following wounding and inoculation (Kupila and Stern, 1961). This increase reached a peak about 2 days after treatment and then began to decrease. After 3 days, the DNA level began to increase again in the inoculated internode and continued until a high level was reached. This is visualized in Text-Fig. 1 E. Thus, an increase in DNA occurs with a stimulus for cell division such as associated with wounding and tumor formation.

A relationship appeared *via* measurements of the enzymatic oxidation of ascorbic acid and catechol in tomato (Spurr *et al.*, 1962). The use of thermal manipulation and different bacterial strains to produce tumors with different growth rates demonstrated a direct relationship between tumor growth rate and oxidase activity. Also, the stimulus of wounding and tumor formation was related to the oxidase activity of the tissue (Text-Fig. 1 E, F). This oxidase activity was the first physiological reaction to be correlated with the factors involved in crown gall tumor formation.

The significance of using different bases for the biochemical comparison of normal and tumor tissues recently was re-emphasized in a study of normal and tumor tissues of red beet-roots (Scott *et al.*, 1962). On a DNA basis (per μg. DNA), the two tissues had similar cell numbers, protein contents, and respiratory rates; however, wet and dry weights, acid-soluble phosphorus, and inorganic phosphate were higher in normal tissue while tumor tissue contained more RNA. Comparisons on a DNA, protein and per cell basis were urged.

Caution seems necessary in applying the findings with one host species to another species. First, per cell or per DNA comparisons are most convincing when tissues are fairly uniform in cell composition. However, we have discussed the diversity of stems and tumors in relation to size, shape, and nucleo-cytoplasmic content of the cells. These factors tend to complicate results. In a comparative study of normal, wounded and inoculated tomato stems, there was no observable change in dry weight for 8 days after treatment (Spurr *et al.*, 1962). This was true for wet weight through day 4. Total nitrogen increased slightly and oxidase activity increased significantly by day 4, the time when cell division was just beginning and the nucleo-cytoplasmic ratio had not appreciably changed. Thus, with practically the same number of cells, there was a marked increase in oxidase activity.

Knowledge concerning the nature of the metabolic changes within cells making them tumorous increased with the development and use of tissue culture techniques. Specifically, it was established that tumor cells had a greater than normal capacity to synthesize compounds essential for growth. This increased synthetic capacity may be ion-activated through an increased or improved ability of tumor tissue to obtain and use certain inorganic ions (Wood and Braun, 1961; Braun and Wood, 1962).

Very little specific information has been presented thus far concerning the bacteria. What is their action? Consider this view point:

some action of the bacteria blocks, interrupts or changes any one or a combination of the steps of the nucleic acid sequence within a normal cell at a critical time such as when the cell is responding to a wound. The result is the normal cell is changed to a crown gall tumor cell. Thus, the continual production of modified wound cells forms a crown gall tumor. The tumor cells are commonly called undifferentiated. However, there is a possibility that they are differentiated, not into cortex, stem and leaves, but into tumor cells and in this way are as much differentiated from growing points as other tissue.

Fortunately, extensive cultural studies have made available much information. The bacteria are able to ferment practically all the common sources of carbon whether in the form of sugar, alcohol or organic acid with the production of H_2CO_3 and little if any other acid. Also they use NH_3, NO_3 and practically all the common organic sources of nitrogen. often with the production of an alkaline reaction (Sagen *et al.*, 1934; Hendrickson *et al.*, 1934). An alkaline reaction appeared also when the bacteria were grown in various types of tomato stem extracts (Riker, 1926; Spurr, unpublished results).

The main culture product besides CO_2 is a bacterial gum (Conner, Peterson and Riker, 1937; Conner, Riker and Peterson, 1937) the properties of which have been extensively studied without being related to tumor formation (Hodgson *et al.*, 1949). It is interesting that more gum is produced by a virulent strain than by an attenuated strain (Hodgson *et al.*, 1945).

At the critical time for a plant cell to change from a normal cell to a tumor cell at least these conditions prevail. The cell finds itself in a condition of reduced oxygen tension from the normal as a result of several situations: (1) the flooding of the intercellular spaces reduces the free exchange of gas from the surface; (2) the metabolism of the host cells and particularly that of the bacterial cells uses considerable oxygen; (3) any localized change in the reaction in an alkaline direction reduces the oxygen tension. Although the bacterial gum is physiologically inert, if it should pass into the cells and mix with the cytoplasm, its viscous nature would slow down the movement of cytoplasm and thereby reduce metabolic activity. Thus, the bacteria may encourage cell division in the host through the modification of physiological conditions.

In addition, the bacteria within the host are competing with the host for various minerals and compounds necessary for growth. At this time the bacteria are producing enzymes that further alter the local milieu and the host cell, and some may react within the host cell. Attenuation studies give credence to this relationship. Glycine-attenuated bacterial strains grew better and were enzymatically more active in the presence of glycine than were unacclimatized strains (Van Lanen *et al.*, 1952).

Once the necessary changes occur the bacteria are no longer needed since the further growth of the tumor cells is autonomous. One can

expect that the change in some cells would be greater than in other cells. Thus, cells with different capacities for growth would be expected. This has been demonstrated with clones of crown gall tumor tissue derived by the isolation of single cells from the same parent tissue (Muir *et al.*, 1958).

Numerous reports have stated that crown gall tumors contain more oxidizing enzymes than normal tissue (*e.g.*, Nagy *et al.*, 1938). With the development and use of the oxygen electrode the determination of many enzymes was possible. Two enzymatic reactions studied, *i.e.*, the enzymatic oxidation of ascorbic acid and catechol, had the same temperature relations as those associated with crown gall tumor formation (Spurr *et al.*, 1962). This emphasized the changes in process during the critical time for tumor formation and helped distinguish the contributions of the host and the bacteria to tumor formation, *i.e.*, initiation of enzyme activity from wounding and retention of enzyme activity from the bacterial action. This is strong evidence that we are approaching an understanding of the etiological factors in the formation of these plant tumors.

Several research observations have been described and an attempt was made to relate these to the mechanism of crown gall tumor formation. The observations were related as much as possible by the time of their appearance and to their origin, *i.e.*, host or bacterial. Although many observations cannot be related in this manner they contribute to the over-all understanding of the problem. It is clear that both the host and the bacteria contribute to tumor formation. The use of several controls such as normal, wounded and wound-inoculated stems, helps to distinguish the source of the contribution. It seems clear also that several contributions of both the host and the bacteria complex the problem. Hopefully, the current developments in the field of nucleic acids may help clarify this problem.

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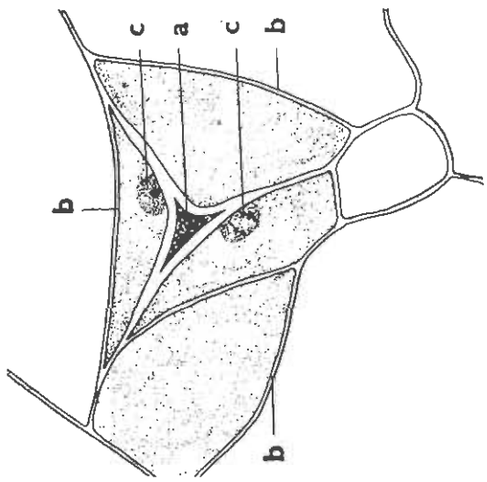
EXPLANATION OF PLATE I

FIG. 1. An intercellular space in the pith of a tomato stem inoculated with tumor-producing bacteria (Riker, 1923 *b*). The intercellular space (*a*) contains many bacteria. New cell walls have formed (*b*) cutting off the stippled cells from the original cells. The nuclei (c) are in a position near the bacteria.



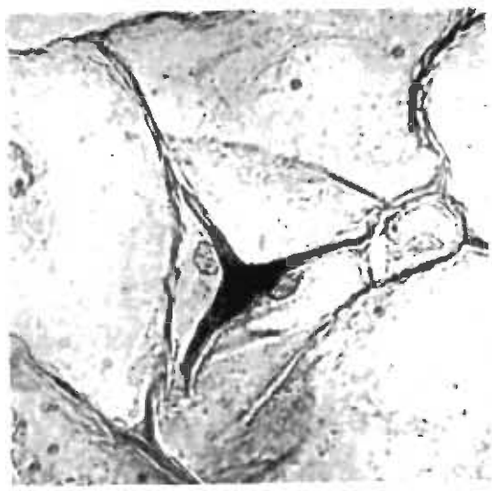


FIG. 1

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MORPHOGENETIC STUDIES ON *OSMUNDA CINNAMOMEA* L. THE SHOOT APEX

By T. A. Steeves

Department of Biology, University of Saskatchewan, Saskatoon, Canada

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Prior to the extensive descriptive and experimental studies of Wardlaw, investigations of the shoot apices of ferns were concerned almost entirely with the presence or absence of an apical cell and with the segmentation pattern and the immediate derivatives of such an initial cell if present. Wardlaw's descriptions of the apex of *Dryopteris* (1947, 1949, 1950) and several other ferns emphasized the importance of the sub-apical region in which the beginnings of tissue differentiation may be detected and made possible a comparison with apices of seed plants in which histogenesis has been extensively investigated. The resulting comparisons, however, seem to have led to confusion and misunderstanding rather than to agreement in interpretation, particularly with reference to the cellular configuration of the sub-apical region and its significance (Esau, 1954).

The predominant view of the initiation of vascular tissue in seed plants seems to be that procambium is the first identifiable stage of vascular differentiation and that procambium arises specifically in relation to developing leaf primordia at the shoot apex (Esau, 1954). Thus, in these megaphyllous plants, the vascular system is regarded as distinctly leaf-oriented in its development. It is often stated (Kaplan, 1937; Esau, 1954; Clowes, 1961) that procambium arises from a recognizable region of residual meristem (Restmeristem) which precedes it in appearance; but this region is regarded as one which has retained the active meristematic state in contrast to other regions around it rather than as the first stage in vascular differentiation. On the other hand in several ferns, Wardlaw (1944) has presented a very different interpretation of vascular initiation. Immediately below the superficial layer of the apex, Wardlaw recognizes a central core of potential vascular tissue which is set apart from surrounding regions by the fact that it has undergone the first phases of vascular differentiation and is, in this sense, potentially vascular. Subsequently the actual vascular tissue differentiates from among the cells of the potential vascular tissue; but a considerable portion of this tissue gives rise to parenchyma in the pith and leaf-gap areas. Experimental evidence has shown the important influence exerted by leaf primordia in this subsequent differentiation of vascular tissue, but not in the initial differentiation.

The differences reported between ferns and seed plants may well represent real differences between the two groups, although, since both are megaphyllous, a higher degree of similarity might be expected. Esau (1954) has expressed the view that the situation in ferns is not materially different from that described for seed plants and that procambium developing in relation to leaves is the first stage of vascular differentiation in both cases. Moreover, Wardlaw (1950) has extended his experimental techniques to the dicotyledon *Primula* and found evidence to suggest a high degree of similarity with his earlier findings on ferns. The problem appears to have reached the stage at which only further investigation can be expected to contribute to a solution. It is with this thought in mind that an investigation of the shoot apex of *Osmunda cinnamomea* L. is presented here.

Material and Methods

In this study, only shoot apices of adult plants were used. Most of the rhizomes were collected in the vicinity of Cambridge, Massachusetts, and collections were made at intervals throughout the growing season to facilitate an evaluation of seasonal changes in the structure of the apex. These collections were supplemented by rhizomes kept over winter in a cold room, forced in a greenhouse and fixed at various intervals from the time of forcing.

Before fixing, the apical bud and a short piece of mature rhizome were cut off and most of the leaves were removed from the bud. The apices were fixed in a chrom-acetic-formalin fixative, Craf III, and, after a minimum of 24 hours, were washed in running tap-water. In some cases, the pieces were treated briefly with hydrofluoric acid to soften sclerotic tissues. After washing, the material was dehydrated in successive changes of ethyl alcohol up to 50%, following which it was carried through an *n*-butyl-ethyl alcohol series to absolute *n*-butyl alcohol. It was then infiltrated with paraffin wax, the final embedding being in 56–58° Fisher tissuemat. In general the procedures followed the technique of Pratt and Wetmore (1951).

Longitudinal and transverse serial sections of the stem tips were cut eight or ten microns in thickness. The sections were stained with Haidenhain's iron-alum hematoxylin and counterstained with safranin.

The Structural Configuration of the Shoot Apex

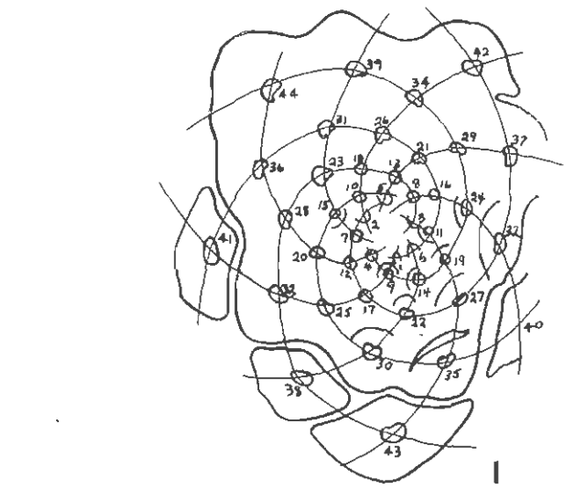
In this consideration of apical organization in *O. cinnamomea* the concepts and the terminology adopted by Esau (1953) for vascular plants in general will be followed as far as possible. The term *shoot apex* is used in a non-specific sense to designate the formative region at the shoot tip, and as such would include the undifferentiated, self-perpetuating meristem and the partly differentiated tissues subjacent to it which, while remaining actively meristematic, have their future course of development at least tentatively determined. Within this complex, the

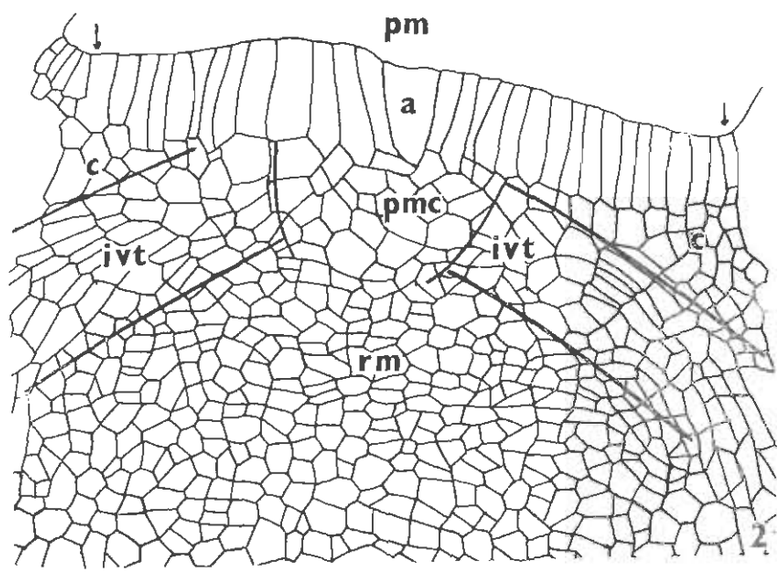
promeristem may be thought of as consisting of the initial cell or cells and their immediate derivatives. As such it contrasts functionally with the partially determined meristematic tissues of the subjacent region of initial differentiation within which the three tissue systems of the primary body (vascular, dermal and fundamental) are beginning to take form. The morphological boundary between promeristem and region of initial differentiation, however, is seldom a sharply recognizable one. In this context the term *apical meristem*, which in the past has had a variety of meanings, is dispensed with.

The apex of *O. cinnamomea* has the shape of a low cone in most cases, although it may sometimes be nearly flat (Plate 1, Fig. 1). The apical cone ordinarily has a diameter, within the inner limits of the youngest visible leaf primordia of 400–600 microns. Around its base are placed the closely spaced young leaf primordia which form a continuous series with a large number of progressively older but still immature leaves in various stages of development (Plate 1, Fig. 2). These primordia and young leaves, together with the apex itself, make up what has been termed the apical bud (Steeves and Wetmore, 1953). The presence of a large number of leaves in this region, 60–80 in plants of average size, reflects the extremely slow rate of development which is characteristic of leaves of this species (Steeves and Briggs, 1958).

The leaves of *O. cinnamomea* are arranged in a close helix ascending to the base of the apical cone. New leaves are produced in succession at the rim of the apical mound, each primordium arising between and only slightly nearer the apex than two primordia five and eight plastochrons older than itself (Text-Fig. 1). In transverse sections of the apical region it has been determined that there are 5 + 8 contact parastichies, which corresponds to a phyllotactic fraction of 5/13 (Esau, 1953). Of 125 species in which phyllotaxis was studied, 71 (57%) had right-handed (clockwise) helices and 54 (43%) left-handed (counterclockwise) helices. Since observations were made at the shoot apex, the directional terms used here refer to the helix passing from younger to successively older leaf primordia, that is, the opposite of the genetic spiral. Thus somewhat more than half of the plants were dextrorse. Since the number of individuals examined was small, however, it seems unwise to conclude that there is any significant deviation from the expected equal occurrence of the two patterns. De Bary (1884) recorded a 5/13 phyllotaxis for *O. regalis* and a similar conclusion was reached by Van Tieghem (1891) and Zenetti (1895). In sporelings of *O. cinnamomea* Faull (1909) observed a phyllotaxis of 1/2 for the first 5–8 leaves, changing with age to a 3/8 pattern which persisted for the next 15–20 leaves. He did not report upon leaf arrangement in adult plants.

In the course of the study just oulined 6 additional instances have been found in which the phyllotaxis deviated from the usual pattern in that the leaves arose in pairs, the members of which had a divergence of somewhat less than 180°, that is, were not quite opposite. In transverse sections of the apical region of one of these specimens, there





TEXT-FIGS. 1-2. Fig. 1. Diagrammatic representation of leaf arrangement as seen in transverse section of the shoot apex at the level of the promeristem. Leaves are numbered in basipetal sequence. This plant is dextrorse and shows the characteristic 5 - 8 contact parastichies (5 long, 8 short). Fig. 2. A tracing of a photograph of the section shown in Plate I, Fig. 3 with the structure indicated diagrammatically. *a*, probable apical cell; *pm* promeristem (delimited by arrows); *pmc*, pith mother cells; *ivt*, incipient vascular tissue; *rm*, rib meristem; *c* cortex.

appeared to be 4 + 6 contact parastichies, or 2 (2 + 3). If this analysis is correct, these unusual cases would conform to the bi-jugate type of phyllotaxis (Richards, 1948). Voeller and Cutter (1959) have reported the occasional occurrence (8·6% of cases studied) of such a system in natural shoots of *Dryopteris aristata* in England, and from experimental studies have concluded that it has no genetic basis but rather results from physiological conditions in the shoot apex. In the *Osmunda* sample examined the frequency of occurrence of bi-jugate phyllotaxis is about one-half that reported for *Dryopteris*.

The cellular construction of the shoot apical region has been investigated in serial transverse and longitudinal sections of numerous apices collected throughout the growing season and during the period of dormancy. In median longitudinal section (Plate I, Figs. 1, 3; Text-Fig. 2) it may be seen that the apical mound is covered by a single layer of large, rectangular cells (90–100 microns in length) whose cytoplasmic density appears to be low and whose nucleo-cytoplasmic ratio is small. In transverse section (Plate I, Fig. 2) this superficial layer of large cells may be seen extending down the flanks of the apical mound to the adaxial margins of the youngest leaf primordia and somewhat further down between the primordia. Thus the surface layer has a rather wavy marginal outline, being terminated by the cell divisions which mark the initiation of leaves or, between leaves, by the divisions which subdivide the cells preparatory to the beginnings of differentiation.

In the Leptosporangiate Ferns, the center of the superficial layer is occupied by a tetrahedral or a bi-facial apical cell. In the Eusporangiate Ferns, on the other hand, there is usually a small group of apical initials rather than a single one (Bower, 1923). The Osmundaceae, which in many respects are intermediate between the two, appear to be rather variable in their apical segmentation pattern. Bower (1889–90) has reported that in *Osmunda regalis* there is, in most cases, a tetrahedral apical cell which cuts off segments from its three lateral faces in ordinary succession. He found, however, that the form and size of the cell may not be regular and that, in some cases, it is not possible to refer all of the cells of the superficial layer to the segmentation of a single initial. He has also pointed out that in longitudinal section it is very difficult to recognize an apical cell. In *O. regalis* Iossa (1914) reported that she could always find a single apical cell in median sections of well-preserved apices. The same author reached a similar conclusion for *O. cinnamomea*. Cross (1931) has described a definite apical cell in the sporeling of *O. cinnamomea*, although its segmentation is not so regular as in typical Leptosporangiate Ferns. In older plants, however, Cross reports that the segmentation pattern becomes progressively less regular, and ultimately the original apical cell is replaced by a group of initials.

In the present investigation observations were made upon adult plants collected at various times during the year. In most cases it has been possible to recognize a single apical cell which is triangular or nearly so in outline; but frequently this initial cell is no larger than some

of its immediate derivatives. Moreover, as Cross (1931) has pointed out, the segmentation is often not regular; and the evidence suggests that there are seasonal differences in the degree of regularity. During the period of dormancy the apical cell is always clearly visible and all of the superficial cells around it appear to be traceable to segments of the single initial (Plate I, Fig. 2). The identity of the segments, however, is soon obscured by subsequent divisions. In the early part of the growing season when the shoot apex appears to be extremely active, the segmentation pattern becomes irregular, primarily, it appears, because derivatives of the apical initial themselves cut off segments, that is, function temporarily as initial cells. In mid-summer, the segmentation pattern becomes more regular again, and the apical cell is relatively distinctive for the remainder of the growing season. With the onset of dormancy, it is always clearly recognizable. In longitudinal section it is seldom possible to distinguish the apical cell from its immediate derivatives with absolute certainty (Plate I, Figs. 1 and 3). It is, however, much easier to do this during late summer than during the early part of the growing season. During the period of dormancy the apical cell is difficult to observe in longitudinal section because of the general lack of differential staining in the shoot apex.

It thus appears that the pattern of segmentation in the superficial layer of the shoot apex of *O. cinnamomea*, and consequently the distinctness of the apical cell, is a phenomenon which exhibits seasonal variation. A reasonable conclusion would be that the pattern of segmentation varies with changes in the activity of the apex, being less regular during periods of greatest activity. However, it would be unwise to accept this as an established conclusion until such time as careful quantitative studies of cell division in various regions of the shoot apex throughout the year have been carried out. Such investigations are now in progress. It may be noted in passing that the fluctuating structural pattern of the center of the superficial meristematic layer gives support to the conclusion that this region is active in the growth of the shoot, in agreement with Gifford's (1960) results obtained by more direct methods.

In several of the Leptosporangiate Ferns and in one Eusporangiate Fern, Wardlaw (1944, 1946, 1947, 1950) has described the initial differentiation of vascular tissues immediately below the superficial layer and has designated the first stage of this differentiation as *incipient vascular tissue*. Similarly, the earliest morphological evidence of tissue differentiation can be detected immediately below the superficial layer in *O. cinnamomea*. This is most clearly seen in median longitudinal section of the shoot apex (Plate I, Fig. 3; Text-Fig. 2). In *Osmunda* it is not possible to demonstrate a homogeneous zone of incipient vascular tissue beneath the superficial layer as Wardlaw has done in *Dryopteris*. Directly beneath the center of the superficial layer there is a cluster of relatively large and lightly staining cells of isodiametric shape. Around this central zone there is a ring of smaller and denser cells which appear to be derived from the more peripheral cells of the superficial layer. The cells of the ring are cut off by unequal divisions of the

superficial cells, and the subsequent divisions of these cells are predominately, if not entirely, longitudinal and are inclined about half-way between the vertical and the horizontal. In cases in which the apex is very flat, the plane of division may be almost horizontal. The result of this orientation of the plane of cell division is that the cells of the ring are elongate, that is, longer than they are wide, but not necessarily longer than other cells around them. The cells of the ring are progressively more elongated away from the central zone and are continuous basipetally with obvious procambium of the vascular system (Plate I, Fig. 1). The peripheral ring, which may be likened to a short truncated cone with its apical end pointed towards the center of the superficial layer, is considered to constitute vascular tissue in the initial state of differentiation and is designated *incipient vascular tissue* according to Wardlaw's terminology. In view of the elongate character of the cells, it might be appropriate to consider this tissue to be *procambium* (Esau, 1954). The advantages and disadvantages of such a designation will be considered in the discussion.

The central group of enlarged isodiametric cells (Plate I, Fig. 3; Text-Fig. 2) is somewhat more difficult to interpret. Its origin appears to be comparable to that of the incipient vascular tissue; that is, its cells are cut off basipetally from cells of the superficial layer, but only from those in the centre of the superficial layer, immediately adjacent to the apical cell, rather than from those at the periphery. Moreover, the boundary between the central zone and the ring of incipient vascular tissue is not a sharp one, at least morphologically. Immediately below the superficial layer evidence may be found for the segmentation of peripheral cells of the central zone and their addition to the incipient vascular tissue. Conversely near the base of the central zone, where it shows some increase in transverse width, cells of the incipient vascular tissue appear to contribute to this widening. Final verification of these relationships, however, must await the results of a careful analysis of cell division patterns and cell lineages now in progress. At its base the central zone is bounded by a cup-shaped zone of small, densely staining cells in which are found evidence of active cell division, both transverse and longitudinal. In the lower part of this zone, at least, transverse divisions seem to predominate, and the cells are in vertical files as a result. Although these files are less regular and less distinct than those found in many gymnosperms and angiosperms, the cup-shaped zone in *O. cinnamomea* conforms to the concept of *rib meristem* and is so designated.

The initiation of the cortex and the epidermis is much less easily recognized because of the interruption of these tissues by numerous leaf primordia and their developing traces. The ground meristem of the cortex is initiated by cells of the surface layer at or near the periphery of the layer in much the same way that the incipient vascular tissue is formed (Plate I, Fig. 3; Text-Fig. 2). The cells of the cortex, however, do not divide predominantly in a longitudinal fashion and remain isodiametric. At the margins of the superficial layer, the large

cells themselves become subdivided, and the layer of cells at the surface becomes the protoderm. This can only be seen where there is no leaf primordium at the edge of the apical cone and can be followed only with difficulty.

The initial stages of differentiation are less easily detected in transverse sections of the shoot apex than in longitudinal sections because the distinctive shape of the cells of the incipient vascular tissue is less clearly seen. A transverse section cut just at the base of the superficial layer (approximately 100 microns from the summit of the apical mound) shows the central zone of enlarged cells surrounded by a relatively narrow band of cells identifiable as incipient vascular tissue by the occurrence of numerous oblique or longitudinal recently formed cell walls (Plate I, Fig. 4, arrows). In active apices, mitotic figures showing the same orientation may be recognized. This ring of incipient vascular tissue is much more clearly seen in apices which are relatively flattened than in those which are markedly conical. Peripheral to the incipient vascular ring is a broad region of developing cortical tissue in which may be seen, in a regular pattern, the traces of the leaf primordia which surround the apical mound. At a slightly lower level in the apex (25–50 microns) the incipient vascular tissue consists of a broad zone of distinctly elongate cells extending from the central zone at the center to the ring of leaf traces at the periphery and merging with the traces (Plate I, Fig. 5). The oblique or nearly horizontal orientation of the cells of the incipient vascular tissue greatly facilitates the recognition of this tissue in transverse sections. Thus an examination of serial transverse sections of the shoot apex substantiates the interpretation based initially upon longitudinal sections.

Discussion

In the description of the shoot apex of *O. cinnamomea* given above, no attempt has been made to apply a precise terminology to the various regions of the apex. It would seem to be desirable to do this now as a means of facilitating future work on this apex which is now in progress. The superficial layer of prismatic cells has been designated by Wardlaw (1943, 1947) in *Dryopteris* and several other ferns the *apical meristem*. Because of the diverse meanings given to this term in various plant groups and by various authors (Clowes, 1961) it does not seem advisable to retain it here. Rather it would seem that the term *promeristem* could be more appropriately applied to this distinctive layer in ferns which includes the initial cell and its immediate derivatives and is distinct from the partially determined meristematic tissues of the immediately subjacent region. In this sense it corresponds to the widespread usage of the term in the seed plants (Esau, 1953) with the difference that in ferns the promeristem is structurally recognizable whereas it is not in most seed plants. On the other hand some authors (Clowes, 1961) believe that the term promeristem loses its significance and value unless restricted to the initial cells, and the term as used here does not conform to this definition.

Immediately below the promeristem and derived from it is the region of initial differentiation in which the tissue systems of the stem are beginning to take form. Protoderm and the ground meristem of the cortex may be detected here, but they are difficult to observe clearly because of the close packing of leaf primordia around the apex. The tissues which represent the first stage in the differentiation of the stele, however, are readily distinguishable by morphological criteria. The peripheral tissue of this region has been designated *incipient vascular tissue*, although it is by no means irrevocably determined as vascular. Because the cells of this tissue are somewhat elongate from the outset, it would perhaps be possible to designate them as procambium, particularly since they are continuous below with obvious procambium; but there are reasons for not following this course. The term procambium is generally used to designate tissue which is well committed to the formation of xylem and phloem, whereas the incipient vascular tissue of *Osmunda* is less specifically designated. Moreover it appears that the comparable meristematic tissue in some other ferns does not necessarily possess the structural characteristics which would permit it to be called procambium; and it is desirable to maintain a degree of uniformity in terminology. Further investigation, however, may require a subsequent change in the designation of this tissue.

The center of the incipient stelar region is occupied by larger, isodiametric and more vacuolated cells which are developmentally related to the pith through a rib meristem. In the incipient stelar region these cells have been interpreted as representing the first stage in the differentiation of the pith and this interpretation is supported by experimental evidence to be reported elsewhere that when a pith is not formed, that is, when a protostele is differentiated, these cells are absent from the incipient stelar region. It would seem appropriate to designate the cells of this central zone as *pith mother cells*. The incipient vascular tissue and the pith mother cells together constitute the stele in the initial stages of differentiation and may collectively be designated as *prestelar tissue*, adopting the terminology used by Wardlaw (1950), and by Wetmore (1956). As originally used (Wardlaw, 1950), the term prestelar tissue replaced incipient vascular tissue; but there would seem to be no difficulties involved in its more inclusive use here particularly since interchanges between its two components have been noted.

Although some differences have been noted, the general pattern of the shoot apex in *O. cinnamomea* corresponds closely to Wardlaw's descriptions for other fern species and certainly supports his interpretation of the initial differentiation of vascular tissue immediately below an undifferentiated surface meristem. The prestelar tissue is set off from surrounding cortical ground meristem immediately behind the promeristem, not, however, as a solid core of incipient vascular tissue, but rather as a ring of incipient vascular tissue surrounding a central group of pith mother cells. The structural changes involved in the segregation of prestelar tissue in *Osmunda* lend support to the interpretation that this process represents the earliest stage in the

differentiation of the stele and not the mere retention of meristematic activity while surrounding tissues are tending to lose it. Thus, whatever may be its applicability in the seed plants, the concept of residual meristem does not appear to be meaningful in the case of *O. cinnamomea*, although Kaplan (1937) has applied it to several ferns including *O. regalis*. To regard the distinctive cells of the prestelar tissue of *O. cinnamomea* as representing a mere residual meristematic derivative of the promeristem would be to ignore the striking dissimilarity between these cells and those of the promeristem as well as the obvious developmental relationship of the prestelar tissue to the later stages of stelar differentiation.

This interpretation of the structure and activity of the shoot apex of *O. cinnamomea* is in many respects a preliminary one and it is intended that this study be followed by more detailed analyses of specific problems. Before valid comparisons with seed plants can be made, it will be necessary to carry out a complete analysis of vascular differentiation including a study of the influence of developing leaf primordia upon this process. It seems probable that a fuller understanding of the dynamic organization of the shoot apex could be achieved by a careful study of the distribution of cell divisions and an analysis of cell lineages as well as by the application of various histochemical and cytochemical methods to the problem of initial differentiation. Surgical experiments on the shoot apex coupled with careful studies of development in operated apices might be expected to be revealing of causal factors in differentiation as well as of latent developmental potentialities which are not normally expressed. Some of this work is now in progress. Finally, it would be desirable to extend these investigations to other ferns, particularly to some species in which the prestelar tissue is less distinct morphologically than it is in *O. cinnamomea*.

SUMMARY

The shoot apex of *Osmunda cinnamomea* consists of an apical cone surrounded by closely packed leaf primordia in a 5 + 8 (5/13) phyllotactic pattern. The apical mound is covered by a single layer of large cells which are regarded as constituting the promeristem. The center of the promeristem is occupied by an apical cell, but its pattern of segmentation is rather variable, being less regular during periods of greatest growth activity. Immediately beneath the promeristem, in the region of initial differentiation, the prestelar tissue may be detected. This consists of a ring of incipient vascular tissue consisting of elongate cells surrounding a central mass of isodiametric pith mother cells. The incipient vascular tissue is continuous basipetally with procambium of the developing vascular system and the pith mother cells give rise to the pith *via* a rib meristem. The bearing of these observations upon the problem of the initial differentiation of vascular tissue in the vascular plants is discussed.

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EXPLANATION OF PLATE I

FIG. 1. Median l.s. of shoot apex showing apical cone between 2 leaf primordia, ×35.

FIG. 2. T.s. of dormant shoot apex just below summit of apical cone. Note promeristem with apical cell surrounded by leaf primordia. Positions of first 5 primordia numbered, ×35.

FIG. 3. Median l.s. of shoot apex showing details of structure. Note surface promeristem with probable apical cell, elongate cells of incipient vascular tissue surrounding pith mother cells, and rib meristem. *See* Text-Fig. 2 for same section with labels, ×170.

FIG. 4. T.s. of shoot apex approximately 120 microns below summit of apical cone, slightly oblique. Note pith mother cells in center, incipient vascular tissue (arrows) on the left and leaf traces at the periphery, ×60.

FIG. 5. T.s. of apex shown in Fig. 4 approximately 170 microns below summit of apical cone. Incipient vascular tissue seen extending from pith mothe cells to ring of leaf traces, ×60.

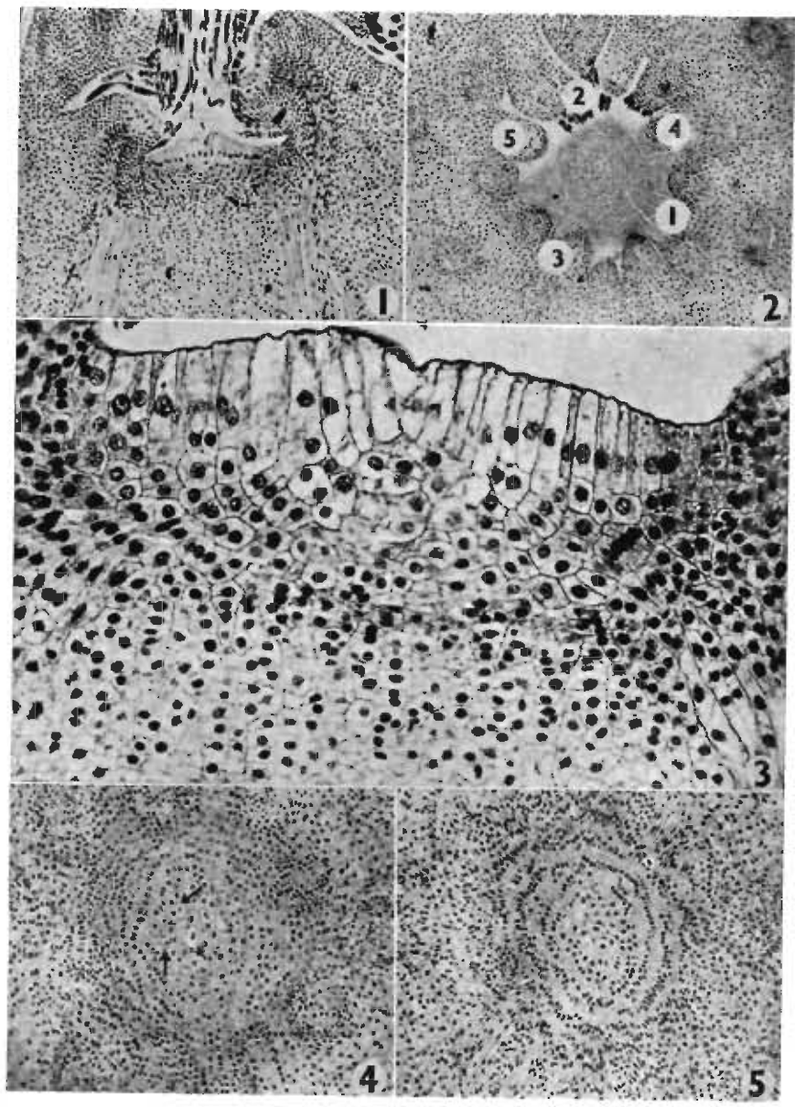

FIG. 1–5

T. A. Steeves

THE TOTIPOTENCY OF CULTURED CARROT CELLS: EVIDENCE AND INTERPRETATIONS FROM SUCCESSIVE CYCLES OF GROWTH FROM PHLOEM CELLS

By F. C. Steward and Marion O. Mapes

Department of Botany, Cornell University, Ithaca, N.Y., U.S.A.

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Daucus carota L. is normally a biennial plant. The familiar storage root of its common cultivars is formed in the first cycle of its growth. After an appropriate period of dormancy and perennation a flowering shoot bears seeds formed in the second cycle of this biennial growth. And so successive life-cycles may be bridged by the formation of seed and viable embryos. As in many biennials temperature (exposure to 5° C.) may promote, to some extent, the premature bolting of the flowering shoot. In some instances the compounds known as gibberellins may substitute for the effect of low temperatures on bolting (Lang, 1957).

Experiments into the culture of the secondary phloem from the storage root, and of free cells derived from it, have shown that this normal sequence of events may now be changed. Growth may be induced in minute explants of the secondary phloem, cut so far from the cambium that they consist only of storage parenchyma that normally would not grow again. When the new growth, stimulated by a medium which contains coconut milk, occurs around a preformed explant, rapid proliferation, without organization, is all that normally occurs. However, by appropriate devices, free cell suspensions can be obtained from the surface of these explants, and the cells so freed will divide when they are suspended in the liquid medium. Such cells give rise to a wide range of growth forms which are not normally encountered either in the cultured explant or the tissue as it occurs normally in the plant body. Two previously unknown responses have been noted with these cells. First, they have been maintained in long continued cultivation as successive liquid cultures, inoculated with freely-suspended cells in the manner of transfer more familiar with micro-organisms. Secondly, the free cells grow into small clusters which readily organize to form roots and, with the formation of organized shoot tips, they form small "embryoids" from which whole plants have been raised. Thus a vegetatively produced "embryoid" grown from cultured phloem cells may bridge two vegetative growth cycles in the manner normally attributable to the setting of seed and the formation of a zygotic embryo.

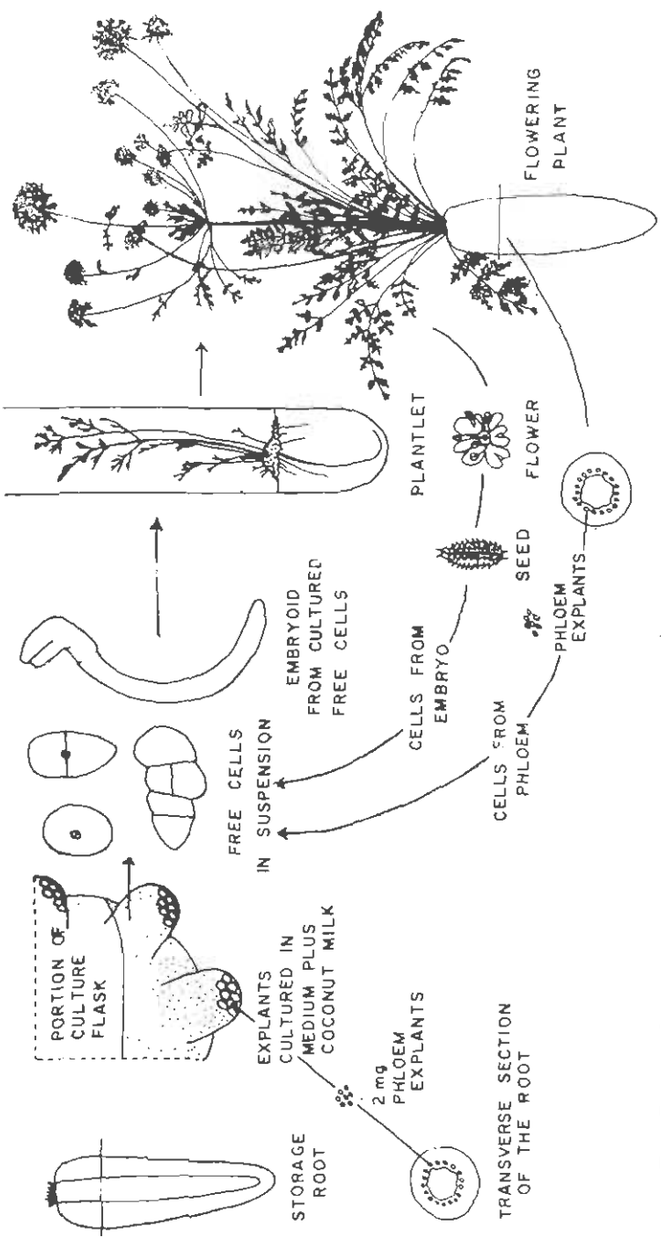
To the first observations of this kind may now be added further examples. Notably, it will be shown that plants bearing carrot roots can be grown from free cells, as heretofore, but that from these storage roots, grown not from seed but from embryoids developed from free cells, the whole process may be repeated. Thus, although the periodic incidence of storage roots may repeatedly give rise to the formation of flowers which set seed, the continuing culture of the strain through successive "life-cycles" may be maintained through vegetatively developed embryoids which originate from cultured cells, instead of from zygotes as in the usual way. The fact that this process may be repeated in yet another cycle of these events is still further evidence of the inherent totipotency of the cultured phloem cells. Moreover, since genetically "pure lines" of carrot are not normally feasible, this may be a technique by which they could be maintained indefinitely and independently of, though concomitant with, the recurrent production of seed.

The sequence described above may be represented by the following series of diagrams in Text-Fig. 1, which will serve as the background against which the new observations may be reported.

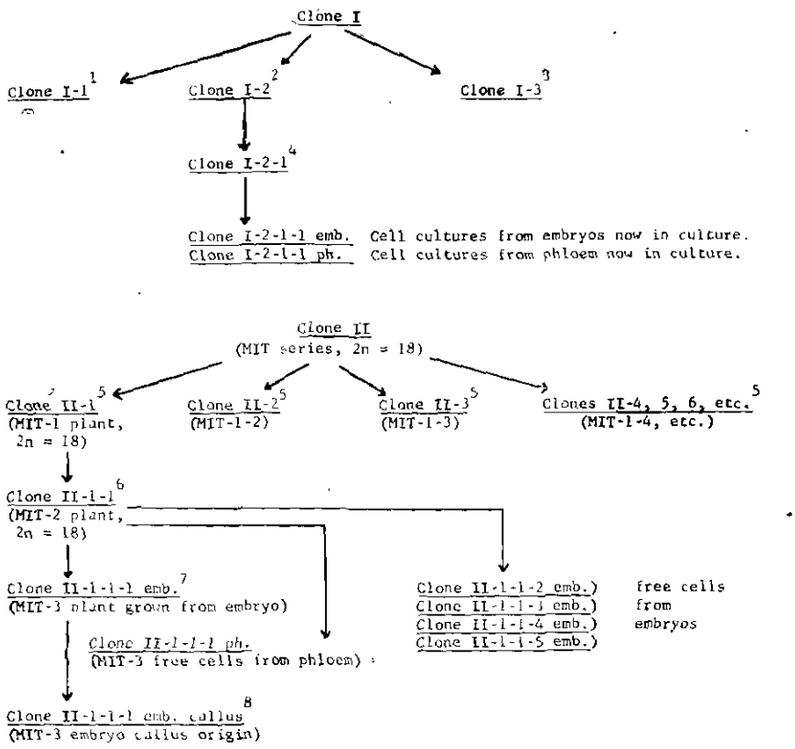
This complete life-cycle of the carrot, which nevertheless bypasses sexual reproduction entirely, has now been traversed several times using different clones of material. These are arbitrarily numbered, as shown in Text-Fig. 2, and summarized as follows:

Clone I-1.—This was derived from a strain of carrot that had been used for purposes of routine assay of the growth factors in coconut milk and by chance it furnished the first cell cultures which were grown freely suspended in the liquid medium. The cytology of the "parent" carrot root was not known. This clone was noteworthy, however, because the freely-cultured cells retained their totipotency and ability to reconstitute whole plants through many (approximately 30) subcultures and a long period of separate cultivation (3 years). Photographs of this first cultured plant, with a small but distinctly orange-colored tap root, were taken and reported by Steward *et al.* (1958 *a*). The storage root was sectioned for photographing and later preserved in alcohol. No derived cultures from this specimen were established.

Clone I-2.—Several other plantlets derived from Clone I were also used to repeat the whole cycle. The objective here was to obtain many plants from one cell suspension to see whether they were constant in their morphology. Two of the older plants (designated Clone I-2 and Clone I-3) were carried through to mature plants bearing inflorescences. Clone I-2 is of particular interest as it was the first plant, cultured from cells, that was successfully grown to the flowering stage (Plate I, Fig. 1 *a*-*b*) and also because cell cultures were again started from the storage root (Plate I, Fig. 1 *c*) to begin a new cycle. Although the cytology of the parent stock was unknown, the chromosome number of this reconstituted plant was determined by Dr. J. Mitra as that of a normal diploid ($2n = 18$).



TEXT-FIG. 1. Growth cycles from freely-suspended cells which were derived from the phloem explants and grown to organized cell clusters and mature plants with inflorescences; the cycle then repeated from the free cells derived from the phloem explants and excised embryos from the reconstituted plant.



1 First cell culture to develop an organized tap root (Amer. Jour. Bot. 45: p. 706).
2 First cell culture to develop into plant with inflorescence. (Referred to in Fig. 3a-d.)
3 Second plant from cells to produce an inflorescence.
4 Another growth cycle from phloem cells leading to *plant with inflorescence* (Fig. 3h).
5 Plants grown from cells from phloem explants -- known cytology (Fig. 4e).
6 Plants raised from cells to maturity. Plant used to start a new growth cycle from phloem and embryo cells (Fig. 4f).
7 Zygotic embryo from 6 raised to mature plant (Fig. 4g).
8 Embryoids from callus grown from the embryo culture of 7 (Fig. 4h and i).

TEXT-FIG. 2. Nomenclature of clones under investigation.

In order to establish a cell culture from this derived plant of Clone I–2 (Plate I, Fig. 1 *a–d*), small explants (3·5 mg) from the phloem of the storage root were again grown in White's basal medium supplemented with coconut milk (10% by volume). These were first grown in culture tubes on June 30, 1959 and were later transferred to large flasks with 250 ml of the medium to obtain a larger population of freely-suspended cells (Plate I, Fig. 1 *e*). Transfers of the rooted clusters were made to agar tubes containing coconut milk medium and the

tubes were kept stationary in the culture room. Shoots with typical carrot leaves appeared after a few months (Plate I, Fig. 1 *g*). The plantlets were maintained in stock culture and transferred to a fresh agar medium when needed. The plants were later (fall of 1961) grown in vermiculite and, when older, transferred to soil in the greenhouse. During this period the plants were nourished occasionally with Hoagland's solution. In January 1963, when the storage root was well developed, the plant produced a flowering stalk (Plate I, Fig. 1 *h*). This shift to the flowering stage without any prior cold treatment or vernalization has been noted, and, although the first plants flowered coincident with the flowering of the wild carrot (*Daucus carota*). the plants did so only after the large storage root was formed.

Clone II–1.—This strain was established from a carrot root of precisely known origin and of determined cytology. A garden variety (Nantes) was selected for this study, and the plant used and the cell cultures derived from it were arbitrarily designated MIT–1. Cultures were started on October 23, 1958 from the phloem tissue which was explanted at a distance of 1–2 mm from the cambium. Free cells (Plate II, Fig. 2 *a*) and cell clusters were obtained in the usual manner and their cytology studied and reported (Mitra *et al.*, 1960).

The first visible root from a cell cluster was observed on February 3, 1959. When the cultures grown from free cells began to show some large cell clusters, one of the flasks was placed in total darkness in the cold room (4–5° C.) on March 5, 1959. After a period of 5 months the flask was brought out for examination and the cell clusters showed abundant roots (Plate II, Fig. 2 *c*). From this flask and from several others rotated on the wheels of the culture apparatus (*cf.* Steward and Shantz, 1956), about 100 of the rooted clusters were transferred to a semi-solid medium containing 2%, 5% or 10% coconut milk on August 26, 1959. The first shoots were noticed in cultures grown in 2% or 5% coconut milk. Some of the cultures transferred to the medium which contained 10% coconut milk continued to proliferate. The plants grew slowly, and as they outgrew the tubes and flasks they were transferred, as required, to vermiculite in the greenhouse.

At least 100 plants were grown in the laboratory and greenhouse in order to record any variations in gross anatomy and chromosome complement. Thus far the chromosome numbers of the organized roots grown from cultured cells have all been of normal diploid number ($2n = 18$), similar to that in the original cells from which the cultures were grown in the first place. Plate II, Fig. 2 *e* shows an assortment of cultured plants, from older ones showing inflorescences and the newly transplanted ones from agar. Probably due to lack of active pollination under greenhouse conditions, only a few flowers per umbel developed seeds.

Clone II–1–1.—This strain of cells was established from the carrot storage roots grown at the end of the first growth cycle of one of the reconstituted plants of MIT–1 (Clone II–1) as these were raised from

cells. This strain was designated MIT-2 in the laboratory record. It will be shown that whole plants, carrot storage roots, and, in the second cycle of growth, an inflorescence bearing viable zygotic embryos have all been grown from the free cells of this strain. Therefore, starting with a carrot root of known origin and of defined cytology the complete "life-cycle", illustrated in the diagram (Text-Fig. 1) has twice been carried completely through. Thus vegetatively produced embryoids grown from free-cell suspensions have twice linked successive "life-cycles" of the carrot plant in the manner normally achieved by fertilization and seed formation. When the plants so grown form flowers they also bear viable embryos. There can, therefore, be no doubt that the cultured carrot phloem cells which linked one "generation" of carrot to another retained the full totipotency equivalent to that in a zygote.

The evidence upon which these statements are based is as follows. In March 1959 one of the first rooted clusters from the cell culture of MIT-1 (Clone II-1) was transferred to a semi-solid medium containing coconut milk. Applying the now usual procedure for growing freely-suspended cells and cell clusters, the rooted cluster and eventually the plantlet derived from it was transferred to vermiculite and then to soil under greenhouse conditions. In September 1960 cell cultures (Plate II, Fig. 2*b*) were started from the storage root and maintained in stock as MIT-2 (*i.e.*, Clone II-1-1). Again when the cell clusters showed roots and later typical carrot shoots (Plate II, Fig. 2 *d*), one of the plantlets was transferred to vermiculite and nourished with Hoagland's solution until it could be grown in soil in the greenhouse. In June 1962 the first flowers opened and the flowering period continued through the summer (Plate II, Fig. 2*f*).

Clone II-1-1-1.—On September 21, 1962 several seeds from a plant in flower (Clone II-1-1), mentioned above and shown in Plate II, Fig. 2*f*, were brought into the laboratory for examination. The fertile seeds in the umbel that were swollen and larger than the rest were excised aseptically for transplanting the embryos to agar plates containing coconut milk. The embryos developed roots and shoots in the normal manner and produced normal-appearing plants which were designated MIT-3 (*i.e.*, Clone II-1-1-1 emb.). One of them is shown in Plate II, Fig. 2*g*. Thus another cycle from cultured root, to freely-suspended cells, to organized clusters and eventually to a plant in flower and bearing normal seeds has been completed; in this clone, therefore, two full "life-cycles" have been completed.

A recent and particularly interesting development is as follows. Embryos, such as those borne on the plant (Clone II-1-1, Plate II, Fig. 2*f*) which was itself grown from free cells, can give rise to a proliferating culture when they are grown aseptically in a medium which contains coconut milk. These embryo cultures readily give rise to cells and these seem to form embryoids with remarkable ease. In fact, as they do this, heart-shaped and torpedo-stage embryoids (Plate II, Fig. 2 *h* and *i*), indistinguishable from those that develop from zygotes, have been seen.

DISCUSSION

The results here described represent the fulfilment of Haberlandt's vision of raising higher plants from cells. In Braun's experiments with tobacco tumors another example is to be seen (Braun, 1958). Single cells of tumorous origin were grown into small clusters which were then grafted on the decapitated shoots of normal plants. By repeated grafts the tissues which originated from tumorous cells retraced their development from the tumorous state until they could give rise again to a normal shoot. This only occurred under some environmental conditions contributed by the normal plant. In the case of carrot the conversion of free cells of adult origin to embryoids requires first a measure of freedom from any of the restrictions superimposed by organic contact with preformed and differentiated tissue, and secondly it requires the nearest equivalent of the biochemical environment of a zygote. Under these circumstances any diploid carrot cell is potentially totipotent and can behave like a zygote. The parallelism with embryogeny is close because, *enroute* from carrot cell to carrot plants, embryoids are formed (*cf.* Fig. 6 of Steward and Mohan Ram, 1961). The data here reported show that plants grown from embryoids originating from cells are normal in every respect and the growth cycle of the carrot plant may be repeatedly passed through without the intervention of seeds. Moreover the early stages of development in the vegetatively produced embryoids resemble key stages in normal embryogeny.

While the totipotency is present in any cell, its full expression it evoked more easily if the cell has access to a medium which is, as is were, "conditioned" by the substances released from other growing cells. Unpublished work of this laboratory shows that free cells, spread thinly on an agar medium like bacteria, spring into growth more readily if, over and above the coconut milk in the medium, they are in the near proximity of some previously cultured explants. This shows that a vigorously growing colony of carrot cells will release to the medium something which is even more potent than is coconut milk itself (work of Blakely with Steward). However, it is now very clear that the conditions under which cells divide most rapidly (*i.e.*, when they are attached to an existing piece of explanted tissue) are *not* those best for their morphogenetic development since this occurs when they can be grown free, albeit at a slower rate. Thus the environment of the cultured explant superimposes some controls or limitations upon the otherwise totipotent cell.

One might suppose that the cells which grow so freely into plants have been changed fundamentally (*e.g.*, cytologically) from those that existed in the carrot plant from which they were derived. While this may yet be so, in detail, all the evidence is against this. Cytological examination of carrot plants derived from free cells always shows (at least as yet) that they are normal diploids ($2n = 18$) and no visible morphological variations exist between them. This is the more surprising because different clones or strains of cells cultured from free

cells, do show spontaneous variations (Steward *et al.*, 1958 *b*). Presumably the variants are not viable enough as organized structures to be raised to whole plants by existing methods.

On this point of variation in the cultured cells it is significant that by long cultivation a strain of free cells (which originated from MIT–1, *i.e.*, Clone II) has tended to lose its ability to organize although *not* its ability to grow freely. This change has occurred after 14 months and 12 transfers, for this strain only occasionally produces roots and forms shoots with even greater difficulty. It may well be that the conditions of long cultivation tend to select some aberrant or less than totipotent cells. However, if one passes the organism through the vegetative cycle shown in Text-Fig. 1, it seems that the free cells derived from the storage organ in each successive vegetative cycle do retain their full totipotency.

Two other possibilities suggest themselves. The free cells that are derived from different tissues and organs that have already differentiated, such as pith, cambium, cortex, xylem parenchyma, phloem parenchyma of root or shoot, etc., may reveal their genetic totipotency with greater or less difficulty according to the tightness of the control which their differentiation imposed upon them. Although this point is under investigation, data cannot yet be given. A corollary is, however, that cell cultures derived from embryos, particularly immature embryos, might well be an exceptionally free source of totipotent cells that will grow, *via* embryoids, into plants. The first culture of this sort which was examined was one derived from tobacco (*Nicotiana rustica*) embryo and supplied by Dr. Mohan Ram of the University of Delhi, India. Although this culture has grown as a free-cell culture, under our conditions, with great vigor it has failed to organize, possibly because of its long history in culture. It is, therefore, of great significance that a free-cell culture from carrot embryos (or plants also grown from cells) does readily organize and grow (*see* Text-Fig. 1 and Plate II, Fig. 2 *h* and *i*). Furthermore, the comparative ability of the wild *Daucus carota*, unmodified by breeding, to exhibit these morphogenetic responses in its various cells is also being investigated.

Now that one can produce embryoids from free cells of vegetative origin, another intriguing possibility exists. If by environmental or growth substance treatment the minute embryoids could be vernalized, the normal biennial growth cycle of the carrot could be by-passed. This has, as yet, only been subjected to preliminary tests.

This work clearly raises some profound problems with respect to the controls which must exist to limit or determine the full expression of the genetic totipotency which is clearly inherent in any diploid carrot cell. Although this point is made here with respect to morphogenetic expression it could be made with equal effect in terms of biochemical and metabolic characteristics. The different organs, derived ultimately from free cells, have different biochemical composition and metabolism.

A current view is that there are mechanisms which in effect "turn on or off" the action of specific genes, or groups of genes. Some believe that histones play a part in this respect (Gifford and Tepper, 1962; Huang and Bonner, 1962). Some also regard it as an adequate explanation to attribute this role to particular parts of the genetic machinery which in effect act as the controls that determine whether certain genes are active or quiescent. Whatever the machinery, however, the facts of plant development require that both environment and exogenous growth substances must have access to the centers where these controls operate. In fact, a feasible but as yet unproven explanation is that the growth-regulating substances which evoke cell division, promote cell enlargement and control morphogenesis may activate particular areas of the genetic apparatus. Unfortunately we cannot yet associate visible areas of activity along plant chromosomes with known stages of morphogenetic development in the manner that has been so dramatically possible in the salivary gland chromosome of certain insect larvae (Breuer and Pavan, 1955). These visible effects on the chromosome, as shown by the now well-known "puffs", are evidence enough of activity evoked by, or concomitant with, some developmental stimuli. In plants, similarly localized activity on the chromosomes may not be accompanied by the same degree of visible expression; even so it may exist.

On these views one could regard a given morphogenetic stimulus as the cause of a temporary release of some particular form of RNA which would make its imbalanced impact upon metabolism and development.

It would have been a happy outcome of the work on the coconut milk stimulus to cell division and morphogenesis if cells synthesized different RNA's in their activated and quiescent states. So far as present data in this laboratory go, this is unlikely to be so. (The base ratios of the RNA from quiescent and activated cells do not seem to be different.) Moreover, even in a complete basal medium, unsupplemented by coconut milk, cells which do *not* divide synthesize RNA to a high content per cell. It seems, however, that the RNA so synthesized cannot be effective as a template for synthesis and thus lead on to cell division and growth. What the coconut milk growth factors do is to allow the RNA so made to become effective in synthesis, growth and the division of cells. Thus there is here an area in which exogenous growth-regulating substances may, without activating genes directly, nevertheless determine indirectly the extent of their effect. This may be so inasmuch as they control the degree to which the RNA's which the genes produce will affect metabolism. Conversely, inhibitors of growth factors, like the factors in differentiation which limit morphogenetic expression, may not exert their effect upon the action of the genes directly, so much as upon the expression of the RNA's through which the genes normally mediate their effect.

Thus, although the totipotency of single diploid carrot cells is now amply demonstrated, the biochemical means by which this is controlled

and constrained during differentiation is now a more pressing problem than ever.

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EXPLANATION OF PLATES I AND II

PLATE I

FIG. 1. Sequence of events from the Clone I-2 plant (*i.e.*, in the first vegetative cycle V_1), which was grown from freely-suspended cells and cell clusters, culminating in a plant of a second cycle (V_2) bearing an inflorescence. *a*. Plantlet grown from cell cluster derived from free cells, grown in agar medium containing coconut milk; *b*. Plantlet shown at (*a*) transferred to vermiculite and grown in the greenhouse; *c* and *d*. The same at maturity showing a massive root system at (*c*) and inflorescence at (*d*): *e*. Freely-suspended cells grown from the phloem explants taken from the tap root shown at (*c*); *f*. Cell clusters with roots grown in flask in a liquid medium containing coconut milk; *g*. Plantlet derived from an organized cell cluster grown in agar; *h*. Flowering carrot plant derived from the plantlet shown at (*g*).

PLATE II

FIG. 2. Sequence of events from free cells, originating from the phloem of carrot with known cytology ($2n = 18$) to organized plants. Clone II series. *a*. Freely-suspended cells from the tap root (phloem) of Clone II-1 (MIT-1 plant); *b*. Freely-suspended cells from the tap root (phloem) of Clone II-1-1 (MIT-2 plant); *c*. Abundant roots formed in cell clusters

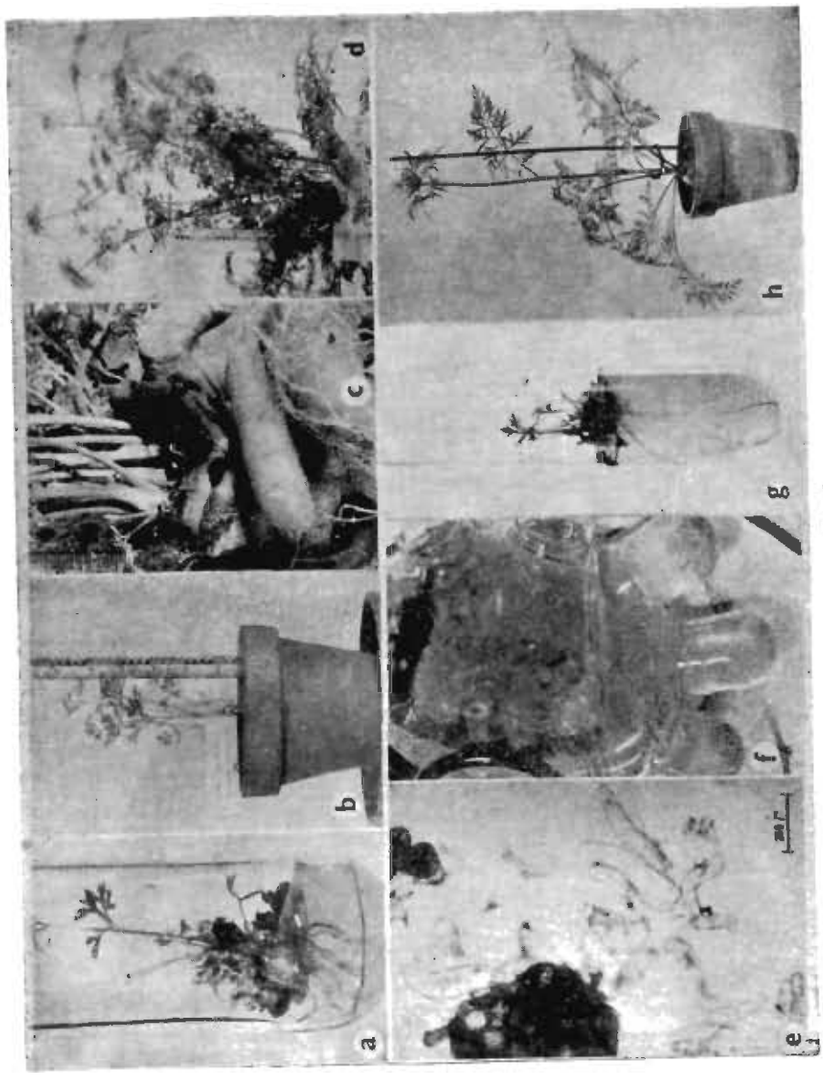

FIG. 1

F. C. Steward & Marion O. Mapes

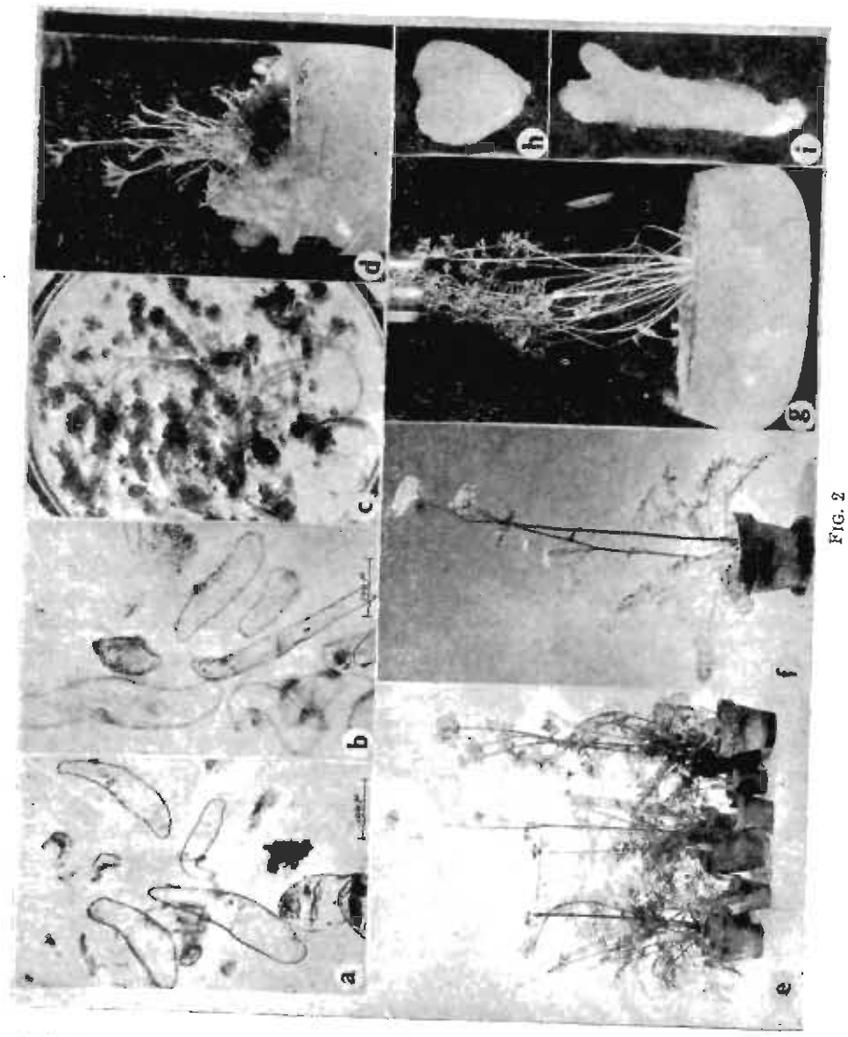

FIG. 2

F. C. Steward & Marion O. Mapes

from the Clone II free-cell cluster after growth in the dark and at low temperature (5° C.) for 5 months; *d.* Plantlet from an organized cell cluster derived from (*b*); *e.* Population of Clone II-1 (MIT-1 plants) of different ages and grown from free cells derived from phloem explants of Clone II plant; *f.* Clone II-1-1 (MIT-2) plant grown from free cells derived from phloem explants of Clone II-1 (MIT-1) plant; *g.* A carrot plant (MIT-3) grown from an embryo taken from a young seed on an umbel of a Clone II-1-1 plant, shown at (*f*); *h* and *i.* Heart-shaped (*h*) and torpedo-shaped (*i*) embryoids among the proliferating embryo cells derived from Clone II-1-1-1 emb. (MIT-3) liquid culture containing coconut milk.

GENERIC CONCEPTS IN THE HYPHOMYCETES

By C. V. Subramanian

Department of Botany, University of Rajasthan, Ja'pur, India

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Recent work on Hyphomycetes has evoked much sound thinking about their classification and we are on the threshold of significant changes in generic concepts and nomenclature in this fascinating group. I am indeed delighted to be able to present this brief account of kaleidoscopic changes in generic concepts of the Hyphomycetes in this volume intended to honour one so distinguished as Professor Maheshwari.

Impact of Saccardo's Classification on Generic Concepts

The classification proposed by Saccardo in 1880 and later elaborated by him in the *Sylloge* was in many ways an outstanding contribution and the fact that it has been in use for the past eighty years is itself a magnificent tribute to its great author. The generic concepts developed during this period reflect the emphasis on the criteria used by Saccardo to delimit taxa. Naturally, in delimiting genera, much emphasis has been placed in the past on the colour of the mycelium and/or the spores and on the formation of sporodochia and of synnemata, features which Saccardo used to separate the four families (Moniliaceae, Dematiaceae, Tuberculariaceae, Stilbaceae). Although the value of these criteria has been doubted from time to time by some students, few of them have succeeded in presenting a definite alternative classification founded on more suitable criteria.

Pertinent to this discussion is the report of albinism in certain dematiaceous fungi. Stover (1956) reported "albino sectors" in both single spore gray wild type cultures as well as single spore brown wild type cultures of *Thielaviopsis basicola*; albino sectors were common in the former, rare in the latter. Reversion of albinos to parent gray wild type was observed, but reversion of albinos from dark brown wild types to their parent type was rare. All albino colonies were identical morphologically to the parent except in loss of pigment and a retarded thickening of the chlamydospore walls. Further, albinism itself was of no parasitic advantage to the pathogen (Stover, 1950). Similar albino mutants have been obtained, by irradiation, from normal brown wild types of *Bipolaris sorokiniana* (= *Helminthosporium sativum*). These albinos are morphologically similar to the brown parents except in the absence of pigments, and appear to be equally pathogenic to wheat plants. Here again, reversion of albinos to the dark parent type has not so far been observed. Studies aimed at

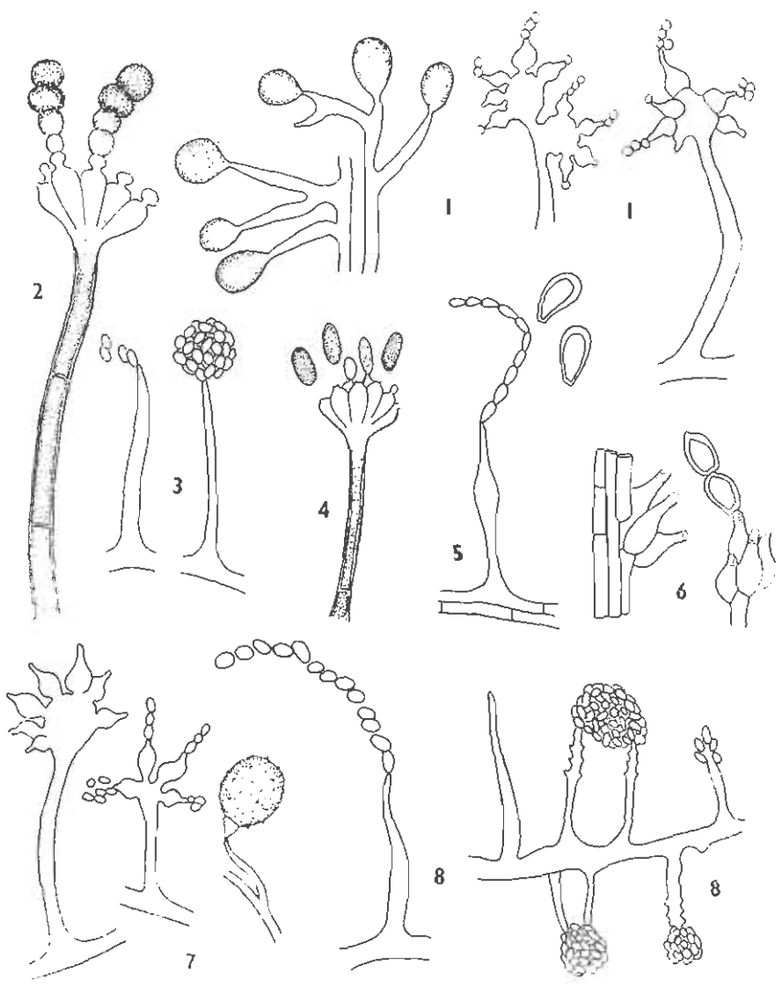
throwing further light on their physiology are under way. It is not known whether such albino forms commonly occur in nature; but, another well-known example, the "pink *Stachybotrys*" of Bisby (1943), which is the moniliaceous counterpart of *Stachybotrys atra* Corda, has been isolated from the rhizosphere of sugarcane plants and has been recently classified, on Saccardoan principles, in a new genus, *Hyalostachybotrys* (Srinivasan, 1958).

There is little work on the physiology of synnema formation to enable us to assess the value of this criterion in the formulation of generic concepts. It is well known that the wild types of several stilbaceous fungi when brought into culture may sporulate without forming synnemata. Thus, Hughes (1953 *a*) studied the cultural behaviour of *Phragmocephala cookei* and *P. atra*; he found that the stilbaceous character of the former was partially lost in culture and that of the latter completely. In the same way, several Hyphomycetes placed in the Tuberculariaceae under the Saccardo system (*e.g.*, *Fusarium* spp.), when cultured, do not form sporodochia and may then be placed in the Moniliaceae. Naturally, Appel and Wollenweber (1910) introduced the valuable concept of *hochkultur*, by which is meant a cultural state most nearly comparable to what is found in a "spontaneous" specimen—the specimens as they are found in nature; this implies that the laboratory specimens in culture tubes may often be in *abkultur* and, as Mason (1940) rightly suggested, should be reserved for purposes other than classification. Loughheed's (1961) study of synnema formation in the entomogenous *Hirsutella gigantea* indicated that, although this fungus grew well on artificial media, it remained sterile and formation of synnemata appeared to be a prerequisite for sporulation. Complex mixtures of certain nitrogen sources and, among carbon sources, a "crude glucose" aided the formation of synnemata; two other compounds, phosphoglyceric and gibberellic acids, were stimulatory. Loughheed concluded that the diversity of factors influencing formation of synnemata indicated that the morphological differentiation is a multistep process which may be affected at several points. Thus, there is need for further work on this problem.

Cytogenetical studies aimed at the elucidation of the behaviour of albino mutants and wild types of dematiaceous Hyphomycetes, coupled with investigations on their physiology with special reference to the formation of pigments, are likely to throw much light on the value of pigmentation in delimiting genera. From what little we know of this and other problems raised here, it would appear unwise to discard the criteria used by Saccardo for separation of taxa completely. What would appear logical is the subordination of these criteria to the morphological picture which emerges from a consideration of spore initiation and development—a point which is discussed below.

Morphological Spore Types

Not long after Saccardo published the fourth volume of his ***Sylloge*** in which he presented the Hyphomycetes, Costantin (1888) proposed a



TEXT-FIGS. 1–8. Conidiophores and conidia of Hyphomycetes. Fig. 1. *Acremoniella atra*: gangliospores (left) and phialospores (right). Fig. 2. *Memnoniella echinata*. Fig. 3. *Cephalosporium* sp. Fig. 4. *Stachybotrys atra*. Fig. 5. *Monocillium indicum*. Fig. 6. *Cephalotrichum stemonites* (= *Stysanus stemonites*). Fig. 7. *Chlamydomyces palmarum*: phialospores (left) and gangliospore (right). Fig. 8. *Gliomastix convoluta*. (Figs. 1, 7, 8 after Mason; Fig. 3, after Funder; Fig. 5, after Saksena; Fig. 6, after Hughes; the rest original.)

system in which primary importance was given to the mode of development of spores and their insertion on the conidiophore. Notwithstanding its distinctive merits, Costantin's work has not been widely known,

but it certainly stimulated serious work in France during the first decade of this century. Thus, a distinct contribution to generic concepts in the Hyphomycetes emerged when Vuillemin (1910 *a*, *b*; 1911) studied morphological spore types and suggested several terms to distinguish them. Vuillemin's most notable contribution is, of course, his characterisation of phialides and phialospores (Text-Figs. 2–5). Mason (1933, 1937, 1941) further elaborated Vuillemin's ideas. More recently, Hughes (1953 *b*) proposed further terms some of which were incorporated in a new classification by Subramanian (1962, 1963). Among the new morphological features in spore initiation and development elucidated by Hughes, particular mention may be made of his concept of annellophores (Text-Fig. 6), porospores (Text-Fig. 9) and meristem arthrospores (Text-Fig. 15). Hughes discarded the term "aleuriospores" of Vuillemin and often used the term "chlamydospores" instead. The former term has been misinterpreted by many workers in the past and the latter connotes a "sedentary spore" (Gregory, 1952), commonly found in almost all genera. I have, therefore, preferred to use the term "gangliospore" instead. Apart from these, special emphasis has been placed on the growth behaviour of conidiophores in distinguishing between genera. Indeed, some of these new concepts form the basis of segregation of the graminicolous species of *Helminthosporium* from *Helminthosporium* Link (*see* below). Several genera believed to produce spores from phialides are now known to produce the spores from annellophores instead of phialides (*e.g.*, *Scopulariopsis* Bain., *Cephalotrichum* Link ex Fries, *Trichurus* Clem. and Shear). It now appears imperative to formulate generic concepts primarily on those morphological features which are presented to us during spore initiation and development. Features in conidiophore growth and development, especially in their relation to spore initiation, are nearly as important.

Biological Spore Types

When Mason (1937) emphasised the distinction between *dry* and *slimy* spores in generic delimitation, he introduced the concept of a "biological spore type" and initiated a new approach to an old problem, although this distinction had been appreciated earlier by Costantin (1888). Wakefield and Bisby (1941) accepted this approach when they listed British Hyphomycetes under the Xerosporae and the Gloiosporae. This concept is particularly applicable to the separation of several genera of the Tuberculariaceae Ehrenb. as emended by Subramanian (1962) which produce phialospores. Thus, in the genera *Cephalosporium* Corda (Text-Fig. 3) and *Monocillium* Saksena (Text-Fig. 5), hyaline amerospores are successively abstricted from phialides, but in the former the spores are slimy and form "false heads" but not chains; in the latter the spores are dry and so form basipetal chains. Similarly, the two dematiaceous genera *Memnoniella* Hoehnel (Text-Fig. 2) and *Stachybotrys* Corda (Text-Fig. 4) are separable by the fact that the dry spores form chains in the former, whereas those of the latter being slimy do not form chains. Separation of genera on this basis has been

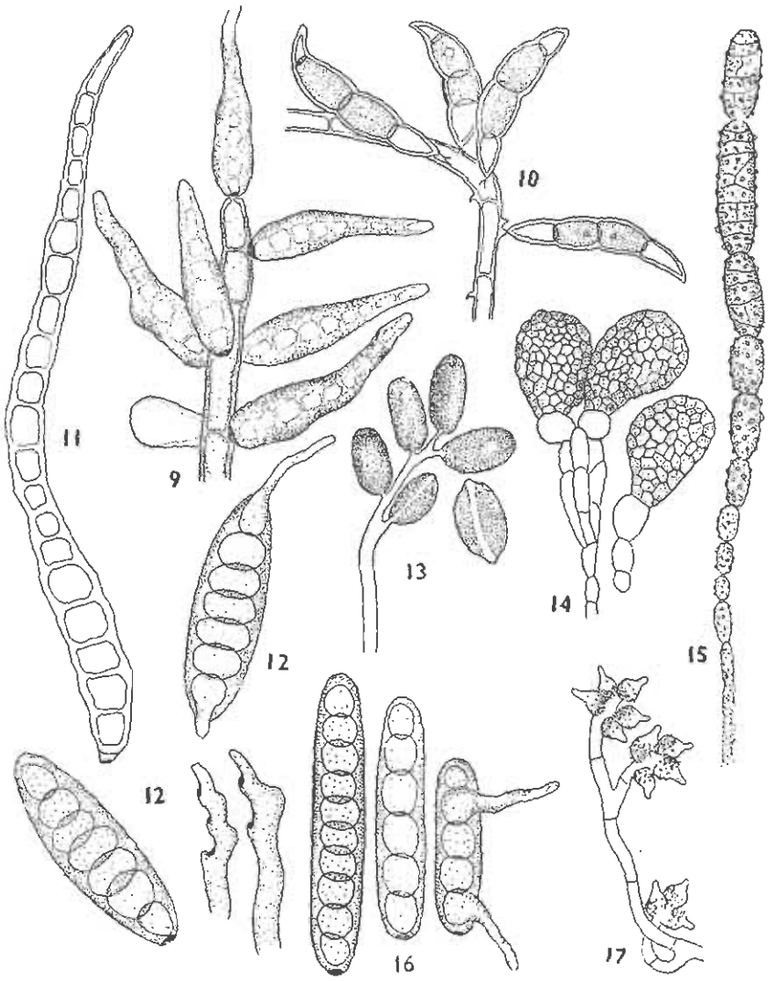
sometimes questioned, mainly because in some genera, and even in a single species of a genus, the dry-spored and the slimy-spored conditions may co-exist, varying only with the cultural conditions. Thus, in the case of *Gliomastix convoluta* (Text-Fig. 8) the spores form chains under dry conditions in culture, but slime down to heads when there is sufficient moisture (Mason, 1941). Similarly, the genus *Paecilomyces* Bainier may show both conditions: when the spores are dry there is similarity with the dry-spored *Penicillium*; when slimy, the genus presents features seen in *Gliocladium* Corda (Brown and Smith, 1957). While one might hesitate to base the major classification of the Hyphomycetes on biological spore types, it appears necessary to retain this concept for generic separation wherever justified.

Polymorphism

Generic concepts present peculiar, and often insurmountable, difficulties when a hyphomycete produces different spore forms. Such is the case with several genera which are based on their gangliospore states but produce phialospores also, *e.g.*, *Acremoniella* Sacc. (Text-Fig. 1), *Chlamydomyces* Bain. (Text-Fig. 7), *Humicola* Traaen. Their phialospore states have not been named and these generic names are based on their respective gangliospore states. In the case of the ascomycetous *Microascus pedrosoi*, hyphomycetous states corresponding to "*Hormodendrum*" and *Acrotheca* (both blastospores) and *Phialophora* (phialospores) occur in the same life-cycle. Again, *Cephalotrichum* is now known to form its spores on annellophores (Text-Fig. 6), but this fungus has another state (Text-Fig. 17) corresponding to the genus *Echinobotryum* Corda. Numerous other examples of this type are known. In all these cases, each fungus cannot have more than one name. The problem, therefore, is the proper choice of a name for the hyphomycete producing different spore types. No ideal solution is available; the choice of a generic name would be arbitrary, as has been the case in the past. In this and in the use of biological spore types to delimit genera, little guidance can be given and I can only quote Rensch and say: "the golden rule is: there is no golden rule".

Nomenclatural Types

One of the pressing problems in the taxonomy of the Hyphomycetes is the consistent and precise application of generic names and specific epithets. In the fulfilment of this aim, we are guided by two lines of approach. We have, on the one hand, generic concepts deeply rooted in conventional usage, and those, on the other, that claim preference and adoption in conformity with the Code. Thus, we have generic concepts derived from Saccardo's *Sylloge* and ingrained in the extensive mycological literature of the subsequent period. There is no trouble if these concepts are validated by provisions in the Code, but that is not feasible and is not the case. The Code not only fixes the starting-point date as 1st January 1821, but also provides that "the application of names of taxa of the rank of family or below is determined by means



TEXT-FIGS. 9–17. Conidiophores and conidia of Hyphomycetes. Fig. 9. *Helminthosporium velutinum*. Fig. 10. *Vakrabeeja sigmoidea*. Fig. 11. *Sporidesmium vagum*. Fig. 12. *Bipolaris sorokiniana* (: *Helminthosporium sorokinianum*). Fig. 13. *Papularia sphaerosperma*. Fig. 14. *Monodictys paradoxa*. Fig. 15. *Coniosporium* state of *Hysterium insidens*. Fig. 16. *Drechslera avenacea*. Fig. 17. *Echinobotryum atrum*. (Figs. 9, 14, 15, after Hughes; Fig. 10, after Tullis; Figs. 13, 17, after Mason; Fig. 16, after Drechsler; the rest original.)

of nomenclatural types". Some problems relating to generic concepts partly arise from the conflicting interpretations based on continued usage *versus* those based on nomenclatural types.

The case of *Coniosporium* Link will illustrate how restudy of the type may necessitate a new generic concept. *Coniosporium* (originally spelt *Conisporium*) was established by Link (1809, *Mag. Ges. natürf. Freunde, Berlin* **3:** 8) for *C. olivaceum* Link, the type species. Since the spores were stated to be non-septate in the generic diagnosis and were so figured by Nees in 1817, the genus was compiled in the Dematiaceae-Amerosporae by Saccardo (1886). However, Mason (1933) examined Link's material and stated that the spores are dictyospores. Hughes (1951) confirmed Mason's observations and illustrated *C. olivaceum* from type material. In keeping with this concept, *Sporidesmium paradoxum* Corda (1838, *Icon. fung.* **2:** 6) and *Sporidesmium peziza* Cooke and Ell. (1876, *Grevillea* **4:** 178) were both transferred to *Coniosporium* as *C. paradoxum* (Corda) Mason and Hughes and *C. peziza* (Cooke and Ell.) Mason and Hughes, respectively. Subsequently, Hughes (1953 *b*) stated that in *C. peziza* the dictyospores are formed in basipetal chains and that these spores are "meristem arthrospores"; accordingly, this fungus was re-classified as *Sirodesmium peziza* (Cke. and Ell.) Hughes. Five years later, on the basis of further studies of type specimens of the various taxa, Hughes (1958) placed *Coniosporium paradoxum* (Text-Fig. 14) in his new genus *Monodictys* and listed *Sirodesmium peziza* as a synonym of *Coniosporium olivaceum*; the genus *Sirodesmium* de Not. (1849, *Mem. Accad. Sci. Torino* **10:** 347) was made a synonym of *Coniosporium*. The type species of *Sirodesmium* produces meristem arthrospores as in *S. peziza*, which Hughes now cited as a synonym of *Coniosporium olivaceum*. The generic concept which emerges from these valuable studies is one where *Coniosporium* may be considered a dematiaceous dictyospored hyphomycete producing basipetal chains of meristem arthrospores (Text-Fig. 15).

Therefore, *Coniosporium* Link cannot be used for those dematiaceous fungi with one-celled spores which were compiled under this generic name in the *Sylloge*. Mason (1933) suggested conservation of *Coniosporium* Sacc. against *Coniosporium* Link, but Saccardo did not exclude *C. olivaceum*, the type of Link's genus and, therefore, the only course open is to re-classify forms not congeneric with *C. olivaceum* elsewhere. Fortunately, the genus *Papularia* Fries (1849, *Summa veg. Scand.*, sect. post., p. 509) is available for these fungi with one-celled spores (Text-Fig. 13) which have been classified in *Coniosporium* by Saccardo (Mason, 1933).

Another genus which has been widely misunderstood is *Sporidesmium* Link ex Fries (Link, 1809, *Mag. Ges. natürf. Freunde, Berlin* **3:** 41; Fries, 1832, *Syst. Mycol.* **3:** 492). Ellis (1958) has reviewed the history of this name. Link established the genus for a fungus he called *Sporidesmium atrum*. In the illustrations provided by Ditmar in the same work, the conidia were shown as having 3–5 transverse septa and borne singly at the tips of simple conidiophores. No type material appears to exist, but yet this is the type species of Link's genus. According to Ellis, there is in Persoon's Herbarium at Leyden a specimen labelled by Ehrenberg "*Sporidesmium fusiforme* Nees ab Essen.—*Sporidesmium atrum* Link. var. *teste* Link", and it is fairly certain that

Link had seen this specimen. In the absence of the type specimen, this specimen may perhaps serve as authentic for Link's concept of his genus. Ellis examined the Ehrenberg specimen and also type material of *S. fusiforme* and found that they are congeneric but specifically distinct. Besides *S. fusiforme*, the Nees brothers described one other species in 1818, *viz.*, *S. vagum* (Text-Fig. 11). In 1825 Link [in Linne's *Spec. Plant.*, ed. 4 (Willdenow's), **5** (2): 120] considered *S. fusiforme* and *S. vagum* as synonyms of his *S. atrum* and emended his diagnosis. In 1829 in Sturm's *Deutschl. Flora*, Heft 7, Corda wrote about these fungi and said that *S. atrum* was distinct from *S. fusiforme* and that *S. vagum* was only a large form of *S. fusiforme*. Later, in *Icon. fung.* **4**: 23 (1840), Corda gave a description of a fungus he collected on conifer wood near Vienna: this he rightly called *S. vagum*. Fries accepted Link's genus, but included *S. cellulosum* Fries, which produced dictyospores. This mistake he rectified in 1849 in *Summa veg. Scand.*, sect. post., p. 506, wherein he excluded *S. cellulosum* from *Sporidesmium* and placed it under *Stilbospora*. Thus, even in 1849 *Sporidesmium* was accepted as a genus with phragmospores which was essentially the concept of Link and corresponds to the type of the genus. In 1882, however, Saccardo (*Michelia* **2**: 23) emended the genus to include fungi with muriform spores and cited *S. cellulosum* Klotsch (in error for Fries !) as the typical example. The type species, *S. atrum*, was excluded; it was disposed under the genus *Clasterosporium* Schw. (*see* Saccardo, 1886, *Sylloge* **4**: 389). Since 1882, most workers have followed Saccardo and the use of the name *Sporidesmium* for phragmospored fungi had been discontinued and phragmospored fungi corresponding to *Sporidesmium* were invariably disposed under *Clasterosporium*, a genus quite distinct from *Sporidesmium* and possessing hyphopodia. Saccardo also altered the spelling of *Sporidesmium* to *Sporodesmium*. According to the Code, *Sporidesmium* Link ex Fries has to be re-established for forms congeneric with *S. atrum* Link ex Fries.

Since the main aim of this paper is to summarise some of the kaleidoscopic changes in generic concepts, I shall cite one other example of a similar nature, but one which may not be allowed to pass without wide comment. This relates to the genus *Helminthosporium* Link (original spelling *Helmisporium*, altered to its present widely used form by Persoon in 1822) which has been used in the past to classify several plant pathogens, especially of cereals, the names of which therefore are deeply rooted in the literature of plant pathology. Several years ago, Mason made the interesting discovery that in the type species of Link's genus, *viz.*, *H. velutinum* Link ex Fries (Link, 1809, *Mag. Ges. natürf. Freunde, Berlin* **3**: 10; Fries, 1832, *Syst. Mycol.* **3**: 354), the phragmospores are produced apically and laterally on the erect conidiophore in exactly the same way as in *Spondylocladium atrovirens* Harz [*S. atrovirens* = *Helminthosporium atrovirens* (Harz) Mason and Hughes]. Hughes (1953 *b*) interpreted these spores as porospores (Text-Fig. 9). It is well known that in the graminicolous species of *Helminthosporium* the porospores are formed differently, being produced singly at the apex of the conidiophore and its new growing points formed sympodially. Further, *H. velutinum* and congeneric species

are usually lignicolous. Accordingly, the graminicolous species of *Helminthosporium* have to be removed and classified elsewhere, not because they are not lignicolous, but because of the characteristic method of spore initiation and conidiophore development. These species have long been known to fall into two groups (Drechsler, 1923): (1) those, like *H. avenae* Eidam [= *Drechslera avenacea* (Curt. ex Cke.) Shoemaker] having cylindrical spores which germinate producing germ tubes from the polar and middle cells (Text-Fig. 16); and (2) those like *H. sativum* Pamm. King and Bakke (= *H. sorokiniana* Sacc.) having fusiform spores which show only bipolar germination (Text-Fig. 12). Ito (1930) classified the former group in a separate genus *Drechslera* Ito and retained the second group in *Helminthosporium* as then understood. Our new understanding of the nomenclatural type of *Helminthosporium* has made the retention, in this genus, of these species with fusiform spores also untenable. Accordingly, Shoemaker (1959) established the new genus *Bipolaris* for these species with fusiform spores; he accepted Ito's genus for the forms with cylindrical spores. Notwithstanding the nomenclatural changes involved, the separation of the graminicolous species from *Helminthosporium* appears quite justified. I am not quite sure about the desirability of distributing them in two separate genera (*Drechslera* and *Bipolaris*), although it must be admitted that the distinct spore morphology claimed for these genera is easily recognised. In several species of *Bipolaris* spore germination would appear to be mostly unipolar rather than bipolar and the mode of germination is not invariable. On the other hand, *Drechslera*-like conidial stages are found in the ascomycetous genus *Pyrenophora*, whereas conidial stages corresponding to *Bipolaris* are seen in *Cochliobolus* and *Trichometasphaeria*. Yet another graminicolous *Helminthosporium* quite distinct from those classified in *Drechslera* and *Bipolaris* is *Helminthosporium sigmoideum* Cav. The phaeophragmospores are nearly falcate with the middle cells darker than the end cells as in *Curvularia* Boedijn, but the spores are borne acropleurogenously on distinct denticles (Text-Fig. 10). It has been reclassified in a new genus *Vakrabeeja* as *V. sigmoidea* (Cav.) Subram. (Subramanian, 1956); it is conidial *Leptosphaeria salvinii*. The nomenclatural changes proposed for the graminicolous forms are not likely to be received favourably by plant pathologists whose attitude rightly reflects the general opposition to frequent and irksome nomenclatural changes for well-known plant pathogens. However, the alternative is to expand the genus concept of *Helminthosporium* Link to include the features seen in the graminicolous species. Such broadening of the generic concept is not without precedent as may be seen from the fact that forms entirely different from *Helminthosporium velutinum* and properly disposed under various other genera (such as *Brachysporium* Sacc., *Corynespora* Güssow, *Dendryphion* Wallr., *Curvularia* Boedijn, etc.) have in the past been classified in *Helminthosporium*. However, it would be unwise to undo what has been done in regard to the sorting out of these forms from the heterogeneous assemblage of Helminthosporia. I also believe that the principle of nomenclatural types, as incorporated in the Code, aims at stability of nomenclature, and such stability can be achieved only when

the principle is strictly and consistently applied. A postponement of the application of this principle will only delay the proper understanding of the genera and evolving of generic concepts on the basis of nomenclatural types. Therefore, here, as indeed in every field of science, considerations of desirability or convenience should not override considerations of precision.

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EMBRYOLOGY OF *SEDUM TERNATUM* MICHX.

By K. Subramanyam

Botanical Survey of India, Calcutta, India

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Sedum ternatum, a member of the Crassulaceae, is placed in section Sedа genuina by Praeger (1921) and Berger (1930), while Fröderström (1930, 1931, 1932, 1935) treats it in section Americana kyphocarpia. The previous work on the embryology of the family has already been reviewed by Subramanyam (1955) and more recently its affinities with such families as Saxifragaceae, Podostemaceae, Hydrostachyaceae and several families of Ranales have also been discussed (Subramanyam, 1962). So far, however, there is no information on the microsporangium and male gametophyte of any species of this genus; further, the development and behaviour of the basal suspensor haustorium has also not been described. These and other aspects of the embryology of *Sedum ternatum* are presented here in detail.

Material and Methods

S. ternatum is a glabrous or finely papillose perennial with obovate or round-spatulate leaves, arranged mostly in whorls of three; the white, tetramerous flowers are borne in 3-parted cymes; the pistils are spreading in fruit. Flowers and fruits in all stages of development were collected in May and June 1953, from the plants growing in the test gardens of the Cornell University, Ithaca, New York. They were fixed in chromo-acetic mixture (Randolph, 1936), as it proved far superior to formalin-acetic-alcohol, dehydrated in N-butyl alcohol and embedded in paraffin. Transverse and longitudinal sections 10–14 microns in thickness were stained in Heidenhain's iron alum haematoxylin with erythrosin in clove oil as a counterstain.

Microsporangium and Male Gametophyte

The stamens which are twice as many as petals occur in two whorls, the outer being antipetalous and the inner antisepalous. The linear anthers borne on long filaments dehisce by longitudinal slits. The young anther in a cross-section is quadrilocular to begin with; at a later stage, however, it becomes bilocular as a result of the degeneration of the sterile tissue separating the two locules in each anther lobe. A transverse section shows a plate of four to six hypodermal archesporial cells. They divide periclinally to form the primary parietal and primary sporogenous cells. The primary parietal layer undergoes

further periclinal divisions to form the anther wall and, after undergoing a few more divisions, the sporogenous cells give rise to microsporocytes. The wall of the anther consists of five layers, including the tapetum. The outermost layer is the epidermis, next comes the endothecium, then the two middle layers which later become flattened, and finally the glandular or secretory tapetum. The tapetal cells are uninucleate throughout. The microsporocytes undergo normal meiotic divisions and form quartets of microspores. Quadripartition takes place by peripheral cleavage furrows and the microspores are arranged tetrahedrally. In the mature anther the tapetum and one or both the middle layers are disorganised and the endothecium develops the usual fibrous thickenings (Text-Fig. 1). At the time of dehiscence the epidermal cells in the region of the groove are small and narrow.

The nucleus in the young microspore is situated in the centre surrounded by a dense mass of cytoplasm (Text-Fig. 2). A vacuole is formed and the nucleus soon moves towards the wall, where it divides (Text-Fig. 3) to form a small lenticular generative cell and a large tube cell (Text-Fig. 4). No further division takes place and the mature pollen grains, which sometimes show the presence of starch grains, have thus only two cells at the time of shedding (Text-Fig. 5). In a solitary case, however, three nuclei in the pollen grain were noticed (Text-Fig. 7); of these, one was a small lenticular cell, close to the wall and the other two were large and of the same size. Possibly they represent two tube nucleii, formed as a result of the division of the single tube nucleus.

The mature pollen grain is spheroidal with the exine of the same thickness or slightly thicker than the intine. It is usually tricolpate (Text-Fig. 5), sometimes quadricolpate (Text-Fig. 6) and the colpi are tenuimarginate.

Pistil

The pistil is superior and apocarpous with four carpels slightly connate at the base. The apex of each carpel is continued into a long, slender stylodium which in turn ends in the inconspicuous stigma (Text-Fig. 8). In the very young ontogenetic stages the carpellary margins remain unsealed for a time, but at a later developmental stage the margins distinctly cohere at certain points with the result the older carpels are sealed. The wall of the carpel is made up of a variable number of loosely arranged parenchymatous cells (Text-Fig. 17) and dense brownish contents are present in the inner epidermal cells.

Due to two swollen flap-like outgrowths with a groove between them, a characteristic gibbosity is seen just above the base on the abaxial side of each carpel (Text-Figs. 8, 9). It appears in the mature flower and can be distinctly seen during later stages when the carpels become kyphocarpic.[1] Serial transverse sections passing from the base to the top (Text-Figs. 10–15) of such a carpel showed that in the region of

[1] Carpels gibbous and divergent at maturity.

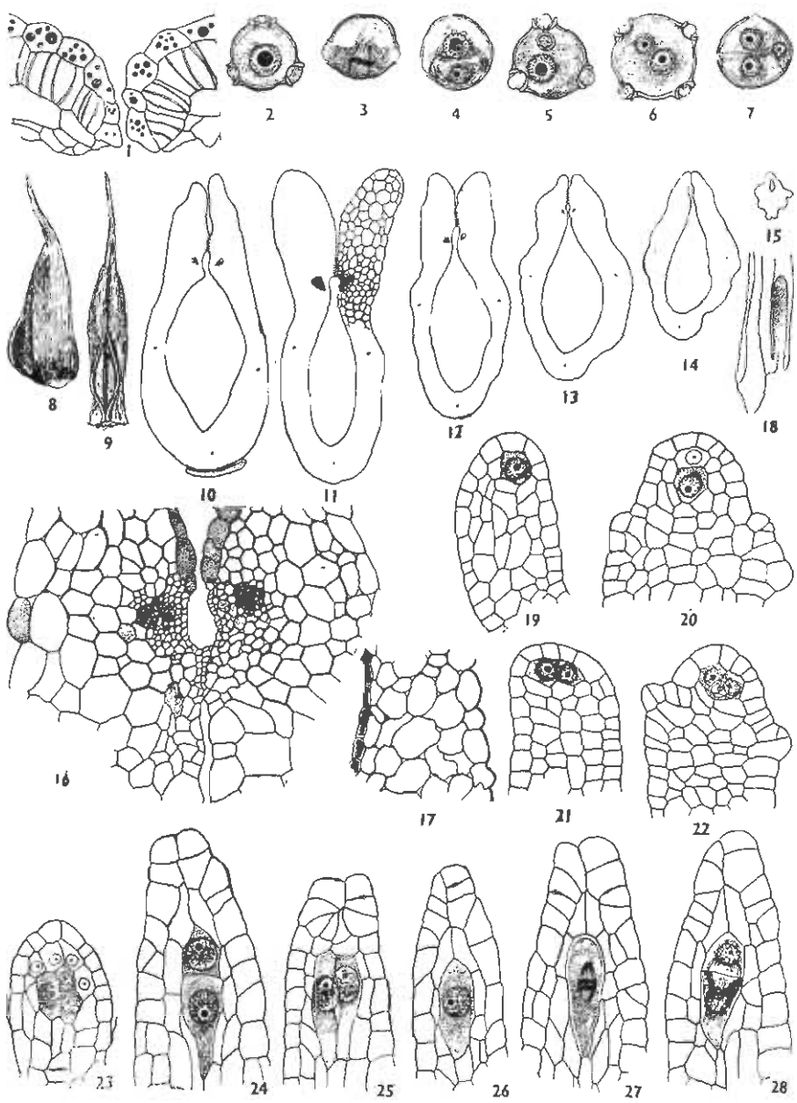
gibbosity small submarginal zones on the abaxial sides of the carpel are prominently swollen. The cells in this region are large and thickened (Text-Fig. 16) and these features are well seen when the sections are counterstained with erythrosin. The narrow zone of closure of the carpellary margins can be noticed at the base of the swollen flaps. The position and structure of the gibbous region indicates that it probably represents an outgrowth of the submarginal regions of the carpel on the abaxial side.

Nectaries are present and they correspond in number with the carpels. Each nectary is in the form of an appendage, attached to the base of the carpel on its dorsal side (Text-Fig. 18). In the upper portion it is free from the dorsal side of the carpel. It is narrowly elongated, clavate in longitudinal section and ovate to oblong in a transverse section. It is broad at the base, gradually tapering towards the apex and consists of a compact mass of closely arranged thin-walled parenchymatous cells with prominent nuclei and dense, vacuolate cytoplasm. From the dorsal carpellary traces procambial strands are produced at the lower region. These extend into the base of the nectary, where they sometimes even branch.

Megasporangium and Female Gametophyte

Each carpel encloses an indefinite number of anatropous, crassinucellar, bitegminal ovules arranged in two or more rows on the smooth, parietal placenta. With reference to the placenta they are oriented obliquely.

The ovule arises as a conical outgrowth on the placenta. It consists of a group of parenchymatous cells surrounded by the nucellar epidermis. Soon the initials of the integuments originate at the base of the nucellar primordium, the inner appearing first. Both the integuments grow around the nucellus and the gradual curvature of the latter results in the formation of the anatropous ovule. The micropyle is narrow and long and is formed by both integuments. The integuments are two-layered to start with but at a later stage the outer integument becomes three-layered as a result of periclinal division in its inner layer. There is a deposition of cuticle on the epidermal layer of the outer integument. In the micropylar region both the integuments are enlarged and consist of more layers of cells. A vascular strand from the placenta traverses the funiculus and finally terminates in the chalaza. The nucellus is narrow and elongated and the small embryo sac is situated at its extreme apex, thus giving it a very characteristic shape. Some cells of the nucellar epidermis usually undergo a periclinal division (Text-Figs. 24, 27, 28, 30, 37, 39, 41). In the lower portion of the nucellus, the epidermal cells are large in size. A distinctive feature noticed all along the length of the ovule and extending from the chalaza to the base of the embryo sac is a strand of elongated thin-walled cells with elongated nuclei. Its position and structure, along with the location of the small embryo sac at the extreme apex of the



TEXT-FIGS. 1–28. Fig. 1. Portion of mature anther wall at region of dehiscence showing epidermis and endothecium, ×350. Fig. 2. Uninucleate microspore, ×750. Figs. 3, 4. Stages in division of microspore nucleus. ×750. Fig. 5. Two-nucleate tricolpate pollen grain, ×750. Fig. 6. Two-nucleate tetracolpate pollen grain, ×750. Fig. 7. Pollen grain showing two tube nuclei and a lenticular generative cell near the wall, ×750. Fig. 8. Side view of carpel showing gibbosity, ×3. Fig. 9.

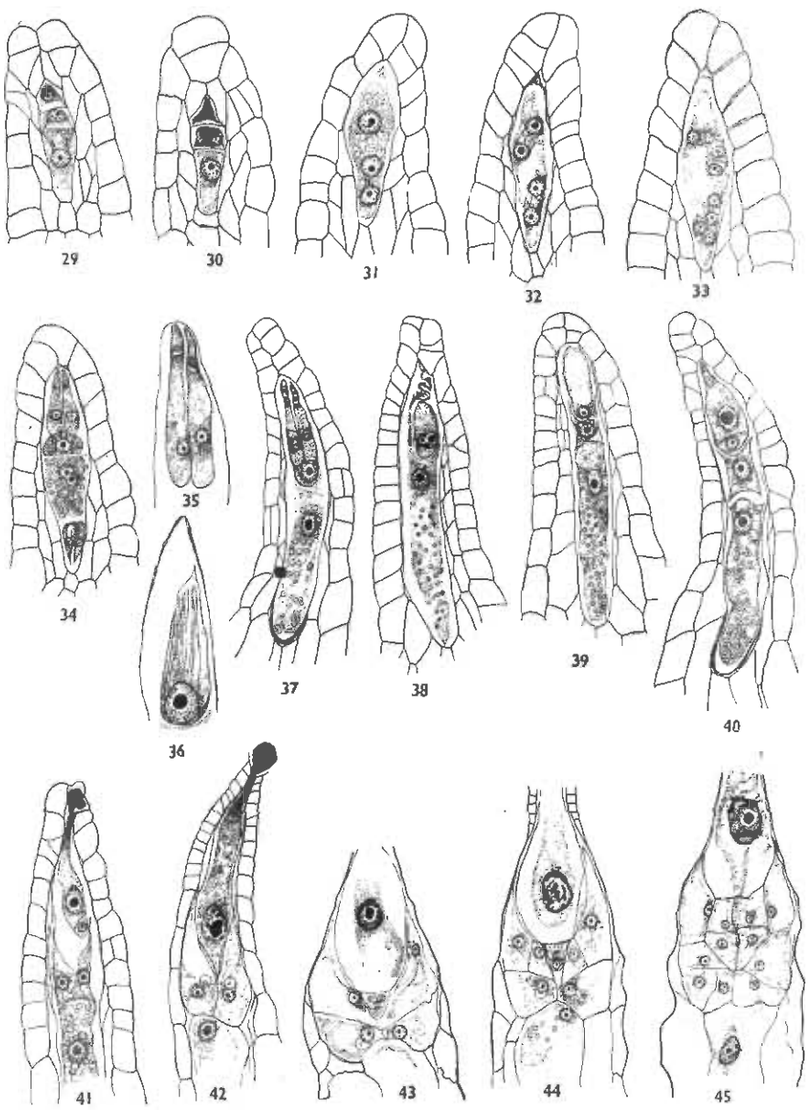
Front view of gibbous region, ×3. Figs. 10–15. Serial transverse sections of the carpel at various levels, passing from the base towards the top: note the section in the region of gibbosity in Fig. 11, × 10. Fig. 16. A portion at the region of gibbosity enlarged to show its histology, ×45. Fig. 17. Portion of carpellary wall enlarged, × 160. Fig. 18. L.s. nectary, ×20. Fig. 19. Hypodermal archesporium, ×350. Fig. 20. Megasporocyte and parietal cell, ×450. Fig. 21. Double archesporium, ×350. Fig. 22. Three archesporial cells, ×350. Fig. 23. Three megasporocytes and parietal cells, ×350. Fig. 24. Two superposed megasporocytes, ×350 Fig. 25. Two juxtaposed megasporocytes, ×350. Fig. 26. Megasporocyte, ×350. Figs. 27, 28. Stages showing division of megasporocyte to form cells of dyad, ×350.

nucellus, suggests that it has probably some conducting function. The nucellus corresponds to the Sedum type (Mauritzon, 1933) observed in all the subfamilies of the Crassulaceae, except the Crassuloideae and Kalanchoideae. At about the stage of fertilization a group of cells at the chalazal region becomes conspicuous by their dense contents and prominent nuclei, simulating the chalazal hypostase seen in some other families of angiosperms.

A hypodermal cell in the young nucellar primordium becomes conspicuous by its dense contents and prominent nucleus even before the differentiation of the integumentary primordia. This is the female archesporium (Text-Fig. 19); sometimes it is multicellular (Text-Figs. 21, 22) and by further development multiple megasporocytes are formed (Text-Figs. 23–25). It soon divides transversely forming the upper primary parietal cell and the lower megasporocyte (Text-Fig. 20). The primary parietal cell next divides either anticlinally or transversely, forming a parietal tissue of one or two layers of cells.

The megasporocyte due to its enlargement coupled with elongation (Text-Fig. 26) becomes very conspicuous in the apical region of the nucellus, exhibiting a prominent, ovoid or ellipsoidal nucleus embedded in a dense mass of cytoplasm. It divides transversely (Text-Fig. 27) to produce a dyad (Text-Fig. 28). The upper dyad cell, which is invariably smaller, never divides as in the usual case, but always degenerates forming a degenerating cap (Text-Fig. 29). The larger lower dyad cell divides into two superposed cells, of which the upper later degenerates (Text-Fig. 30), while the lower functions further. Since only one megaspore takes part in the formation of the embryo sac, the development conforms to the monosporic Polygonum type. The eight-nucleate embryo sac is formed as a result of three divisions of the functioning chalazal megaspore (Text-Figs. 32, 33). The two-nucleate and four-nucleate embryo sacs are surrounded on the sides by two or three layers of nucellar cells. As the mature embryo sac enlarges in the centre, some of these cells get crushed so that it is surrounded directly by the nucellar epidermis. A case was observed where the primary chalazal nucleus in the two-nucleate embryo sac had divided a little earlier than the micropylar giving rise to a three-nucleate embryo sac for a short duration (Text-Fig. 31).

Of the eight nuclei in the mature embryo sac, three at the micropylar end give rise to the egg and two synergids; three at the chalazal



TEXT-FIGS. 29–45. Fig. 29. Degenerating upper dyad cell and dividing lower dyad cell, ×350. Fig. 30. Degenerating dyad cell and upper megaspore; note enlarging chalazal megaspore, ×350. Fig. 31. Three-nucleate embryo sac, ×350. Fig. 32. Formation of four-nucleate embryo sac, ×350. Fig. 33. Formation of eight-nucleate embryo sac, ×350. Fig. 34. Mature embryo sac; note starch grains in embryo sac, egg and synergids, ×350. Fig. 35. Synergids enlarged showing

apical caps, ×500. Fig. 36. Egg enlarged to show cytoplasmic strands, ×500 Fig. 37. Development of chalazal embryo sac haustorium, ×250. Fig. 38. Double fertilization; note triple fusion has taken place earlier than syngamy, ×250. Figs. 39–45. Stages in development of cellular endosperm, Figs. 39–41, ×250. Figs. 42–45, ×160.

end organise into the antipodal cells; and the remaining two polar nuclei, one from each pole, fuse near the egg to form the secondary nucleus. The mature embryo sac (Text-Fig. 34) is small, spindle shaped and is situated in the apical region of the nucellus. The synergids are elongated and their apices are provided with distinct apical caps (Text-Fig. 35) and these take a characteristic rosy stain with erythrosin. The elongated egg is situated between the synergids or towards one side in the upper portion of the embryo sac. It shows fine cytoplasmic strands (Text-Fig. 36) simulating the filiform apparatus met with in synergids. The small antipodals which have slender pointed ends are located in the narrow chalazal end of the embryo sac. They begin to degenerate at about the time of the fusion of the two polars. The secondary nucleus is situated above the centre of the embryo sac and nearer the egg. Starch grains are often seen in the mature embryo sac (Text-Figs. 34, 37) and are present even in the egg and synergids (Text-Fig. 34).

Double fertilization has been observed (Text-Fig. 38). Entry of the pollen tube is porogamous and the pollen tube appears coiled in the micropylar region of the embryo sac. Syngamy and triple fusion occur as normal processes during fertilization, the latter taking place lightly earlier than the former.

Immediately after the fusion of the polar nuclei or at about the time of fertilization, the chalazal end of the embryo sac becomes active. It now begins to elongate and grows towards the chalaza in the form of a tubular structure, crushing the narrow elongated cells of the conducting strand. It thus acts as a chalazal embryo sac haustorium and its active basal end is blunt, thick-walled (Text-Fig. 37) and takes a reddish stain with erythrosin.

Endosperm

Endosperm is *ab initio* cellular. The primary endosperm nucleus which is situated just above the centre of the embryo sac and nearer the egg (Text-Fig. 38) divides by the laying down of a transverse wall (Text-Fig. 39). This results in the formation of a smaller upper micropylar chamber and a larger lower primary chalazal chamber (Text-Fig. 40). There are no further divisions in the lower chamber. It soon becomes long and tubular and functions as the uninucleate chalazal endosperm haustorium. The endosperm tissue is formed by divisions in the upper primary micropylar chamber. A vertical wall is laid down in this chamber (Text-Fig. 41) and this is soon followed by another vertical wall at right angles to the first and thus four cells are formed which surround the upper and basal part of the proembryo (Text-Fig. 42). These four cells next divide transversely forming eight cells

arranged in two tiers of four cells each (Text-Fig. 43). Starch grains are usually present in the young endosperm cells and in the chalazal endosperm haustorium (Text-Figs. 39–44).

In the upper tier of four cells longitudinal walls are laid down, resulting in the formation of an inner layer of four endosperm cells surrounding the proembryo and an outer endosperm layer of four cells (Text-Fig. 44). Transverse walls are next laid down in the layer of inner four endosperm cells (Text-Fig. 45), and later by further divisions in both the outer and inner endosperm layers, the cellular endosperm tissue is produced. The development of the endosperm closely resembles the Acre type described by Mauritzon (1933) for a number of other species of *Sedum*. There are, however, certain differences between the course of endosperm development in the present taxon and Acre type and these are pointed out in the discussion.

The further growth of the endosperm is from the micropylar end towards the chalaza. The endosperm cells are large, thin-walled, polygonal in outline, uninucleate and enclose scanty granular cytoplasmic contents. The growing embryo displaces the endosperm tissue with the result the mature seed is exalbuminous.

The lower chamber, transformed into the chalazal endosperm haustorium, grows in the form of a long, narrow, tubular structure towards the chalaza, crushing the cells of the conducting strand and a few surrounding nucellar cells. It is uninucleate throughout and contains granular contents. At a later stage the haustorium becomes broad and finally it is compressed by the growing endosperm from the micropylar end. The haustorium closely resembles the Sempervivum type of chalazal endosperm haustorium reported by Mauritzon (1933) for the other taxa of *Sedum*.

Embryo

The development of the elongated fertilized egg starts slightly later than the endosperm. The zygote divides transversely forming two unequal cells (Text-Fig. 46). Of these, the upper is the small lenticular terminal cell *ca* and the lower, which is large and vesicular, is the basal cell *cb*. All the embryonal parts are derived from the terminal cell. The basal cell, on the other hand, becomes much swollen and it occupies the entire micropylar portion of the embryo sac; its further development into an aggressive haustorium will be dealt with at the end of this account.

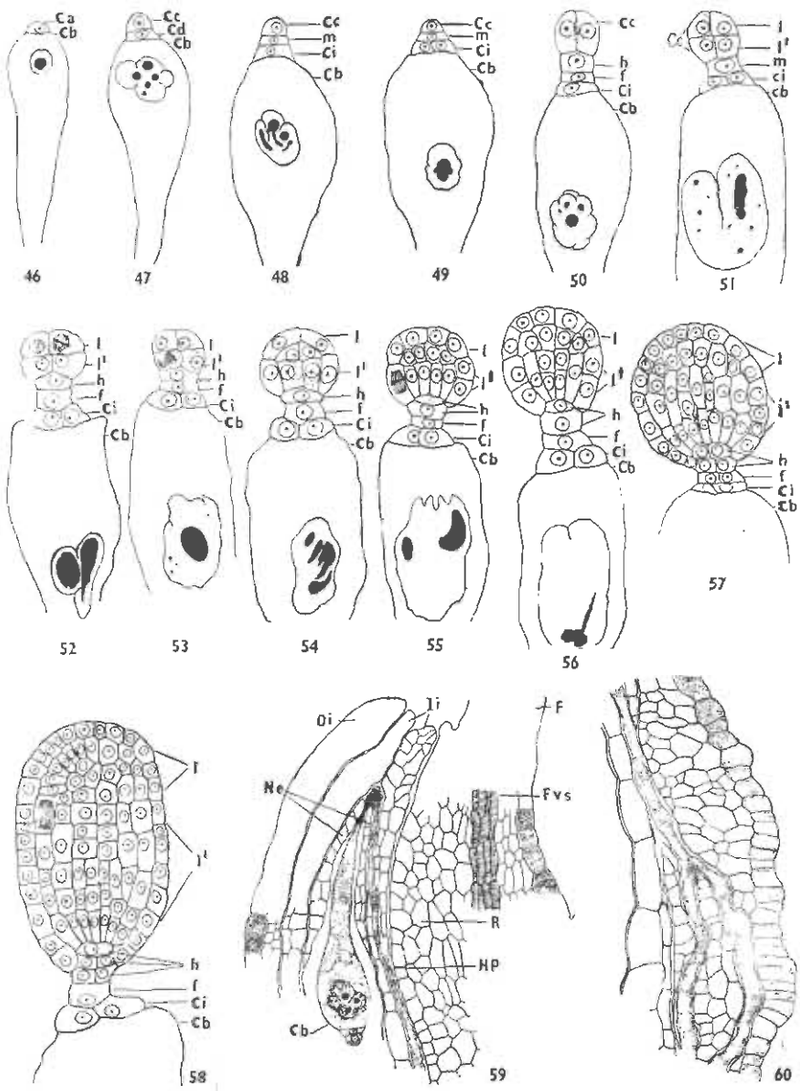
The terminal cell *ca* divides transversely into two superposed cells *cc* and *cd* (Text-Fig. 47); by one more transverse division in one of these cells, three cells *cc*, *m* and *ci*, lying one over the other, are formed (Text-Fig. 48). Cell *ci* divides vertically forming two juxtaposed cells (Text-Fig. 49) and these can be well seen during the developmental stages of the embryo (Text-Figs. 51–58). They do not divide again but

at a later stage become greatly flattened and stretched out in the horizontal plane, constituting a part of the suspensor. The topmost cell *cc* divides vertically and by another vertical division at right angles to the first results in the quadrant (Text-Fig. 50). This is followed by the octant stage which is derived by the transverse division of the cells of the quadrant (Text-Fig. 51). The four upper and four lower cells of the octant constitute the superior (*l*) and inferior cells (*l'*) of the octant respectively. In the superior cells (*l*) oblique walls (Text-Figs. 52, 54) delimit the initials of the protoderm. The cells underlying the protoderm become partitioned by vertical walls and all these cells together form the ground meristem (Text-Figs. 55–58). The inferior cells of the octant (*l'*) divide by longitudinal walls and thus the outer protoderm initials are separated from the inner initials of the ground meristem (Text-Figs. 53, 54).

The intermediate cell *m* divides transversely forming cells *h* and *f* (Text-Fig. 50). These are very much flattened in the horizontal plane. The upper cell *h* more or less gets wedged into the base of the spherical embryo and constitutes the hypophysis (Text-Fig. 54). This cell by further transverse divisions, followed by vertical ones (Text-Figs. 55–58), results in a group of cells which complete the epidermis and the ground meristem at the basal region of the developing embryo.

In the spherical embryo (Text-Figs. 56, 57), as a result of a series of anticlinal divisions in the outer protoderm layer, the epidermis is formed. At the same time longitudinal, followed by transverse walls, are laid down in the cells of the ground meristem. The embryo now becomes elongated (Text-Fig. 58) and as it develops further, *l* gives rise to the cotyledons and stem tip, *l'* to the hypocotyl, *h*, to the root tip, *f* and *ci*, along with the enlarged basal cell *cb* to the suspensor. The development of the embryo closely resembles *S. acre* described by Souèges (1925, 1927) and fits into Sedum-variation of the Caryophyllad type (Johansen, 1950). Certain minor differences from *S. acre* noticed in the further divisions of *ci* in the plant under investigation are indicated in the discussion.

The behaviour of the basal cell is very characteristic. After the first transverse division of the zygote the lower basal cell becomes large and vesicular with a prominent nucleus and dense granular contents (Text-Fig. 40). Soon it grows out of the nucellar epidermis (Text-Fig. 41) and lodges itself as a vesicular structure at the lower region of the micropylar canal (Text-Fig. 42). From here, and usually along the funicular side, it begins to grow in the form of a tubular structure between the nucellar epidermis and the inner integument. During its growth it crushes some cells of the inner integument in the upper region. Finally it pierces through the inner integument (Text-Fig. 59) and then begins to grow for some distance in the narrow space between the inner integument and the raphe. Later it branches extensively into the raphe and grows through the parenchymatous cells in an intracellular manner like the fungal hyphae (Text-Fig. 60). They are tubular and contain dense cytoplasmic contents. The large vesicular basal cell thus persists as a prominent part of the growing embryo for a long time in the



TEXT-FIGS. 46–60. Figs. 46–58. Stages in development of embryo, ×250; *ca*, terminal cell of the two-celled embryo; *cb*, basal cell of the two-celled embryo; *cc* and *cd*, cells derived from the terminal cell *ca*; *m* and *ci*, cells derived from *cd*; *h* and *f*, cells derived from *m*; *h*, hypophysis; *l* and *l'*, upper and lower groups respectively in a two-tiered octant stage. Fig. 59. Haustorial process from the basal cell of the embryo, ×100; *cb*, basal cell of embryo; *F*, funiculus; *Fvs*, funicular

vascular strand; *HP*, haustorial process; *Ii*, inner integument; *Ne*, nucellar epidermis; *Oi*, outer integument; *R*, raphe. Fig. 60. Portion of raphe enlarged showing branching lateral processes from basal cell, ×100.

developing seed. Moreover, its nucleus becomes very large (Text-Figs. 42–45) and greatly hypertrophied (Text-Figs. 47–56, 59) during the developmental stages of the embryo.

The mature embryo is erect and dicotyledonous. The cotyledons are lateral and the stem tip arises in the deep notch between them. The procambial strands, consisting of elongated cells, now become evident in the axial region of the hypocotyl. Below the stem tip they branch and each branch traverses up into a cotyledon. The cells of the stem tip and procambial strands are vacuolated and get less densely stained when compared with the remaining cells of the embryo, which show plenty of darkly stained contents. At the basal region of the embryo, the epidermal cells undergo a series of periclinal divisions followed by anticlinal ones and the resulting tissue thus formed constitutes the root-cap.

Mature Seed

The mature seed is elongated, has longitudinal ridges and is very small in size. Since the inner integument is completely destroyed, the seedcoat is mainly formed by the outer integument. It consists of three layers of which the outermost is the prominent epidermis; the remaining two layers become greatly flattened. The embryo fills up the entire cavity of the seed. The mature seed is exalbuminous, but remnants of the endosperm are sometimes seen in the form of a thin delicate layer around the hypocotyledonary region of the embryo. The internal morphology of the seed thus corresponds to the dwarf type (Martin, 1946).

Discussion

A survey of the previous literature on the embryology of *Sedum* has shown that there has been almost no work on the development of the anther and the male gametophyte. The development of the microsporangium and anther in the present and other species of *Sedum* (Subramanyam, 1955) shows great uniformity. The wall of the anther consists of the epidermis, fibrillar endothecium, two middle layers and finally the tapetum. The epidermal cells are large in size, except at the line of dehiscence and enclose brownish contents. The tapetum is of the glandular or secretory type. The tapetal cells remain uninucleate throughout and correspond to the first type recognised by Cooper (1933) in his classification of tapetal cells in Angiosperms. The mature pollen grain is binucleate, consisting of a large tube nucleus and small ovoid generative cell. Elfving (1879) had also noticed binucleate condition of the pollen grain in *Sedum hybridum*. According to him the sperm nuclei in *S. spurium* undergo elongation and lie side by side or close to one another during their course in the pollen tube.

The mature pollen is spheroidal in outline, with the exine thicker than the intine. It is usually tricolpate, sometimes quadricolpate and the colpi are tenuimarginate. A similar morphology of pollen has been recorded for other genera of Crassulaceae, like *Bryophyllum*, *Echeveria*, *Sempervivum* and *Aeonium* (Erdtman, 1952).

The pistil is superior, apocarpous and the carpels are slightly connate at the base. The stylodium is long, slender and is of the solid type (Hanf, 1935). In its axial region there is a transmitting tissue. A very characteristic gibbosity is present on the abaxial side of each carpel and just above its base. This gibbosity begins to appear in the open flowers and is well seen during post-fertilization stages. The cells at this region are large and thickened and morphologically it seems to represent submarginal outgrowth of the abaxial surface of the carpel.

The carpel of *Sedum* appears to have been formed by upfolding or uprolling and by the ontogenetic fusion of the carpellary margins. Anatomically this is indicated by the complete inversion of the ventral bundles (Eames, 1931; Quimby, 1939; Tillson, 1940; Subramanyam, 1955). Histologically it is supported by the distinctness of the epidermis of the two fused *margins* in some of the species examined. The latter fact in conjunction with the unfused condition during the early ontogeny in some other species of this genus (Subramanyam, 1955) and also the limited degree of fusion (the epidermal layers still persisting) of some of the carpels indicates that the genus shows relatively primitive carpels.

In transverse section, the carpel of *S. ternatum* recalls the conduplicate carpels of Lardizabalaceae and other primitive families (Bailey and Swamy, 1951). The latter is said to have originated by a simple upward folding of the carpel so that the adaxial surfaces on either side of the midrib approximate one another. The true margins do not fuse as in *Sedum*. Bailey and Swamy (1951) postulate a phylogenetic restriction of the ventral edges of the conduplicate carpel until a condition resembling a simple carpel like that found in *Sedum* is produced. In view, then, of the gross similarity between the outgrowths of *S. ternatum* and the primitive conduplicate carpels one is tempted to speculate that the prolonged ventral edges of the conduplicate carpel might be similar submarginal outgrowths. Data from careful ontogenetic studies and critical examination of the histological details of ventral bundles in conduplicate and other types of carpels are, however, essential for drawing any definite conclusion.

A characteristic feature of the family is the presence of a nectary in the form of a clavate outgrowth from the basal region of the carpel on its dorsal side. The nectaries have been referred to variously by different taxonomists. They are thus described as scales (Praeger, 1921), nectar scales (Fröderström, 1935), scales or nectaries at the base of carpels (Gunderson, 1950), nectaries (Clausen, 1951), hypogynous scale-like nectariferous glands (Lawrence, 1951) and hypogynous scales Rendle, 1952). Ontogenetic studies of the nectary in *S. aizoou*

(Subramanyam, 1955) have revealed that it arises as a result of periclinal divisions of some hypodermal cells in the basal region of the very young carpel. Further, each nectary receives at its base delicate procambial strands from the dorsal carpellary trace. It also receives procambial strands branching from the region where the lateral carpellary traces diverge into adjacent carpels (Quimby, 1939; Tillson, 1940; Subramanyam, 1955). So, the evidences from ontogeny as well as floral anatomy point to the fact that the nectaries are of the nature of clavate outgrowths or appendages from the basal regions of the carpels and not inter-carpellary structures. Puri (1951, p. 529), while reviewing the morphological nature of nectaries, remarks that "The inter- and extra-staminal nectaries of the Crassulaceae have been regarded as appendages of the carpels rather than formations of the axis".

Mauritzon (1933) while discussing the embryological features of Crassulaceae states that a whole series of delays occur in the division of *the upper dyad cell, which may at times even fail to divide. The situa*tion in *S. ternatum* well illustrates the case where the upper dyad cell always fails to divide and the lower dyad cell alone divides transversely and further development takes place as in the Polygonum type. A similar degeneration of the upper dyad cell has been reported by Mauritzon (1933) for *Echeveria rosei* and *Umbilicus intermedius*. Further, he regards the row of three cells, consisting of the undivided upper dyad cell and the megaspores formed as a result of the division of the lower dyad cell, as a "triad". A delayed division is sometimes seen in other species of this genus and other genera of this family like *S. palmeri* and *S. stenopetalum* (Subramanyam, 1955), *Crassula bolusii*, *Aeonium bethencourtianum*, *Sempervivum alpinum* and *Pachyphytum amethystinum* (Mauritzon, 1933).

Development of the endosperm follows the Acre type (Mauritzon, 1933) with certain minor differences. In the present plant at the eight-celled stage of the endosperm oblique longitudinal walls are laid down in the upper tier of four cells and this results in the formation of an inner layer of endosperm cells surrounding the proembryo, and an outer endosperm. In the Acre type of endosperm oblique longitudinal walls are laid down first in the lower tier of four cells.

The embryogeny of this plant resembles *S. acre* (Souèges, 1925, 1927) and some other species of this genus (Mauritzon, 1933). In the scheme of classification of embryo types in Angiosperms by Johansen (1950), it fits into the Caryophyllad type, Sedum-variation. The first transverse division of the fertilized egg results in a lower, large basal cell and an upper very small terminal cell. The basal cell takes no essential part in the formation of the embryo; it does not divide further but forms a large vesicular cell which at a later stage develops into an aggressive haustorium. The terminal cell divides by a transverse wall during the second cell generation and by one more similar division three cells, arranged in a row, are formed. Of these, the uppermost takes part in the formation of the embryo. The lowermost divides vertically forming two juxtaposed cells and these are characteristically seen

during the different stages of embryo development. In *S. acre* (Souèges, 1927) these two cells undergo another vertical division at right angles to the first, resulting in a group of four cells. The middle cell of the row divides transversely forming two cells; of these the superior daughter cell functions as a hypophysis which behaves as in the embryo of the Onagrad type; the inferior daughter cell, along with the two juxtaposed cells and the vesicular basal cell form the suspensor.

An outstanding feature of the embryo is the behaviour of the basal cell. It enlarges, becomes vesicular and soon occupies the major portion of the micropylar region of the ovule. Its nucleus becomes prominently hypertrophied and is surrounded all round by dense granular cytoplasm. It next pushes its way through the nucellar epidermis and lodges itself at the base of the micropylar canal in the form of a vesicle. It then grows further in the form of a tube between the nucellar epidermis and the inner integument on the funicular side. In *S. ochroleucum* (Subramanyam, 1955) it is sometimes noticed that the extra-nucellar vesicle of the basal cell develops tubiform branches both on the side of the funiculus and the integument. By further growth it crushes the cells of the inner integument and after penetrating through the latter, grows between the inner integument and the raphe. Here it produces a system of haustorial branches and these in turn ramify freely in an intracellular manner through the parenchymatous cells of the raphe, very much resembling the branched hyphae of a fungal mycelium. These haustorial branches are thin-walled and contain granular cytoplasm. Usually the nucleus in the basal cell never migrates into its extra-nucellar haustorial branch system. In a solitary instance in *S. ochroleucum* (Subramanyam, 1955) it was found that the nucleus of the basal cell had migrated out into one of these haustorial branches and Mauritzon (1933) has reported a similar condition in *Crassula orbicularia*. In other species studied by Mauritzon (1933), while the haustorial processes grow into the funiculus in *S. anopetalum*, they come out of the micropyle and thus become extra ovular in *S. crassipes* and *Pistorinia hispanica*. The development of haustorial processes has been observed in a number of genera of the Crassulaceae and hence can be regarded as a characteristic feature of the family. It may also be pointed out that in the closely allied families Podostemaceae (Magnus, 1913; Razi, 1949; Mukkada, 1962) and Hydrostachyaceae (Warming, 1882; Palm, 1915) a similar behaviour of the basal cell has been recorded.

The nutritional mechanism of the embryo sac and embryo in the family Crassulaceae deserves some consideration. The vascular supply to the ovule is formed by the funicular strand, which terminates at the base of the chalaza. A strand of elongated cells traverses the length of the nucellus in the axial region and connects the antipodal end of the small apically situated embryo sac with the chalazal region of the ovule, thereby helping in the translocation of food material from the chalaza to the antipodal end of the embryo sac. The presence of starch grains in the mature embryo sac and their occurrence even at the megaspore stage might also be regarded as a nutritive device. As the polar nuclei

fuse and when the embryo sac is ready for fertilization, the antipodal end of the embryo sac becomes active and soon begins to grow towards the chalaza forming a chalazal embryo sac haustorium. Mauritzon (1933) ascribes a haustorial role to the tubes or processes developed from the quartets of megaspores in *Rosularia pallida* and *Sedum sempervivoides*. He has also reported the presence of antipodal haustoria in *S. fabaria*.

Again, during post-fertilization stages two more haustoria are organized, one from the endosperm and the other from the basal cell of the embryo. After the first division of the primary endosperm nucleus, the lower chalazal chamber grows out into a long tubular chalazal endosperm haustorium. At the same time the basal cell of the embryo becomes very active and produces aggressive haustorial processes which penetrate into the seedcoat and sometimes even into the funiculus. These haustorial processes perhaps absorb nutrients from the cells of the seedcoat and transfer them to the developing embryo.

Finally, the endosperm tissue itself that surrounds the embryo acts as a nutritive tissue, at the expense of which the embryo undergoes its further growth and development. It thus becomes evident that the nutritive devices of the embryo sac and embryo in the Crassulaceae are very varied and extremely interesting.

SUMMARY

The wall of the anther consists of five layers of cells. The uninucleate tapetal cells are of the secretory type. The mature pollen grain is binucleate, tricolpate, sometimes tetracolpate, with the colpi tenuimarginate.

The carpels are of a primitive type, sealed on the ventral side. They show a gibbosity at their base which represents a submarginal outgrowth of the abaxial surface of the carpel. The nectaries are of the nature of clavate outgrowths from the basal regions of the carpels.

The ovules are crassinucellar and bitegminal. The nucellus is of the Sedum type. The archesporium consists of single or more cells and triads are invariably formed. Development of the embryo sac follows the Polygonum type.

Synergids have apical caps and the egg shows cytoplasmic strands. Starch grains are present in the mature embryo sac, egg, synergids and pollen grains.

Endosperm is *ab initio* Cellular and follows the Acre type; the chalazal endosperm is of the Sempervivum type.

Development of the embryo conforms to the Sedum variation, Caryophyllad type. The basal cell of the suspensor becomes vesicular and develops haustorial processes which penetrate into the seedcoat.

The mature seed is exalbuminous and the seedcoat is formed by the outer integument. The internal morphology of the seed is of the dwarf type.

The nutritional mechanism of the embryo-sac and embryo in the family Crassulaceae is discussed.

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INDUCED MUTATIONS IN RELATION TO PHYLOGENETIC ANALYSIS IN *TRITICUM*

By M. S. Swaminathan

Indian Agricultural Research Institute, New Delhi, India

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Introduction

It is well known now that spontaneously occurring mutations provide the raw material for natural selection to build upon and that through selection operating upon "blind" mutations microbes have evolved into men over millions of years. This process has been aptly termed by Muller (1960) as "muddling through". While the slow accumulation of small differences provides the basic mechanism for evolution of both intra- and inter-specific categories, the scrambling and repatterning of polarized sequences of chromosomal sections may occur occasionally in a single event and may lead, if viable, in one step to a major evolutionary deviation (Goldschmidt, 1955). Goldschmidt (1955) has designated such a change as "systemic mutation" and has cited several examples from both animals and plants in support of his view that such changes could have been important in macro-evolutionary divergence. Swaminathan (1961) has shown that in angiosperms polyploidy is a particularly potent mechanism for facilitating the viability of macro-mutations. Since it is now possible to induce a high frequency of mutations in most plants through the use of a wide array of physical and chemical mutagens, the study of induced mutations provides an additional source of information in reconstructing the phylogenetic history of plant species. The value of induced mutations in studies of this kind is enhanced by the identity of most of the experimentally induced mutations with those occurring spontaneously. The present paper provides data from a study of this kind carried out in the genus *Triticum*.

Cytogenetic History of *Triticum*

Members of the genus *Triticum* belong to a polyploid series with $2n = 14$, 28 and 42. The cytogenetic studies of Kihara and his co-workers (Kihara and Lilienfeld, 1949), McFadden and Sears (1946), Sarkar and Stebbins (1956), and Riley *et al.* (1958) have clearly established that the tetraploid ($2n = 28$) and hexaploid ($2n = 42$) species of *Triticum* arose respectively by chromosome doubling in hybrids between einkorn wheat ($2n = 14$) and *Aegilops speltoides* ($2n = 14$) and emmer wheat ($2n = 28$) and *Aegilops squarrosa* ($2n = 14$). Among

tetraploid *Triticum* species there are both free-threshing and non-free-threshing forms. McFadden and Sears (1946) synthesised the non-free-threshing hexaploid species, *T. spelta*, from the cross *T. dicoccoi 'es* × *A. squarrosa*, while Kihara and Lilienfeld (1949) obtained plants similar to the hexaploid free-threshing bread wheat, *T. aestivum*, from the cross *T. carthlicum* × *A. squarrosa*. Thus, the divergence existing at the tetraploid level with regard to the threshing character has also been transmitted to the hexaploids.

The hexaploid *Triticum* group comprises the following species (Plate I, Fig. 1).

1.	*Free threshing:*	*T. aestivum* L.
		T. sphaerococcum Perc.
		T. compactum Host.
		T. amplissifolium Zhuk.
2.	*Non-free-threshing:*	*T. spelta* L.
		T. macha Dek. *et* Men.
		T. vavilovi Jakub.
		T. zhukovskyi Men. *et.* Er.

Mac Key (1954 *a*) has shown that *T. aestivum* and *T. spelta* differ by one major gene *Q* situated on the long arm of chromosome 5 A (nomenclature of Sears, 1959 *a*). Sears (1947) and Unrau *et al.* (1950) found that *T. sphaerococcum* and *T. compactum* are each separated from *T. aestivum* by a single gene: *S* located on chromosome 3 D and *C* on 2 D respectively. Thus, the *sphaerococcum* and *compactum* types could have arisen only after the origin of the first hexaploid *Triticum*. *T. amplissifolium* is a little known Chinese wheat with compactoid ears of the inflatum type. Among the non-free-threshing species, *T. macha* has been considered by Kihara (1959) as the probable ancestral hexaploid species. Singh *et al.* (1957) found that *T. vavilovi* differs from *T. aestivum* only in 2 genes. *T. zhukovskyi* is also a little known species found only in Georgia in the U.S.S.R. It resembles the tetraploid *T. timopheevi* in phenotype and is believed to have been derived from it (Jakubziner, 1959). The available data thus show that the key characters separating most of the hexaploid *Triticum* species are governed by only 1 or 2 genes. Hence, Mac Key (1954 *b*) and Sears (1959 *b*) have suggested that all the hexaploid species should be regarded as subspecies of *T. aestivum* L. Since phenotypically, each of the subspecies possesses a whole constellation of morphological traits, systematists have had sufficient grounds for describing them as distinct species.

From a developmental standpoint, the origin of several distinctive character st cs from the effect of one gene would imply that the mutant action affects one of the early determinative processes. In this way, one primary action could have manifold consequences. If, however, the single unit of recombination is in fact a string of tightly linked genes,

the possibility is open to get the different phenotypic effects separated. From the view-point of the plant breeder, the latter situation would be more desirable as otherwise he cannot hope to separate the constellation of characters. Studies on induced mutations would facilitate an understanding of the prevailing situation, since owing to the low resolving power of genetic analysis in higher plants, conventional crossing procedures may not throw much light.

Induced Mutations of Phylogenetic Interest in Hexaploid *Triticum* Species

All the hexaploid species were treated with several dosages of X-rays, gamma-rays, Fast Neutrons and Ethyl Methane Sulphonate (Swaminathan and Rao, 1960; Rao, 1962). Different types of mutations were found in the M_2 and subsequent progenies. Those of phylogenetic interest alone are referred to in this paper. The ears of mutations in *T. aestivum* resembling the naturally occurring subspecies are given in Plate I, Fig. 2.

(*a*) *T. aestivum*

(i) *Speltoid mutations.*—A commonly occurring mutation is the "Speltoid" type in which the glumes get strongly keeled and threshing becomes difficult as in *T. spelta*. Associated with this change there may be others such as laxity of the ear, narrowness of leaves and lateness in maturity. Mac Key (1954 *a*) has shown that the speltoid mutation arises from the loss or inactivation of the free-threshing gene *Q*. This is the principal gene differentiating *T. spelta* and *T. aestivum*.

(ii) *Vavilovoid mutation.*—Mutations characterised by the elongation of rachillae as in *T. vavilovi* occur in speltoid types of *T. aestivum*. *Q* suppresses the expression of this character and it is hence that Singh *et al.* (1957) found a 2-gene segregation in crosses between *T. aestivum* and *T. vavilovi* (Rao and Swaminathan, 1963).

(iii) *Sphaerococcoid mutation.*—Mutations giving rise to rigid and bayonet-like flag leaf, inflated and hemispherical glumes and spherical grains occur rarely in irradiated material and more frequently in the progenies of plants treated with ethyl methane sulphonate. These mutants thus possess the key characteristics of *T. sphaerococcum*. When varieties of *T. aestivum* and *T. sphaerococcum* are crossed, segregates combining the spherical grains of the latter species with the plant characters of the former never occur (Singh, 1946). In mutation experiments, however, such phenotypes are found. Thus, the "S" locus seems to be a compound one with several recons and mutons.

(iv) *Compactoid mutation.*—Plants resembling *T. compactum* occur frequently in irradiated progenies. The strong keeling of glumes found in *T. compactum*, however, is absent in such mutants. Mac Key (1954 *a*) has shown that an overdose of *Q* gives rise to compactoid ears. The Chinese wheat *T. amplissifolium* appears to have arisen in this way.

(*b*) *T. sphaerococcum*

Mutations resembling *T. aestivum* frequently occur in this species following treatment with mutagens. The suggestion of Ellerton (1939) that *T. sphaerococcum* arose through a chromosome deletion in *T. aestivum* is thus rendered improbable. The constellation of characters associated with *T. sphaerococcum* is broken up in irradiated material thereby making available combinations of characters not usually found in nature.

(*c*) *T. compactum*

The *aestivum* mutation occurs commonly when this species is subjected to treatment with mutagens. The *C* locus can be either deleted or broken up through suitable treatments.

(*d*) *T. spelta*

It has not been possible to obtain a free-threshing mutation from *T. spelta* so far. Obviously, this mutation must be an extremely rare one and might have occurred only once at the tetraploid level in the phylogenetic history of the polyploid *Triticum* species. The speltoid suppressor factor *Q* has played a dominant role in the evolution of the widely cultivated *durum* and bread wheats. *Q* not only inhibits spelting but also brittleness of rachis, a development of obvious importance in cultivated forms. It also inhibits various forms of spikelet sterility and vavilovoid expression.

(*e*) *T. vavilovi*

Forms resembling *T. spelta* can be obtained in this species through the loss of *v*, the factor responsible for the elongation of rachillae.

(*f*) *T. macha*

This species is basically a spelt type with a factor for ear compactness. When the gene for compactness is removed, spelta-like forms are produced. This species is a very old one having been found in the ancient remains in Colchis. It appears to have been derived from the tetraploid Georgian wheat *T. palaeocolchicum*.

Discussion

The few mutations described in this paper make it obviously clear that mutation analysis will be rewarding from the phylogenetic angle. What have been described as distinct species by competent systematists in the genus *Triticum* are seen to have arisen through single step mutations. Such phylogenetically important mutants have been described in *Bromus* (Cugnac, 1939), *Zea-Euchlaena* hybrids (Langham, 1940), *Chlamydomonas* (Moewus, 1940), *Streptocarpus* (Oehlkers, 1940), *Marchantia* (Burgeff, 1943), ferns (Andersson-Kottö and Gairdner, 1936), maize (Singleton, 1951), *Pisum* (Rosen, 1944; Lamprecht, 1948),

Linaria (Schwanitz, 1955), *Secale* (Stutz, 1957) and *Antirrhinum* (Stubbe, 1959). In *Antirrhinum*, mutants having the key characters of the genera *Hemimeris*, *Mohavea*, *Ixianthes*, *Verbascum*, *Veronica*, *Lagotis* and *Wulfenia* have been isolated (Stubbe, 1959). Stubbe (1959) has also shown how through hybridization with other types such macromutants can easily acquire the necessary modifiers for normal vitality. Gustafsson (1954) and Wettstein (1954) have found that in the erectoides mutants of barley, a single mutant step leads to several pleiotropic effects. Goldschmidt (1955) has given examples of this kind in *Drosophila*. It thus seems that though the process of evolution through mutation is an extremely slow one, jumps of larger quanta in the evolutionary scale are occasionally possible. In polyploid plants, the buffering effect caused by the presence of duplications facilitates the survival of such large changes.

Maheshwari (1950) has elegantly shown how embryological research is many times useful in solving taxonomic puzzles. The availability of potent tools for inducing a high frequency and wide spectrum of mutations has opened up yet another path for reconstructing the phylogenetic history of the plants and animals with which we have the good fortune to live.

Summary

Mutants resembling *T. spelta*, *T. vavilovi*, *T. compactum*, *T. sphaerococcum* and *T. amplissifolium* occur in progenies of *T. aestivum* treated with different doses of physical and chemical mutagens. Similar macromutants also occur in the other hexaploid species of *T. aestivum*. With a single-step mutation, a constellation of new characteristics may sometimes arise thereby giving scope for a systematist to designate the mutant as a new species. Induced mutation analysis provides information of value in understanding the nature of genetic change involved in species differentiation and in reconstructing the phylogenetic history of plant species.

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EXPLANATION OF PLATE I

FIG. 1. Ears of hexaploid *Triticum* species. (a) *T. aestivum*. (b) *T. amplissifolium*. (c) *T. compactum*. (d) *T. sphaerococcum*. (e) *T. spelta*. (f) *T. vavilovi*. (g) *T. macha*. (h) *T. zhukovskyi*.

FIG. 2. Ears of *T. aestivum* and some induced mutants. (a) *T. aestivum* var. N.P. 798, (b) to (e) Induced mutants in *T. aestivum*. (b) Sphaerococcoid mutant. (c) Compactoid mutant. (d) Speltoid mutant. (e) Vavilovoid mutant.

FIGS. 1-2

M. S. Swaminathan

PHYSIOLOGICAL STUDIES ON CERTAIN FUNGI CAUSING LEAF-SPOT DISEASES

By R. N. Tandon

Department of Botany, University of Allahabad, India

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Introduction

Leaves are the chief centres of activity which discharge various physiological functions in the chlorophyllous plants. These plant structures have received much attention of plant morphologists and physiologists, but the plant pathologists have not devoted full attention towards them. A keen and constant observation of any apparently healthy plant reveals that at some stage or the other, some of its leaves exhibit distinct leaf spots. The magnitude of this expression depends upon several factors including the nature of the host, invading capacity of the pathogens, environmental conditions and the age of the leaves, etc. The total exposed area of the entire foliage system is enormously large enough to trap the fungal spores. Bald (1952) observed that stomatal droplets induce the germ tube of the leaf invading fungi to pass into the stomata and infect the leaf.

Recent results of many other investigators also amply indicate that several nutritive substances ooze out over the leaf surface, promote spore germination and facilitate the entry of germ tube into the host tissue. These findings indicate that in many cases host ingredients are responsible for determining the initiation of infection. Further propagation of disease depends upon the capacity of the pathogen to derive its food from the host tissue. This paper deals only with the role of carbohydrates on the growth of some pathogenic fungi causing leaf spot diseases. Carbohydrates, being the chief metabolic products of the photosynthetic plants, have special importance in the nutrition of disease causing organisms. Inside the host they exist in two states: (i) simple easily utilizable forms; (ii) complex forms.

The food of the first category is assimilated as such and the pathogens have not to strain on that account while the utilization of complex substances is accomplished by those micro-organisms only which can satisfactorily break them.

The utilization of some common carbohydrates by a number of pathogenic fungi representing diverse groups of Deuteromycetes has been studied.

Materials and Methods

The following fungi were isolated from the leaves of their respective hosts:

1. *Pestalotia banksiana* from *Grevillea robusta*
2. *Pestalotia citri* from *Citrus maxima*
3. *Phyllosticta artocarpina* from *Artocarpus heterophyllus*
4. *Phyllosticta mortoni* from *Mangifera indica*
5. *Phyllosticta morifolia* from *Morus alba*
6. *Phyllosticta carica-papayae* from *Carica papaya*
7. *Alternaria tenuis* from *Lycopersicon esculentum*
8. *Colletotrichum capsici* from *Scindapus pictus*
9. *Colletotrichum gloeosporioides* from *Punica granatum*
10. *Pestalotiopsis glandicola* from *Thea sinensis*
11. *Cercosporina ricinella* from *Ricinus communis*
12. *Curvularia penniseti* from *Pennisetum typhoideum*

A medium containing 10·0 g glucose, 3·5 g KNO_3, 1·75 g KH_2PO_4, 0·75 g $MgSO_4.7\,H_2O$, 100 μg thiamine, 10 μg biotin dissolved in one litre of double distilled water was employed for physiological studies. All the experimental glassware was of Pyrex-make. Extra pure chemicals supplied by B.D.H., E. Merck or Difco were employed. The substrate consisted of autoclaved or steam-sterilized liquid nutrient. Superficially cut agar discs from 6–10 days old colonies were used as inocula. The inoculated flasks were incubated at 25° C. At the end of each incubation period the fungal colonies were thoroughly washed and were subsequently filtered on previously dried and weighed filter-paper No. 42. Fungus containing filter-papers were subjected to 65° C. temperature (in electric ovens) for two days after which they were cooled and accurately weighed. Dry weight results were used for comparative purposes. Each set was run in triplicate. Average of three dry weights was taken as standard value for comparison of growth. The efficiency of growth has been grouped in three broad categories, *viz.*, good, moderate and poor. The medium was chromatographically analyzed in order to study the daily changes in the sugar contents of the medium. The technique of Giri *et al.* (1953) was employed for this purpose. *n*-Butanol-acetic acid-water (4 : 1 : 5) or *n*-butanol-pyridine-water (4: 6: 3) were used as solvents. The chromatograms with single central wick were run for about 7 hours. They were subsequently dried at room temperature for three hours and were then sprayed with a mixture of aniline-diphenylamine phosphoric acid. The sprayed chromatograms were again dried in air for about 45 minutes and were finally

suspended in electric oven at 110° C. for 60–90 seconds. After appearance of bands the Rf values of different sugars were calculated. Occasionally the solutions of known sugars were also placed on the test chromatograms to facilitate the identification of various sugars.

In order to determine the nature of synthetic oligosaccharides, the approximate area containing the new oligosaccharide was separated and eluted in 5·0 c.c. of hot water. The eluted solution was divided into two equal parts. One fraction was partially hydrolysed while the other part was totally hydrolysed with conc. HCl. The hydrolysed solutions were again assayed chromatographically.

Results

The three grades of growth (*viz.*, good, moderate and poor) of different fungi attained on various carbohydrates is recorded in Table I.

A review of Table I shows that starch, sucrose, maltose, glucose and fructose were invariably good sources for all the pathogens while their response on cellulose and melibiose was poor. The efficiency of raffinose, lactose, galactose and mannose ranged from good to poor. The results also express that different species of the same genus could behave alike or differently towards a particular source of carbon.

Chromatographic Observations

The chromatographic analysis of the starch-medium revealed that in all cases except in *Colletotrichum capsici* maltose and glucose were distinctly traced in the culture solution. An oligosaccharide (Rf 0·42) was also produced in the culture solution. *Phyllosticta artocarpina* and *P. mortoni* synthesized two additional oligosaccharides. Neither the hydrolytic products of cellulose (*viz.*, cellobiose and glucose) nor any synthetic oligosaccharides were spotted during the utilization of this substance. Trisaccharide, raffinose was gradually transformed into a mixture of melibiose and fructose by all the organisms except *Colletotrichum capsici* which used it at a very slow rate. Invariably all the fungi converted sucrose into a mixture of glucose and fructose and in every case the former component was consumed earlier. This evidently shows their comparative preference for glucose. At least one oligosaccharide was synthesised during the growth of these fungi on sucrose solution. Most of the species of *Phyllosticta* were also capable of forming an additional oligosaccharide (Rf 0·30).

Maltose medium showed distinct presence of glucose. The rate of its conversion was generally rapid by all the isolates but the corresponding rate of consumption of glucose varied with different isolates. One or two transient oligosaccharides were also produced in the medium. The other glucose-yielding disaccharide, *viz.*, cellobiose, was disintegrated at a very slow rate and the hydrolytic product (*viz.*, glucose) was detected only occasionally in very feeble quantity. Synthetic oligosaccharides were not spotted in any of the culture flasks. None of the

TABLE I

Representing the growth of twelve leaf spot causing fungi on various carbon sources

	Pestalotia banksiana	*Pestalotia citri*	*Phyllosticta artocarpina*	*Phyllosticta mortoni*	*Phyllosticta morifolia*	*Phyllosticta caricapapayae*	*Alternaria tenuis*	*Colletotrichum capsici*	*Colletotrichum gloeosporioides*	*Pestalotiopsis glandicola*	*Cercosporina ricinella*	*Curvularia penniseti*
Starch ..	G	G	G	G	G	G	G	M	G	G	G	G
Cellulose ..	P	P	P	P	P	P	..	..	..	..	..	..
Raffinose ..	G	G	M	M	G	G	M	P	G	G	..	..
Sucrose ..	G	G	G	G	G	G	G	G	G	G	G	G
Maltose ..	G	G	G	G	G	G	G	G	G	G	G	G
Cellobiose ..	P	M	P	P	P	P	..	..	..	..	..	..
Lactose ..	P	P	M	P	P	P	G	G	M	P	P	P
Melibiose ..	P	P	P	P	P	P	..	..	..	..	..	..
Glucose ..	G	G	G	G	G	G	G	G	G	G	G	G
Fructose ..	G	G	G	G	G	G	G	G	G	G	G	G
Galactose ..	G	M	M	G	M	G	G	G	P	M	G	G
Mannose ..	M	G	P	G	M	M	G	P	G	P	M	P

G has been used for 'good' ; M for 'moderate' ; P for 'poor'.

organisms under study were able to consume the entire cellobiose within the incubation time and a significant portion was left unfinished in the medium even at the end of the incubation period. The hydrolytic products of melibiose and lactose (*i.e.*, glucose and galactose) were spotted infrequently on lactose medium while they were not produced on melibiose medium. In all the cases a significant fraction of melibiose was left unconsumed in the medium. No trace of any synthetic oligosaccharides was recorded on either of the above media.

Amongst the hexose sugars the rate of utilization of glucose and fructose was quite rapid by all the pathogens. Glucose was finished within 4–6 days while fructose was exhausted by the 8th day. The rate of consumption of galactose and mannose varied with the organism and in some cases they were left unfinished even at the end of the incubation period. The comparative rate of utilization in every case corresponded with the hyphal output.

A correlative study of dry weight and chromatographic results showed that the rate of utilization of oligo- and polysaccharides was dependent on their rate of disintegration. Those complex carbohydrates which were hydrolysed quicker (*e.g.*, starch, sucrose or maltose) were always more favourite sources. Distinct formation of oligosaccharides was also a consistent feature in the above cases.

Discussion

Studies dealing with leaf-spot diseases are very spotty and, therefore, no co-ordinated account dealing with physiological aspects is available. The plant pathogens mostly come in contact with complex type of food in the host tissues. The survival and further propagation of the disease depends upon the success of the growing mycelium in absorbing the nutrients. An analysis of the hosts showed that at least four carbohydrates, *viz.*, starch, sucrose, glucose and fructose, were present in all of them. The results confirm that invariably they were satisfactory sources for the present pathogens, which were, therefore, well adapted to utilize those carbohydrates which existed in the hosts. Maltose was a good source even though it was lacking in the healthy tissues. Better response on maltose can be attributed to the capacity of these pathogens to use starch satisfactorily because maltose is produced during its breakdown. Most of the present organisms attained comparatively better growth on starch and maltose than on its basic structural constituent, *i.e.*, glucose. Similar preferentiality was exhibited by several other fungi including *Saprolegnia delica* (Bhargava, 1945), *Macrochytrium botrydioides* (Crasemann, 1954), *Fusarium oxysporum* f. *nicotianae* (Wolf, 1955), and species of *Phyllosticta* (Bilgrami, 1962). The fungi included here failed to adapt themselves to cellulose and this evidently shows that *cellulase* activity was limiting in the pathogenic microorganisms. With the exception of *Colletotrichum capsici* all other fungi were capable of disintegrating raffinose satisfactorily. The formation of only melibiose and fructose during the utilization of

this trisaccharide shows efficient β-fructosidase activity. Rapid utilization of fructose and accumulation of melibiose has also been reported for *Ceratocystis fimbriata* and *Thielaviopsis basicola* (Wilson and Lilly, 1958). It was interesting that with the exception of melibiose all other components of raffinose were individually good sources.

Wilson and Lilly (1958) as well as Wilson (1960) have also listed several fungi which are able to synthesize transient oligosaccharides during the utilization of maltose. This distinctly shows that cleavage of maltose is accomplished by transglycosidases. Pan *et al.* (1953) and Giri *et al.* (1953) have shown that along with hydrolysis, transglycosidases also cause replacement reaction of glycosidic bond due to which there may be synthesis of certain oligosaccharides as intermediates during the utilization of oligo- or polysaccharides. Conversion of starch and maltose media to a mixture of carbon sources might be one of the reasons for the superiority of these substances over glucose. Instances are, however, not lacking where certain fungi are completely incapable to thrive on maltose, *e.g.*, *Polychytrium aggregatum* (Ajello, 1948) and *Chytridium* spp. (Crasemann, 1954). The fungi included in the present study expressed only a mild liking for cellobiose but a number of imperfect fungi including *Chalara quercina* (Beckman *et al.*, 1953), *Memnoniella echinata* (Perlman, 1948 *a*), *Colletotrichum phomoides* (Lilly and Barnett, 1953) attained better vegetative growth on cellobiose than on glucose.

It has been found that the organisms which are adapted to saprophytic mode of living, *e.g.*, some members of Chytridiales (Crasemann, 1954), Saprolegniales (Bhargava, 1945 ; Ram Dayal, 1961) and Mucorales (Raizada, 1957) failed to utilize sucrose while most of the pathogenic fungi were fully capable to assimilate it. Sucrose has been reported superior even to glucose for a number of fungi studied by Perlman (1948 *b*) as well as Misra and Mahmood (1960). Due to transglycosidation, the synthetic oligosaccharides are formed on sucrose medium also. On the basis of the analysis of these synthetic oligosaccharides Bilgrami (1962) suggested unequal availability of its component sugars (*i.e.*, glucose and fructose).

Like most of the present organisms a number of other imperfect fungi including *Sphaeropsis malorum*, *Fusarium niveum*, *Melanconium fuligineum* (Lilly and Barnett, 1953) and *Fusarium oxysporum* f. *nicotianae* (Wolf, 1955) are also known to use lactose incompletely. Limits ing melibiase activity by leaf-spot pathogens appears to be responsible for poor availability of melibiose to them.

The formation of oligosaccharides on media of various carbohydrates is evidently carried by enzymes which catalyse the rupture of glycosidic linkage, uniting the various monosaccharide components. This is accomplished by introduction of a molecule of water which splits the glycosidic bond. The dissociated monosaccharide again gets attached to the original oligosaccharide and in this manner the oligosaccharide of varying chain lengths may be produced in the culture-medium.

SUMMARY

The utilization of carbohydrates by 12 pathogenic imperfect fungi was studied. The carbohydrates or their derivatives which existed in the host tissues were generally suitable sources. Synthesis of oligosaccharides on solutions of oligo- or polysaccharides was also quite common. There was no consistency in response on those substances which were lacking in the host. Factors responsible for the formation of synthetic oligosaccharides have also been discussed.

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MORPHOLOGY OF THE PACHYTENE CHROMOSOMES OF MAIZE

BY J. VENKATESWARLU

Department of Botany, Andhra University, Waltair, India

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DETAILED studies of the morphology of maize chromosomes may be said to have begun with the discovery by McClintock (1929) that every one of them is individually identifiable at the first division of the microspore by such features as length and position of the primary and secondary constrictions. Very soon after this discovery, McClintock (1930, 1931 *b*) found that the mid-prophase (pachytene) chromosomes in the first meiotic division of the pollen mother cells were even more suitable for morphological studies and that these stain very well and readily with acetocarmine. The morphology of the prophase chromosomes of corn has been subjected to intensive study by McClintock (1931 *a*, *b*), Burnham (1932), Rhoades (1933 *a*, *b*), Longley (1938, 1939) and others. Particularly due to the pioneering and patient research for many years by McClintock on various commercial and derived genetic strains and by Longley on thirty-three strains grown by North American Indian tribes and forty-one varieties from isolated regions of Mexico, the chromosomes of maize rank as morphologically the best known in any plant. In 1941, Longley gave a review of the morphology of the standard chromosomes in maize and its relatives and later Rhoades (1950) gave a brief and illustrated account of meiosis in maize based on his own observations and on the published accounts of Longley, Burnham, Randolph, Beadle, Anderson, McClintock and others.

The ten chromosomes in the haploid complement are individually recognizable at pachytene by their relative lengths, position of the centromere, knobs and prominent chromomeres in characteristic positions and by deep staining pycnotic concentrations adjacent to the centromere. One of the chromosomes of the set is characterized by the presence of a satellite and a nucleolus organizing body near the secondary constriction.

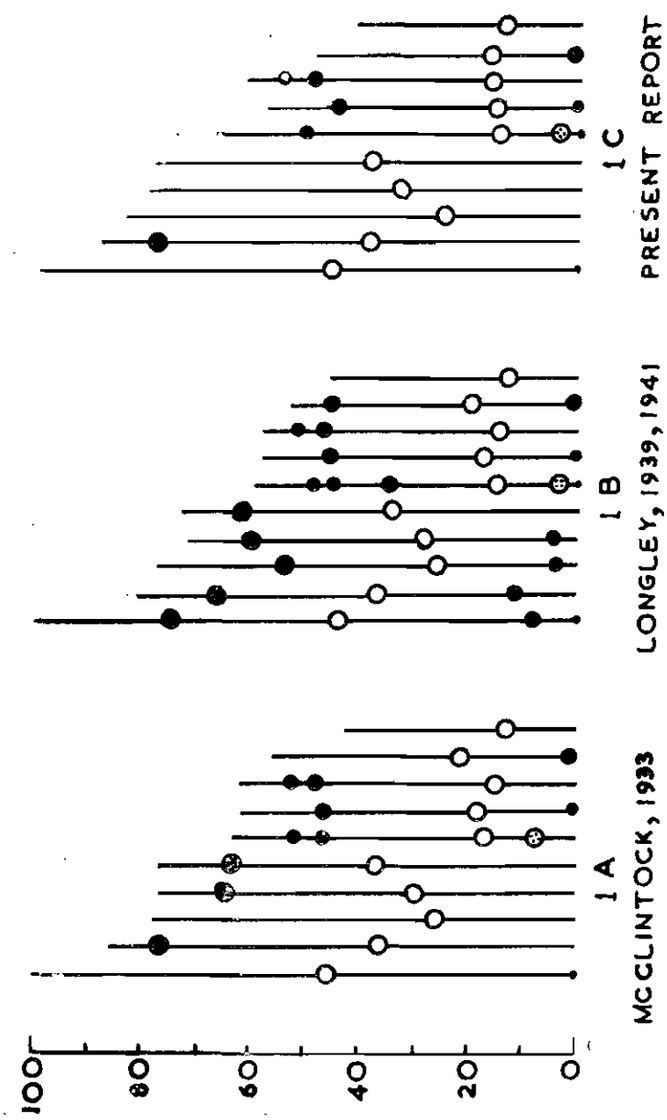
Pachytene chromosome morphology was studied in detail in an inbred strain of maize and a variance analysis of the average values of total length of chromosomes, length of arms, position of centromeres and arm ratios and knob positions has been made with a view to compare the values obtained in the present study with those of other investigators (McClintock, 1933; Longley, 1939, 1941) and to determine the reliability of observations and to judge the bias in the observations of the individual workers. The results of these studies are given below.

Lengths of the chromosomes.—The chromosomes of maize have been arranged in order of their length and given the reference numbers 1–10, chromosome 1 being the longest and chromosome 10 the shortest member of the complement. Length alone does not offer an entirely satisfactory criterion for identifying all the chromosomes with certainty because of the difficulty of having all the chromosomes of the complement and even the different sections of the same chromosome equally extended. In general there is a progressive reduction in the length of the chromosomes from 1–10 but it was often found that chromosomes 5 and 8 are on an average slightly longer than chromosomes 4 and 7 respectively. This was also the experience of Longley (1939).

For working out the relative lengths measurements of chromosomes in the pollen mother cells at mid-pachytene were used. Only a few cases of cells that could be completely analyzed were available for the purpose. However, in a number of cells chromosome 1 could always be followed and in these cells a number of other chromosomes, different ones in different cells, could also be followed from end to end. The length of chromosome 1 was arbitrarily taken to be 100 units and the lengths of the rest of the chromosomes in the cell were measured in equivalent units with reference to this one as a standard. The average of the observed lengths of each chromosome, expressed in these units, is used in constructing Text-Fig. 1 C, which shows the various morphological features of the complement in the strain used in this investigation. The average measurements used for it are given in column 2 of Table I.

Besides analyses, from the same cell of four or more chromosomes including chromosome 1, the lengths of various single chromosomes of the complement were also measured in many different cells each in terms of the average length of chromosome 1 taken as 100 units. The average of these are shown in column 4 of Table I. In columns 6 and 7 are given, for comparison, the measurements calculated in the same way as above, from diagrams published by McClintock (1933) and Longley (1939, 1941). Also, diagrams constructed from the last mentioned measurements are given in Text-Figs. 1 A and B alongside Text-Fig. 1 C for the haploid complement of the maize strain used in this investigation.

A variance analysis of the average values of the measurements obtained by the different investigators (Table I) has been made with a view to compare those obtained in the present study with the values of other investigators and to determine the reliability of the observations. The details are presented in Table I *a*. The ratio between the variance within chromosomes (degrees of freedom 2) and that due to sampling or residual error (degrees of freedom 18) is 1·9396. A value of 3·55 is significant at the 5% level and a value of 6·01 at the 1% level for the corresponding degrees of freedom, *i.e.*, 2 and 18 (*see also* Table V; Fisher and Yates, 1949). The observed value is smaller than these and, therefore, the measurements obtained in this study are judged to be in



TEXT-FIG. 1. Diagrams of the ten chromosomes of maize showing relative mean length in microns at the pachytene stage, positions of knobs (black circles), nucleolus-organizing body (shaded circle), and centromeres (circles).

TABLE I

Relative lengths of meiotic mid-prophase (pachytene) chromosomes in maize

Chromosome	Present report				McClintock (1933)	Longley (1939, 1941)
	Measurements of Chromosomes					
	From same cell		*From different cells*			
	Mean	Number measured	Mean	Number measured		
1	100·0	21	100·0	35	100·0	100·0
2	88·4	18	88·4	47	85·3	81·1
3	83·4	11	76·2	69	76·9	78·0
4	79·9	6	73·8	20	75·9	71·7
5	78·5	7	80·3	22	76·0	73·2
6	67·5	13	70·3	118	61·4	59·8
7	57·5	13	57·6	42	61·2	58·5
8	62·1	13	63·4	63	61·2	58·5
9	49·0	10	56·4	90	55·2	53·0
10	41·4	9	40·7	54	42·8	45·7

TABLE I *a*

Analysis of variance (relative lengths of chromosomes)

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance)	Variance ratio (F)
Between chromosomes	9	6285·4502	698·3833	158·2394
Within chromosomes	2 (n_1)	17·1035	8·55175	1·9396
Residual error	18 (n_2)	79·4243	4·41346	..
Total	29	6381·9780	..	..

n_1 (small variance) = 2 significance at 5% level = 3·55

n_2 (greater variance) = 18 significance at 1% level = 6·01

agreement with those of the previous investigators (McClintock, Longley, 1941).

Lengths of the arms and positions of the centromeres.—The centromere divides each chromosome into two arms. The ratio of the lengths of the two arms in each of the ten chromosomes is different and is one of the characters that is useful for the identification of the individual chromosomes. The position of the centromere is fixed for each chromosome of the set. It is conveniently expressed by a fraction which shows the length of the short arm relative to that of the long arm. These values, for the various chromosomes of the set, are given in column 2 of Table II. The values obtained from the diagrams published by McClintock (1933) and Longley (1939, 1941) are given in columns 4 and 5 of Table II for comparison. The measurements of the arms in each chromosome were based on camera lucida drawings made from the pollen mother cells.

Since the observations have been made on chromosomes at different stages of contraction, it is possible that the position of the centromere may be affected, to some extent, by differential contraction. A much more troublesome source of error is differential stretching of parts of chromosomes and chromosomes in different nuclei as a consequence of the mechanical process of preparing the squashes.

In constructing Text-Fig. 1 C, the relative lengths of the arms were used to divide, at the position of the centromere, the lines drawn to represent the chromosomes proportionately. The centromere is represented as a circle.

Table II *a* summarises an analysis of variance of the data presented in Table II. The ratio between the variance within the chromosomes (degrees of freedom 2) and that due to sampling or residual error (degrees of freedom 18) is 0·0788. A value of 3·55 is significant at the 5% level and 6·01 at the 1% level for the corresponding degrees of freedom (2 and 18) (*see also* Table V; Fisher and Yates, 1949). The observed value is smaller than both the above values and, therefore, the arm length ratios obtained in this study are in agreement with those obtained by McClintock (1933) and Longley (1939).

Knobs.—In the pachytene chromosomes of many maize varieties deeply stained and enlarged regions of various sizes may be observed. Careful observation has shown that they occur at definite points along the chromosomes. McClintock (1931 *b*, 1933) demonstrated the presence of knobs on all except one of the ten chromosomes of corn. Later on, Longley (1939) investigated many strains and found that knobs occur in eighteen different positions on the corn chromosomes. Since then four more positions have been reported to make the total twenty-two (*see* Weatherwax and Randolph, 1955; p. 33). However, all of them were not found together in any single strain examined. The fact that knobs, when present, occupy a constant position has afforded a very useful aid in quick identification of the chromosomes. Also, the

TABLE II

Relative lengths of the arms and the position of the centromere as expressed by length of short arm/length of long arm

Chromosome	Present report: Mean	Present report: Number of cases measured	McClintock (1933)	Longley (1939, (1941)
1	0·801	14	0·839	0·782
2	0·783	22	0·706	0·821
3	0·389	58	0·505	0·493
4	0·688	14	0·637	0·638
5	0·921	15	0·933	0·875
6	0·266	75	0·343	0·324
7	0·351	12	0·428	0·411
8	0·342	38	0·320	0·315
9	0·477	80	0·584	0·581
10	0·476	45	0·400	0·388

TABLE II *a*

Analysis of variance in the arm ratio of the corn chromosomes

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance)	Variance ratio (F)
Between chromosomes ..	9	1·283942	0·142660	10·74
Within chromosomes ..	2 (n_1)	0·002095	0·0010475	0.0788
Residual error	18 (n_2)	0·239188	0·01328	
Total ..	29	1·521035	..	

n_1 (small variance) = 2 significance at 5% level = 3·55
n_2 = (greater variance) = 18 significance at 1% level = 6·01

TABLE III

Position of knobs and nucleolus organizer expressed as:*
Distance of the knob or nucleolus organizer from the end of the chromosome concerned

		The length of the chromosome concerned										
		Present report										
Chromosome	Arm concerned	Mean	Number of cases measured	Mean	Number of cases measured		McClintock (1933)			Longley (1939, 1941)		
2	Long	0·138	29	..	..		0·102			0·188		
6	Long	k_1 ..	.. ..	k_2 0·26	105	k_1	k_2 0·261	k_3 0·170		k_1 0·428	k_2 0·257	k_3 0·195
6	Short	†N.O. 0·06	102	..	..	..	N.O. 0·127			N.O. 0·051		
7	Long	0·24	29	..	..	..	0·257			0·229		
8	Long	k_1 0·22	50	k_2 0·13	50	k_1 0·228	k_2 0·157			k_1 0·208	k_2 0·135	

* K = Knob, wherever there are 2 or 3 knobs on a chromosome they are numbered as k_1, k_2 and k_3, k_1 being that nearest the centromere.

† N.O. = Nucleolus organizer.

different strains of maize differ from each other, to some extent, in the number of knobs as also in their size. In the strain used by me, one knob occurs on the long arm of each of the chromosomes 2, 6 and 7, and two knobs on the long arm of chromosome 8. The short arm of chromosome 9 bears a terminal knob. Besides these, it was also found that the short arms of chromosomes 1 and 7 have a little larger terminal chromomere. Chromosome 6 has a nucleolus-organizing body situated near the secondary constriction in the short arm. This chromosome is attached at this region to the nucleolus during the prophase and thereby is easily identified. The knob positions, each expressed as a ratio of its distance from the end of the long arm to the whole length of the chromosome concerned, are given in columns 3 and 5 of Table III. The values obtained for the same knobs and given in the diagrams of *McClintock (1933) and Longley (1939, 1941)* are also shown in columns 7 and 8 of Table III for comparison.

TABLE III *a*

Analysis of variance of knob positions

Sources of variation	Degrees of freedom	Sum of squares	Mean square (variance)	Variance ratio (F)
Between chromosomes ..	4	0·037255	0·009313	11·699
Within chromosomes ..	2 (n_1)	0·000025	0·0000125	0·0157
Residual error ..	*8 (n_2)*	*0·000796*	*0·0000995*	..
Total ..	14	0·042008	..	..

n_1 (smaller variance) = 2 significance at 5% level = 5·79
n_2 (greater variance) = 8 significance at 1% level = 8·65

An analysis of variance of the data on knob positions presented in Table III is summarised in Table III *a*. The ratio between the variance within chromosomes (degrees of freedom 2) and that due to sampling or residual error (degrees of freedom 18) is 0·0157. A value of 5·79 is significant at the 5% level and 8·65 at the 1% level for these degrees of freedom (*see also* Table V; Fisher and Yates, 1949). The observed value is smaller than these and, therefore, the data obtained in the present study are in agreement with those of McClintock (1933) and Longley (1939).

Below are given the diagnostic morphological features which enabled me to identify the ten chromosomes of the complement in the

strain used in the present study with a fair amount of certainty. No B-type chromosomes were found in this strain.

Diagnostic features of the ten pachytene chromosomes of the haploid complement:

Chromosome 1. Longest of the complement with an average length of 84·5 μ. A slightly enlarged chromomere terminates the short arm. Arm ratio is 0·801 : 1·0 (44·5 : 55·5).

Chromosome 2. This is distinguishable from the rest of the long chromosomes (numbers 1–5) of the complement by the presence of a rather prominent knob (diameter about two to two and a half times the thickness of the chromosomes) on the long arm situated at a distance of about one quarter the length of the arm from its end. The arm ratio is 0·783 : 1·0 (43·9 : 56·1) and the average length is 74·7 μ.

Chromosome 3. The long arm is about twice the length of the short arm. This feature distinguishes it from the rest of the long chromosomes of the complement. The arm ratio is 0·389 : 1·0 (28 : 72) and the average length is 70·5 μ.

Chromosome 4. This is difficult to distinguish from chromosome 1 unless one of the longer chromosomes is clearly traceable in the same cell. However, the ratio of the short arm to the long arm is different from that in chromosome 1. It is 0·688 : 1·0 (40·8 : 59·2). The average length is 67·5 μ.

Chromosome 5. This is distinguishable by the nearly equal length of its arms. The arm ratio is 0·921 : 1·0 (48 : 52) and the average length is 66·3 μ.

Chromosome 6. This is distinguishable by its attachment to the nucleolus by means of a deeply-staining nucleolus-organizing body, about three to four times the thickness of the chromosome. A small knob is present on the long arm situated at a distance of a little more than one-third the length of the long arm from its end. The arm ratio is 0·2666 : 1·0 (21 : 79) and the average length is 57 μ.

Chromosome 7. The region of the long arm adjacent to the centromere is deep-staining. There is a knob on the long arm at a distance of rather more than one-third the length of the arm from its end and the short arm has a slightly enlarged chromomere at its end. The arm ratio is 0·351 : 1·0 (26 : 74) and the average length is 48·6 μ.

Chromosome 8. This is distinguished by the presence of two small knobs on the long arms, the distal knob being smaller than the proximal one. The proximal knob is situated rather more than one-third the length of the arm from its end and the distal knob is rather less than one-sixth the length of the arm from its end. This feature was most helpful in identification as this chromosome was found to be longer than chromosome 7. The arm ratio is 0·342 : 1·0 (25·5 : 74·5) and the average length is 52·5 μ.

Chromosome 9. This is readily distinguished by the presence at the end of the short arm of a prominent terminal knob having a diameter rather more than twice the thickness of the chromosome thread. The arm ratio is 0·477 : 1·0 (32·3 : 67·7) and the average length is 41·4 μ.

Chromosome 10. This is the shortest member of the complement with an average length of 35 μ. The region of the short arm next to the centromere stains more deeply than the other parts of the chromosome. The arm ratio is 0·476 : 1·0 (32·5 : 67·75).

I am grateful to Professor D. G. Catcheside, F.R.S., for his advice and encouragement and for providing me with the inbred strain of maize used in the work which was carried out at the Botany School, Cambridge, England, some years ago in his laboratories.

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REFLECTIONS ON ORGANIZATION IN PLANTS

BY C. W. WARDLAW

Department of Botany, University of Manchester, Manchester, England

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ORGANIZATION A *Continuum*

A LONG time ago Lyell noted that ancient fossils were generally like contemporary living organisms in their basic organization. This he described as the principle of continuity. One may now recognize that some aspect of organization can be perceived in *all* the materials with which science is concerned—the elements, inorganic crystals, organic macromolecules, and progressively up the scale of increasing biological diversification, from the amoeba to the most highly evolved angiosperm. In this general sense, organization is a *continuum* in the physical world. Organization is also a *continuum* in the ontogenesis and reproduction of the individual organism and of the phyletic line of which it is a component.

Since metabolism, growth and structural development are considered to take place according to physico-chemical laws, it would be surprising if living entities did not manifest organization, since many physico-chemical processes have organizing effects. In some instances it is possible to indicate the physico-chemical factors, or the mathematical relationships, that are directly or indirectly involved in, or indicative of, organizing processes; but, in others, the developmental situations are so complex, and our knowledge of the kinetics and reactions of large organic molecules in the protoplasm and between cells so inadequate, that reasoned conjecture must still take the place of validated interpretation. Such difficulties led earlier workers to formulate vitalistic hypotheses, concepts of the 'emergent' properties of more complex substances, emergent evolution, and so on.

While some problems of organization are yielding to the application of physico-chemical ideas and techniques, and many others may be expected to do so in the coming decades—especially if biophysics and biochemistry become established in biology departments, as they ought to be—the higher levels of organization call for concepts of an organismic kind. The concepts of 'organismic biology', as distinct from 'molecular' or 'atomistic biology', are not only justifiable: they are essential. The development of an organism as an integrated whole, the relation of its parts to the whole and to each other, its co-ordination and functional unity, and many aspects of its life-history, all require concepts that lie outside the physical sciences as presently formulated. Thus while biologists should use to the full the ideas, results and

criteria of the physical sciences, they must go further and formulate their own distinctive and necessary concepts. In fact, the aim should be to work towards a philosophy of biology; *i.e.*, a philosophy of *organisms* as distinct from the analysis of individual processes that take place in them. These views are in no sense new but a re-emphasis of them seems timely.

Organization a Heritable Character

Organization in plants is a *continuum* in the sense that its basis is heritable. At some stage during the phase of chemical evolution, a persisting arrangement of substances that could be specified as "living", in contrast to "non-living", was attained. For convenience, we may accept this stage as the beginning of organismal organization. The essential features were that the "living substance" had considerable stability, that it could increase quantitatively, and that it could reproduce itself so that its distinctive features, the cumulative result of the previous phase of chemical change and selection. were perpetuated. In other words, the nascent organization was heritable; the "living substance", or entity, consisted of a compatible system, or "consociation", of molecules, capable of growth and self-reproduction.

In considering the phenomena of organization in plants, we are typically concerned with the development of a zygote, a spore or other reproductive cell, or with the active apices of shoots, buds, leaves or roots. Since there is no evidence that runs contrary to the aphorism: 'all cells from cells, all life from life', every cell or tissue region possesses some characteristic organization from the outset. Accordingly, all the phenomena of metabolism, growth, development and progressive organization which may be observed during the ontogenesis of a moss or a vascular plant, involving sequences of chemical reactions, take place in embryonic cells in which some organization is already present.

Integration an Essential Condition

Integration is an essential condition for organization. It presents itself as a logical necessity, in that organization could scarcely emerge from the action of forces causing disintegration. Even in the simplest living system, certain physico-chemical and biological conditions must be satisfied. Thus the molecules and macromolecules in the system must tend to cohere rather than disperse in the particular environment; their reactions must be such as to impart stability and equilibrium, or steady state, to the system; and they must be disposed in some characteristic spatial or geometric arrangement so that they can maintain and reproduce themselves and react with each other. All this is as much as to say that, collectively, the molecules must constitute a system which has functional efficiency. In more complex organismal systems, it may be assumed that similar physico-chemical relationships also obtain; for organisms in general are characterized by functional efficiency, by stable relationships with the environment, and by the

arrangement and compatibility of their components, making for harmony of development.

Organismal Reaction Systems

Theoretical considerations together with observations and experiments support the general idea that the primary organizational features in plants are due to the functioning of *organismal reaction systems*. These cannot yet be defined with any precision, but they are held to be present in individual cells. as in zygotes and spores, and in the integrated groups of cells that constitute embryonic or meristematic regions, *e.g.*, the shoot apex. In each instance, an inherited cellular organization determines the nature and potentiality of the reaction system. The activity of the reaction system is affected by all its components, *i.e.*, by the specific physico-chemical properties of its inherent reacting substances and by external factors such as heat, light or nutrient supply. In fact, the reaction system admits of continuity between organism and environment.

The idea of reaction systems in embryonic cells and regions makes possible at least a tentative approach to some of the more fundamental aspects of organization and evolution in plants. Among the things we can say, at least tentatively, are: (i) that the basis of the reaction system is transmitted in heredity, *i.e.*, in the protoplasmic organization of the ovum; (ii) that individual components of the system include specific gene-determined substances and nutrient and other substances obtained from the environment or from contiguous cells or tissues; (iii) that the reaction system admits of the interaction of genetic factors, factors in the environment, *e.g.*, heat, light nutrient supply, etc., and organismal factors, *e.g.*, those involved in correlation; (iv) that the reaction system in a shoot apex typically gives rise to a patternized (or patterned) distribution of metabolites, this process preceding and underlying organogenic developments and tissue differentiation; (v) that, since the reaction system is physico-chemically an open system which tends towards a steady state, and is the primary locus for the interaction of the several categories of factors indicated above, the organization which results is typically characterized by unity. integration, specificity and adaptation; (vi) that as the reaction system in a shoot apex becomes more complex during ontogenesis, so also does the pattern of organs and tissues to which it gives rise; but the transition from one morphological "stage" to another is usually characterized by physical and developmental harmony; (vii) that the specific substance determined by a mutant gene must either be compatible with the other components of the reaction system, or simply non-reactive; otherwise the system may be disrupted to a greater or less extent, *i.e.*, the gene will be lethal; (viii) that, in relation to (vii) above, selection begins in the reaction system of the ovum, zygote, or embryonic tissue; and that, however random gene mutation may be, the selection of mutants is to some extent canalized by the constitution of the reaction system; (ix) that evolutionary concepts such as those of orthogenesis, or of the inheritance of acquired characters of adaptive

value, may be re-examined in the light of the reaction system theory, (x) that although each species or variety has its own specific reaction system, which gives rise to many specific characters, the number of basic kinds of pattern in plants is really quite small. Reaction system theory can thus contribute to a better understanding of differences in specific organization on the one hand and of the prevalence of homologies of organization on the other.

The Interpretation of Physiological Processes

In theories of correlation in plant development, *e.g.*, the inhibition of lateral buds by the main apex, it cannot be said that truly satisfying "explanations" have not yet been proposed, though the reality of the phenomenon itself is not in doubt. The general idea in correlation is that an auxin, or a hormonal substance, moves from one region of the plant into another, *e.g.*, from the shoot apex to an axillary bud or bud site, and there exercises some characteristic effect on growth and organogenesis. It is not unusual in such studies to find that the state of the tissue acted upon is not specified, *e.g.*, in respect of its histology or other aspects of its initial organization. Such information is now seen to be essential. Again, studies of organ formation in undifferentiated tissue cultures, *e.g.*, the induction of buds and/or roots, suggest that certain growth-regulating substances, alone or in combination, have definite organizing and morphogenetic effects. Such substances are certainly essential to the observed developments. Nevertheless, explanations of specific developments in terms of metabolism alone are usually quite inadequate—a view that has long been entertained by Sinnott and other workers in this field. It is now suggested that a growth-regulating, "organizing" or "morphogenetic" substance exercises its characteristic effects by becoming a component of the reaction system of the meristematic tissue or nascent organ acted upon. It may then affect the inherent organization in characteristic ways. The development which ensues must accordingly be referred to *all* the factors in the situation: the "morphogenetic substance", though it may be quite essential, is only one factor in the situation.

External and Internal Factors

As J. B. S. Haldane once remarked: "It is hard to disentangle the effects of nature and nurture". At any particular stage in development, *e.g.*, of an angiosperm, the manifestations of organization are due to genetic, organismal and environmental factors. This is well illustrated by the transition from the vegetative to the reproductive phase in flowering plants: genetic factors fundamentally determine the nature and scope of the general and specific morphological developments; but the stage reached in ontogenesis, and the incidence of external factors such as day-length or temperature, are usually closely involved in the inception of the flowering process. One of the important results of experimental taxonomy has been to show how important both genetic and environmental factors can be in different circumstances.

Specificity and Homology of Organization

In studying any considerable range of organisms, either within the same circle of affinity or in different major groups, one becomes aware of the dual nature of organization. On the one hand, the evolution of plants, in all groups and at all levels, has been marked by active differentiation of new varieties from the parental stock, yielding abundant specificity of organization. On the other, the Plant Kingdom is characterized by much homology of organization. Specificity of organization, *e.g.*, in related varieties or species, can be referred to genotypic differences. Homologies of organization, by contrast, raise problems of a different order of magnitude. In the simplest case, *i.e.*, that of genetical homology, comparable features may be expected in plants which have originated from the same ancestral stock. On the other hand, closely comparable organizational features may be produced by quite differently constituted genetical systems. Other homologies of organization may be ascribed to the action of self-organizing factors which may not be closely gene-controlled, to extrinsic factors, to size-and-form correlations, or to other mathematical relationships.

In searching for general ideas relating to organization in plants, it may be emphasized that while the number of specific genetical systems in the Plant Kingdom is very large, the number of basic organizational patterns, or plans, is quite small. For example, viewed as geometrical constructions, all vascular plants are essentially alike, *i.e.*, they consist of an axis with regularly-disposed lateral appendages. In addition to genetical homology, the prevalence of homologies of organization may be accounted for on various grounds: (i) that all heritable organizational changes must obey physico-chemical laws; (ii) that the successive organizational innovations, which mark the course of evolution, were those which were most likely to take place; these may include the evolution of important parallelisms in genetical systems; *i.e.*, some kinds of system will tend to have been perpetuated and others to have been eliminated; and (iii) that environmental factors and certain limiting factors tend to canalize development. In plants, it appears that the number of major organizational innovations has been quite small; out the number of variations of these innovations has been very large indeed.

Since the principal metabolic processes are common to all green plants, their reaction systems may be expected to have features in common and to yield some similarities of pattern. This may account for some aspects of parallel or homoplastic development. In fact, certain general configurations or patterns are common to all the classes of higher plants. But within any one kind of pattern, there may be more or less extensive variations. These may be attributed to specific gene-controlled metabolic substances acting as components of the reaction system.

A MORPHOGENETIC LOOK AT THE ALTERNATION OF GENERATIONS

By R. H. Wetmore,[1]

Harvard University, Cambridge, Mass., U.S.A.

A. E. De Maggio[2]

College of Pharmacy, Rutgers, The State University, Newark, N.J., U.S.A.

AND

G. Morel

Station Centrale de Physiologie Végétale, C.N.R.A., Versailles (S & O), France

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Introduction

One by one modern morphologists are challenging the classic concept of an obligate alternation of generations in plants. Their dissent, contradictory as it may seem, is but a logical step in a century-old study.

Hofmeister's demonstration (1851) of fundamental parallelism in the alternation of vegetative and sexual generations in life cycles of the different groups of plants has been profound and lasting. His theory, illuminated by the doctrine of evolution, was clearly reflected in writings of the latter part of the century. Its effect was enhanced by Strasburger's report on the general phenomenon of the periodic reduction of chromosomes (1894) and the rediscovery of Mendelism (1900). The cytological basis for segregation of Mendelian characters, on the one hand. and, on the other, for the interjection of variation through the potentialities of meiosis seemed to provide a ready justification for survival of the alternate generations in the evolutionary history of the different groups of plants. The haploid generation became a prothallus, varying in form, in pattern of development and even in degree of independence in the life cycle, but it was always more or less thalloid and without vascular tissue. By contrast, the diploid generation, developing from the fertilized egg,

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acquired the organs, leaf, stem, root and flower, of which the phenotypic characteristics have become basic for taxonomic identification of groups and species.

That this generalization was not interpreted the same way by all botanists became clear in the first quarter of the present century. At the Discussion on the Alternation of Generations before the Linnean Society of London (1909) and in the following decades, persisting arguments revealed that there were believers in the clean-cut separation between the gametophytic and vegetative or sporophytic generations. These were the adherents of the Antithetic Theory of Alternation vigorously lead by Bower (1894, *et seq.*). To this group, the sporophytic generation was an innovation in evolution, a gradual, progressively more important generation which in the evolutionary sequence of plants gave rise to groups with a variety of elaborations of photosynthesizing shoot systems, the metabolic reserves of which were expended sooner or later as meiotic spores. The "new" or "interpolated generation" became the symbol of advance in the vascular plants. The advent and evolution of the land plant was attributed to the phenomenon of sex and the postponement of meiotic reduction to that time in the life cycle when reserves from photosynthesis gave rise to myriads of spores with their statistical fulfilment of the potentialities of multiple genes in sets of chromosomes (*e.g.* Svedelius, 1927).

Unrest over the interpretation of the alternation of generations increased among botanists in this early part of the century. Evidence was accumulating that did not seem to support the idea of a new and distinct spore-producing generation. Many botanists were disturbed by the discovery of apogamy (Farlow, 1874; De Bary, 1878) and by the production of sporophytes directly from prothallial tissue without fertilization (Lang, *1898; see reviews by* Steil, 1939, 1951; Manton, 1950). Moreover, Goebel's demonstration of the aposporic production of prothalli directly from leaves of young fern plants (1905, 1907, 1908) was soon supported by additional reports from other workers. These reports were quite in keeping with the much earlier revelations of aposporic phenomena in mosses by Pringsheim (1876) and Stahl (1876). When, in turn, investigation of life histories of algae revealed case after case of isomorphic gamete-bearing and spore-bearing generations, the arguments for the Homologous Theory of the Alternation of Generations became arresting (Discussion on *Alternation of Generations*, 1909; Svedelius, 1927; text-books on algology).

In the past fifty years one finds decreasing emphasis given to any concept of antithesis in the two generations. As Lang pointed out in his now classical, retiring presidential address to the British Association for the Advancement of Science (1915), one must look for answers in causal morphology rather than in heredity and chromosome numbers. One must explore the range of possibilities within which the plant *can* express itself under its hereditary potential, rather than be insistent upon a fixed routine of alternation by which it *must* express itself. Again the question of why and how the plant can achieve more

than one pattern of development with the same genome becomes the dominant issue. Interpretations of the foundation of morphogenetic pattern rest upon response to this question.

Current Observations and Discussion

During the past decade, one important factor making for unexpected progress on botanical problems has been recognition of the potentialities of sterile nutrition culture techniques. For example, whereas in nature, prothalli of ferns, lycopods, horsetails, etc., are rare, in *in vitro* cultures, on no more than a mineral salt agar medium, with exposure to uniform illumination each day, growth of prothalli can be maintained and studied for month upon month (*e.g.*, Steeves *et al.*, 1955; Hurel-Py, 1950, 1951). The authors utilize these culture techniques in setting up their problems. Thus spores of *Todea barbara* (L.) Moore will grow into prothalli with mature sex organs in about 4–6 weeks. Fertilization occurs regularly in the water of condensation, when the temperature in the culture room drops a few degrees at night, and embryos develop. Each embryo can be carefully dissected out and planted on nutrient agar when its first leaves reach a few centimeters in height. Experience indicates some sugar added to the medium is advantageous—perhaps 0·5% sucrose—at least until the first transfer. Embryonic fern plants can thereby be obtained as separate plants. Or, the early leaves can be excised from the embryos and planted on the agar surfaces of slanted culture medium—with no more than 0·1% sucrose in the medium—for 2–3 months for aposporic growth of prothalli from the leaf margins or sides of petioles (Plate I, Figs. 1, 2). These new prothalli, diploid like the embryonic leaves from which they are derived, again soon produce embryos by fertilization, clearly tetraploid. Repetition of the process has enabled us to have tetraploid prothalli which give octoploid embryos upon fertilization. Thus, in about a year of culturing stocks of prothalli from spores, we have cultures of prothalli of *Todea barbara* of 1 N, 2 N and 4 N chromosome numbers and of young sporophytic fern plants of 2 N, 4 N and 8 N numbers. It is clear that the procedure can be extended until the problem will unquestionably arise concerning size of nucleus, with an ever-increasing deoxyribonucleic acid (DNA) content. No effort has yet been made to resolve this problem. In its present form, the polyploid sequence of prothallus and sporophyte, in entirety or in part, has been successfully produced in other ferns, e.g. *Onoclea sensibilis* L., *Osmunda cinnamomea* L., *O. regalis* L., *Athyrium filix-femina* (L.) Roth., *Pteridium aquilinum* (L.) Kuhn, var. *latiusculum* (Desv.) Underwood ex Heller, and *Phlebodium aureum* J. Smith.

Examination of any such series has led to consistent conclusions. Prothalli of 1 N, 2 N and 4 N are inseparable phenotypically. Chromosome counts (Torrey *et al.*, 1962). or presumably DNA determinations, are necessary to distinguish one from the other. The same similarities exist among the young 2 N, 4 N, and 8 N young plants and therefore

the same procedures must be used for their determination. The 2 N prothallus is and remains consistently a prothallus whereas the 2 N sporophytic plant, conceived by fertilizatien and initiated in archegonia on nourishment provided by the prothallus, continues in sporophytic pattern. In the past three years hundreds of fern plants in the polyploid series of prothalli and sporophytic plants of *Todea* have been grown in adequate quantity for biochemical analyses. No inconsistent variation has disturbed our progress. No obvious mutations have appeared. Genetic stability in this species and in *Onoclea sensibilis* seems secure, as cytological uniformity during reduction divisions would support. A study is not yet completed of cytological variation in the polyploid series of prothalli or of sporophytic plants in *Todea*. This will be informative but is not necessary for the recognition that there is no gross measurable phenotypie ratio of difference in the prothalli of different chromosome levels or of embryonic plants of the polyploid series. Clearly differences in the two generations exist, persisting differences. When do these differences originate in the development of the two generations and is there an interpretation available for their origin?

Lang (1909) has invoked environmental influences on the respective generative cells, the spore and the fertilized egg, to account for the early pattern of sporeling and embryo development. The germinating free spore, growing without restraint, produces a filament which ordinarily develops into a heart-shaped, sex organ-bearing prothallus. By contrast, the fertilized egg or zygote was not the exposed free cell exemplified by the spore but was enclosed in the venter of an archegonium which in turn always developed in the cushion part of the prothallus. Inherent in its position within this organ of incubation is the potentiality of restraint upon the embryo envisioned by Lang. This shrewd suggestion seems justified but to what degree is not at all clear. Ward and Wetmore (1954) have shown that the initial obvious aftermath of fertilization in *Phlebodium aureum* seems to be observed in the venter jacket, cells there being stimulated to cell division in periclinal planes. Within the 5–6 days between fertilization and the first mitosis of the zygote, jacket cells often divided more than once. The resulting calyptra quickly became 2 or 3 cells thiek around the egg. If one assumes a normal turgor pressure of prothallial cells—as yet we know nothing of its absolute dimensions—it is clear that the egg must develop against a kind of turgor strait-jacket of surrounding cells. To what end? Is the effect, if there be an effect, on the orientation of the mitotic spindle at the time of mitosis? Are the spindle fibres of long chain molecules such as would take a determined position in this environment? So far as the writers know, this information is not yet available. Nor have the authors found a method foi checking it. At least three attempts have been made to determine the existence and, if possible, the effect of archegonial constraint upon the development of the contained embryo. All point to the presence of pressure but how this acts upon the physiology of development is as yet only a matter of conjecture.

Ward and Wetmore (1954) reported that surgical removal of portions of the calyptra resulted in a less vigorous pattern of cell divisions in the embryo. In certain experiments, a protocorm-like structure commonly resulted which gave rise to numerous leaf-like structures, the earliest having no veins, that is, no vascular tissue. No root was formed. But eventually the unorthodox shoot system gave rise to stems with leaves.

De Maggio and Wetmore (1961) discovered that embryos of *Todea barbara* could be removed from their archegonia by careful manipulation and grown *in vitro*. Recognizable *Todea* plants resulted but the uniform pattern of cell division characteristic of the embryology of this plant did not follow, and development was correspondingly retarded. The younger the stage at which the embryo was excised, the more the early pattern deviated and the more the development was slowed down. It is interesting and probably significant that one-celled fertilized eggs became prothalloid embryos and none has attained the pattern, form or potentiality for continual growth which characterizes the excised later stages.

Jayasekera and Bell (1959) found that careful removal of the archegonial neck of *Thelypteris palustris* resulted in a gaping slit into the venter so that pressure on the embryo was lessened. Thereby the pattern of development of the embryos was modified and differentiation delayed.

In most of these cases, while the embryo eventually produced its own species of fern embryonic plant, it did so by a distinctly unorthodox sequence of cell divisions. Moreover, as pointed out, it achieved development only after a longer period of time. The authors suggest that this lack of uniformity in pattern and in time outside of the usual archegoniate site of development of the embryo is significant in itself. May it not be that ordinary embryological pattern reflects the orderliness which environment, both physical and nutritional, exerts upon sequential cell divisions of the embryo? The embryo, excised from the organ in which it ordinarily develops, does not follow the same orderly pattern. It is certainly less economical of time and energy than in the streamlined routine which results from the evolutionary setting of the archegonial stage for fertilization and development of the embryonic fern sporophytic plant.

Growth of Prothalli

No serious consideration can be given to the body of literature on fern prothalli without a realization that physical environmental factors such as light, gravity, and contact with the substrate exert formative as well as nutritive influences on the germination, shape, size, development of sex organs, production of embryos, and life-span of the prothallial phase of the fern. The same consideration must add that, even for these simple-appearing plants, there is no simple interpretation of the separate and/or collective roles played by morphogenetic factors in the environment. Rather vigorous and refined experi-

mentation must be invoked as is evidenced by the elegant studies of Mohr and his co-workers (1962; Mohr and Barth, 1962; Mohr and Ohlenroth, 1962). In these investigations, light at the blue end of the spectrum shorter than 500 mμ is necessary to change the growth pattern of a prothallial filament of *Dryopteris filix-mas* to a heart-shaped two-dimensional plant. This morphogenetic blue light does not produce its effect through immediate photosynthetic products. Red light can provide the latter but will not induce the pattern change. Filaments remain as filaments. It is interesting as Mohr (1955) had earlier pointed out that germination of spores of this fern only occurred after exposure to red light of a maximum of 650–670 mμ. This phenomenon is now known to be a part of the phytochrome red-far-red light reacting system.

Subsequent growth of prothalli has not been subjected to the same discriminating investigations as have the younger stages. However, in the process of watching the growth of prothalli of many species of ferns over recent years in our laboratories, it has become clear that single mature prothalli or prothallial colonies grown in sterile culture are very subject to formative influences. Thus prothalli of *Onoclea* (Plate I, Fig. 4), *Osmunda*, or *Todea* (Plate I. Fig. 5) planted erect on the usual Knudson's mineral salt-agar medium with 1% sucrose; and grown in white light for 12 hours per day appear after some months as tall, almost cylindrical, upright, sturdy growths. Moreover, from many and varied experiments, all such prothalli have been demonstrated to possess vascular tissue in a more or less complete strand or sometimes in interrupted short strands throughout the length (Plate II, Figs. 8, 9). Available evidence (De Maggio, Wetmore and Morel, 1963) indicates that so long as sugar and auxin are of an adequately high concentration, whether native or supplied in the medium, the vascular tissue will be present. Characteristically this tissue is comprised of xylem only (Plate II, Figs. 7, 10). The special techniques necessary for recognizing phloem have not yet been invoked.

By contrast, if prothalli are planted on the same medium, whether prostrate or erect, without sucrose or with only 0·25% sucrose and grown under similar conditions, they continuously remain thin, membranous and without vascular tissue. If the medium contains 1% or more of sucrose—up to 6% has been employed—the cushion of the prothallus thickens, vascular tissue develops, and the membranous margin gets narrower progressively as the thickening process involves more and more of it (Plate I, Fig. 3). Contrarily, if more or less cylindrical prothalli are transferred to a medium with little or no sugar, membranous margins appear and increase with time (Plate I, Fig. 6). Any of these prothalli may proliferate and colonies may result under these conditions as elsewhere in culture. This colony formation is much more unusual *in vivo* than *in vitro*.

Many predictable variations of these extremes have been grown. It seems clear that prothalli respond promptly to environmental and

nutritional stimuli. Efforts are now underway to resolve some of the difficulties. A special chamber has been constructed which hopefully will permit a preliminary analysis of variables involved. Clearly position of growth is a variable. Bell (1958) has suggested that the prostrate prothallus has more uncutinized surface in contact with the medium and thus permits more loss to the medium of diffusible and possibly significant substances. This suggestion needs further study. The definite effect of adding sugar to the medium is a variable which can now be employed with culture techniques; this was not possible for early workers who grew prothalli on soil, sphagnum or flower pots. Presumably it adds expendible material to that available from photosynthesis and can be and is utilized in part or in entirety as metabolic substrate for energy and/or synthesis of cellular components. Whatever the answers may be, it seems a reasonable inference and perhaps a permissible working hypothesis to assume that specific genes in the prothallus may, under similar conditions, induce the same chain reactions with the production of the same enzyme systems and inhibitors as do the corresponding genes in the fern plant. The assumption does not seem unwarranted in view of the very similar response in its production of vascular tissue to what we begin to believe is the case in sporophytic plants. In fact, the experimental production of vascular tissue in prothalli resulted from a deliberate attempt to produce it by utilizing the techniques that had proved successful with callus tissue.

Apogamy.—Thus far in our studies, it has not proved possible to obtain apogamous plants from prothalli of *Todea*. All young plants have developed as products of fertilization. Every embryo examined was housed in the venter of an archegonium and was connected to prothallial tissue by the nutrition-providing foot. It is generally recognized that neither of these conditions is present in the so-called apogamous embryos, whether natural or induced.

Recent investigations have supplied information pertinent to this report. The background against which this recent work has been initiated lies in Farlow's early discovery (1874) of natural apogamy in *Pteris cretica*, and in Lang's (1898) induction of apogamy in eight species of ferns by the simple and still effective expedient of withholding surface-watering. Thereby the natural opening of antheridia and archegonia was made possible, with subsequent fertilization. Lang also commented upon the necessity of growing prothalli under strong illumination as well to induce apogamous embryos. Characteristically these plants developed from cylindrical projections near the apical notch, projections which bore archegonia, and within which were found vascular elements. In a number of cases he obtained separate organs—roots, leaves, or even sporangia—directly from the cushion or even from the margins of the prothalli. Not infrequently he found a normal sexually produced embryo on the same prothallus with apogamous embryos.

Excellent reviews of the considerable body of literature of fascinating observations concerned with apogamy are available (Steil,

1939, 1951; Manton, 1950). Manton, in her summary, concludes (p. 185, 1950) "that, at least as regards the ferns, hybridization is.... a cause and not merely the occasion for the manifestation of apogamy. whatever the physiological mechanism may be". Hybridization may be the background for correlation of the cytological instability with sterility and the stimulation of alternative methods of reproduction, but the investigation of the "physiological mechanism" involved has opened a door to alternative and simpler methods of inducing apogamy.

Whittier and Steeves (1960, 1962) and Whittier (1962) have brought the subject of apogamy to a focus by careful studies based upon Lang's (1898) original observations. If bottom watered only, and grown on nutrient medium supplemented by sugar instead of illuminated by intense light, prothalli of *Pteridium aquilinum* var. *latiusculum* produced apogamous plants, the number being directly proportional to the concentration of sugar within the effective range, that is, before osmotic balance of medium and cells was disturbed. With glucose, from 0·8 to 2·5%, a continuing and striking increase in apogamous embryos was shown. Sucrose, fructose and maltose proved to be as efficacious as glucose Whittier's original induction of apogamy in the bracken fern has since been extended to nine other species of ferns by similar techniques.

Whittier (1962), after careful investigation of the histological details concerned with the physiological changes of induced apogamy, reports that under extra nutrition with sugar, as probably with the intense illumination of Lang (1898) and Duncan (1941, 1943), certain changes have taken place. He has noted first the thickening of the cushion and its extension towards the lateral margins until almost no margins may remain. Centres of mitosis seem activated well within the depth of the cushion though it is not clear whether the initiation is in a single cell or a group of cells. The activity progressively involves more and more of the surrounding cells until surfaces are reached. Then only are apical meristems produced, both shoot and root, if the activated nest of cells has reached both upper and lower surfaces of the prothallus. If the zone of activation is small and near the upper surface, an organ only may be produced; a leaf is very common. Like Steward (1958), Whittier (1962) interprets the behaviour as indicative of gradients of formative substances set up in tissue centres, gradients which are important in the differentiation of tissues but become effective in the production of meristems only upon reaching the surface. He infers that factors "which initiate cell division must be different from those which produce apical organization" (p. 19). His interpretation is that a high level of energy-providing substrate, whether photosynthetic in origin or provided as sugar directly, enables the prothallus to shift from the genetically determined prothallial pattern to the production of equally genetically determined sporophyte pattern.

Apospory.—While apospory may occur in Nature, it is probably rare. As pointed out earlier, apospory can originate from the margins of young leaves and from petioles of such leaves when they are placed

on a wet surface and kept sterile for some time. In our experience, leaves from young fern plants, placed on the surface of an agar-mineral salt medium with no or very little sucrose, *e.g.*, 0·1%, in two or three months will develop filaments from marginal or surface cells of blade or petiole. These filaments prove to be prothalloid and, if illuminated, will become heart-shaped and bear sex organs and rhizoids as usual. The interesting situation, apparent to anyone who has tried to develop aposporous growths on ferns, is that during the two or three months of waiting the small leaves look progressively more and more moribund. At about the time when one tends to discard the culture as being dead, small protuberances, soon filamentous, develop from the leaf margins or petioles (Plate I, Fig. 1). These grow rapidly and become colonial (Plate I, Fig. 2). They prove to have the same chromosome numbers as the leaf from which they were derived. The obvious query is "Why is proliferation so delayed?" Ito (1960) has shown that if he pricks, with a very fine glass needle, many cells of a young prothallus, he can get active cell proliferation from the isolated unpricked cells. Pending other information, present knowledge suggests that when enough cells are dead, for any reason, integration within the organ fails and the cells still alive then behave as individual cells and not as part of a whole. Dependent upon available nutrition, the cells proliferate as individual cells and give rise to filaments. The opinion of the authors, as yet untested, is that similar experiments to those of Ito on prothalli with young leaves may give comparable results. A corresponding explanation for natural death of numerous cells of young fern leaves may well permit eventual cellular expression of the remaining living cells. Such free cells give rise to prothalli always, early as filaments, later as heart-shaped structures (Plate I, Figs. 1, 2; Bell and Richards, 1958, Fig. 1). In the hundreds of aposporous prothalli produced we have never obtained a leaf from a leaf. It is of interest that after two months or more of waiting for aposporous growths, the appearance of the first filament is usually an indication that others will occur promptly from the same leaf. One should recall that whereas apogamous entities have their origin from cells within the tissues, aposporous growths consistently originate from marginal or superficial cells.

General Discussion

Alternation of generations in Nature.—The alternation of generations in ferns, as in the other vascular cryptogams, is ordinarily considered a reality in Nature. It is so treated in our text-books and in much of our teaching. In consequence, many botanists fail to realize how difficult and often impossible it is to find prothalli of ferns, lycopods, and horsetails in nature. Consider an ordinary fern. A single plant may reasonably produce some millions of spores per season. If even one per cent of these spores were to germinate and grow, from the 9,000 or so known species there would be little space on earth for the potentially very numerous prothalli of the other vascular cryptogams, club mosses and horsetails, to say nothing of their sporophytes and those of gymnospermous and angiospermous plants. Actually, for ferns, at least,

Mohr's (1956) studies show necessity for red light for germination of those spores tried, and (Mohr, 1962) necessity for blue light below 500 mμ for the change of the early filamentous prothalli to the heart-shaped form of the later mature stage with its sex organs. In Nature, therefore, only those spores would germinate which could get enough red light in the forest shade. Presumably these might not be many since blue light has a better chance to penetrate than the longer, more easily filtered rays of the remainder of the spectrum. Of those spores which did germinate, the ordinary uncutinized prothalli would certainly be desiccated by very little exposure to direct sunlight. The senior author saw this desiccating effect upon a large colony of fern prothalli, species unknown, by removal of a few shading trees in a wooded area in New England. The colony was wiped out within a fraction of an hour after exposure to the sun. Whether the greater percentage of spores germinate and later succumb before producing offspring or whether the majority never germinate can only be conjectured. The suggestions made here for ferns seem equally valid for lycopods and horsetails according to the studies of Freeberg and Wetmore (1957) and Walker (1921, 1931). That the alternation of generations for these three groups of homosporous vascular cryptogams appears to play a minor part in their maintenance as persisting parts of the present flora of the world in no way obviates the probability that it may have been of significant evolutionary importance for their survival under climatic or seasonal conditions different from those of the present. Certainly today these groups, being characteristically rhizomatous plants, depend more upon vegetative than upon sexual reproduction. One might well question whether their survival in existing numbers of species would have been realized under natural selection, if the alternation of generations had been continuously or even periodically required.

Morphogenesis of the alternation of generations.—Whatever the significance of the alternation of generations has been or is in Nature, it has morphogenetic implications (Bell, 1959). If spore and egg are genetically like germ cells or initials, and evidence from studies on apospory, apogamy, and polyploidy bear this out, then the differences must have become imposed upon the respective initials early in their development, as Lang (1909) has suggested. Thereafter it certainly proves difficult, if not impossible, to change the prothallus to the sporophytic plant. The converse is equally true. But it is not difficult to cause the prothallus to produce sporophytic plants almost at will, either by fertilization or by apogamy, really alternative procedures. Neither is it difficult to effect the production of aposporous prothalli from the leaves of young sporophytic plants. Whether there are basic and fundamental physiological differences between the shade-tolerating prothalli and the sun-loving sporophytic fern plant remains to be seen.

It is clear from observations *in vivo* and from studies *in vitro* that growth in prothalli is slow and that growth of young fern plants separated from their prothallial parents is much faster. So far as the writers know, dry weight comparative studies during fixed periods of time have

not been made. Subjective visual findings are so obvious to those who have grown the two stages that the current query is always, "Why?". A partial answer suggests that light is limiting to the prothallus. When cultures of prothalli are grown side by side with cultures of young fern plants under light conditions favourable to the fern plants, there is a gradual change in the nature of the prothallus, both physically and potentially. Lang (1898) had noted that the potential for induced apogamy was expressed only when prothalli were grown under more intense light than was ordinarily available to them in native habitats. Others have confirmed this finding. With the adoption of culture techniques, it has become apparent that the physical changes resulting from illumination of prothalli by more intense light, undoubtedly with increased photosynthetic output, can be duplicated by adding 1% or more of sucrose—or another sugar—to the medium. The prothalli lose their tendency to extend two-dimensionally, the blue-light response of Mohr (1962; Mohr and Barth, 1962; Mohr and Ohlenroth, 1962), and instead thicken until they become essentially cylindrical in cross-section (Plate I, Figs. 4, 5) including what was earlier the membranous margins. Also, they develop vascular tissues in a continuous or interrupted strand of scalariform or reticulate-scalariform tracheids (Figs. 8, 9). The situation is much as described for *Psilotum* prothalli by Holloway (1938, 1939) and Bierhorst (1953). In other words, one can believe that the shape and degree of organization of the prothallus is limited by light and its photosynthetic products, certainly those necessary for energy and probably those necessary for the materials required in intermediate metabolism and differentiation of the sporophytic fern plant. Even with the addition of sugar up to 8%, the prothallus is still a prothallus. Evidence again supports the thesis that a prothallus once initiated tends to continue as a prothallus.

It would appear that the determination of what might be called the prothallial or the sporophytic pattern is not a commitment because of hereditary or DNA differences in spore and zygote. Accumulating evidence indicates that the milieu in which spore and zygote develop may have a profound effect on their early pattern. The unenclosed spore grows into a filament if and when exposed to formative blue light which somehow influences the planes of its cell divisions so that it gives rise to a two-dimensional prothallus with its rhizoids and sex organs. By contrast the fertilized egg is enclosed within its jacket of turgid archegonial cells. These cells collectively may well exert a determining influence on planes of cell division of the zygote, divisions which follow a consistently regular pattern. This enclosed embryo becomes multi-dimensional, adequately massive to acquire its own gradients. Gradients produce differential distributions in cell masses with the evident effects of such differentials upon inductions and inhibitions. At least these entities early acquire a leaf, a stem and a root, each with its apical meristem and capacity to extend itself.

In tobacco callus, Skoog and Miller (1957, and later) have shown that multi-dimensional entities, chemically provoked from local centres

of cell division, become buds which develop leaves, axis and roots—normal young plants. Whittier (1962) induces that apogamous fern embryos also originate as multi-dimensional entities of cells in the cushion part of a prothallus. These entities acquire apical meristems, vascular tissues, leaves and roots and also become young plants.

Morphogenetically, it seems reasonable to consider that, at least in the Vascular Plants, this sporophytic pattern of development is a multi-dimensional pattern, and that the formation of a young plant involves a sequence of biochemically motivated changes which culminate in the appearance of localized morphological aggregations of cells, recognizable by familiar fundamental patterns of organization. Among these early entities are the shoot and root apical meristems with their initial appendages. From existing studies, it is not clear that there is a definite orderliness in occurrence of root before shoot or the converse. Nor it is clear that the occurrence of these apical meristems always precedes the initiation of vascular tissues. In substance, it appears to be a fair conclusion that outside of the regularized early pattern of embryology, developmental sequences are not rigidly sequential in their biochemical motivation. Morphologically and physiologically, single cells or small cell groups, which can give rise to cell aggregates by regularized pattern or by statistically haphazard cell division, all produce sporophytic or multi-dimensional types of plant. Deviations in the pattern of achieving this end may morphogenetically be considered as incidental and inconsequential. Whenever cell development is free on a surface which supplies moisture and nutrients, development is mainly two-dimensional and the prothallus-like plant is formed. Once initiated, neither of these two types is easily changed; either can be varied or may even assume characters of the other type, or may give origin to plants of the other type. But each tends to remain in its own pattern. In mosses, in other vascular cryptogams, and in seed plants, recent and current investigations seem to be pointing in similar directions. For the miscellany of organisms with a cellular or thallus plant body, the resources for morphogenetic investigation have hardly been opened. Are we not justfied in considering the alternation of generations as primarily a morphiogenetic problem?

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EXPLANATION OF PLATES I & II

PLATE I, FIGS. 1–6

FIG. 1. Stages in the development of aposporous prothalli from portions of excised young leaves and petiole fragments of *Todea*, ×2·6.

FIG. 2. Colonies of prothalli originating aposporously from detached petioles of young leaves of *Todea*; note that petioles are still visible, ×2·3.

FIG. 3. Single prothallus of *Onoclea* originally planted erect on a medium with 0·5% sucrose and transferred to a medium with 1·0% sucrose. Note that the increased thickening of the cushion is gradually eliminating the membranous margins, ×2·0.

FIG. 4. Single prothallus of *Onoclea* (6 cm. in length) grown erect on a medium with 1·0% sucrose and later transferred to a medium with 0·5% sucrose. The terminal, forking portion of the prothallus has become thinner and more membranous, ×1·3.

FIG. 5. Single prothallus of *Todea* grown erect on a medium with 1·0% sucrose for more than 1 year. Membranous prothallial margins are almost completely lacking due to the expansion and thickening of the cushion, ×1·7.

FIG. 6. Single prothallus of *Onoclea* grown erect on a medium with 1·0% sucrose for some months and then transferred to a medium with 0·25% sucrose. The membranous new growth is in sharp contrast to the thickened, cylindrical growth obtained with 1·0% sucrose, ×1·7.

PLATE II, FIGS. 7–10

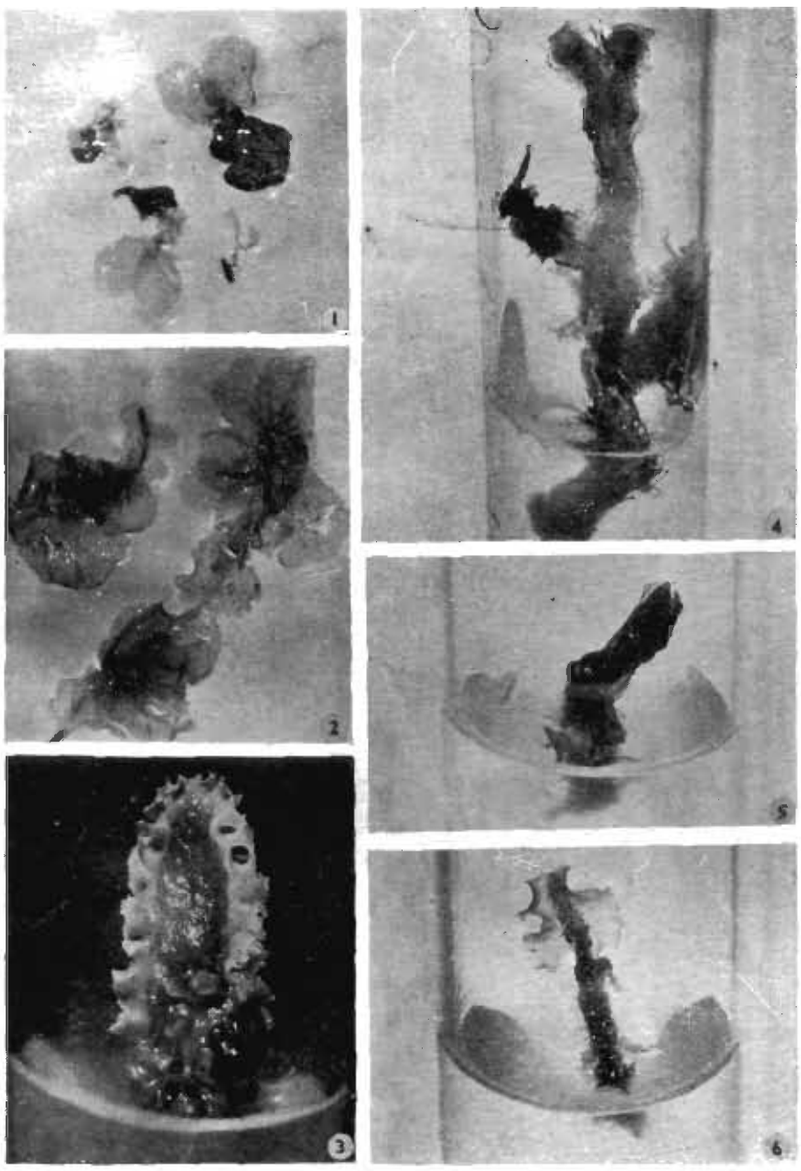
FIGS. 7–10. Vascular tissue induced in prothalli of *Todea* by supplying 5·0% sucrose and 10·0 mg/l. NAA

FIG. 7. Section of a prothallus with a compact "nest" of tracheids to the right with a branch extending to the left, ×93.

FIG. 8. Elongated tracheids continuous for some distance through the cushion of the prothallus, ×362.

FIG. 9. Isolated group of tracheids showing scalariform or scalariform-reticulate thickenings on their walls, ×258.

FIG. 10. Group of tracheids in c.s., showing thickened lignified secondary walls, ×603.



FIGS. 1–6

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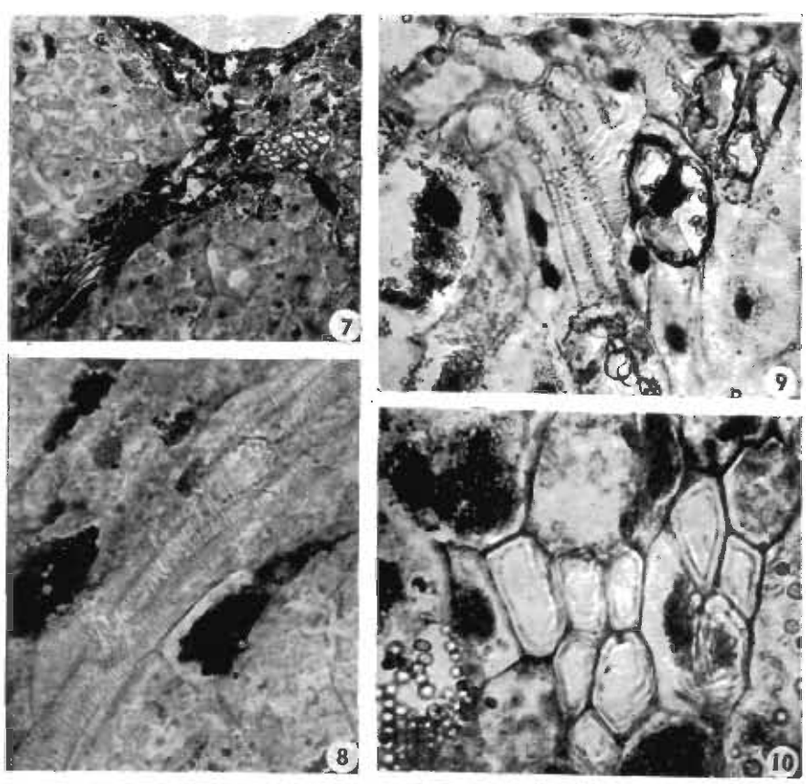

FIGS. 7–10

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THE *POGOSTEMONEAE*—A DEBATABLE GROUP OF *LABIATAE* (SOME REMARKS ON THEIR TAXONOMIC POSITION WITH REGARD TO THEIR POLLEN GRAINS)

By Rosalie Wunderlich

Vienna, Austria

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Introduction

The *Pogostemoneae* sensu Briquet (1897) are a debatable group of *Labiatae*. A taxonomical comparison of the systems from Bentham (1832–36) to Briquet (1897) shows this clearly:

Bentham (1832–36): *Labiatae*. II. *Menthoideae :*

Pogostemon, Dysophylla, Elsholtzia, Tetradenia, Colebrookia, etc.

Endlicher (1836–40): *Labiatae*. II. *Menthoideae :*

1. *Pogostemoneae : Pogostemon, Dysophylla.*
2. *Elsholtzieae : Elsholtzia, Tetradenia.*
3. *Mentheae : Colebrookia*, etc.

Bentham and Hooker (1876): *Labiatae*. II. *Satureineae :*

1. *Pogostemoneae : Pogostemon, Dysophylla, Colebrookia, Tetradenia.*
2. *Menthoideae : Elsholtzia, Keiskea*, etc.

Baillon (1892): *Labiatae*. III. Menthées:

..... *Elsholtzia, Comanthosphace, Keiskea.*

Sous-série Pogostemonées: *Pogostemon, Dysophylla, Colebrookia, Tetradenia.*

Briquet (1897): *Labiatae*. VI. *Stachyoideae*:

12. *Pogostemoneae : Elsholtzia, Comanthosphace, Keiskea, Pogostemon, Dysophylla, Tetradenia, Colebrookia.*

Of the seven genera (Briquet, 1897) Bentham (1832–36) quotes five (*see* above), all of which he integrates into his "*Menthoideae*".

Endlicher (1836–40), who classified Bentham's (1832–36) system more strictly, separates those five genera and arranges them in three different sub-groups of his "*Menthoideae*" (which, however, according to him, comprise more genera than in Bentham's system). He arranged *Pogostemon* and *Dysophylla* in a special sub-group, the "*Pogostemoneae*" and *Elsholtzia* and *Tetradenia* also in a special sub-group, the "*Elsholtzieae*". *Colebrookia* he counts among the sub-group "*Mentheae*".

Bentham and Hooker (1876) accepted Endlicher's (1836–40) classification only to a certain extent. They retained the group of "*Pogostemoneae*", but—in contrast to Endlicher (1832–36)—included the genera *Tetradenia* (according to Endlicher within "*Elsholtzieae*") and *Colebrookia* (according to Endlicher within "*Mentheae*"). The "*Elsholtzieae*" were not acknowledged as a separate group and the two genera were put into two different groups: *Elsholtzia* within their "*Menthoideae*" beside *Keiskea*; *Tetradenia*, however, as I mentioned above, within their "*Pogostemoneae*". Both groups—the *Pogostemoneae* and the *Menthoideae*—belong, according to Bentham and Hooker (1876), to their "*Satureineae*".

Baillon (1892) places *Elsholtzia*, *Keiskea* and the new genus *Comanthosphace* in the "*Menthées*". The *Pogostemoneae* (comprising the same genera as with Bentham and Hooker) he regards it to be a special "sous-série" of his "Menthées".

Briquet (1897) retains the tribus "*Pogostemoneae*", but he includes in it also the genus *Elsholtzia* (which since Endlicher had been felt to be different) and besides, *Keiskea* and *Comanthosphace*. Thus, he returns to Bentham's original concept. But he neither subordinates his "*Pogostemoneae*" to the "*Menthoideae*" (Endlicher, Baillon) nor to the "*Satureineae*" (Bentham and Hooker), but he ranges them as the last (twelfth) tribe within his extraordinarily comprehensive and much divided "*Stachyoideae*".

Briquet's classification has met with contradiction. According to F. Mayer (1909) "*Pogostemon* differs anatomically from other *Pogostemoneae* as *Tetradenia*, *Colebrookia* and *Elsholtzia*" (quoted from Erdtman, 1945).

Investigations of Pollen Grains

That the *Pogostemoneae* sensu Briquet are not a natural tribe is also confirmed by Leitner (1942). She stated that in some species the pollen grains, at the time of shedding, are binucleate, whilst in other species they are trinucleate. Leitner suggests that perhaps a comprehensive comparative investigation will make it necessary to divide the genera of *Pogostemoneae* into different sub-tribes, as the

two characteristic features (binucleate or trinucleate pollen grains) are elsewhere constant in larger taxonomical groups. This taxonomic importance of the number of nuclei in the pollen grains at the time of shedding has repeatedly been pointed out by Schnarf (1937, 1939) and by Maheshwari (1949, 1950).

Further, Erdtman (1945, 1952) stated tricolpate as well as hexacolpate pollen grains within *Pogostemoneae*. He was the first to point out "that in *Labiatae* tricolpate grains are binucleate, and hexacolpate grains trinucleate (which, by the way, possibly may be due to coupled genes)". According to Erdtman (1952), who following the above-mentioned statements divides the whole family of *Labiatae* into two large groups, the *Pogostemoneae* sensu Briquet (1897) belong partly to the first group (*i.e.*, "*Pogostemon* only," with binucleate tricolpate pollen grains), partly to the second group ("*Pogostemoneae*, except *Pogostemon*", with trinucleate hexacolpate pollen grains).

Leitner (1942), however, had stated binucleate pollen grains also in other genera beside *Pogostemon*, namely, in *Colebrookia oppositifolia*, *Keiskea japonica*, *Comanthosphace japonica* (in *C. stellipila*, though, trinucleate pollen grains!), and in two species of *Dysophylla* (*D. auricularia*, and *D. linearis*). Just in the last genus, however, (though in two other species: *D. crassicaulis* and *D. stellata*) Risch (1956) found hexacolpate pollen grains of the *Satureia*-type.

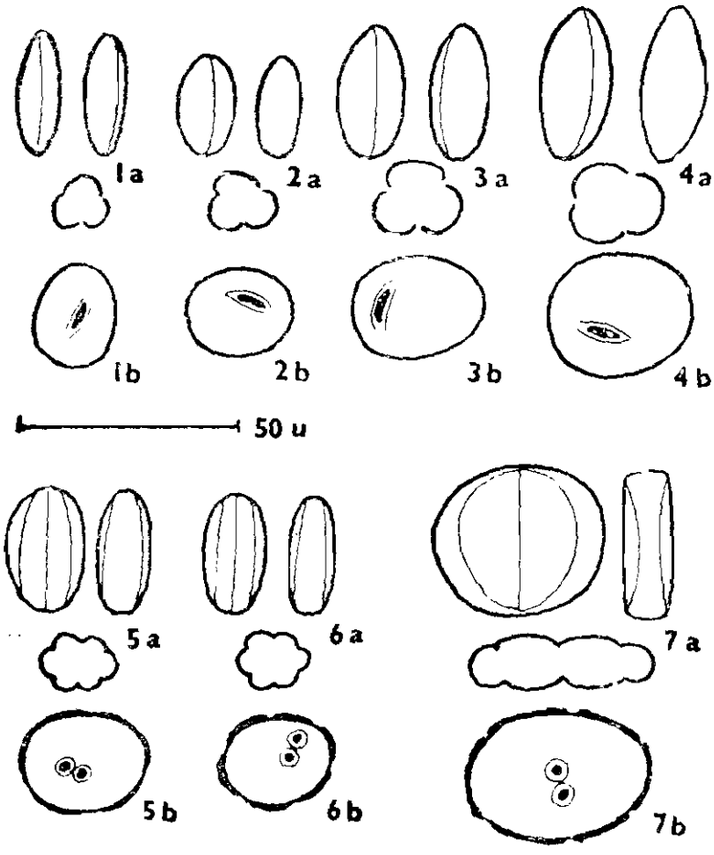
It appeared, therefore, to be necessary to investigate the pollen grains regarding the number of their colpi as well as the number of the nuclei (at the time of shedding) in as many species of *Pogostemoneae* as possible. Above all, it had to be ascertained if really also in this tribe the binucleate pollen grains are tricolpate, and the trinucleate hexacolpate, and further it was necessary to try to explain the contradictory statements of various authors.

I examined pollen grains taken from herbarium plants† using various methods. For colouring the nuclei I used carmine acetic acid (Leitner, 1942), for finding the number of colpi partly concentrated sulphuric acid, partly paraffin oil or thickened Canada balsam, sometimes various chemicals in the same species.

Again and again I am astonished to see how well preserved the cells of the pollen grains are in the herbarium plants, at least in most cases. Even around the nuclei the light zone is to be seen, which proves the presence of generative cell (all "b" in Text-Figs. 1–4) or sperma cells respectively. The latter always lie beside each other (all "b" in Text-Figs. 5–7). The vegetative nucleus is never to be seen, or sometimes only as

† On this occasion I should like to express my best thanks to Prof. L. Geitler, Head of the Botanical Institute of the University of Vienna, for kindly placing all facilities of the Institute at my disposal, to Prof. K. H. Rechinger, Head of the Naturhist. Museum in Vienna, for the material of the herbarium, and to Dr. Hilde Nietsch, Vienna, for various help.

a shadow, as it is the case in fresh pollen grains. The vegetative cell (which constitutes the whole contents of the pollen grains) swells up when treated with water, carmine acetic acid, etc., in the same way as that of the fresh pollen grain. The colpi open. The results are—according to which medium is used—differences in size and shape (*cf.* "a" and "b" in Text-Figs. 1–7). If the pollen grain swells up, the diameter



TEXT-FIGS. 1–7. Pollen grains of *Pogostemoneae*—tricolpate binucleate in Figs. 1-4, hexacolpate trinucleate in Figs. 5-7; mounted in canada balsam (in dry condition) all Figs. marked "a" (two equatorial views and the polar view) and in carmine acetic acid (colpi are not to be seen or only indistinctly) all Figs. marked "b". Figs. 1 a, b. *Colebrookia oppositifolia.* Figs. 2 a, b. *Dysophylla stellata.* Figs. 3 a, b. *Comanthosphace stellipila.* Figs. 4 a, b. *Rostrinucula dependens.* Figs. 5 a, b. *Elsholtzia communis.* Figs. 6 a, b. *Tetradenia goudotii.* Figs. 7 a, b. *Keiskea japonica.*

of the equator increases considerably. Repeatedly I observed that the vegetative cell as a whole "slips out" of the pollen grain, when treated with chemicals which cause swelling. Afterwards the shape of the empty pollen grain is different from before, as the pressure on the exine has stopped. This could be seen very well in *Keiskea*.

Table I gives a survey of the statements of various other authors and my own.

TABLE I

The pollen grains of Pogostemoneae

(Enumeration according to Briquet, 1897. The incorrect, or probably incorrect statements are enclosed within brackets [ ].)

Species	Number of nuclei. cells (*c*) respectively	Number of colpi
125. *Elsholtzia*		
argyi Lév.	3 *c*	6
bodinieri Vaniot = *heterophylla* Diels	3 *c*	6
ciliata	..	6 Erdtman (inform. by letter), Risch (1956)
cristata	..	6 Fischer (1890)
communis (Coll. *et* Hemsl.) Diels	3 *c*	6
fruticosa (D. Don) Rehd.	3 *c* Schnarf (1937)	6
patrini (Lepech) Garcke	3 *c* Leitner (1942)	6 Risch (1956)
stauntoni Benth.	3 *c* Leitner (1942)	6 Risch (1956)
Rostrinucula dependens (Rehd.) Kudo = "*Elsholtzia dependens* (Rehd.)"	2 *c*	3
	2 *c*	3
126. *Comanthosphace*		
japonica (Miq.) S. Moore	2 *c* Leitner (1942) Wunderlich	3
ningpöensis (Hemsl.) Hand. Mazz.	2 *c*	3
stellipila S. Moore	[3 *c* Leitner (1942)] 2 *c* Wunderlich	3
127. *Keiskea japonica* Miq..	[2 *c* Leitner (1942)] 3 *c* Wunderlich	6

TABLE I—(*Contd.*)

Species	Number of nuclei, cells (*c*) respectively	Number of colpi
128. *Pogostemon*		
auricularia	..	3 Erdtman (1952)
glaber Benth.	2 *c*	3
mollis Benth.	..	3
parviflorus Benth.	2 *c*	3
patchouli Pell.	2 *c* Leitner (1942)	..
plectranthoides Desf.	2 *c*	3
speciosus Benth.	2 *c* Leitner (1942)	..
129. *Dysophylla*		
auricularia (L.) Blume	2 *c* Leitner (1942)	3
crassicaulis	..	[6 Risch (1956)]
cruciata Benth.	2 *c*	3
gracilis Dalz.	2 *c*	3
linearis Benth.	2 Leitner (1942)	3
stellata Benth.	2 *c*	3 [6 Risch (1956)]
verticillata Benth.	2 *c*	3
130. *Tetradenia*		
fruticosa Benth.	3 *c* Leitner (1942)	6
goudotii Briq...	3 *c*	6
131. *Colebrookia*		
oppositifolia (Poir.) Sm.	2 *c* Leitner (1942) Wunderlich	3 Erdtman (inform. by letter), Wunderlich

This survey shows that in the species of *this* tribe also the tricolpate pollen grain at the time of shedding is always binucleate, the hexacolpate pollen grain trinucleate. These two coupled features are constant in every natural genus of *Labiatae*. It is most interesting that Kudo (1929) has separated "*Elsholtzia dependens* Rehd." as a special genus "*Rostrinucula dependens* (Rehd.) Kudo" from *Elsholtzia* for other reasons. This separation, therefore, is also with regard to the mature pollen grain (binucleate, tricolpate) perfectly justified. Material which was taken from the herbarium of the Naturhist. Museum of Vienna, and which was still labelled as "*Elsholtzia dependens* Rehd." agreed, regarding the pollen grain, with *Rostrinucula dependens*, but not with *Elsholtzia* (*cf.* Text-Figs. 4 a, 5 b with 5 a, 5 b).

Leitner's (1942) statements that the mature pollen grain in *Comanthosphace stellipila* was trinucleate, in *Keiskea japonica*, however, bi-

nucleate, are certainly wrong, as follows from the reinvestigation of the same material (*cf.* Text-Figs. 3 a, 3 b; 7 a, 7 b). Perhaps she has exchanged them by mistake, or it is a misprint.

It will perhaps be possible to clear up in a similar way Risch's (1956) statement that *Dysophylla crassicaulis* has hexacolpate pollen grains (whereas all other investigated species of *Dysophylla* are tricolpate). For *D. stellata* (Text-Fig. 2 a) I was able to correct his statement. This species possesses a very clear binuclear tricolpate pollen grain. I am sorry that for lack of material it was impossible for me to investigate also *D. crassicaulis*.

Systematic Considerations

From the present results it may be concluded with certainty that the genera of *Pogostemoneae* sensu Briquet (1897) split into two separate groups. To the first group (with binucleate, tricolpate pollen grains) belong not alone "*Pogostemon* only", as Erdtman believes, but besides also *Rostrinucula*, *Comanthosphace*, *Dysophylla*, and *Colebrookia* (Text-Figs. 1–4); to the second group (with trinucleate, hexacolpate pollen grains) do not belong "*Pogostemoneae*, except *Pogostemon*" (Erdtman, 1945, 1952), but only *Elsholtzia*, *Keiskea*, and *Tetradenia* (Text-Figs. 5–7).

If we now compare these two groups with the views of the taxonomists before Briquet (1897), we are struck by a surprising agreement. Since Endlicher (till Baillon) *Pogostemon* and *Dysophylla* were always placed beside each other, but separated from *Elsholtzia*. Also *Keiskea* (a new genus since 1874) was placed beside *Elsholtzia* by Bentham and Hooker (1876), who were followed by Baillon, which regarding the pollen grains is more correct than the position between *Comanthosphace* and *Pogostemon* (Briquet, 1897).

Some uncertainty is shown only as to the taxonomic position of the three other genera (*see* Introduction). So *Tetradenia* was first placed beside *Elsholtzia* (Endlicher, 1836–40), which is correct regarding their pollen grains; by Bentham and Hooker (1876) and Baillon (1892), however, it was placed within their "*Pogostemoneae*".

Colebrookia, which had been placed by Endlicher quite apart from the other genera, was placed by Bentham and Hooker, and also Baillon, within their "*Pogostemoneae*", which is in agreement with the pollen grains.

Comanthosphace (a new genus since 1877) was placed by Baillon (1892) between *Elsholtzia* and *Keiskea*, which regarding the pollen grain is incorrect.

On the whole it is evident that the investigations of the pollen grains confirm—in some respects—the old classifications before Briquet.

Of course, there arises another question: Where are these two groups of *Pogostemoneae* sensu Briquet—*after* their separation—to be

placed correctly within the family of *Labiatae*? Erdtman (1945, 1952) was the first to point out in his survey of the taxonomy of *Labiatae* that the differences between the two groups are greater than the old taxonomists before Briquet supposed, who simply placed the two groups (the one with *Pogostemon*, the other with *Elsholtzia*) beside each other, though separately.

It is to be regretted that so little is known about the embryology of these two groups of *Pogostemoneae*. But also from the few investigations made about the seed development in *Elsholtzia cristata* and *Pogostemon Patchouli* (Junell, 1937) it may be concluded that the difference at least between those two genera is considerable. As to the seed development of *Elsholtzia cristata* the big chalazal haustorium is a characteristic feature, which has only rarely been found within *Labiatae*, namely, in *Lallemantia iberica*, *L. peltata* (Créte, 1942), *Horminum pyrenaicum* (Junell, 1937) and *Lavandula multifida* (Junell, 1937). All these genera mentioned have trinucleate hexacolpate pollen grains like *Elsholtzia*; besides, according to Risch (1956), the shape of the pollen grain of *Lallemantia* belongs to the same type ("*Satureia*-Formtypus" as *Elsholtzia*. If Risch considers also the pollen grains of *Horminum* and *Lavandula* to represent special types of shape ("*Horminum*-" and "*Lavandula*-Formtypus"), I think he goes too far; for the differences between the types in question are only very slight. Erdtman (1945) also thinks that in Risch's work "the importance of certain minor pollen-morphological features seems to be overemphasized".

My own observations show that the pollen grains of *Elsholtzia* and *Tetradenia*, also regarding the shape, agree exactly (Text-Figs. 5, 6). The pollen grain of *Keiskea* (Text-Fig. 7), however, differs in its shape strikingly from the other two genera, although all three of them are trinucleate and hexacolpate. But *Keiskea* agrees very well, on the other hand, with Risch's (1956) *Coleus*- and *Plectranthus*-type, and, according to Leitner (1942), *Coleus* and *Plectranthus* (both in the subfamily of *Ocimoideae*) are also trinucleate. In this connection Briquet's statement gains special importance; he says that the *Pogostemoneae* are a "Übergangsgruppe zwischen den *Stachyoideae* and *Ocimoideae*" (Briquet, 1897, p. 326) and that the latter can "nur schwer gegen die *Stachyoideae-Pogostemoneae* begrenzt werden" (Briquet, p. 206). Perhaps future investigations will show that *Keiskea* after all belongs to the *Ocimoideae*.

In contrast to *Elsholtzia cristata* the chalazal haustorium of *Pogostemon patchouli* (Junell, 1937) is only small. The micropylar haustorium, however, which consists of two (or occasionally more, up to eight) cells, is developed most conspicuously. Though, according to Junell it does "not reach a higher development", his Fig. 6 f shows a well-developed micropylar haustorium of a very characteristic shape. Up to now a micropylar haustorium of a similar shape was found only in *Anisomeles indica* (Murthy, 1946; Ganguly, 1948), *A. malabarica* (Murthy, 1946), *Prostanthera lasianthos* and *Westringia Dampieri* (Junell, 1934). It is most remarkable that in these genera the pollen grains

are binucleate and tricolpate as in *Pogostemon*. As to *Anisomeles indica*, *A. malabarica*, *A. ovata* and *Westringia rosmariniformis* I could observe this myself, as to *Prostanthera* Leitner found binucleate pollen grains in *P. marifolia*, Erdtman (information by letter) found tricolpate pollen grains in *Prostranthera cuneata*, *P. denticulata*, *P. nivea*, *P. ovalifolia* and *Westringia glabra*. Risch (1956) in six species of *Prostanthera* and two species of *Westringia*.

Crété (1942) supposes that the seed development of *Pogostemon patchouli*—according to Junell's (1937) investigations—shows primitive features, which is also my opinion.

Whereas the *Elsholtzia*-group might retain its place after the *Satureieae* (*Keiskea* perhaps even in the subfamily of *Ocimoideae*), the *Pogostemon*-group ought to be placed more at the beginning of *Labiatae*, perhaps near the *Prostantheroideae*.

But in order to clear up the taxonomic position of the *Pogostemon*-group, further thorough investigations will be necessary especially of seed development, and not only within the *Pogostemon*-group, but also in those groups which are supposed to be related to it.

SUMMARY

The *Pogostemoneae* sensu Briquet (1897) should be split into two distinctly separate groups; the first (*Pogostemon*-group) with binucleate, tricolpate pollen grains (*Rostrinucula*, *Comanthosphace*, *Pogostemon*, *Dysophylla*, *Colebrookia*), the second (*Elsholtzia*-group) with trinucleate, hexacolpate pollen grains (*Elsholtzia*, *Keiskea*, *Tetradenia*). Also, the seed development shows the great difference between the two groups, at least between *Pogostemon patchouli* and *Elsholtzia cristata*, according to Junell (1937). Whereas the position of the *Elsholtzia*-group after *Satureieae* (according to Briquet, 1897) may be correct, the *Pogostemon*-group ought to be placed more at the beginning of the *Labiatae*.

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