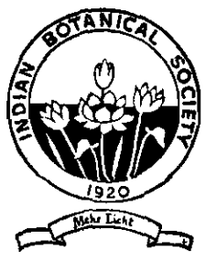
Memoirs

of

The Indian Botanical Society

MEMOIR 4



Editor

T. S. SADASIVAN

1963

BANGALORE CITY
PRTNIED AT THE BANGALORE PRESS, MYSORE ROAD
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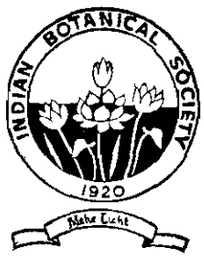

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PREFACE

WE have much pleasure in presenting *Memoir* 4 as a Special Publication of this Society.

There are four Symposia, three on Evolutionary Problems in the Pteridophytes, Gymnosperms and Angiosperms and a fourth on Parasitism dealt with in its broadest sense. Much thought and effort have gone into the preparation of these papers and I extend warmest thanks to all Chairmen of the Symposia and to the individual participants.

These Symposia were held in 1961 and 1962 at the Roorkee and Cuttack Sessions of the Indian Science Congress. We offer our grateful thanks to the authorities of the Indian Science Congress Association for the use of their forum for holding these Symposia.

University Botany Laboratory,
MADRAS, *August* 1963.

EDITOR.

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ROORKEE

SYMPOSIUM I

PHYLOGENY AND EVOLUTION IN PTERIDOPHYTES

CYTOLOGY AND PHYLOGENY OF THE PTERIDOPHYTES

By A. Abraham

Professor of Botany, University of Kerala, Trivandrum

The use of cytology as a valuable tool in elucidating phylogenetic relationships of plant groups has been well recognised, though this has not till recently been possible in the pteridophytes due to the technical difficulties inherent in the cytological study of this group. Manton (1950) in her pioneering work on the pteridophyte flora of Britain and Madeira followed by that on Malaya (1954) and Ceylon (Manton and Sledge, 1954) has demonstrated that cytological study of the ferns may yield information of great value in evolutionary, phylogenetic and taxonomic considerations of the group. Since then pteridophyte cytology has received a great deal of attention in other countries including India. Investigations on the South Indian Pteridophytes were started in 1952 in the Botany Department of the Kerala University, and important findings on the microphyllous pteridophytes and fern allies have already been published (Abraham and Ninan, 1954, 1958; Ninan, 1955, 1956 *a, b, c, d, e*; 1958 *a, b, c*). In addition, cytology of over 100 species of ferns have also been studied. In the present paper discussion on some aspects of phylogeny of the Pteridophytes is attempted in the light of informations gathered from this study.

Phylogenetic relationships of ferns.—In Ophioglossaceae, *Ophioglossum* and *Botrychium* with the lowest haploid numbers $n = 120$ and 45 respectively are traceable in origin to a common basic number 15. Though the haploid number $n = 94$ in *Helminthostachys* is slightly higher than an exact multiple of 15, it is also in ultimate origin referable to the same number (Ninan, 1958 *c*). This indicates that these three genera might have arisen from a common ancestor in the distant past, thus lending support to the contention of Eames (1936) that *Ophioglossum*, *Botrychium*, and *Helminthostachys*, though unlike in appearance are closely related. Bower (1926) has treated Ophioglossaceae as having some degree of affinity to the Marattiaceae and Osmundaceae. But cytologically the Ophioglossaceae with the basic number 15 indicates relationships neither to the Marattiaceae nor to the Osmundaceae which show basic numbers 13 and 11 respectively.

There exists much disagreement among pteridologists as to which of the three genera in Ophioglossaceae is primitive and which is advanced. Bower (1926) considers *Helminthostachys* as the most primitive, and *Ophioglossum* as the most advanced, while Nishida (1952) regards *Helminthostachys* as the most advanced. Cytological study of the three genera indicates that *Ophioglossum* with very high chromosome

numbers like $n = 120, 240, 480$, c.570 and c.630 in the different species is extremely specialised in comparison to *Helminthostachys* ($n = 94$) and *Botrychium* ($n = 45, 90$).

Bower (1926) has suggested Marattiaceae to have analogy in the structure of the sorus to the Gleicheniaceae and Matoniaceae. Cytological evidence supports this view in as much as members of all these three families have chromosome numbers in multiples of 13. In this connection it may also be noted from evidence of chromosome numbers in ancient genera like *Psilotum*, *Marattia*, *Matonia*, *Hymenophyllum*, *Dicranopteris*, etc., that chromosome number 13 might have been widely prevalent in the past in primitive groups of Pteridophytes (Manton, 1950; Ninan, 1956 *b*, *c*).

Schizaeaceae is supposed to be the ancestral stock from which advanced leptosporangiate families like the Pteridaceae and the Marsiliaceae might have arisen (Bower, 1926; Copeland, 1947). Cytological situation in the Pteridaceae shows that *Pteris* and subsequent genera form a cytologically uniform group with haploid numbers 29 or 30, or their multiples. Certain genera like *Adiantum*, *Cheilanthes*, *Doryopteris*, *Pityrogramma* and *Pellaea* show both $n = 29$ and $n = 30$ (as in *Lygodium*, the most primitive genus of the Schizaeaceae). Manton and Sledge (1954) have suggested that the haploid numbers 29 and 30 characteristic of the advanced Pteridaceae might be primitive numbers which are still retained in these genera through some direct ancestor in the Schizaeaceae in close relation to the genus *Lygodium*, and this view is supported by evidences from the S. Indian Pteridophytes also.

Haploid chromosome numbers $n = 38$ in *Anemia* and $n = 19$ in *Regnellidium* offer strong support to the suggested alliance between the Schizaeaceae and Marsiliaceae. The Marsiliaceae might have arisen from the Schizaeaceous stock in close relation to *Anemia*.

Holttum (1947) has suggested a line of evolution of the Pteridaceae from *Dennstaedtia* ($n = 34$). In the presence of consistent base numbers like 29 and 30 in the Pteridaceae this relationship is not supported by cytological evidence.

Ceratopteris has been described by Copeland as having originated from indusiate ancestors, most probably from Pteroid ferns. Ninan (1956 *e*) reported $n = 77$ and $2n = 154$ in *C. thalictroides* (= *C. siliquosa*) and Pal (1959) further reported $2n = 80$ in this species. The Schizaeoid affinity of the genus is clear from the presence of a common haploid number of $n = 77$ in *Schizaea* (Mehra, 1961) and *Ceratopteris*. Ninan (1956 *e*) suggested that in the possession of haploid number in multiples of 11 *Ceratopteris* and *Osmunda* ($n = 22$) might be related (*see* also Bower, 1928). The report of $n = 40$ and $n = 77$ in the same species of *Ceratopteris* however makes it difficult to trace the relationship of this genus. In view of this, further work on this problematic genus is much to be desired before its relationship can be more definitely established.

The primitive members of the Aspidiaceae are considered to have an origin cognate with the Cyatheaceae (Copeland, 1947). Bower (1928) regards the *Woodsia* and *Dryopteris* group of genera as having originated from the Cyatheaceae, while Holttum (1947, 1949) advocates a Dennstaedtioid origin for this. Bower's suggestion of relationship between *Dryopteris* and allies with the Cyatheaceae is not supported by cytological evidence in that the number 41 common in *Woodsia* and *Dryopteris* cannot be related to the haploid numbers 69 and 70 in the Cyatheaceae. Holttum's suggestion is also not in agreement with cytological evidence as the haploid numbers 40 and 41 in the Dryopteroid group are quite distinct from that in *Dennstaedtia*.

Holttum places *Thelypteris* and near allies in a family Thelypteridaceae suggesting that they may be allied to Gleicheniaceae and Cyatheaceae. The relationship of the Thelypteridaceae to the Cyatheaceae is supported on cytological grounds as the haploid number $n = 35$ in *Thelypteris* may be related to $n = 70$ in the Cyatheaceae.

Regarding the relationships of the Blechnaceae Copeland has suggested an origin of *Blechnum* from *Athyrium*. Bower (1928) is of the view that *Blechnum* and its allies have originated from the Cyatheaceae by way of a reduced ancestor, *Metteuccia intermedia*. Cytological evidence is against Copeland's view, since the base numbers 40 and 41 in *Athyrium* are evidently unrelated to those in *Blechnum* (28, 31, 32, 33 and 34). Holttum (1947) places *Blechnum* near *Asplenium* deriving it ultimately from the Dennstaedtioid stock. In the possession of common haploid numbers 33 and 34, *Blechnum* may be related to *Dennstaedtia*. But the relationship of *Blechnum* to *Asplenium* seems rather remote as it is not easy to relate the base numbers in *Blechnum* with $n = 36$, so characteristic of the genus *Asplenium*. *Asplenium* has been associated with *Athyrium* by certain authors because of the similarity in soral structure (Bower, 1928)., Holttum (1947) proposed that *Asplenium* is derived from something like *Davallia*, and he regards that the similarity in soral structure of *Asplenium* and *Athyrium* is the result of convergent evolution. Cytological evidence indicates that *Asplenium* ($n = 36$) is neither related to *Athyrium* ($n = 40$ and 41) nor to *Davallia* ($n = 40$).

Polyploidy, hybridisation and apogamy.—In addition to being a useful tool in assessing phylogenetic relationships of families, cytology also offers valuable clues regarding the probable manner of evolution of species and groups of species in the Pteridophytes. Most of the evolutionary mechanisms described in the flowering plants like polyploidy, hybridisation, etc., have also been found operative in speciation in the Pteridophytes.

Polyploidy, more than any single factor, is seen to have affected the Pteridophyte flora as a whole. A good number of pteridophytes are found to be high polyploid strains, and in the attainment of high levels of polyploidy, the pteridophytes have gone far beyond any other group of plants. Chromosome numbers like $n = 120$, 240, 480, c.570, c.630,

etc., in species of *Ophioglossum* (Abraham and Ninan, 1954; Ninan, 1958 *c*), $n = 104$ in *Psilotum* (Ninan, 1956 *c*), $n = 108$ in *Equisetum* (Manton, 1950 and Ninan, 1955), $2n = 408$–420 in *Tmesipteris* (Barber, 1955), $2n = 502$–510 in *Phylloglossum* (Blackwood, 1953), etc., are examples of this. On the contrary, low chromosome numbers like $n = 9$ in *Selaginella* (Manton, 1950), $n = 10$ and 11 in *Isoetes* (Manton, 1950; Ekstrand, 1920; Takamine, 1921 and Abraham and Ninan, 1958) and $n = 13$ in *Hymenophyllum* (Manton, 1950; Mehra and Singh, 1957) indicate that in the distant past the cytological situation of Pteridophytes has been very simple, starting from fundamentally low numbers, and that the high numbers observed in some of the modern genera might have been the cumulative effect of polyploidy working for millions of years, and maintained through the interpolation of vegetative modes of reproduction.

As regards the grade of polyploidy attained by South Indian ferns, the commonest chromosomal type in them is the tetraploid, while hexaploids, octoploids and even still higher levels of polyploidy also occur, though less frequently. Data of chromosome numbers from Ceylon (Manton and Sledge, 1954) also indicate the preponderance of polyploids. Regarding the type of polyploid changes, it is to be noted that allopolyploidy and aneuploidy have played significant roles in the evolution of species. Of all pteridophytes so far studied, *Psilotum* is the only clear case in which a natural autopolyploid series has been demonstrated (Manton, 1950 and Ninan, 1956 *c*), the positive criterion of this being multivalent pairing. Mehra and Loyal (1959) have also studied the role of autopolyploidy in the diversification of the genus *Marsilea*. It is quite probable that multivalent pairing, during the course of millions of years, might have been replaced by bivalent pairing consequent on the accumulation of inter- and intra-chromosomal changes. This indicates that all species exhibiting regular bivalent pairing may not necessarily be of allopolyploid origin. What we are currently studying merely represent upper members of a series whose bases have been lost, and as such we cannot decide whether auto or allopolyploidy or a combination of both might have been operative at different stages in their evolutionary history.

Several clear instances of aneuploid changes resulting in speciation could also be noticed. Haploid chromosome numbers like 29 and 30 in species of *Adiantum*, *Doryopteris*, *Cheilanthes*, etc., are examples of aneuploid changes (from 30 to 29) resulting in speciation at the diploid level. Chromosome numbers like $n = 58\ (29 \times 2)$ and 57 (58–1) observed in *Adiantum* further illustrate aneuploidy coming in at the tetraploid level.

Cytological data on the South Indian ferns have further revealed that hybridisation has also been a significant factor in evolution of species. Several clear instances of hybrids with irregular pairing behaviour at meiosis as well as apogamous forms (diploids, triploids and hexaploids), which might be related to hybrid origin, have also been encountered. The various species of the genus *Adiantum* investigated

in this laboratory with chromosome numbers $n = 30$ ($2n = 60$), $n = 60$, $n = 57$ ($2n = 114$), $2n = 90$ (irregular pairing), $2n = 171$ (irregular pairing) and $n = 2n = 171$ illustrate how polyploidy, hybridisation, aneuploidy and apogamy account for the evolutionary diversification of a single genus.

Chromosome size in relation to phylogeny.—Litardiere (1921) has shown that there is a phylogenetic reduction in the absolute size of chromosomes in the leptosporangiate ferns. Among the ferns, according to him, the Osmundaceae and Hymenophyllaceae have relatively larger chromosomes, the Cyatheaceae and Polypodiaceae have medium-sized chromosomes and the vegetatively reduced and reproductively specialised heterosporous family, Salviniaceae, have the smallest chromosomes. Chromosome studies in the South Indian ferns show the smallest chromosomes occurring in *Marsilea* ($1 \cdot 7$–$2 \cdot 8\,\mu$) and the largest in *Osmunda* ($6 \cdot 6$–$10\,\mu$). Considering the pteridophytes as a whole, starting from *Psilotum* ($4 \cdot 5$–$18\,\mu$) there is definite decrease in chromosome size as we go on to the more specialised leptosporangiate ferns. Most of the ancient genera as a rule show large chromosomes, while in the advanced genera they are relatively smaller in size. Pierce (1937) has noticed that lack of phosphorus in the nutrition of *Viola* results in considerable reduction in the size of chromosome. Tobgy (1943), from his studies on *Crepis neglecta* and *C. fuliginosa*, found that difference in the degree of coiling of chromonemata and distribution of heterochromatin may be responsible for the size differences of chromosomes. In *Psilotum* also certain chromosomes have been found to carry large blocks of heterochromatin. It is known that heterochromatin is not actually inert in a genetical sense, but that it is non-specific or indiscriminative in its activity (Darlington and Mather, 1950). Evidences from *Zea mays*, *Cimex*, *Sorghum*, *Secale*, etc. (Darlington and Mather, 1950 and Darlington and Thomas, 1941) suggest that in certain cases it is possible to eliminate the inert chromosome or parts of chromosomes, which are useful and at the same time not indispensable, without upsetting the normal cell functions, and that in still other cases the inert chromosomes are preserved by positive selection, as in some way they might be useful to the cell and the organism containing them. This would indicate that in the course of evolution inert segments of large chromosomes might have been eliminated by natural selection resulting in a decrease in size of chromosomes, while in others they might have been retained by positive selection; and this may well account for the differences in the size of chromosomes in the pteridophytes as well.

Regarding the relation between chromosome size and chromosome number in closely related forms like the different species in a genus, two tendencies are met with. The more common of these is the increase in absolute chromosome size corresponding with an increase in the chromosome number. The genus *Isoetes* (Ninan, 1958 *b*), which shows relatively larger chromosomes in the triploid *I. coromandelina* ($3 \cdot 3$–$6\,\mu$>) when compared with those in the diploid form (2–$4\,\mu$), furnishes convincing evidence in favour of this. In other genera like *Ophoglossum*, *Adiantum*, *Blechnum*, etc., also this tendency is very

marked. The opposite tendency of decrease in chromosome size associated with increase in chromosome number is observed in a few instances among the South Indian ferns. In *Doryopteris concloor* with $2n = 60$, the chromosomes vary from 2–4 μ in length, while in *D. luddns* with $2n = 232$ they show comparatively smaller dimensions ranging from 1·3–3·3 μ. Therefore it may be seen that the generally accepted idea that within closely related species higher chromosome numbers are associated with relatively smaller size of chromosomes (Sharp, 1943) is of no general validity as far as the pteridophytes are concerned.

It is clear from the foregoing that cytological studies can help to throw valuable clues to phylogeny and evolution in the pteridophytes also.

The observations mentioned in this paper have been drawn from an extensive work on the cytology and phylogeny of the pteridophytes in progress in the Botany Department of the Kerala University. The co-operation and assistance of Dr. C. A. Ninan and Mr. P. M. Mathew in this work have been most valuable.

REFERENCES

ABRAHAM, A. AND NINAN, C. A. 1954. Chromosomes of *Ophioglossum reticulatum* L. *Curr. Sci.* **23**: 213–14.

———. 1958. Cytology of *Isoetes*. *Ibid.* **27**: 60–61.

BARBER, H. N. 1955. *Biol. Abstr.* **3**: (17257).

BLACKWOOD, M. 1953. Chromosomes of *Phylloglossum drumondii* Kunze. *Nature, Lond.* **172**: 592.

BOWER, F. O. 1926. *The Ferns* **2**: Cambridge University Press, London.

———. 1928. *Ibid.* **3**: Cambridge University Press, London.

COPELAND, E. B. 1947. *Genera Filicum*. Waltham, Mass., Chronica Botanica Co.

DARLINGTON, C. D. AND MATHER, K. 1950. *Genes, Plants and People*, Allen and Unwin Ltd., London.

—— AND THOMAS, P. T. 1941. Mobid mitosis and the activity of inert genes in *Sorghum*. *Proc. roy. Soc.* **130 B**: 127.

EAMES, A. J. 1936. *Morphology of Vascular Plants (lower groups)*. McGraw-Hill Co., New York, pp. 433.

EKSTRAND, H. 1920. Über die, mikrosporanbildund Von. *Isoetes echinosporum*. *Svensk. bot. Tidskr.* **14**, 312–18.

HOLTTUM, R. E. 1947. A revised classification of Leptosporangiate ferns. *J. Linn. Soc. (Bot.)* **53**: 123–58.

———. 1949. The classification of ferns. *Biol. Rev.* **24**: 267–96.

LITARDIERE, R. DE. 1921. Recherches Sur l' element chromosomique des Filicinees. *Cellule* **31**: 255–473.

MANTON, I. 1950. *Problems of Cytology and Evolution in the Pteridophyta*. Cambridge University Press.

MANTON, I. 1954. Cytological notes on one hundred species of Malayan ferns. Appendix to Holttum's *Flora of Malaya*, **2**, *Ferns*, Singapore.

—— AND SLEDGE, W. A. 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. *Phil. Trans.* **238 B**: 127–85.

MEHRA, P. N. 1961. *Cytological Evolution of Ferns with Particular Reference to Himalayan Forms.* Presidential Address, 48th Indian Sci. Congr. Roorkee.

—— AND GURDIP SINGH. 1957. Cytology of Hymenophyllaceae. *J. Genet.* **55**: 379–93.

—— AND LOYAL, D. S. 1959. Cytological studies in *Marsilea* with particular reference to *M. minuta* L. *Res. Bull. Punjab Univ.* (*N.S.*) **10**: 357-74.

NINAN, C. A. 1955. Cytology of *Equisetum debile*. *J. Indian bot. Soc.* **34**: 112-14.

———. 1956 *a*. Cytology of Ophioglossaceae. *Curr. Sci.* **25**: 161-62.

———. 1956 *b*. Studies on the cytology and phylogeny of the pteridophytes. I. Observations on the Marattiaceae. *J. Indian bot. Soc.* **35**: 232-39.

———. 1956 *c*. Cytology of *Psilotum nudum* L. *Cellule* **57**: 307-18.

———. 1956 *d*. Studies on the cytology and phylogeny of the pteridophytes. III. Observations on *Osmunda regalis* L. *J. Indian bot. Soc.* **35**: 248–51.

———. 1956 *e*. Studies on the cytology and phylogeny of the pteridophytes. IV. Systematic position of *Ceratopteris thalictroides* (L) Brongn. *Ibid.* **35**: 252-56.

———. 1958 *a*. Studies on the cytology and phylogeny of the pteridophytes. II. Observations on the genus *Lycopodium*. *Proc. Nat. Inst. Sci., India* **24B**: 54-66.

———. 1958 *b*. Studies on the cytology and phylogeny of the pteridophytes. V. Observations on the Isoetaceae. *J. Indian bot. Soc.* **37**: 93–103.

———. 1958 *c*. Studies on the cytology and phylogeny of the pteridephytes. VI. Observations on the Ophioglasaceae. *Cytologia* **23**: 291–316.

NISHIDA, M. 1952. Studies on the systematic position and constitution of the Pteridophyta. A new system of Osphioglossales. *J. Jap. Bot.* **27**: 271–78.

PAL, S. 1959. Chromosome number in *Ceratoptertis siliquosa* (L.) Cope. *Curr. Sci.* **28**: 455.

PIERCE, W. P. 1937. The effect of phosphorus on chromosomes and nuclear volume in a *Vipla* species. *Bull. Torrey bot. Cl.* **64**: 345-54.

SHARP, L. W. 1943. *Fundamentals of Cytology*. McGraw-Hill Co., New York.

TOGGY, H. A. 1943. A cytological study of *Crepis fulginosa, C. neglecta* and their hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. *J. Genet.* **45**: 67–111.

TAKAMINE, H. 1921. Some observations in the life-history of *Isoetes*. *Bot. Mag., Tokyo* **35**: 184-90.

PHYLOGENETIC STUDIES IN POLYPODIACEAE (*SENSU* COPELAND, 1947)

By G. Panigrahi* and S. N. Patnaik‡

Botanical Survey of India, Shillong

The family Polypodiaceae (*sensu* Copeland, 1947) is characterized by epiphytic ferns, very rarely terrestrial, rhizome usually covered with peltate scales (bristles or hairs in a few primitive members), fronds simple to pinnate, very rarely more compound, venation usually reticulate with free included veinlets in the areoles, sori typically exindusiate and round, rarely elongate to form linear coenosori and sometimes acrostichoid, spores without epispore, or rarely with thin epispore.

Cytology of nearly 40 species of the family was studied by Manton (1950, 1954) and Manton and Sledge (1954), the majority of the species coming from Ceylon and Malaya. Recently, 36 species have been cytologically investigated by us (Panigrahi and Patnaik, 1961 *b* and Patnaik and Panigrahi, 1963; and Panigrahi, 1962) out of nearly 100 Indian species studied as herbarium specimens at the Regional Herbarium of Botanical Survey of India, Shillong, Herbarium of the Forest Research Institute, Dehra Dun and Central National Herbarium, Sibpore, Howrah. Several other species of the family have also been worked out cytologically by other Indian workers, *viz.*, Nayar (1958), Bir (1960), Ghatak (1961), Malhotra (in Mehra, 1961 *b*) and Pal (1961).

While competent fern taxonomists have made serious attempts to postulate the phylogenetic evolution in ferns at the family or order levels from the time of Bower (1928) to Mehra (1961 *a*), there are serious disagreements between them with regard to the relationships between different genera included within the family and on the question of the relative primitiveness of different genera included amongst the four families of Polypodiales (Pichi-Sermolli, 1958). This paper, therefore, attempts to review our existing knowledge on the formal taxonomy in the group, particularly with reference to the genera occurring in India (Chart 1) and to re-evaluate the various hypotheses on the affinity between genera on the basis of available cytological and taxonomic data on the family Polypodiaceae (Copeland, 1947).

The genus *Dipteris*, whose fossil history extends to Rhaetic, is considered to be the most primitive genus on account of its terrestrial habit, bristle-like appendages and solenostelic condition of the rhizome

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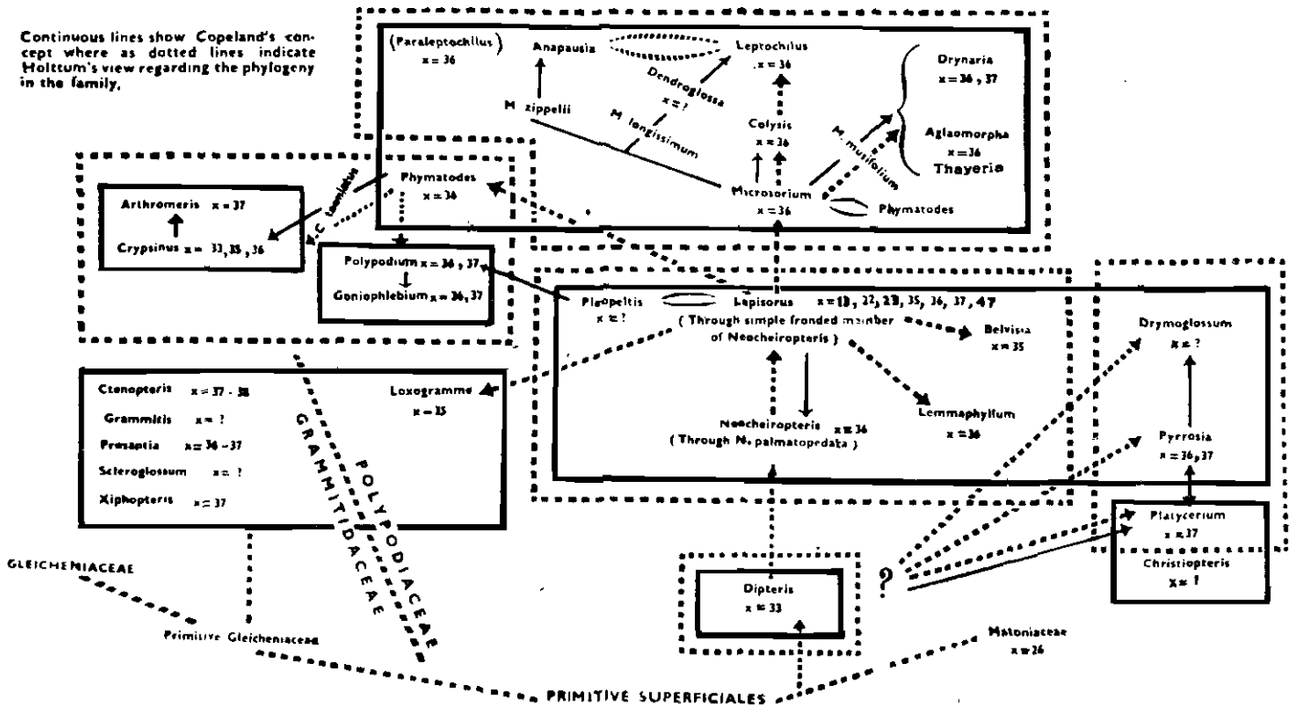


EVOLUTIONARY TRENDS IN POLYPODIACEAE

CHART 1

and dichotomous branching of the main veins. A genus of eight known species, it is characterized by discontinuous distribution, within its range, three species being endemic, *viz.*, two species *D. nieuiwenhuisii* and *D. quinquefurcata* (Bak.) Christ to Borneo and *D. wallichii* (R. Br.) Moore to Eastern India. Considering the possession of primitive characters and the relict nature of its distribution Bower (1928), Ching (1940) and Pichi-Sermolli (1958) have erected the family Dipteridaceae comprising of only one genus, *Dipteris*. Its affinity with *Matonia* and *Gleichenia* was stressed by Bower (1928) on the basis of venation, anatomy, soral characters, and gametophyte. Pichi-Sermolli (1959) also attributes its probable origin with Matoniales from Gleichenioid ancestry. The affinity of *Dipteris* is also suggested with another primitive genus *Cheiropleuria* of the family Cheiropleuriaceae (*cf.* Nakai, 1928; Ching, 1940; Pichi-Sermolli, 1958). Stokey and Atkinson (1954 *b*), from their studies on the gametophytes of *Cheiropleuria*, not only support its affinity with *Dipteris* but advocate the distinctiveness of the Cheiropleuriaceae from Polypodiaceae. But the possession of certain common features, *viz.*, the reticulation of the smaller veins forming areoles with free included veinlets and the exindusiate sori with a tendency towards mixed condition, which are shared by other members of Polypodiaceae and the anxiety "to establish the probability of the family, *viz.*, Polypodiaceae as a phyletic unit", led Copeland (1947, p. 174) to include *Dipteris* within his Polypodiaceae as its most primitive genus. He, however, recognised its distinctive status by attributing to it an ancestry more remote than the austral origin of the remaining genera of Polypodiaceae (Copeland, 1947, p. 174). The cytology of *Dipteris conjugata* Reinw. from Malaya revealed $n = $ c.33 at meiosis (Manton, 1954). *Dipteris wallichii* (R. Br.) Moore from Khasi hills shows clearly 66 bivalents at meiosis and is, therefore, proved to be a tetraploid species. Thus, cytology of *Dipteris* with this uncommon basic number (*viz.*, $x = 33$) together with its primitive morphological and anatomical features may support the ancestral nature of the genus within the family Polypodiaceae and may justify an independent family rank for it (Pichi-Sermolli, 1958). The discovery of $n = 33$ in two species of *Crypsinus* (Manton, 1954) and $n = 22$ in a single species of *Lepisorus* (Panigrahi and Patnaik, 1961 *a*) is, however, highly significant and suggests very close affinity between the two families Dipteridaceae and Polypodiaceae whose origin may be sought for from a common ancestral stock characterised by $x = 11$.

Seward (1922) considered the four genera *Matonia*, *Dipteris*, *Cheiropleuria* and *Neocheiropteris* as phylogenetically related on the basis of their resemblance to each other in frond habit, venation and restricted and overlapping range of geographical distribution. Similarly, Bower (1928) suggested the genus *Neocheiropteris* [through *N. palmatopedata* (Bak.) Christ with reticulate venation, large-naked sori in rows, one on either side of the main veins as in *Dipteris lobbiana* (Hk.) Moore], as a link between *Dipteris* and other reticulate veined species of *Polypodium* (*sensu lato*). Holttum (1947, p. 126), in agreement with Bower, envisaged *N. palmatopedata* (Bak.) Christ with dichotomous branching of the main veins and peltate scales as the connecting

link and hypothesised that " if *Neocheiropteris* produced offsprings with simple unlobed fronds, they would exactly resemble *Lepisorus* and it is most probable that *Lepisorus* originated in this way. The production of a simple frond from the pedately lobed *Dipteris-Neocheiropteris* type seems to me a main step in the evolution of Polypodiaceae ". But Copeland (*l.c.*, p. 184), while suggesting *Microsorium-Phymatodes* group as more primitive than *Pleopeltis*, considers (*l.c.*, p. 189), *Neocheiropteris* evidently related to *Pleopeltis*, as apparently too recent to be a possible connecting link between Pleopeltideae and Microsorieae, and, therefore, between *Dipteris* and Pleopeltideae. It may, however, be stated that the study of the two Indian species *N. phyllomanes* (Christ) Ching and *N. lancifolia* (Alston) Dickason with their peltate scales, dictyostelic condition of the rhizome, reticulate venation and scattered arrangement of sori (covered when young with peltate paraphyses like *Lepisorus*) around the costa, and the discovery of $n = 36$ and $2n = 72$ in *N. phyllomanes*, collected from Khasi and Jaintia hills, suggests that *Neocheiropteris* is not only characterised by a different basic chromosome number but also possesses too advanced morphological features to have arisen from *Dipteris*-like ancestors, the latter genus characterised by $x = 33$ and solenostelic rhizome, covered with bristle-like hairs and dichotomy of the frond. Thus, the possession of the reticulate venation and large-naked sori on either side of the main vein as in *N. palmatopedata* (Bak.) Christ can no more indicate ancestral relationship (Bower, 1928, p. 219) with *Dipteris lobbiana* (Hk.) Moore than of *N. palmatopedata* (Bak.) Christ with any species of *Lepisorus* or *Pleopeltis*. This suggestion of close affinity between *Lepisorus* and *Neocheiropteris* on the basis of comparative morphology gains support from the discovery of common base number, *viz.*, $x = 36$ in the two genera.

Considering the geographical distance between the American genus *Pleopeltis* [one *P. lanceolata* (Linn.) Klf. extending to South India and Africa] with non-clathrate paleae and peltate scales and the Asiatic genus *Lepisorus* with " often clathrate " paleae and ovate lanceolate scales, Christensen (*cf.* Copeland, 1947, p. 183), Ching (1933 *a*) and Holttum (1947) regard them as two distinct genera. But Copeland (1947) regards these morphological distinctions as rather inconstant and cites *Lepisorus* as synonymous to *Pleopeltis*. Three species of *Lepisorus* studied by Manton and Sledge (1954) showed three different haploid numbers as n=35, 36, 74 whereas two Indian species *L. excavatus* (Bory) Ching, and *L. macrosphaerus* (Bak.) Ching studied by us showed $n = 35$ in each case and a few other species of *Lepisorus* from Khasi and Jaintia Hills showed $n = 22$, 23, 47 and again $2n = 39$. Study of the chromosome number in *Pleopeltis lanceolata* (Linn.) Klf. from South India is of urgent necessity to settle not only the relationships between *Lepisorus* and *Pleopeltis* but also to establish the basic chromosome numbers characterizing the group Pleopeltideae.

Lemmaphyllum, a small genus, comprising nearly 8 species, is represented in India by only 2 species, *viz.*, *L. subrostratum* (C. Chr.) Ching and *L. carnosum* (Wall.) Pr. But Ching (1940) assigns these

two species to the genera *Lemmaphyllum* and *Lepidogrammitis* respectively, assigning round or imperfectly fused sori to the former and linear coenosori to the latter. *Lepidogrammitis* as a valid genus is not accepted by Copeland (1947) or Holttum (1954), since these distinctive soral characters are found to be very inconstant in the non-Indian species, *viz.*, *L. microphyllum* Presl and *L. drymoglossoides* (Bak.) Ching which show completely fused, half-fused and distinctly separate round sori on different parts of the same frond. While the cytological study in *L. carnosum* (Wall.) Pr. with linear coenosori is essential to settle the relationship between *Lemmaphyllum* and *Lepidogrammitis*, *L. subrostratum* (C. Chr.) Ching with round sori on each side of the costa shares its chromosome number, *i.e.*, $2n = 72$ with *Lepisorus longifolius* (Bl.) Holtt. This finding may, therefore, support close affinity between *Pleopeltis-Lepisorus* group on the one hand and the genera, *viz.*, *Lemmaphyllum*, *Neocheiropteris* and *Microsorium*, etc., on the other.

Holttum (1947, p. 127) postulates the derivation of *Microsorium* from *Lepisorus*-like ancestors by the loss of peltate paraphyses and increase in the number of sori, and of *Phymatodes* from *Lepisorus*-like ancestors by the strengthening of main veins and development of lobes in the leathery fronds. Ching (1933 *b*) considered *M. normale* (Don) Ching with simple narrow frond, peltate paraphyses and irregular rows of sori on either side of the costa as the transition between *Lepisorus* and *Microsorium*. Copeland (*l.c.*, p. 184) on the other hand treats *Phymatodes* under *Microsorium* and due to the austral origin of *Microsorium-Phymatodes* group, considers them to be more primitive than *Pleopeltis* and *Lepisorus*. Both Holttum (1947, p. 127) and Copeland (*l.c.*, p. 199) attribute the origin of *Colysis* from *Microsorium*. While Holttum would derive *Leptochilus* from *Colysis*-like ancestors by the contraction of fertile frond, Copeland would derive it directly from *Microsorium* through forms like *M. longissimum*, since, according to him (*l.c.*, p. 198), the terrestrial genus *Colysis* could not have been the immediate fore-bearer of the epiphytic genus *Leptochilus*. But Copeland's (1947) treatment of *Leptochilus* of restricting it to the single species *L. axillaris* (Cav.) Klf. and treating *L. decurrens* Bl. (which occurs both as epiphyte and terrestrial) at first under *Paraleptochilus* (Copeland, *l.c.*) and then under *Anapausia* (Copeland, 1950), the latter derived from *Microsorium* through *M. zippelii*, has not been accepted by later workers like Holttum (1954) and Sledge (1956). The finding of $n = 36$ in *Paraleptochilus decurrens* (Bl.) Copel. (Bir, 1960) agrees with the basic number $x = 36$ in *Leptochilus* (Manton and Sledge, 1954) and together with the unsatisfactory morphological distinctions between the two genera, provide no justification for the separation of *L. decurrens* Bl. from other members of *Leptochilus*. The occurrence of the same basic chromosome number, *viz.*, $x = 36$ in the four genera *Microsorium*, *Phymatodes*, *Colysis* and *Leptochilus* undoubtedly indicates close affinity between them.

The morphology of the three Indian species of *Colysis* together with the discovery of same basic chromosome number in this genus and

Leptochilus shows an interrelationship between them. *C. hemionitidea* (Wall.) Pr. shows round sori with a tendency to elongation and fusion resulting in linear sori in mature condition. *C. elliptica* (Thbg.) Ching as well as its variety *pothifolia* (Don) Ching are characterized by linear sori on pinnately lobed fronds and *C. pedunculata* (Hk. *et* Grev.) Ching, by linear sori on narrow long stipitate fertile frond. More pronounced dimorphism in this direction may ultimately result in the production of narrow acrostichoid fertile frond simulating the condition in *Leptochilus*. The sterile leaf of *L. decurrens* Bl. resembles that of *C. pedunculata* (Hk. *et* Grev.) Ching as well as the frond of *C. hemionitidea* (Wall.) Pr. in texture and venation and the lobed fronds of *L. decurrens* Bl. forma *laciniata* Sledge resemble the pinnate fronds of *Colysis elliptica* (Thbg.) Ching. Thus, *Colysis* occurring in diverse habitats and with variable nature of frond forms and soral characters [unstable soral condition in *C. hemionitidea* (Wall.) Pr.] might have acted as a potential source for the origin and evolution of other allied genera including *Leptochilus*, as postulated by Holttum (1947).

The *Drynaria* group comprising of *Drynaria* and *Aglaomorpha*, etc., are considered both by Copeland (1947, p. 199) and Holttum (1947, p. 127) to be derived from *Microsorium* through a form like *M. musifolium* (Bl.) Ching. Christensen (1938) remarked that the species of *Aglaomorpha* are intermediate between *Phymatodes* and *Drynaria*. The genus *Drynaria* with its two distinct types of fronds, *viz.*, short shallowly lobed ones for humus collection and long deeply lobed ones for bearing sporangia, may be considered less primitive and more specialised than *Aglaomorpha*, *Drynariopsis*, and *Pseudodrynaria* (Copeland, 1947, p. 204) in which both the functions are carried out by the same frond, the base being shallowly lobed and dilated for humus collection and the upper part deeply lobed for bearing sporangia. The occurrence of $n = 37$ in three different species of *Drynaria* (Manton and Sledge, 1954) in contrast to $n = 36$ in *Microsorium*, *Phymatodes*, *Colysis*, *Leptochilus* presented difficulties in the derivation of *Drynaria* group from *Microsorium*-like ancestors. But our discovery of $n = 36$ in *Drynaria propinqua* (Wall.) J. Sm. and in the relatively primitive genus *Aglaomorpha* [*viz.*, in *A. coronans* (Wall.) Copel. in Khasia Hills] may bridge the evolutionary gap between the two. Accordingly, *Aglaomorpha* may serve as the connecting link between *Microsorium-Phymatodes* on the one hand and *Drynaria* on the other. The evolutionary sequence from *M. musifolium* (Bl.) Ching to *A. heraclea* (Kze.) Copel. and then separately to *Drynaria* and *Merinthosorus* has no serious gap in it. The discovery of $n = 36$ in *Photinopteris* (Manton, 1954), devoid of humus collecting adaptations, may confirm Copeland's (1947, p. 203) suggestion of the aberrant nature of the genus in *Aglaomorpha* group.

The genus *Crypsinus* of the group Crypsineae (Copeland, 1947) is presumed to be related to *Microsorium*. *Crypsinus* with 40 or more species shows a great degree of morphological variations from simple or pinnatifid to pinnate fertile fronds and also ranging to typical dimorphic condition. Copeland (1947, pp. 206 and 209) considers several species

of *Crypsinus* as aberrant enough to constitute potential minor genera though he did not feel the necessity to separate them from *Crypsinus* which is, according to him, a natural genus. Out of the six species studied cytologically by Manton (*cf.* Manton and Sledge, 1954) and the present authors, 3 species [including *C. crenatopinnatus* (Clarke) Copel. from Shillong] have haploid number $n = 36$, one species, *viz.*, *C. oxylobus* (Wall. ex Kunze) Sledge studied by us showed $n = 35$ whereas the other two species, *viz.*, *C. enervis* (Cav.) Copel. and *C. wrayi* (Bak.) Copel. from Malaya showed $n = 33$ in each case (*cf.* Manton, 1954). Accordingly, she has aptly marked them as discordant elements within the genus. Thus, the cytological findings corroborate Copeland's observations regarding the diversified nature of the genus *Crypsinus*. Further cytological investigation of the other species of different morphological types is essential before postulating interspecific relationships within the genus *Crypsinus*. But the finding of $n = 36$ in both *Phymatodes* and *Crypsinus* point to the existence of strong affinity between them as postulated on morphological ground by Copeland (*l.c.*).

The genus *Arthromeris* having pinnate fronds with articulate pinnae is combined with *Crypsinus* within the same group Crypsineae (Copeland, 1947) because of both having distinct raised main veins and cartilaginous margin of the pinnae. Accordingly, Copeland expressed the advisability of merging *Arthromeris* with its parent genus *Crypsinus* to form a natural group although he recognised some practical inconveniences by such merger. Our discovery of $n = 37$ in *A. wallichiana* (Sprg.) Ching from Shillong in contrast to $n = 33, 35, 36$ in the genus *Crypsinus* affords cytological proof in favour of the validity of the two genera established on morphological differences between them.

Polypodium and *Goniophlebium* are the only two genera of Copeland's Polypodieae group represented in India. Holttum (1947, p. 127) postulates some Asiatic species with simple venation to bridge the gap between this group and *Phymatodes*. The two genera *Polypodium* and *Goniophlebium* were distinguished from each other by Presl (1836) by the formation of areoles with one excurrent veinlet in *Goniophlebium* and free veins without the formation of areoles in *Polypodium*. But Copeland (1947, p. 181), while admitting hesitatingly the generic distinction of the two genera, considered Presl's criteria of generic distinction as unreliable and assigned the species having pinnately lobed and pinnate fronds with non-articulate pinnae to *Polypodium* and species possessing only pinnate fronds with articulate pinnae to *Goniophlebium*. He (*l.c.*, p. 209), however, would like to merge *Goniophlebium* with its parent genus *Polypodium* to form a natural group of species although he recognized that such merger would result in practical inconveniences. Our unambiguous discovery of $n = 36$ in three species of *Polypodium* and one species of *Goniophlebium* in Khasi hills, *viz.*, *P. amoenum* Wall., *P. lachnopus* Wall. and *P. microrhizoma* Clarke and *G. argutum* (Wall.) J. Sm., considered together with the report of $n = 37$ in *P. amoenum* Wall., *P. lachnopus* Wall., *P. argutum* Wall., *P. erythrocarpum* Mett. and *P. microrhizoma* Clarke from Mussoorie and Darjeeling (*cf.* Mehra, 1961 *a*) and of $n =$ c. 37 in *P. subauriculatum* Bl.,

P. persicifolium Desv. and *P. verrucosum* Wall. from Malaya (Manton, 1954) and of $x=37$ in *P. vulgare* complex from Western Europe, would suggest that we are yet very far from unravelling the cytological picture in *Polypodium-Goniophlebium* complex. Since Malayan species of *Polypodium* studied by Manton do really belong to the genus *Goniophlebium* (*cf.* Copeland, 1947 and Panigrahi and Patnaik, 1961 *a*), cytology provides yet no proof against Copeland's (1947) suggestion for merging the two genera on morphological grounds.

The genus *Platycerium* by its dichotomous branching of the main veins is associated with *Dipteris* and *Cheiropleuria* but is given a separate family rank Platyceriaceae by Ching (1940). Copeland (1947, p. 222), though combines *Platycerium* with *Cheiropleuria* and *Christiopteris* in a single group, remarks that its affinity with these genera as also with *Dipteris* is not intimate. He (*l.c.*, p. 179) considers the resemblance of *Platycerium* as rather more evident to *Pyrrosia*. Holttum (1947, p. 127) also attributes common origin for *Platycerium*, *Pyrrosia* and *Drymoglossum* on the basis of the presence of stellate hairs on their fronds and peltate scales on the rhizomes and also because of some Chinese species of *Pyrrosia* having repeated dichotomous fronds like that of *Platycerium*. *Pyrrosia*, though a genus of nearly 100 species, shows great uniformity in the character of the frond such as simple, fleshy, lanceolate entire fronds with stellate paraphyses and a great degree of structural specialisation in fronds for control of water and, accordingly, Copeland considers the genus "a most natural one". *Platycerium* on the other hand shows a type of specialisation in frond characters as exhibited by *Drynaria* and possesses two types of fronds, normal fronds being stipitate, dichotomously branched and bearing sporangia whereas sterile scale fronds being sessile, broad, almost entire and soon becoming dry to collect debris. Holttum (1947, p. 126), therefore, considers *Platycerium* as a very specialised genus which is not on the main lines of evolution of the majority of Polypodiaceae. The finding of $x = 37$ in both *Platycerium* (*cf.* Manton, 1954) and *Pyrrosia*, however, may support close affinity between the two genera postulated on morphological similarities between them. These together with the evidences from the gametophytes of *Platycerium* and *Cheiropleuria* (*cf.* Stokey and Atkinson, 1954 *a*, *b*) suggest *Platycerium* as a primitive member of the Pleopeltideae group having affinity with *Pyrrosia*. The genus *Drymoglossum* is more specialised than *Pyrrosia* with the combination of dimorphic fronds and continuous linear coenosori. *Pyrrosia nummularifolia* (Sw.) Ching shows distinct dimorphism like *Drymoglossum piloselloides* (Linn.) Pr. but it differs by the possession of scattered sori on the whole surface of the frond characteristic of *Pyrrosia*. But *Pyrrosia confluens* (R. Br.) Ching, with unbroken coenosori on uniform frond type, is presumed to be the meeting point of the two genera (Copeland, 1947, p. 194). However, study of cytology of *Drymoglossum* sp. in India is essential for the discussion on affinity between them.

Loxogramme is another isolated genus within the family with its distinct morphological characters like simple fleshy fronds with linear

and oblique sori. Ching (1940) gave it a separate family rank advocating its affinity with Vittariaceae. Copeland (1947, p. 217) suggested the derivation of *Loxogramme* from *Grammitis* and included it in Grammitidaceae (Copeland, 1952). But Holttum (1947, p. 127) postulated its derivation from *Lepisorus* in an independent line and repudiated any affinity between *Loxogramme* and *Grammitis* because of their differences in the vascular anatomy, scales and venation. The present cytological study of *Loxogramme lanceolata* (Sw.) Pr., *L. avenia* (Bl.) Pr., *L. scolopendrina* (Bory) Pr. from Khasia hills established them as diploid species showing 35 bivalents at diakinesis while *L. involuta* (Don) Pr. from Shillong is a tetraploid species showing $n = 70$. Thus, the genus *Loxogramme* is characterised by a base number of $x = 35$ and not $x = 36$ (*cf.* Manton and Sledge, 1954). Manton, however (personal communication in 1961), accepts our evidence on the base number $x = 35$ for *Loxogramme* and states that this number has now turned up in *Belvisia* and *Selleguea*. Though the cytology of none of the species of *Grammitis* has yet been studied, the *Grammitis* group of genera like *Ctenopteris*, *Xiphopteris* and *Prosaptia* show varying base numbers, *viz.*, $n = 36$–37 and $n = 37$–38 but none of them show $n = 35$ characteristic of *Loxogramme*. Thus, *Loxogramme* cannot be treated as a member of Grammitidaceae both on morphological and cytological grounds. It is, therefore, rightly treated by Holttum (1947) and Pichi-Sermolli (1958, 1959) as a member of the Polypodiaceae with close affinity with *Lepisorus*, the latter along with *Belvisia* of the Pleopeltideae group sharing the base number $x = 35$ with *Loxogramme*. Similarly Ching's (1940) suggestion on the affinity of *Loxogramme* ($x = 35$) with Vittariaceae ($x = 30$) has to be discounted on the basis of different base numbers between them.

The overall cytological picture of the family Polypodiaceae (*sensu* Copeland, 1947) reveals different base numbers (Table I), *viz.*, $x = 13$, $x = 22$, $x = 23$, $x = 33$, $x = 35$, $x = 36$, $x = 37$, $x = 47$ of which 36 and 37 are more frequently prevalent within the family. The percentage of Polyploidy in the family seems to be very low (Table II), in comparison with other families like Aspleniaceae, Blechnaceae, Aspidiaceae, Pteridaceae and Vittariaceae. Out of the total of 85 species studied cytologically, only 14 species are polyploids, the highest grade of polyploidy being only hexaploidy discovered in 4 species. Thus, it appears that polyploidy has played much less effective role in the evolution within the family in which morphological variations might have arisen largely by the origin of different base numbers even within a genus or by genic mutations. But the discovery of a range of haploid chromosome numbers, *viz.*, $n = 22, 23, 35, 36, 47, 74$ in different species of *Lepisorus* and detection of a natural hybrid in *Lepisorus* in Khasia hills showing $2n = 39$ and formation of 17 bivalents *plus* 5 univalents in one cell, 11 bivalents and 17 univalents in another cell together with other types of cytological aberrations in a large number of cells (Panigrahi and Patnaik, unpublished), provides significant evidences to show that the genus *Lepisorus* is in a fluid state of evolution.

TABLE I

Distribution of various genera of Polypodiaceae sensu *Copeland*, 1947, *under different basic chromosome numbers*

$x=13$	$x=22$	$x=23$	$x=33$	$x=35$	$x=36$	$x=37$	$x=36-37$	$x=37-38$	$x=47$
Lepisorus	*Lepisorus*	*Lepisorus*	*Cryptsinus*	*Belvisia*	*Aglaomorpha*	*Arthromeris*	*Prosaptia*	*Ctenopteris*	*Lepisorus*
			Dipteris	*Crypsinus*	*Colysis*	*Drynaria*			
				Lepisorus	*Crypsinus*	*Goniophlebium*			
				Loxogramme	*Drynaria*	*Lepisorus*			
					Goniophlebium	*Platyserium*			
					Lemmaphyllum	*Polypodium*			
					Lepisorus	*Pyrrosia*			
					Leptochilus	*Xiphopteris*			
					Microsorium				
					Neocheiropteris				
					Photinopteris				
					Phymatodes				
					Polypodium				
					Pyrrosia				

TABLE II

Showing the percentage of polyploidy discovered in various families of polypodiaceae, sensu lato, *cytologically investigated up-to-date*

	Aspleniaceae	Aspidiaceae	Blechnaceae	Davalliaceae
Total no. of taxa cytologically studied	49	221	9	18
Total no. of taxa with polyploidy ..	39	87	5	2
Percentage of polyploidy ..	80	39	56	11

	Polypodiaceae	Pteridaceae	Vittariaceae
Total no. of taxa cytologically studied	85	142	9
Total no. of taxa with polyploidy ..	14	62	8
Percentage of polyploidy ..	17	44	89

SUMMARY

1. A review of our existing knowledge on the formal taxonomy and phylogenetic trends of evolution in Polypodiaceae (*sensu* Copeland, 1947) is presented in this paper.

2. On the basis of cytological data available on 85 species investigated up-to-date, an attempt has been made to re-evaluate the various hypotheses on the affinity and relative primitiveness between genera, particularly with reference to the 27 genera of Polypodiaceae occurring in India.

3. The discovery of only 17 per cent of polyploidy in the family appears significant against the finding of different base numbers even within a genus, *viz.*, *Crypsinus*, *Lepisorus*, *Pyrrosia* and *Polypodium*. But the discovery of $n = 22$ and the detection of a natural hybrid with 11 bivalents and 17 univalents in one cell in *Lepisorus nudus* complex, provide clearest proof of the occurrence of the lowest base number, *viz.*, $x = 11$ so far known in Polypodiaceae.

ACKNOWLEDGEMENTS

Grateful thanks are due to the authorities of the Council of Scientific and Industrial Research, New Delhi, for the award of a Junior Research Fellowship and other financial assistance to the junior author for carrying out cytotaxonomic studies on East Indian Polypodiaceae

and to Rev. Father H. Santapau, Chief Botanist, Botanical Survey of India, Calcutta. for his keen interest in the progress of the research scheme. The junior author expresses his thanks to the Education Department, Government of Orissa, for granting him leave during the period of this investigation.

REFERENCES

BIR, S. S. 1960. Cytology of some "Acrostichoid" ferns. *The Nucleus* **3**: 121-24.

BOWER, F. O. 1928. *The Ferns* **3**: University Press, Cambridge, England.

CHING, R. C. 1933 *a*. The studies of Chinese Ferns—IX. *Bull. Fan Inst. Biol.* **4**: 47-113.

———. 1933 *b*. The studies of Chinese Ferns—X. *Ibid.* **4**: 299.

———. 1940. On natural classification of the family "Polypodiaceae". *Sunyatsenia* **5**: 201-69.

CHRISTENSEN, C. 1938. *Verdoorn's Manual of Pteridology*. Nijhoff, The Hague, 522-50.

COPELAND, E. B. 1947. *Genera Filicum*. Waltham, Mass., U.S.A.

———. 1950. Additions and corrections to the genera *Filicum*. *Amer. Fern. J.* **40**: 16-21.

———. 1952. Grammitidaceae of New Guinea. *Philipp. J. Sci.* **81**: 81-119.

GHATAK, J. 1961. Some meiotic counts on the Ferns from the Khasia-Jaintia Hills. *Proc. 48th Indian Sci. Congr.* 270.

HOLTTUM, R. E. 1947. A revised classification of Leptosporangiate ferns. *J. Linn. Soc. (Bot.)* **53**: 123-58.

———. 1954. *A Revised Flora of Malaya*, **2**: *The Ferns of Malaya*, Government Printing Office, Singapore.

MANTON, I. 1950. *Problems of Cytology and Evolution in the Pteridophyta*. University Press, Cambridge, England.

———. 1954. Cytological notes on one hundred species of Malayan ferns. *Appendix in Holttum's Flora of Malaya* **2**: 623-28.

—— AND SLEDGE, W. A. 1954. Observations on the cytology and taxonomy of the Pteridophyte flora of Ceylon. *Phil. Trans.* **238 B**: 127-85.

MEHRA, P. N. 1961 *a*. Cytological evolution of ferns with particular reference to Himalayan Forms. Presidential Address. *Proc. 48th Ind. Sci. Congr.* Part II. Section Botany, 130-53.

———. 1961 *b*. Chromosome numbers in Himalayan ferns. *Res. Bull. Punjab Univ.* (*N.S.*) **12**: Parts I-II, 139-64.

NAKAI, T. 1928. Notes on Japanese ferns. VII. *Bot. Mag., Tokyo* **42**: 203-18.

NAYAR, B. K. 1958. Studies in Polypodiaceae. V. Cytology of *Colysis pedunculata* (Hk. et Grev.) Ching. *Sci & Cult.* **24**: 181-82.

PAL, S. 1961. Chromosome number in some genera of Polypodiaceae. *Ibid.* **27**: 499-501.

PANIGRAHI, G. 1962. Cytology and its Contribution to Fern Systematics. *Proc. Summer School of Botany—Darjeeling*. June 2-15. 1960: 261-275.

PANIGRAHI, G. AND PATNAIK, S. N. 1961 *a*. Polypodiaceae—Enumeration of the species with correct nomenclature. *The Indian For.* **87**: 242-47.

———. 1961 *b*. Cytology of a few genera of Polypodiaceae in Eastern India. *Nature (Lond.)* **191**: 1207-08.

PATNAIK, S. N. AND PANIGRAHI, G. 1963. Cytology of a few genera of Polypodiaceae in Eastern India—II. *Amer. Fern. J.* (in Press).

———. Cytological studies in *Lepisorus nudus* complex (Unpublished).

PICHI-SERMOLLI, R. E. G. 1958. The higher taxa of the Pteridophyta and their classification in *Systematics of Today, Uppsala Univ., Arsaskrift*, 70–90.

———. 1959. *Pteridophyta in "Vistas in Botany"*, Pergamon Press, Ltd., London.

* PRESL, C. B. 1836. *Tentamen Pteridographie*, Haase, Prague.

SEWARD, A. C. 1922. Hooker Leeture. A study in contrasts: The present and past distribution of certain ferns. *J. Linn. Soc. (Bot.)* **64**: 210-40.

SLEDGE, W. A. 1956. The Ceylon species of *Leptochilus*. *Ann. Mag. nat. Hist. Ser.* 12, **9**: 865-77.

STOKEY, A. G. AND ATKINSON, L. R. 1954 *a*. The Gametophyte of five species of *Platycerium*. *Phytomorphology* **4**: 165–72.

———. 1954 *b*. The Gametophyte of *Cheiropleuria bicuspis* (Bl.) Presl. *Ibid.* **4**: 192–201.

* Not seen in original.

EXPLANATION OF PLATES

PLATE I

FIG. 1. *Lepisorus excavatus* $n = 35$ (×1,000).

FIG. 2. *Lepisorus macrosphaerus*, $n = 35$ (×1,000).

FIG. 3. *Lepisorus pseudonudus*, $n = 11^{II}-17^{I}$ (×1,000).

FIG. 4. *Lepisorus sp.*, $n = 22$ (×1,000).

FIG. 5. *Pyrrosia mannii*, $n = 37$ (×1,000).

FIG. 6. *Pyrrosia mollis*, $n = 74$ (×1,000).

PLATE II

FIG. 1. *Loxogramme avenia*, $n = 35$ (×900).

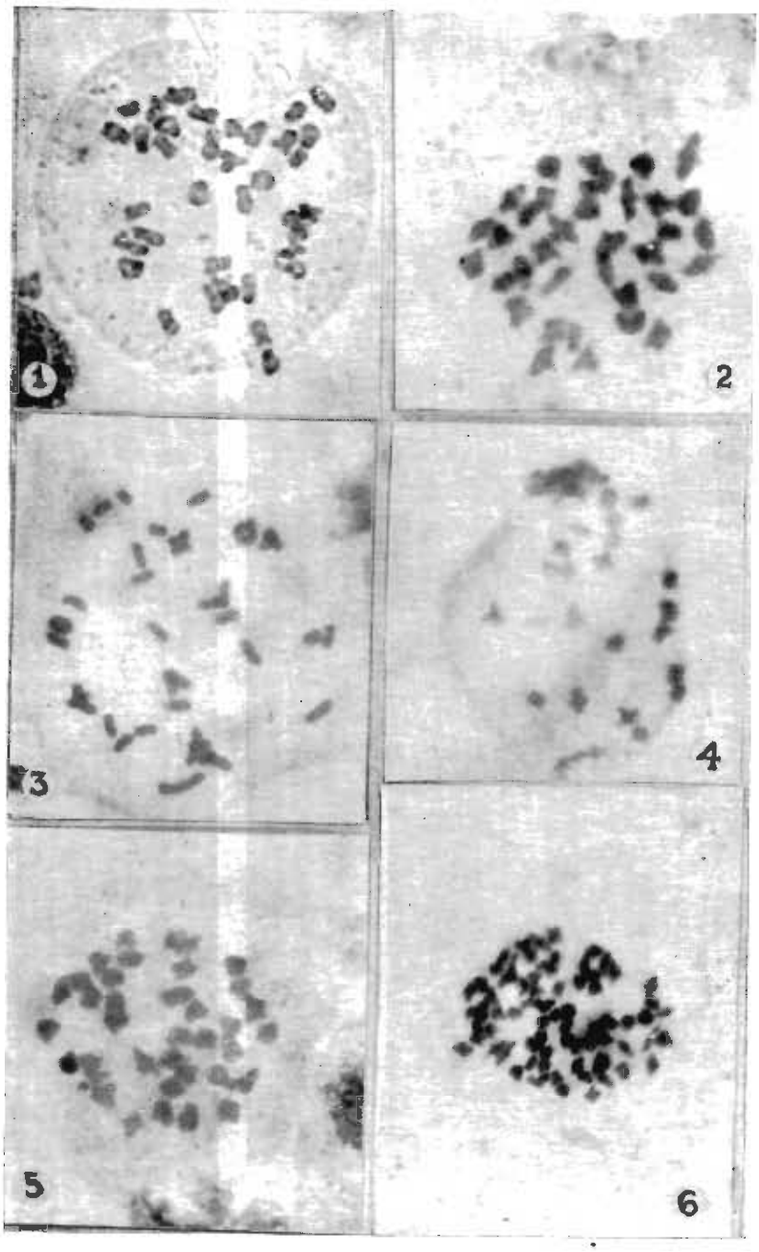
FIG. 2. *Loxogramme lanceolata*, $n = 35$ (×900).

FIG. 3. *Loxogramme scolopendrina*, $n = 35$ (×1,000).

FIG. 4. *Crypsinus crenatopinnatus*, $n = 36$ (×900).

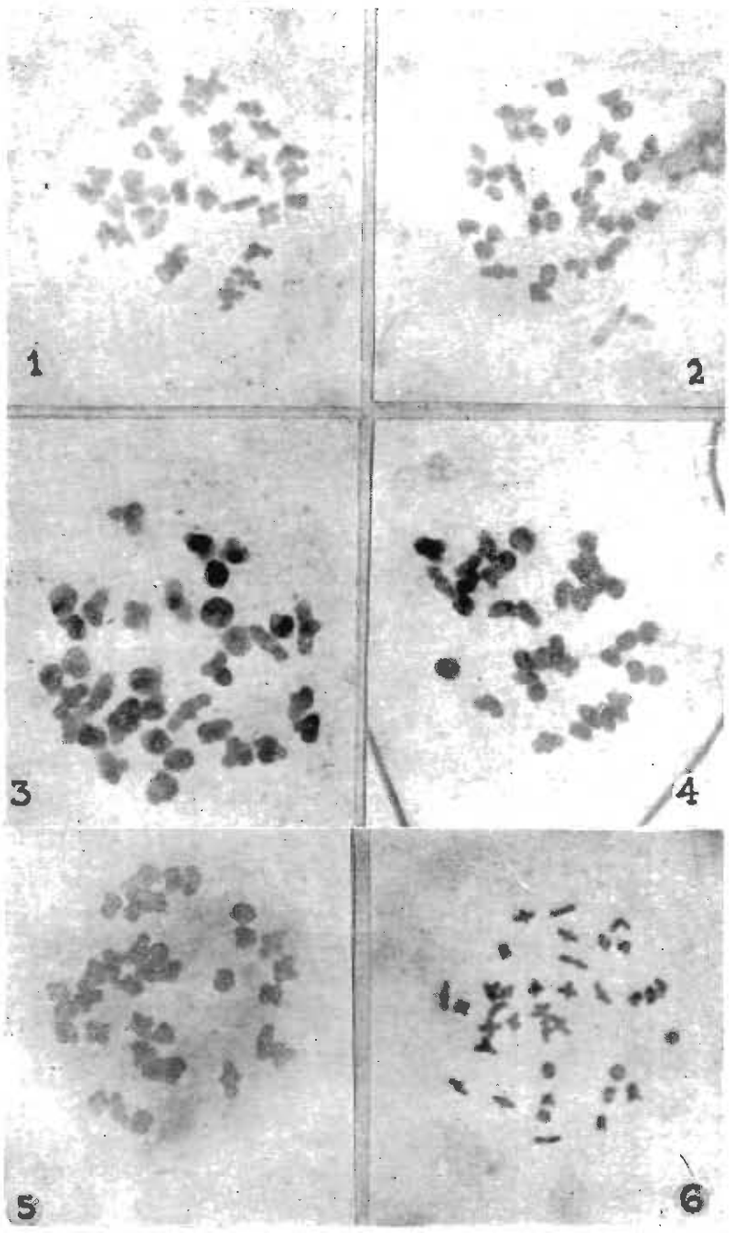
FIG. 5. *Microsorium superficiale*, $n = 36$ (×900).

FIG. 6. *Microsorium membranaceum*, $n = 36$ (×700).



FIGS 1-6

G. Panigrahi and S. N. Patnaik



FIGS. 1-6

SOME EVOLUTIONARY TRENDS IN FAMILY THELYPTERIDACEAE WITH PARTICULAR REFERENCE TO HIMALAYAN SPECIES

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THE family Thelypteridaceae represents a group of advanced leptosporangiate ferns, chiefly distributed in the tropics and only a few are met with in the temperate regions. According to the traditional taxonomic treatment, these ferns were usually grouped under the old composite genus *Dryopteris* due to their morphological similarities especially of the sorus and indusium. These features of resemblance with *Dryopteris* (*sensu stricto*), according to some modern workers, represent a case of parallel evolution between the two groups rather than true phyletic relationship.

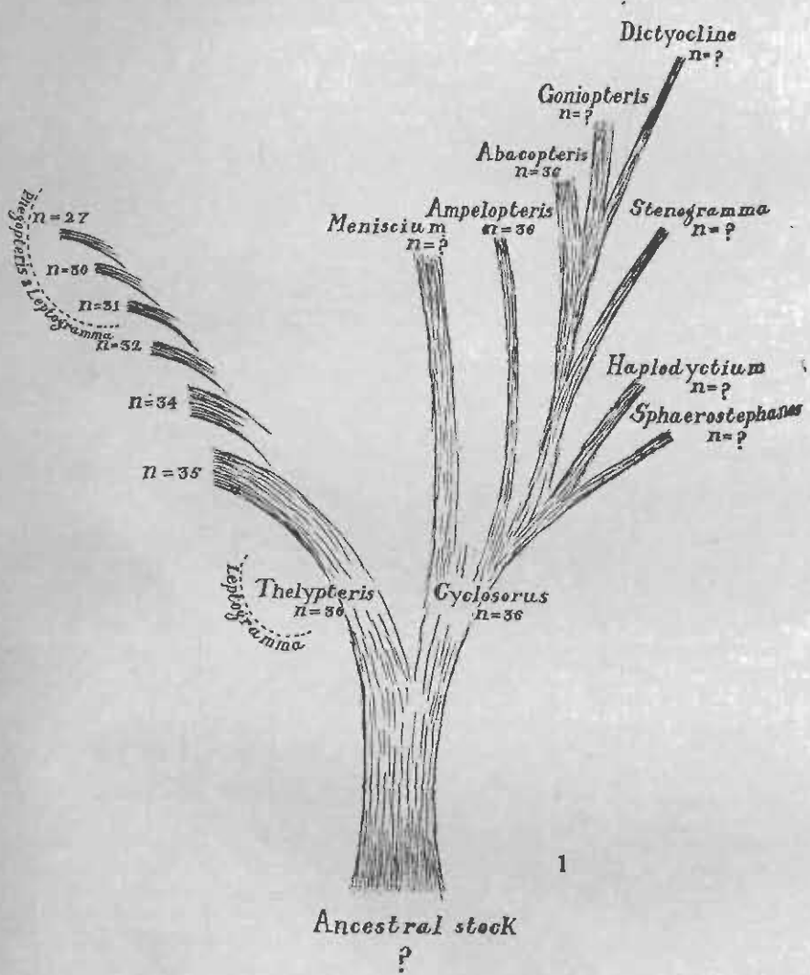
Christensen (1938) was the first to group them in the tribe Thelypterideae, subfamily Dryopteridoideae of his family Polypodiaceae. Ching (1940) raised it to the family rank and this has been upheld by Dickason (1946) as well as Holttum (1947). The recent studies of morphology, anatomy and cytology of the principal genera, indicate conspicuous naturalness of this assemblage and Ching's treatment of these ferns, therefore, seems justified.

This paper discusses some trends of evolution amongst the thelypteroid genera, chiefly based upon the results, obtained by the present writer on the Himalayan species.

Cytological consideration.—Nothing can be stated at present as to the origin of the thelypteroid ferns. Holttum's suggestion that they belong to the gleichenioid stock (Holttum, 1947), although may be held valid on certain morphological grounds, but the cytological data thus far gathered in the family Gleicheniaceae indicate that at least none of the living forms of this family can be ancestral to thelypteroid ferns.

The cytological information for Thelypteridaceae is now available for 51 species distributed in four genera, namely, *Thelypteris*, *Cyclosorus*, *Abacopteris* and *Ampelopteris*. Out of these 51 species, 32 possess a gametic set of 36 chromosomes. The last three genera are exclusively based on $n = 36$. In view of the preponderance of this number the writer is inclined to believe that forms with this number perhaps represent the ancestral condition. It is suggested that from this stock, along two parallel lines, arose various species of *Thelypteris* ($n = 36$)

on the one hand and on the other a prominent stock of *Cyclosorus* with the basic set of 36 chromosomes (Text-Fig. 1). A single morphological character, which probably has brought about the segregation of these two lines, is the increase of laminar extent which resulted in the anastomosis of the basal one or more pairs of veinlets—a condition seen in *Cyclosorus*. This view is borne out by the occurrence in certain species of *Cyclosorus* of both the thelypteroid as well as goniopteroid venation.



TEXT-FIG. 1. The interrelationships and probable evolutionary course within the family Thelypteridaceae as visualized on the basis of cytomorphological data.

The evolution within the genus *Thelypteris* has presumably been through aneuploidy because there occur seven basic numbers, *viz.*, 36, 35, 34, 32, 31, 30 and 27. Of these the base numbers 32, 30 and 27 are not represented in the Himalayas. It is interesting to note the nearly sympatric distribution of the species with various base numbers in the different parts of the world. Some of the species with base number 31 and some with lower numbers, as well as those with 36 have been previously placed under such genera as *Phegopteris* and *Leptogramma* because these possess non-indusiate sori and in some the sori elongate along the veinlets. On morphological grounds too, the species with base number lower than 36, seem to have evolved from the main thelypteroid stock because of certain important unifying characters of the frond form, rhizome, stelar pattern, leaf-trace, dermal appendages, spores and gametophyte (Loyal, unpublished data). In view of the above the present writer believes that the reasons for including all these variants under the genus *Thelypteris* are stronger than those for the retention of old genera *Phegopteris* and *Leptogramma.*

It is of interest to point out here that there exists strong isolation barriers between the various chromosomal lines, for, not a single hybrid has been discovered so far even when species with such close numbers as $n = 36$ and 35 grow as mixed populations.

From the stock to which *Cyclosorus* belongs, have descended such genera as *Abacopteris, Ampelopteris, Goniopteris* and some more. doubtfully referred here by Copeland (1947). But the evolutionary mechanisms involved seem to have been different from that seen within the genus *Thelypteris.* The presence of $n = 36$ in all the species of *Cyclosorus, Abacopteris* and monotypic *Ampelopteris* indicates complete chromosomal stability in this assemblage and this in turn strongly suggests that perhaps the evolution of this group of plants has occurred chiefly through mutations, gene and/or chromosomal. From morphological standpoint, however, the differentiation of these genera from *Cyclosorus* involves the same factor which segregated *Cyclosorus* from *Thelypteris*, namely, the increase in the extent of lamina followed by the anastomosis of veinlets. This tendency seems further augmented in these various genera and can best be observed in *Abacopteris, Meniscium, Goniopteris* and in the monotypic *Dictyocline.* The latter genus possesses a complete anastomosis of the veinlets in the expanded lamina. The cytology of many of the genera in this group is still unworked, and in all probability may turn out to be the same as in *Cyclosorus.*

In the matter of speciation and taking into consideration the entire cytological data available at present, it may be suggested that, besides aneuploidy and mutations, the role of euploidy is well marked because of the presence of 57·69%, 41·17% and 28·5% polyploid species in *Thelypteris, Cyclosorus* and *Abacopteris* respectively. Another important point that emerges from the present study is the complete absence of the phenomenon of apogamy in these ferns. This is in

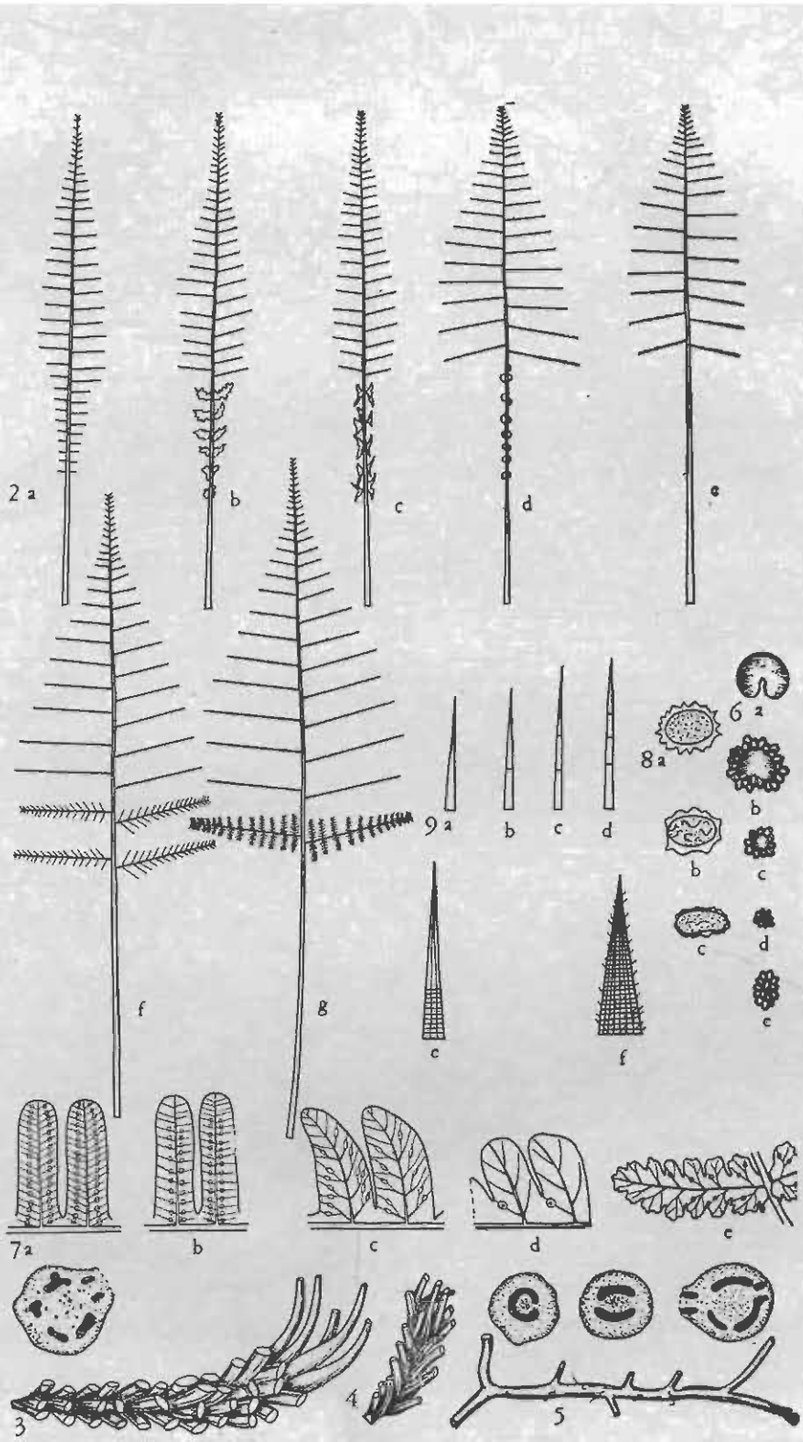
strong contrast to a number of genera of advanced leptosporangiate ferns notably *Pteris* and *Dryopteris*.

Morphological consideration.—The interpretation of the morphological data has certain obvious limitations especially when taken in the light of newer approach which is based on the idea that the evolutionary processes occurring in a species are influenced by the genetic make-up of the species on one hand and by the physiological and ecological environments in which it lives on the other. To the writer's knowledge this approach in understanding morphological variation in any particular group of ferns has been applied but by very few students of morphology. Some evolutionary trends discussed here are chiefly based on the Himalayan species of the genus *Thelypteris* and these are considered at present to be of tentative nature.

As stated earlier, these ferns are essentially the tropical forest dwellers and from ecological standpoint the Himalayan species of *Thelypteris* fall in the following four groups:

1. Mountain ferns, 6,000 ft. and above:
   - (i) *T. aurita* (Hook.) Ching
   - (ii) *T. brunnea* (Wall.) Ching
   - (iii) *T. levingei* (Clarke) Ching
   - (iv) *T. elwesii* (Baker) Ching
2. Species of lowland forests, 4,000–6,000 ft.:
   - (i) *T. esquiroli* (Christ) Ching
   - (ii) *T. repens* (Hope) Ching
   - (iii) *T. flaccida* (Bl.) Ching
   - (iv) *T. subvillosa* (Moore) Ching
3. Sun ferns:
   - (i) *T. xylodes* (Kze.) Ching
   - (ii) *T. erubescens* (Wall.) Ching
4. Lowland tropical ferns, from plains to 3,000 ft.:
   - (i) *T. uliginosa* (Kze.) Ching
   - (ii) *T. ornata* (Wall.) Ching
   - (iii) *T. oppositipinna* (v.A.v.R.) Ching
   - (iv) *T. serra* Swartz (cultivated in the gardens of unknown wild origin)

A comparison of the forest ferns (2) with those of sun ferns (3) and inhabitants of lowland open valleys (4) shows that these two types of habitat reflect the origin of two types of frond forms (characterizing these groups), namely linear-lanceolate with gradually shortened basal pinnae in the forest ferns (Text-Figs. 2 *a*, *b*, *c*, *d* and *e*) and deltoid or triangular form in those which live in the open unshaded localities (Text-Figs. 2 *f*, *g*). The linear-lanceolate form of the frond is met with in the largest number of the species with $n = 36$ and 35 and also in various



TEXT-FIGS. 2–9

TEXT-FIGS. 2–9. Diagrammatic representation of the various organs of the sporophyte in the Himalayan species of *Thelypteris*. Figs. 2 *a–g*. Frond form; Figs. 3–5. To show the shoot posture and external morphology, C.S. also shown; Figs. 6 *a–e*. Soral types; Figs. 7 *a–e*. Soral position and shape of the ultimate pinnules; Figs. 8 *a–c*. Show variation in the spore structure. Figs. 9 *a–f*. Dermal appendages.

species of *Cyclosorus* growing in the similar situations. This tendency to have basal pairs of pinnae gradually or abruptly shortened, is quite understandable in situation where light conditions are rather difficult so far as the basal part of the frond is concerned. This tendency is discernible not in thelypteroid ferns alone but in some other species of diverse phyletic affinity as well. This frond form may be considered as the derived condition from a linear-lanceolate one which is observed in a sizable number of species.

The creeping posture of the shoot seems the basic type (Text-Fig. 3), for it is present in almost all the species except one (*T. oppositipinna*) which grows in the small crevices of rocks, that it is short and more or less erect (Text-Fig. 4). However, from physiological standpoint concerning the growth rate in length of the shoot in relation to the number of leaf primordia differentiated during one active growth period, there are two types distinguishable. In the first type the growth rate of the shoot in length is comparatively slower but the rate of leaf-primordia differentiated is comparatively faster during the same period. This results in a stout creeping shoot with very compactly arranged leaves and the leaf primordia form a crown around the growing apices of the shoots (Text-Fig. 3). In the second type the shoot grows in length at a much faster pace than in the former type and the number of leaf primordia differentiated during the same period is much less than in the former case. This results in comparatively fragile shoots with sparsely arranged leaves and generally a single leaf-crozier may be present in contrast to many in the first type (Text-Fig. 5). It seems logical to conclude that the thelypteroid ferns possess only the creeping type of shoot with minor departure in those occupying special habitats.

A comparison of the soral types in the genus *Thelypteris* indicates that a non-indusiate sorus and its slight elongation along the veinlet is a derived condition from a zygomorphic, lopsided sorus with a horse-shoe-shaped indusium (Text-Figs. 6 *a-e*). This view, however, is not in line with that of Holttum (1947) who considers this condition as primitive in these ferns. In the writer's opinion his assumption is primarily based on his ideas regarding the phylogenetic relationship of these ferns with Gleicheniaceae which possess non-indusiate rounded sorus. The writer's view is based on a number of species as intermediates, which show a well-marked tendency to have fugacious indusium—a condition between the non-indusiate sorus on the one hand and a completely naked one on the other. Secondly, the writer is inclined to assume that the rounded, naked sorus in the living members does not imply geological primitiveness because wherever this condition occurs due to geological primitiveness, it has perhaps been retained as such up to the present day as seen in Gleicheniaceae. Furthermore, wherever

this condition has arisen secondarily in the higher members of leptosporangiate ferns, the sorus in such cases tends to elongate along the veinlet as seen in thelypteroid ferns and in many other families of higher leptosporangiate ferns. This tendency has led to the origin of acrostichoid condition seen in many advanced families and is a glaring instance of parallel and convergent evolution (Copeland, 1946). In short, the writer believes that a lopsided sorus with a horse-shoe-shaped persistent indusium is the "parental" or ancestral type amongst the living forms. The persistent nature of this horse-shoe-shaped indusium, which envelop the sporangia on all sides up to the stage when the spores are ready for dispersal, perhaps hinders their adequate dispersal. Therefore, the forms with either the small-sized indusium or with its fugacious nature seem to have overcome the difficulty of proper spore dispersal. A completely naked, rounded sorus thus evolved, then started elongation along the veinlet.

With regard to the shape of the pinna, position of the sorus, and the number of sporangia per sorus, the various species fall in two nearly distinct groups. (1) In this group the species live under shade, possess sub-bipinnate fronds with ultimate segments linear or oblong and the sori are in two distinct rows either proximal to the costa or almost along the central line between the margin and the costa (Text-Figs. 7 *a–c*). The number of sporangia per sorus is comparatively large. (2) The ferns of this group grow in low altitudes and comparatively more exposed localities. These show bipinnate, tripinnate or subquadripinnate condition and decrease in the extent of lamina. The ultimate segments are roundish or oblong and the sori come to lie below the sinus (Text-Figs. 7 *d–e*). A comparatively low sporangial output per sorus is a conspicuous feature of these lowland members such as *T. uliginosa*, *T. serra* and *T. ornata*.

The spores of these ferns are of three types so far as their shape and the nature of the exosporium is concerned. (1) The spores are roundish, brownish-black in colour and the perisporium is broken up into more or less sharp spines (Text-Fig. 8 *a*). To this belong species of *Thelypteris* with $n = 36$, 35 as well as some species of *Cyclosorus* all with $n = 36$. (2) The spores are more or less oblong, brownish with verrucose perisporium (Text-Fig. 8 *b*). This type is exemplified both by some species of *Thelypteris* as well as *Cyclosorus*. (3) This type is exclusively met with in species of *Thelypteris* with $n = 31$ and perhaps species with lesser numbers also possess the same type. They are more or less bean-shaped with poorly developed exosporium which may be reduced to the extent of being apparently absent in some cases (Text-Fig. 8 *c*). The spores are yellowish-brown.

A strongly unifying and constant character of these ferns is the presence of unicellular needle-like hairs or bristles on all the various parts of the plant (sporophyte) including sporangium in those cases where the sorus is naked (Text-Fig. 9 *a*). It is interesting to note that in *T. ornata* which grows in the exposed valleys in Northern Sikkim, there

exist all stages from needle-like hairs to the formation of fully formed scales especially on the stipe and the rachis (Text-Figs. 9 *a–f*).

For gametophytic studies species of *Thelypteris* belonging to two different chromosomal lines, *viz.*, 35 and 31 were selected. Broadly, the development and structure are identical in both the groups except that in species with 31 chromosomes these are not strictly cordate (Loyal, unpublished data). The margins of the prothallus are devoid of unicellular, glandular hairs in the early stages but they start appearing on 1–1½ months old prothalli. These develop directly on the marginal cells of the prothallus. No marginal filamentous projections were observed. This character is also shared by other allied genera, *viz.*, *Cyclosorus*, *Abacopteris* and *Ampelopteris*. Thus, it is clear that like the attributes of the sporophyte of this group, the characters of the gametophyte also indicate a close affinity.

References

Ching, R. C. 1940. On the natural classification of the Polypodiaceae. *Sunyatsenia* **5**: 201–70.

Christensen, C. 1938. Filicinae. *Verdoorn's Manual of Pteridology*. The Hague, pp. 522–50.

Copeland, E. B. 1946. Parallel and convergent evolution in ferns. *J. Arnold Arbor.* **27**: 355–60.

———. 1947. *Genera Filicum*, Chronica Botanica Co., Waltham, Mass., U.S.A.

Dickason, F. G. 1946. A phylogenetic study of the ferns of Burma. *Ohio J. Sci.* **46**: 73–108.

Holttum, R. E. 1947. A revised classification of leptosporangiate ferns. *J. Linn. Soc. (Bot.)* **53**: 123–58.

VARIATION AND EVOLUTION IN *ONYCHIUM*

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To be able to postulate the probable course of evolution in a group of species on indirect evidences, like morphology, anatomy and cytology, is one of the aims of modern phylogenetic research. *Onychium* is a small genus with major distribution in the Sino-Himalayan region, which is the primary reason to discuss the extent of variation in this genus and to visualise the evolution of the polyploids in it.

Till recently three species were recognized in the Himalayan region, namely *Onychium siliculosum* (Desv.) C. Chr., *O. japonicum* (Thunb.) Kze. and *O. contiguum* (Wall.) Hope, and their cytology was reported preliminarily earlier by Mehra and Verma (1957). Present Himalayan collections have revealed the existence of at least six distinct 'taxa' and their taxonomic treatment even with the help of Ching's (1934, 1937) excellent works remains uncertain. It is likely that Prof. Ching never saw a fully representative collection from Sikkim and Eastern Himalayas. However, from an overall survey of the various taxonomic accounts including Hope's (1901) and Kümmerle's (1930) monographic study and the present collections, the genus comprises nearly of ten species (*cf.* Table 1) of which one (*O. strictum* Kze.) is endemic in West Indies and one [*O. melanolepis* (Dcne.) Kze.] is common to Africa and Asia. The rest of the species are well represented in the Sino-Himalayan region especially Yunnan-Himalayas, which may aptly be stated as the centre of distribution of the genus. Presently, except for the two S.W. Chinese species, namely *O. moupinense* Ching and *O. tenuifrons* Ching, the entire Sino-Himalayan element is investigated cytotaxonomically.

In spite of the small size of the genus, the study has revealed considerable variation in morphology, stelar anatomy and cytology, which thus justifies an attempt made here to trace the evolution of the polyploid 'taxa' tentatively in cognizance with morphological data. The extent of variation in the genus can conveniently be dealt with under three heads, namely, Morphology, Anatomy and Cytology, and the evolution of the polyploid 'taxa' is taken up at the end under Evolution of Polyploids.

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* The writer is grateful to Prof. P. N. Mehra for constant encouragement and criticism. He is deeply indebted to Dr. R. E. Holttum (Kew) for his keen interest and identification of the specimens.

TABLE I

Distribution of species in Onychium

Species	Distribution
1. *O. siliculosum* (Desv.) C. Chr.	Farmosa, *Yunnan*, *Himalayas*, New Guinea, Malaysia, Philippine Islands and Cochin China
**O. chrysocarpum* (Hk. *et* Grev.) C. Chr.	North India (*Himalayas*)
2. *O. japonicum* (Thunb.) Kze.	Japan, Polynesia, Philippines, *S. W. China* (esp. *Yunnan*, *Szechwan*), *Himalayas*
3. *O. lucidum* (Don.) Spr. [= *O. japonicum* var. *lucidum* (Don) Christ]	*Sikkim*, *Darjeeling*, *S.W. China* (esp. *Yunnan* and Szechwan)
4. *O. moupinense* Ching	*Szechwan* (S.W. China)
5. *O. ipii* Ching (?) Sikkim material	*Hupeh* (S.W. China) *N. Sikkim* (Chungtang)
6. *O. tenuifrons* Ching	*Yunnan*, *Szechwan*
7. *O. strictum* Kze.	Cuba, Porto Rico, Hispaniola
8. *O. melanolepis* (Dcne.) Kze.	Abyssinia, Nubia, Eritrea, Arabia, Sinai Peninsula, S. Persia, East Indies
9. *O. contiguum* (Wall.) Hope	*Yunnan*, *Szechwan*, *Nepal*, *Sikkim*, *Himalayas*, Tibet (Yatung) and Siam
10. **O. plumosum* Ching	*Yunnan*

‡ Reduced to *O. siliculosum* and *O. contiguum* respectively by the present writer.

MORPHOLOGY

The evaluation of various morphological characters like: rhizome posture, scales, colour of stipe, nature of sori and indusia, colour of ripe sori (capsule), size of sori, nature of spores, and texture has indicated that the genus *Onychium* can be grouped into four distinct sections, namely, *Siliculosum*, *Melanolepis*, *Japonicum* and *Contiguum* (*cf.* Table II). The first group corresponds to section *Euonychium* and the rest three to section *Leptostegia* of Ching (1934) based on soral length and presence or absence of yellow waxy powder coating. Ching's (*l.c.*) division of *Onychium* into the above two sections is quite correct and Kümmerle (1930), too, subdivides the genus into two sections. Leaving aside section *Siliculosum* (one representative), section *Melanolepis* (one

representative) and *O. strictum* (less safe in *Onychium*, *cf.* Copeland, 1947), the rest of the species fall in two groups, section *Japonicum* and section *Contiguum* in which the two basic species *O. japonicum* and *O. contiguum* (s.s.) are diploid sexual with $n = 29$.

Anatomy

It is interesting to observe that at least in the Sino-Himalayan element, the morphological grouping holds good admirably on the basis of rhizome posture and stelar organization, thus confirming further their distinctness (*cf.* Table II). It needs to be pointed out at the outset that Ching (1934) describes the stelar structure in the rhizome as typically solenostelic with two comparatively broad leaf traces which unite upwards as a garter-shaped strand as in *Athyrium*. Copeland (1947) describes the rhizome to be creeping and solenostelic or more often short and compact. Present observations are not in perfect agreement with the earlier concept. In order to stress the identity of each of the morphological group, the anatomical observations are dealt with separately for each section.

Sect. *Siliculosum*:

It is characterized by ascending to sub-erect rhizomes and is represented by only *O. siliculosum* (including *O. chrysocarpum*). The plants inhabit dry rocky crevices and the short somewhat ascending to occasionally sub-erect rhizomes are attached by a thick mat of roots. It has a typical solenostele with a central medullary strand which may branch and anastomose. The central strand is encircled by a parenchymatous zone followed by a sclerenchymatous one. The outer broad cylinder is also encircled both internally and externally by a parenchymatous zone, the latter is followed by a broad sclerenchyma (middle cortex). A unitary U-shaped leaf-trace departs from the outer cylinder and its hollow is occupied by sclerenchyma derived from the inner sclerenchymatous zone encircling the central strand. The foliar gap gets closed immediately by a small daughter strand (commonly known as 'compensation strand') from the medullary stele. The plant, in spite of close setting of leaves, thereby retains the solenostelic nature of its outer cylinder by an immediate closure of the foliar gaps. Such a system affords to bear closely set leaves and yet retain large surface for bearing numerous roots for perfect anchorage and absorption. It also provides the necessary strength. This species thus provides an excellent example of a correlation between habit, habitat and internal structure, which is decidedly an advantageous adaptation.

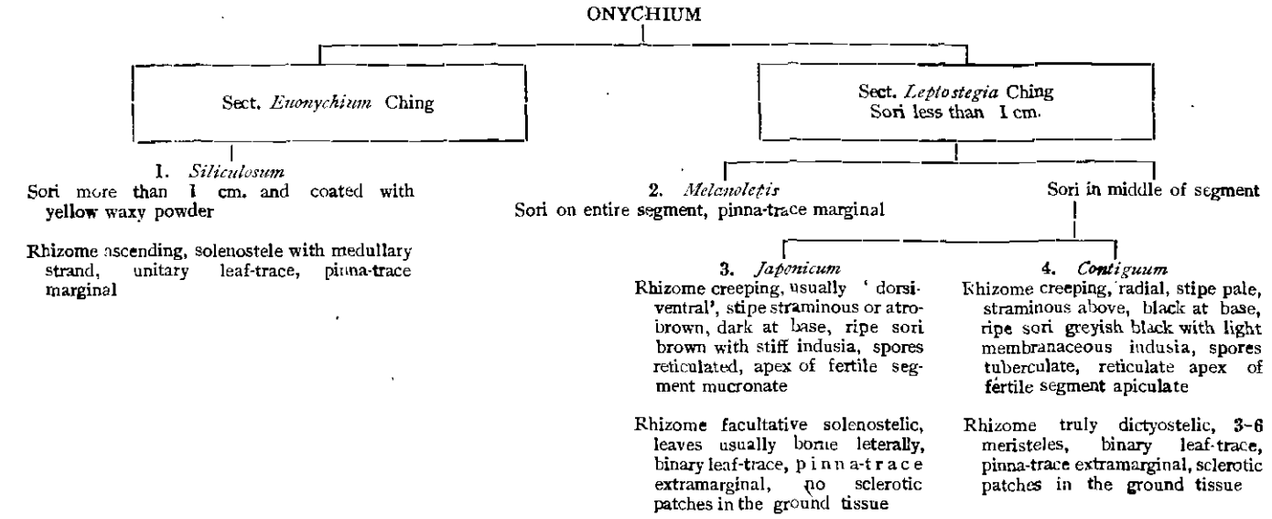
Pinna-trace is marginal and the xylem core in the rhizome is intermixed with sufficient parenchyma.

Sect. *Melanolepis*:

Neither the posture nor the internal structure of the rhizome is as yet investigated, but in its marginal pinna-trace (*cf.* Davie,

TABLE II

Morphological grouping in Onychium



1918) it is certainly not related to *Japonicum* and *Contiguum* groups (the two other segregates of sect. *Leptostegia* Ching, *cf.* Table II) with invariably extramarginal pinna-trace.

Sect. *Japonicum*:

It is characterized by short or long creeping rhizomes, usually with 'dorsiventral' symmetry and alternate lateral bearing of leaves. However, the condition is not strictly dorsiventral since the roots also arise from the dorsal face as well which is most likely the result of submerged nature of rhizomes. Three species are investigated in this group.

Onychium japonicum and *O. lucidum* (= *O. japonicum* var. *lucidum*) have internally two flat ribbon-shaped, or little curved, meristeles lying dorsiventrally, enclosing much elongated foliar gaps laterally. Binary foliar trace departs from almost the middle of a gap. However, initially it is a perfect solenostele from which the mature condition is derived later due to overlapping of the elongated foliar gaps. Usually two foliar gaps are observed in every section. Wetter (1952) reports also 'dictyostelic' condition in *O. japonicum* due to more than two rows of leaves but presently I have not observed it even in specimens from Japan. But all the same it is interesting to note in Abb. 8, II of Wetter (1952) for *O. japonicum* that the additional foliar gaps arise in the dorsal meristele alone, which confirms its 'dorsiventral' nature. This 'dictyostelic' condition may be occasional and such types may aptly be stated as *Facultative Solenostelic*, a term applied by Wetter (*l.c.*). These species are further characterized by the absence of sclerenchyma and by the presence of well-defined and marked endodermis, narrow zone of xylem intermixed with little of parenchyma. Pinna-trace is extramarginal.

The third species *Onychium ipii* (?) from N. Sikkim is truly solenostelic with an irregular arrangement of leaves. Though in general it conforms to *O. japonicum*, yet this type may be strictly transitional to radial types. Foliar gaps are much smaller and a C-shaped structure appears in every section. Even if two foliar gaps per chance may overlap, the stele never gets the *Japonicum* pattern. Foliar trace departs just at the point of initiation of the foliar gap and splits before complete abstriction into a binary trace. Pith region is sclerenchymatous and a portion always extends into the foliar trace. Xylem core is thin, intermixed with little of parenchyma and the pinna-trace is extramarginal.

Sect. *Contiguum*:

It is characterized by wide creeping radially symmetrical rhizomes where the leaf bases on the ventral face are secund. Rhizomes are thicker and truly dictyostelic with usually three to five (occasionally six) small meristeles in a transverse section. Dictyostely is not the result of close setting of leaves but is due to exceptionally elongated foliar gaps from the middle of which binary leaf

trace departs. Meristeles are much smaller, different from the *Japonicum* group in shape, appearance, in having inconspicuous endodermis and sufficient parenchyma admixture in the xylem core. This type is further marked by the presence of sclerotic patches in the ground tissue. The two foliar strands as usual unite high up in the petiole in a Athyrioid manner. Pinna-trace is extramarginal.

The foregoing account reveals that the internal structure is quite distinct in each group and thus can be used reliably in the present cytotaxonomic and evolutionary study of the genus. It seems likely that the original type is solenostelic and dictyostely is derived through facultative solenostelic forms by way of elongated foliar gaps, probably as a natural consequence of the creeping habit.

CYTOLOGY

Chromosome determinations are made only for six Himalayan 'taxa' and the only remaining ones for the Sino-Himalayan region are, *O. tenuifrons* and *O. moupinense* (and also perhaps true *O. ipii*). The latter are S.W. Chinese endemics of the *Japonicum* group, and their study seems essential since all the species of this group are sympatric at least in Yunnan. Chromosome determinations are summarised in Table III.

TABLE III

Chromosome numbers in the Himalayan Onychiums

Species	Locality	*n*-chromosome number	Ploidy
Sect. *Siliculosum :*			
O. siliculosum (Desv.) C. Chr. (syn. *O. auratum* Klf.) (incl. *O. crysocarpum*)	Darjeeling, Manjitar-Teesta-pony road, Badamtam, Rongdong bridge, beyond Teesta (Sikkim)	29	Diploid sexual
Sect. *Japonicum :*			
O. japonicum (Thunb.) Kze.	Mussorie, Karponang (E. Sikkim) and Meerut (cult.)	29	,,
O. lucidum (Don.) Spr.	Darjeeling : Lebong forest	58	Tetraploid sexual
forma *'tenuiseeta'*	Darjeeling : Llyod Botanical Garden	58	,,
O. ipii Ching (?)	North Sikkim : Chungtangh	87	Hexaploid sexual
Sect. *Contiguum;*			
O. contiguum (Wall.) Hope, s.s.	North Sikkim : Lachen	29	Diploid sexual
	Nainital : Laads end		
	Darjeeling : Below Tonglu monastery	'58'	Diploid apogamous
(var. *major*)	Darjeeling : Near Tonglu	'87'	Triploid apogamous

In the group *Contiguum* three cytological races, namely diploid sexual, diploid apogamous, and triploid apogamous, are noticed within the broad limits of the Himalayan species *O. contiguum*. The *Japonicum* group on the other hand contains a diploid sexual (*O. japonicum*), a tetraploid sexual (*O. lucidum*, two forms) and a hexaploid sexual (*O. ipii*). Polyploid 'taxa' in the two groups do not show any intermediate or combination of characters which serve to distinguish the two groups (*Japonicum* and *Contiguum*). This by itself is apparently suggestive of the absence of hybridization between these groups. These groups are perhaps very distinct genetically too and may be a strong barrier to crosss ability exists between them. Furthermore, distinct altitudinal zonation of the two groups, even if occurring in the same region, is perhaps an additional factor (*Japonicum* group occurs usually up to 2200 m., while *Contiguum* group occurs between 2600 m. to 3500 m.). Hence the evolution of the various polyploid 'taxa' presently recorded have to be sought for within the groups themselves.

Onychium siliculosum (incl. *O. chrysocarpum*) in the sect. *Siliculosum* is throughout diploid, $n = 29$. *O. melanolepis* is as yet not investigated.

Evolution of Polyploids

The small size of the genus, limited distributional area, overall distinctness of the two diploids have in fact facilitated the problem. As pointed out above, the evolution of the polyploids is discussed within the groups themselves.

Sect. *Contiguum:*

Ching (1934) includes two species *O. contiguum* and *O. plumosum* in this group, which are distinguished on the basis of length of sori and compact or lax nature of fronds. However, due to inconstancy in Ching's description, it appears that both belong to *O. contiguum*. Secondly *O. plumosum* is based so far only on the type collection from Yunnan and it is not unlikely that it represents a natural variant of *O. contiguum* in Yunnan, where the latter is common.

The three cytological races, $2x$ sexual, $2x$ apogamous, and $3x$ apogamous agree broadly with *O. contiguum* (Wall.) Hope, based on rhizome posture and structure, frond shape, texture, broadly ovate and finely dissected lamina, straminous stipe with black base, tuberculate (girdled) spores, membranaceous contiguous indusia, apiculate, ends of the fertile segments and nature and colour of ripe sori (*cf.* Table II).

Diploid sexual and diploid apogamous types resemble so much that morphologically it is hard to separate them and this is the reason why only diploid apogamous taxon was reported earlier (Mehra and Verma, 1957). The latter is confined to Darjeeling, while the sexual form ranges from Western to Eastern Himalayas. These 'taxa' tally almost exactly with *O. contiguum* (Wall.) Hope (s.s.), though these are more open in branching than Wallich's original specimen. The triploid also belongs to *O. contiguum* but the sori are longer (4–7 mm.)

and the segments are broader. The branching is also more open and lax. This form, because of its morphological distinctness, may be designated presently as var. major. Triploids as well are restricted only to Tonglu in Darjeeling District. Unfortunately 16-celled sporangia are not so far recorded in both the apogamous 'taxa' and thus a part of the direct evidence is lacking.

Except in *Cyclosorus* experimental hybrids (*cf.* Panigrahi and Manton, 1958), all the hybrids and polyploid 'taxa' investigated cytotaxonomically especially by Prof. Wagner and his associates (Michigan, U.S.A.) show always intermediate characters between putative parents. On the basis of morphological intermediacy of the polyploids, Wagner (1954) visualised a reticulate evolution in Appalachian Aspleniums. In fact some of the polyploid 'taxa' have actually been synthesized later by Prof. Wagner and his students (*cf.* Wagner and Darling, 1957; Wagner and Whitmire, 1957) from the previously suggested parents. It is, therefore, logical to rely, apart from experimental evidence, on morphological characters for tracing the evolution of the diploid and the triploid apogamous 'taxa' here.

Apogamous taxa have so far been believed to be of hybrid origin (*cf.* Manton, 1950) and hybrids between distinct taxa are generally intermediate, which condition can admirably be retained by the very nature of reproductive system in apogamous ferns. The broad morphological similarity of the diploid sexual and diploid apogamous forms most likely suggests that the only other diploid in the area, *Onychium japonicum*, is not involved in the origin of the diploid apogamous taxon. *Onychium japonicum* is both morphologically and anatomically very distinct from *O. contiguum* (*cf.* Table II) and it is inconceivable that none of the *Japonicum* characters would be represented in such hybrids. The only other possibility which seems highly probable is the origin of the diploid apogamous taxon in a previously sexual species which still exists and has a wider distribution. The restricted occurrence of the apogamous taxon coupled with its extreme similarity with the sexual taxon strongly supports this hypothesis. No doubt the direct experimental evidence and the nature of chromosomal associations in the 16-celled sporangia are lacking but the small size of the genus, limited distributional area, overall distinctness of the two diploids (*O. contiguum* and *O. japonicum*), permit reasonably good reliance on the indirect evidence.

However, it is not intended to generalise this hypothesis, particularly for those diploid apogamous taxa which resemble very closely their sexual relatives. Each case needs to be evaluated critically on its own merits, but one can safely conclude that apogamy may not be always preceded by hybridity (at the species level). Such a possibility is certainly not new since it has already been referred to by Manton (1950) in *Pteris cretica* L. although rejected in favour of hybridity. Theoretical possibility was, however, suggested by Wagner (1951). Recently Loyal (1960) seems to have hinted at such a possibility, though not explicitly

stated, in case of the Himalayan diploid apogamous *Dryopteris paleacea* (Don) Hand-Mazz. [= *D. wallichiana* (Spr.) Hyld.].

The triploid again presents the same situation. Qualitative analysis reveals it to be a true *Contiguum* representative with no *Japonicum* character in it. Quantitatively the triploid is gigas as compared to the diploid sexual and apogamous taxa. The gigantism is best manifested in the soral length and size of ultimate segments. Sixteen-celled sporangia probably do not occur in sufficient number so as to be detected in few field fixations. The origin of the triploid apogamous form is conceivable since the diploid sexual and apogamous forms occur in the same area. The restricted distribution of the triploid favours this hypothesis and the gigantism in some characters is most likely due to its being an '*autotriploid*'. It is likely that both the apogamous taxa are of recent origin and as yet have not spread appreciably. Further work on the distribution of cytotypes is in progress.

Sect. *Japonicum:*

So far three taxa have been discovered in the Himalayas: *Onychium japonicum* (diploid), *O. lucidum* (tetraploid) and *O. ipii*? (hexaploid). From a morphological comparison of the entire *Japonicum* group, it is possible to trace out the evolution of the two polyploids. Pending their synthesis, the suggestions regarding their parents are to be treated as tentative.

Onychium lucidum [= *O. japonicum* var. *lucidum* (Don) Christ]:— This Himalayan and Western Chinese fern occurs in two morphological forms, both being tetraploid sexual. They undoubtedly share the general pattern of *O. japonicum* from which it is separated by larger size, stouter habit, coarser texture with thick stipe shaded rufo brown (*O. japonicum* may also have such stipes) and somewhat larger sori. Close and critical morphological comparison of diploid *O. japonicum* and tetraploid *O. lucidum* reveals an overall similarity in pattern, and yet the two are quite distinct for treating the latter as a distinct species on cytogenetical grounds. The very fact that the tetraploid has been taken to be a variety of *O. japonicum*, indicates that probably the latter represents one of the parents. Secondly, the somewhat contiguous indusia, pale brown-lemon-coloured ovate scale, somewhat deeply undulated to slightly erosed indusial margin and acute teeth of the sterile fronds (in some populations) in the tetraploid are essentially the characters which characterise *O. tenuifrons* (Western Chinese endemic). Possibly the latter is the other parent involved in the origin of *O. lucidum*. Some of the tetraploid populations come closer to *O. tenuifrons* especially in the acute teeth of the sterile fronds, pale scales and erosed indusial margin but still are intermediate between *O. japonicum* var. *lucidum* and *O. tenuifrons* (typical). The tetraploid shows variations probably connecting the two parents. It is significant that Ching (1937) has referred *O. lucidum* Kümm. *pro parte* as a taxonomical synonym of *O. tenuifrons*. The intermediacy in morphological characters of the tetraploids between *O. japonicum* and *O. tenuifrons*

is expected in genomic alloploids. All the three taxa (*O. lucidum*, *O. tenuifrons* and *O. japonicum*) are sympatric in Western China especially in Yunnan (*cf.* Table 1 and Ching, 1934). A logical conclusion arises that *O. tenuifrons* should be entirely diploid or has diploid races. This awaits confirmation; however, an examination of spores by me shows that the structure and size is like the other diploids in the sect. *Japonicum*.

Onychium ipii? Hexaploid:—It agrees very closely with Yunnan (Hupeh) endemic *O. ipii*, though it still requires confirmation. According to Ching's (1934) key it resembles *O. japonicum* var. *lucidum* but the upper part of the stipe and rachis and also the lamina are darker than Wallich's specimen No. 69 quoted by Ching. Further, it differs from var. *lucidum* in scales, rigid texture and indusial margin; above all in anatomy and less pinnate fronds with lamina narrowly lanceolate on a deltoid base. These characters are equally characteristic of both *O. ipii* and *O. moupinense*, though the latter is dimorphic with the sterile leaves being much smaller. Sori are 5–7 mm. in *O. moupinense* and 2 mm. in *O. ipii*. In the present case these vary between 2 and 3·5 mm. Fronds are much larger than both, rigidly herbaceous and have raised veins. In the absence of a truly dimorphous nature, the present taxon is taken tentatively to be *O. ipii*, which is a totally fresh record in Himalayas, being plentiful in N. Sikkim at Chungthang.

Meiosis in the hexaploid is perfectly normal which possibly implies cytogenetically that three different genomes are involved. Its close resemblance in its rigid texture, general shape of fronds, less pinnation and raised veins to *O. moupinense* is perhaps indicative of the latter being parental to hexaploid *O. ipii*. Both these taxa are met with in S.W. China especially Szechwan (Moupin) and Hupeh. The only tetraploid encountered here and also available in Szechwan (Moupin) is the widely distributed *O. lucidum*, to which incidentally the present taxon can be referred to, following Ching's key (1934). Thus, morphologically it seems probable that *O. lucidum* and *O. moupinense* are parental to *O. ipii* (6 *x*). It is not unlikely that such assumptions may prove incorrect with further search in S.W. China especially Yunnan which may reveal some more elements that could altogether change the pattern of discussion.

Stated in brief, the present results indicate that morphological variation in the *Japonicum* group is intrinsic and speciation in it is possibly the result of genomic allopolyploidy. On the other hand, the sect. *Contiguum* has developed the apogamous habit within itself at the diploid level and the limited extent of variation is due to apogamy followed perhaps by a back-cross with the diploid sexual resulting in the 'autotriploid' apogamous type. Detailed accounts of the investigations shall appear separately.

References

CHING, R. C. 1934. On the genus *Onychium* Kaulf. from the Far Orient. *Lignan Sci. J.* **13**: 493–501.

———. 1937. *Icones Filicum Sinicarum* Fascicle. 4. *Fan. Mem. Inst. Biol.*, Peiping (China).

COPELAND, E. B. 1947. *Genera Filicum.* Chron. Bot. Publ., Walth., Mass.

DAVIE, R. C. 1918. A comparative list of Fern pinna-traces, with some notes on the leaf-trace in the Ferns. *Ann. Bot. Lond*, **32**: 233-45.

HOPE, C. W. 1901. The ferns of North-Western India—Part IIII. The general list. *J. Bombay nat. Hist. Soc.* **13**: 443-61.

KÜMMERLE, J. B. 1930. Has the genus *Onychium* any representative in South America? *Amer. Fern. J.* **20**: 129-38.

LOYAL, D. S. 1960. Some observations on the cytology and apogamy of Himalayan *Dryopteris paleacea* (Don) Hand-Mazz. *J. Indian bot. Soc.* **39**: 608-13.

MANTON, I. 1950. *Problems of Cytology and Evolution in the Pteridophyta*, Cambridge University Press.

MEHRA, P. N. AND VERMA, S. C. 1957. Cytology of Indian species of *Onychium*. *Nature, Lond.* **180**: 715-16.

PANIGRAHI, G. AND MANTON, I. 1958. Cytological and taxonomic observations on some members of the *Cyclosorus parasiticus* complex. *J. Linn. Soc.* (*Bot.*) **55**: 729-43.

WAGNER, W. H. JR. 1951. Cytotaxonomic analysis of evolution in pteridophyta. *Evolution* **5**: 177-81.

———. 1954. Reticulate evolution in Appalachian Aspleniums. *Ibid.* **8**: 103-18.

—— AND DARLING, T. JR. 1957. Synthetic and wild *Asplenium gravesii*. *Brittonia* **9**: 57-63.

—— AND WHITMIRE, R. S. 1957. Spontaneous production of a morphologically distinct fertile allopolyploid by a sterile diploid of *Asplenium ebenoides*. *Bull. Torrey bot. Cl.* **84**: 79-89.

WETTER, CARL. 1952. Untersuchungen uber Anordnung und Anlegung der Wurzeln bei leptosporangiaten Farnen. Akad. der Wissen. und der Lit. Abhandlungen der Math. *Nat. Klasse Jahrgang* (Mainz) **1**: 26-84.

EVOLUTION IN THE INDIAN MEMBERS OF THE GENUS *ASPLENIUM* LINN.

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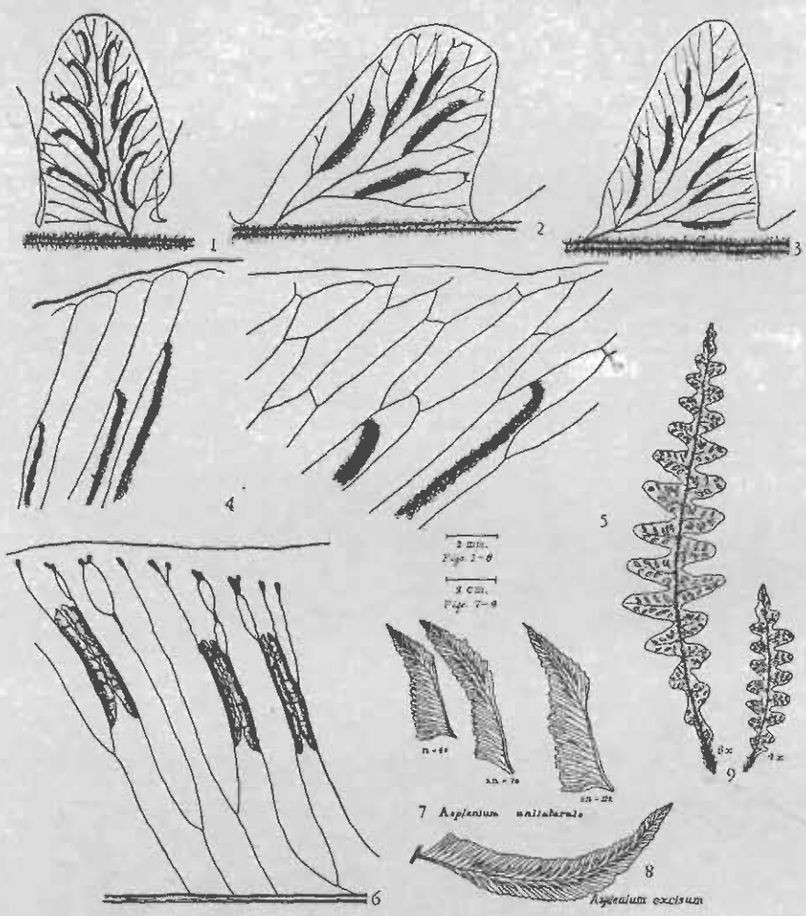
The interest in fern phylogeny has been revived during the last two decades and now sufficient morphological and cytological data on Pteridophytes have accumulated so as to enable us to follow evolution in any particular genus. *Asplenium* Linn. has been taken up particularly because it is the most distinct and is the large central genus of the family Aspleniaceae which has been my main interest. Furthermore, it is one of the earliest recognized genera and was included amongst the fourteen genera of ferns listed by Linnaeus (1753) in *Species Plantarum*. There is one more advantage and that is, the genus *Asplenium* is cosmopolitan in distribution, flourishing practically in all the habitable lands and that is why its study has received so much attention. So far, it is perhaps the best understood genus.

In the present communication the evolution in *Asplenium* has been explained from two aspects, namely, morphological and cytological, with a main stress on the data available from the studies of the Indian members.

Morphological Evolution

The genus exhibits extreme types of diversity as far as the outline of frond, venation and soral characters are concerned. It is such a vast genus that without the study of good amount of material from different regions nothing can be postulated about various groups within it. However, during the course of study of the Indian members some different elements have been noted. A brief outline of these is given below.

(1) *Asplenium Dalhousiae-paucivenosum* group.—This group of *Asplenium* species has only pinnatifid fronds which are thick and opaque, small in size and very similar in outline to *Ceterach officinarum* Lam. *et* DC. Only two ferns, namely, *A. Dalhousiae* Hook. and *A. paucivenosum* (Ching) Bir. are included here. The venation in these is free as a rule, but in *A. Dalhousiae* one or two veinlets near the margin casually anastomose, thus showing an intermediate condition between *Asplenium* and *Ceterach* (Text-Fig. 1). It may be pointed out that in *Ceterach* the veins typically anastomose towards the margin (Text-Fig. 2) but in few samples of *C. officinarum* from Kulu in the Western Himalayas, the veins are free as in *A. Dalhousiae* with only one or two fusions (Text-Fig. 3). Here is a clear indication as to how *Ceterach* has possiby evolved fom *Asplenium* through *A. Dalhousiae*. Ching



TEXT-FIGS. 1–9. Fig. 1. Pinna lobe of *A. Dalhousiae* showing fusion of one or two veinlets near the margin. Fig. 2. Pinna lobe of *Ceterach officinarum* showing normal venation, here almost all the veinlets near the margin anastomose. Fig. 3. Pinna lobe of *C. officinarum* showing abnormal venation where practically all the veinlets are free. Fig. 4. Portion of a frond of *Asplenium nidus* showing the venation near the margin. All the veinlets are connected with each other by intramarginal vein. Fig. 5. Portion of a frond of *A. finlaysonianum* showing the fusion of veinlets near the margin resulting in the formation of areolae without any included veinlet. Fig. 6. Portion of frond of *Phyllitis scolopendrium* showing 'island' type of areolae arising from a single veinlet and geminate sori. Fig. 7. One pinna each from *uni*diploisexual 'diploid' hybrid, and 'triploid' hybrid individuals found in *Asplenium laterale* species complex. Fig. 8. One pinna from *A. excisum*, this is very similar to the pinna line in case of *A. unilaterale*. Fig. 9. Two fronds from octoploid and tetraploid individuals of *A. paucivenosum*.

(1940 *b*) has based a genus *Ceterachopsis* on these two species of *Asplenium* referred to above and *A. Dalhousiae* typifies it. These two species exhibit so many characters of *Asplenium* that they cannot be removed from here and either raised to a new genus or added to *Ceterach* as was done by Christensen (1906, 1938) in case of *A. Dalhousiae*. By so doing the entire definition of *Ceterach* will collapse which shows an advancement in the fact that the indusium is totally abortive. The loss of indusium is possibly due to the fact that the undersurface of the frond is densely covered with scales and the protective function of the indusium has been obliterated. The presence of scales is an adaptation to the xerophytic environments in which the members usually grow.

(2) *Bird's nest fern group.*—In this group there is an advancement over the previous one. It is represented by *A. nidus*, *grevillei* and *simosianum* and is characterised by the presence of a transverse intramarginal vein connecting the apices of veins or veinlets and by the presence of sori on the side of veinlet towards the apex of the frond (Text-Fig. 4). These ferns are simple-leaved. The presence of the connecting vein has the obvious advantage of protection from any tearing-off of the frond which in case of *A. nidus* may reach a metre or more in length and 10–20 cm. in width. Out of these, *A. nidus* typifies *Neottopteris* J. Smith (or *Thamnopteris* Presl).

(3) The third group is represented by *Asplenidictyum* of J. Smith and only one species, *A. finlaysonianum* is met with in India which incidentally typifies the genus. The veinlets in this case anastomose completely towards the margin of the frond so as to form areolae without included veinlets (Text-Fig. 5). This fern has very variable type of frond outline, from simple to pinnate, and when simple sometimes there is no fusion of veins, indicating that this is not a constant feature. However, it points out the advancement over free-veined representatives.

The foregoing evolutionary tendencies in *Asplenium* are recognizable only when the modern concept of the genus is accepted. Each of these elements had at one time or the other been elaborated by the creation of a genus. But these genera, namely, *Ceterachopsis*, *Neottopteris* and *Asplenidictyum*, which represent each group, cannot stand because of plastic characters. Earlier authors like Christensen (1938) and Ching (1940 *a*) recognized these genera but with the advancement of knowledge several modifications have been suggested. Copeland (1947) merged all these in *Asplenium* which is a very sound proposition. The present-day complexities represented by *Neottopteris*, *Ceterachopsis* and *Asplenidictyum* are the cases of specialization over *Euasplenium* and are, therefore, the end-points.

About the group of ferns represented by *Phyllitis* (= *Scolopendrium*) Bower (1928) remarks "*Phyllitis*, even in its most condensed and derivative leaf forms, is a natural genus sprung from a Blechnoid source and that *Blechnum punctulatum* var. *krebsii* gives a true key to its origin". Similar is the origin of *Camptosorus* according to Bower. However, the cytological evidence points otherwise. *Phyllitis* and

Camptosorus have the same basic number 36 as in *Asplenium*, while *Blechnum* has 28, 32, 33, 34. Furthermore, *Phyllitis* and *Asplenium* hybridize freely in nature which shows that genetically these two genera are very close to each other. There is no hybrid so far known between *Phyllitis* and *Blechnum*. This disproves at least on cytological grounds the evolution of *Phyllitis* and *Camptosorus* from a Blechnoid source. I am of the view that in all probability *Phyllitis* and *Asplenium* have evolved from a common source. The morphological similarities of *Phyllitis scolopendrium* and *Blechnum punctulatum* var. *krebsii* are probably due to the homoplastic developments.

Copeland's (1947) contention of complete merger of *Phyllitis* with *Asplenium* does not hold good. *Phyllitis* has a special type of venation and double sori and so far no *Asplenium* is known which has got the venation of the type seen in *Phyllitis*. In *Phyllitis scolopendrium* (= *A. scolopendrium* Linn.) the venation is of most interesting type, each vein bifurcates usually once and rarely twice. The veinlets in turn often bifurcate towards the margin, and then fuse together to form a sort of areolae and this may happen twice on a single veinlet. Hardly there is any fusion of primary veinlets to form areolae (Text-Fig. 6). This 'island type' areolae arising from a single veinlet is characteristic of at least the type species of the genus. The sori in *Asplenium* are sometimes double but they are never in pairs as in *Phyllitis*. On the basis of these two characters of peculiar venation and geminate sori, *Phyllitis* deserves a special status. In order to avoid the multiplicity in the number of genera and to give proper representation to those elements in *Asplenium* that show geminate sori and peculiar venation as pointed above, it is suggested that *phyllitis* may be recognized as a subgenus within large and comprehensive genus *Asplenium*. The elements represented by *Phyllitis* show advancement over *Euasplenium* in the position of sori and peculiar venation.

Cytological Evolution

The Himalayas are one of the important centres where *Asplenium* flourishes very well in number of individuals as well as of species. It is represented by a total of 36 (unpublished data) species in the area, out of which 23 species spreading over 36 taxa have been worked out by the author. A preliminary report on these findings has already been published (*cf.* Bir, 1960). It may be pointed out that there is no other work done so far on the Indian members. In the following pages the details about the interesting species are enumerated:

(1) *Asplenium unilaterale* Lam.—This species presents one of the most interesting cytologieal situations that has been observed in the ferns so far. In this 'species complex' in addition to the sexual race profusely flourishing near Darjeeling in the Eastern Himalayas, two dibasic natural hybrids have been detected. The present species is based on $x = 40$ which is in strong contrast to the rest of the worked-out species of the genus throughout the world.

It is distributed throughout the Himalayas, South India, Ceylon, Burma, Malaya, China, Japan and Tropical Africa. There is good amount of morphological variation in the present species, mainly noticeable being the size and colour of the stipe, the extent to which the midrib runs parallel to the lower margin and finally the size and outline of the pinnae. In Darjeeling area where extensive investigations have yielded interesting results, the typical form grows abundantly on the forest floor near Lebong, 5,000 ft. The individuals cover large areas. The following three 'cytotypes' within the morphological range of the typical form have been discovered.

(i) *A 'diploid' sexual with* $n = 40$.—The course of meiosis is regular and normal viable spores are the result. This is by far the commonest race.

(ii) *A 'diploid' hybrid showing* $2n = 76$.—This is a totally asynaptic form, indicating that the two genomes contributed by the parents are altogether non-homologous. All irregularities of meiosis are noticeable and the abortive spores are the ultimate result.

(iii) *A 'triploid' hybrid showing* $2n = 112$.—Here the pairing phenomenon is somewhat different. The number of univalents varies between 36–44 and bivalents 34–38 indicating that two sets are homologous while the third is non-homologous. This too produces bad spores as the result of irregular meiosis.

Repeated attempts were made to grow the spores of these hybrids but without success. Even the diads produced by the 'diploid' hybrid were not functional.

The two hybrids are very rare. As far as the morphological differences are concerned, the hybrids are not in any way significantly different from the sexual race. In fact, in the field these cannot be segregated from the sexual race (Plate III, Fig. 1). One pinna each from the diploid sexual, 'diploid' hybrid, and 'triploid' hybrid individuals is shown in Text-Fig. 7. In the extreme cases one can make out some differences amongst these 'cytotypes', however, from the amount of variation noticed in sexual race one cannot put forward a case for their morphological distinctness. The details about stomatal size, their distribution pattern and structure are rather uninformative. The question that arises next is how the hybrids have come into being? The diploid sexual race may be represented by AA, 'diploid' hybrid as AB and 'triploid' hybrid as ABB, where A represents a genome of 40 and B of 36 chromosomes. About the parentage of the hybrids nothing can be inferred except that one of the parents is the diploid sexual race of *A. unilaterale* which has played a dominant role as far as the phenotypic characters are concerned. The other parent is definitely based on 36. There is only one species of *Asplenium*, namely, *A. tenuifolium* Don which is diploid ($n = 36$) in the area but that cannot be the other parent because of the large-scale differences in the morphology of frond and the rhizome. So the other parent of these hybrids can only be found

amongst the closely allied members of *A. unilaterale*, which are *A. excisum*, *A. obscurum* and *A. cheilosorum*. Recently Holttum (1954) adds *A. normale* to this group. Out of these, *A. cheilosorum* cannot be one of the parents of the hybrids because it is a triploid apomict with $2n = 108$ (*cf.* Manton and Sledge, 1954; Mehra and Bir, 1960). The second species, *A. normale*, is a tetraploid ($n = 72$) in the Himalayas (*cf.* Bir, 1960) and Ceylon (*cf.* Manton and Sledge, 1954), while a diploid ($n = 36$) in Malaya (*cf.* Manton, 1954). The other two species are still unworked. Attempts were made to cross *A. unilaterale* ($n = 40$) with *A. normale* ($n = 72$) but these were unsuccessful. *Asplenium excisum* is very nearly allied to *A. unilaterale* and often confused with it and is also met with in the Eastern Himalayas (N. Sikkim) growing practically by the side of the other species (Text-Fig. 8). Therefore the two possibilities about the second parent of *A. unilaterale* hybrids are:

(i) There already exists in this 'species complex' hitherto undiscovered a form with $n = 36$ and the species is dibasic or

(ii) The closely allied species, *A. excisum*, may be based on 36 and has served as one of the parents.

For the proper understanding of the problem efforts are being made to work out cytologically *A. unilaterale* throughout its distributional range. The dibasic hybrids with $2n = 76$ $(40 + 36)$ and $2n = 112$ $(40 + 36 + 36)$ referred to above are the first cases known to the author in ferns. This formation of a hybrid with $2n = 76$ $(40 + 36)$ paves a way for the evolution of a new secondary basic number in the genus *Asplenium*.

In addition to the typical variety of *A. unilaterale*, two varieties, namely, *delicatulum* Par. and *udum* Atk. are also met with in the Himalayas. These are much smaller in size as compared to the typical form and are sexual diploids based on $x = 40$ and this confirms the results for *A. unilaterale*. Furthermore, there exists in nature near Darjeeling a triploid hybrid with $2n = 120$. In this case during meiosis multivalents are commonly formed and phenotypically this hybrid is exactly similar to var. *udum* Atk. Therefore, this taxon has been considered to be an autotriploid of var. *udum* Atk.

(2) *Asplenium varians* Hook. *et* Grev. exists in two forms in the Himalayas, of which the diploid ($n = 36$) is the commonest throughout. A tetraploid ($n = 72$) was collected in North Sikkim where a diploid also grows nearby. The quantitative differences in size of the frond spore and stomata are not well pronounced here.

(3) *Asplenium paucivenosum* (Ching) Blir.—Like the previous species, this fern has two 'forms', a tetraploid ($n = 72$) and an octoploid ($n = 144$), the former being rare, having been collected only once, while the latter is quite abundant near Darjeeling and in North Sikkim. There are marked quantitative differences in the $4x$ and $8x$ individuals. The octoploids show broader and larger frond size (Text-Fig. 9) and possess bigger stomata and spores.

(4) *Hybrids.*—Out of the six hybrids detected in the Himalayas, two dibasic hybrids and one autotriploid belonging to the *A. unilaterale* 'species complex' have already been referred to above. The other two are, diploid (*A. griffithianum* with $2n = 72$) and tetraploid (*A. laciniatum* var. *subintegrifolium* with $2n = 144$). *Asplenuim griffithianum* is perhaps the most beautiful fern of *A. ensiforme* group. The individuals of these hybrids survive year after year through rhizomes. The sixth hybrid is, *A. cheilosorum*, a triploid apogamous fern with $2n = 'n' = 108$. This is the only apomictic 'spleenwort' within the Darjeeling-Sikkim Himalayas. From cytological considerations it is a very interesting species and has been worked out in detail by Mehra and Bir (1960). In 16-celled sporangium roughly the pairing position is $36_{II} + 36_{I}$ while in 8-celled sporangium which is the result of failure of premeiotic mitosis, 108 'autobivalents' are present at meiosis. The spores from 8-celled sporangia are normal and viable. Quite often double premeiotic divisions fail leading to the formation of four giant mother cells with 216 'autobivalents'. Some of the spore mother cells from 4- or 8-celled sporangia undergo irregular cytokinesis due to an irregular lobing and cleavage of the nucleus, as a result of which unequal cells with variable number of 'autobivalents' as 39, 43, 47, 63, 72, 113, 116, 117, 124, etc., are discernible in different cells. The species is well spread in the Himalayas and several populations have shown similar results. Populations from Ceylon likewise are triploid apogamous (*cf.* Manton and Sledge, 1954).

Conclusions

The existence of the kind of cytological variations as shown above in the Himalayan members of *Asplenium* is very interesting from evolutionary viewpoint. Moreover, the detection of several 'cytotypes' within various species from the Himalayas reveals that polyploidy and hybridity have played a significant role in the evolution of 'micro-species' in the region. The available data[1] (*cf.* Mehra and Bir, 1957; Bir, 1959, 1960) for the Indian species is presented in Table I.[2]

The analysis of Table I shows that amongst the sexual taxa which are 83·33% of the total investigated, the tetraploids are the most common while in case of the hybrid taxa, the triploids are in abundance. It also indicates that in the Himalayan 'spleenworts' the incidence of hybridity (16·67%) is substantially high. Furthermore, the percentage of polyploidy within the genus in the Himalayas with warm to cold temperate climate[3] is significantly high (77·78%) but polyploidy is

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[1] The two species not reported eerlier are: *A. septentrionale* (L.) Hoffm. (Kashmir), $n = 72$; and *A. sarelii* Hook. (Simla), $n = 72$; both being tetraploid sexual.

[2] All the taxa have been investigated from the Himalayas.

[3] At the foot of the Himalayas particularly in the east, up to 4,000 ft., the climate is tropical or sub-tropical but very few species (*Asplenium falcatum, macrophyllum, indus, finlaysonianum* and *nitidum*) grow in this belt and the majority are found between 4,000–10,000 ft. that is under warm temperate climate.

TABLE I

Summary of the cytological work done on Asplenium *in India*

Grade of Ploidy	2*x*	3*x*	4*x*	8*x*	Total taxa	Percentage
Sexual (fertile) ..	6	..	23	1	30	83·33
Hybrids (sterile) ..	2	3	1	..	6	16·67
Total ..	8	3	24	1	36	100
Percentage ..	22·22	8·33	66·67	2·78	..	..

known only up to an octoploid level. When compared with other genera of ferns from the Himalayas, namely, *Athyrium*, *Diplazium*, *Dryopteris*, etc.. *Asplenium* shows the highest percentage of polyploids in the area which clearly indicates that the genus is in an active state of evolution in this phytogeographically important region whose fern flora has similarities with that of Burma, Malaya and China (Yunnan Province). Further, a comparison of cytological data on *Asplenium* from the Himalayas and other areas, such as Ceylon and Tropical Africa, North America and Europe gives a clear support to Manton's (1953) observation that the grade of polyploidy is definitely higher in the tropics than in the temperate.

The entire data on the genus throughout the world shows that *Asplenium unilaterale* is the only species with $x = 40$ while the rest are based on 36. This detection of $x=40$ in *A. unilaterale* gives some support to Hayata's (1927) segregation of this species into a new genus *Hymenasplenium* which was erected on certain supposed anatomical peculiarities of *A. unilaterale*, the type species of the genus. However, Tagawa's (1938) and Holttum's (1954) reference of *A. cheilosorum* and *A. normale* also to the genus *Hymenasplenium* is untenable on cytological grounds since both of these species are based on 36 (*cf.* Manton and Sledge, 1954; Manton, 1954, Mehra and Bir, 1960; Bir, 1960). The morphological similarities of *A. cheilosorum* and *A. normale* with *A. unilaterale* go to prove that the evolution of all these ferns is from a remote common element in *Asplenium*. Since there are no specific morphological or anatomical characters on which *Hymenasplenium* can stand separately from other *Asplenium* species, therefore a genus cannot be recognized on purely cytological grounds. Hence *A. unilaterale* deserves a place in *Asplenium* as has long been thought. Therefore, the different basic number in *A. unilaterale* clearly shows that the genus *Asplenium* is dibasic, composed of cytologically two distinct elements although not so morphologically. The basic number 40 in all probability has evolved by aneuploidy from 36.

My sincerest thanks are due to Prof. P. N. Mehra for very kindly going through the manuscript and for giving valuable suggestions.

REFERENCES

BIR, S. S. 1959. Cytotaxonomic notes on some Aspleniaceae from Kulu valley. *J. Indian bot. Soc.* **38**: 528–37.

———. 1960. Cytological observations on the East Himalayan members of *Asplenium* Linn. *Curr. Sci.* **29**: 445–47.

BOWER, F. O. 1928. *The Ferns* 3: Cambridge University Press.

CHING, R. C. 1940*a*. On Natural classification of family Polypodiaceae. *Sunyatsenia* **5**: 201–70.

* ———. 1940*b*. *Bull. Fan. Mem. Inst. Biol. Bot. Ser.* **10**: 9.

CHRISTENSEN, C. 1906. *Index Filicum*, apud H. Hagerup, Hafniae.

———. 1938. Filicinae in Verdoorn, F., *Manual of Pteridology*, Martinus Nijhoft, The Hague.

COPELAND, E. B. 1947. *Genera Filicum*, Waltham, Mass., U.S.A.

* HAYATA. 1927. *Bot. Mag., Tokyo* **41**: 712.

HOLTTUM, R. E. 1954. *Flora of Malaya* **2**: *The Ferns*, Govt. Press, Singapore.

LINNAEUS, C. 1753. *Species Plantarum* **2**: Holmiae.

MANTON, I. 1953. The cytological evolution of the fern flora of Ceylon. *Sym. Soc. Exp. Biol.* (Evolution) 7: 174-85.

———. 1954. Cytology of one hundred ferns. Appendix in R. E. Holttum's *Flora of Malaya* **2**: *The Ferns*, Singapore.

—— AND SLEDGE, W. A. 1954. Observations on the cytology and taxonomy of the Pteridophyte Flora of Ceylon. *Phil. Trans.* **238 B**: 127–85.

MEHRA, P. N. AND BIR, S. S. 1957. Cytology of some Indian species of genus *Aspelenium* Linn. *Curr. Sci.* **26**: 151–52.

———. 1960. Cytological observations on *Asplenium cheilosorum* Kze. *Cytologia* **25**: 17–27.

* TAGAWA, M. 1938. *Acta Phytotax. et. Geobot.* 7: 84.

* Not seen in original.

EXPLANATION OF PLATE III

FIG. 1. Fronds from the diploid sexual ($n = 40$), 'diploid' hybrid ($2n = 76$), 'triploid' hybrid ($2n = 112$) individuals discovered in *Asplenium unilaterale* species complex.

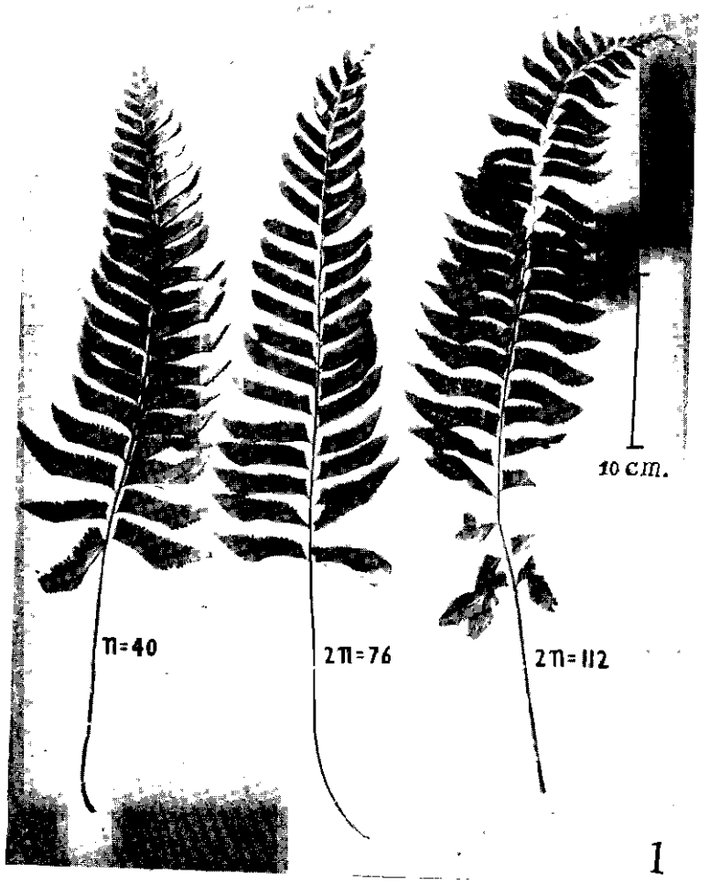

FIG. 1

EVOLUTIONARY TENDENCIES IN THE GENUS *AZOLLA*

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The paper gives an account of the main evolutionary tendencies in the genus *Azolla* which is essentially a Tertiary genus. It is one of the heterosporous ferns the origin of which is still shrouded in mystery. Some botanists trace heterosporous ferns back to Westphalian genus *Mittagia* (Emberger, 1949), some to Jurassic genera (Knowlton, 1919) and some to Carboniferous coenopterid *Stauropteris* (Andrews, 1960). Among living ferns, *Azolla* is considered to be related to *Salvinia*, both of them being thought to show Hymnophyllaceous affinity on account of their peculiar sporocarps, elongate receptacle with basal growth, sporangia with long stalks lacking annulus, thin and papery indusium—the covering of the sporocarp. The latter are often homologised with sori included under thin indusium as in Hymenophyllaceae or Cyatheaceae.

The main parts of this plant that seem to have materially changed in course of evolution appear to be (1) massulae in microsporocarps, (2) float carpuscles associated with megaspores in megasporocarps and (3) vegetative body of the plant and its reduced stem stele. Chronologically the most ancient species of the genus is the Indian member belonging to Eocene period, called *Azolla intertrappea* by Sahni and Rao (1943). In this species the massular segments are 8. The float corpuscles are 8–9 and the stele is a simple protostele with a few tracheids. The long and delicate glochidia are anchor-shaped but apparently with no partition walls in them. Characters similar to these are seen in the living species *A. filiculoides*, *A. maxicana*, *A. macrocarpa* and *A. rubra*, each having some variation in the detail of anchor-shaped glochidia or massulae. But all of them agree in having anchor-shaped glochidia. They constitute a group of species by themselves that may be called *Azolla filiculoides* complex.

In the second group of species the number of float carpuscles is 4–8 and glochidia are simple, hair-like, branching occasionally. The plants are smaller than those of *A. filiculoides* and have much more reduced stele—a protostele made up of one or two tracheids. The living species *A. pinnata* of India, Japan and Australia belongs to this group. But curiously enough it is found in fossils in the late Tertiary formation of Holland and Volga basin. It is largely an Oligocene form continuing since then till today.

However by far the most interesting features are presented by the living species of *A. nilotica* from West Africa described by Demalsy

(1953). In this species plants are much branched and the stele is amphiphloic siphonostele. There are 8 float corpuscles, but surprisingly the number of massulae in a microsporangium is reduced to 4–2 and they have no glochidia. There is thus (1) a progressive reduction in the stele either in size or character, both in *A. filiculoides* complex and *A. pinnata* and (2) in the structure of massulae which have anchor-shaped glochidia in primitive species and have many partitions in them. Progressively they appear to lose both partitions and blade of the anchor and have become hairy as in *A. pinnata*, and eventually become eglochidiate as in *A. nilotica*. The species thus may be arranged in the following series on the basis of reduction in the structure of glochidia.

1. *A. filiculoides*, *A. maxicana*.—Glochidia long, anchor-shaped and with many partitions.

2. *A. rubra*, *A. prisca*, *A. intertrappea*, *A. primaeva*.—Glochidia anchor-shaped, partitions few or probably none.

3. *A. pinnata*.—The blade of the anchor lost; massulae hairy.

4. *A. nilotica*.—Both the blade of the anchor and its stem are lost, massulae becoming eglochidiate.

Reduction in the structure of the glochidia thus seems to have played important part in the evolution of the species. This seems to have been associated with the reduction in the number of massular segments in a micro-sporangium from 8, as in *A. filiculoides* complex, to 4 as in *A. pinnata*, to 2 as in *A. nilotica*. However there does not seem to be a corresponding reduction in the number of float carpuscles in a megasporangium; nor these two characters seem to have moved equally well in the same direction in evolution.

As regards the stem stele, large forms met with in *A. filiculoides* complex and *A. pinnata* have protostele; but in *A. nilotica* which is also a large form, the stele is amphiphloic siphonostele. This character, therefore, seems to be more related with the size of the plants, and their floating habit in deep or shallow waters.

The main evolutionary changes in the genus thus are largely concerned with the male organs, the massulae and glochidia. This is quite understandable as the plants often thrive in small sheets of water in isolated manner in both temperate and tropical countries. Their clones often get repeatedly isolated from each other and their ability to survive depends upon their capacity of reproduction. It is well known that glochidia of massulae attach themselves to long hairy processes on the megaspores below float corpuscles. Unfortunately exact place and mode of fertilization are not known with certainty in different species. We do not know whether the megaspore sinks first to the bottom of lake in which the plants grow before fertilization, forms a megagametophyte and then comes to surface exhausted by the developing female gametophyte making megaspore lighter, so that it could come up to the surface for fertilization, microspores attaching themselves to it by means of glochidia; or whether both male and female

gametophytes are formed at the surface of water and the megaspore with female gametophyte having fertilized archegonia sinks to the bottom and comes to the surface again after the first two leaves of the sporophyte have been formed and food material in the lower part of megaspore exhausted by the developing embryo making megaspore light.

Another important point about the genus is its geographical distribution in the past and present.

The present-day distribution of the genus is peculiar. *A. filiculoides* complex occurs in Central and South America, Europe, Australia (*A. rubra*) and its fossils are found in the Tertiary formations of British Columbia in the North America (*A. primaeva*), in the Miocene strata of the Island of Wight near London in England, and in Tertiaries of Holland, Germany, India, etc. *A. prisca*, a British fossil form from the Island of Wight has typical anchor-shaped glochidia as in *A. maxicana*, but the fossil Columbian form is very small with delicate, short glochidia. The Australian form found in bore core deposits near Melbourne is more like *A. rubra* which is considered to be only a variety of *A. filiculoides* by some. *A. intertrappea* is closely related to *A. filiculoides* living and to Columbian Tertiary species *A. primaeva*. *A. pinuata* occurs in Australia and India, but its fossils are found in the late Tertiaries of Holland and in the Volga basin (Russia) as stated above. A fossil *Azolla* having eglochidiate massulae like those in *A. nilotica* is not yet known.

What must be the causes for such varied discontinuous distribution of the three major complexes in *Azolla* can only be inferred. The chromosome number in all the species is fairly constant (*n*-8) but several races which inhabit either only temperate or tropical waters or both are known. Apogamy also may have played its own part in the evolution of the genus as in *Salvinia* (Mahabalé and d'Mello, 1952). The plants often grow in isolated ponds having a variety of pH, temperature range and other conditions. All these factors seem to have operated in various directions in evolution. Apparently the floating aquatic nature of plants and discontinuous existence in space is largely responsible for their discontinuous distribution facilitated by the capacity of plants to propagate successfully in ponds with varied conditions. The reduction or elaboration in the size of plants and stem stele seem to be due to these reasons. The most crucial stage in their life-cycle is fertilization. The parts that seem to have been affected in this respect are microsporocarps, especially the glochidia, rather than float carpuscles of megaspores. Evidently the male reproductive parts have been affected more than the female reproductive parts either megasporocarps, megasporangium or the float carpuscles. But this is not to be wondered at all, as in many other groups of plants also such as fungi, gymnosperms, angiosperms, the parts reduced or greatly altered seem to be male reproductive organs. For example, in higher plants like gymnosperms and angiosperms the male gametophyte and gametes have undergone more substantial change than female parts. A strange fact that emerges out of this is, it looks, as if the extremes of opposite conditions namely,

lack of water for fertilization as in conifers and angiosperms, or its excess as in the water-ferns seem to have evoked similar response in the male reproductive parts of these plants.

REFERENCES

ANDREWS, H. N. 1960. *Studies in Paleobotany.*

DEMALSY, P. 1953. Le sporophyte *d'Azolla nilotica*. *La Cellule* **56** (1): 1-60.

EMBERGER, LOUIS. *1949.* Les Prephanerogams. *Ann. Des. Sci. nat. Bot.*, 11 Serie, **10**: 1949.

KNOWLTON, F. H. 1919. *Catalogue of the Mesozoic and Cenozoic Plants of North America*, pp. 666.

MAHABALÉ, T. S. AND d'MELLO, JOYCE. 1952. Apogamy in *Salvinia*. *Curr. Sci.* **21**: 227-28.

SAHNI, B. AND RAO, H. S. 1943. Silicified flora from the Intertrappean cherts round Sausar in the Deccan. *Proc. nat. Acad. Sci., India* **13**: 36-75.

SHOOT APEX ORGANIZATION IN LYCOPODIALES*

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Introduction

Shoot apex organization in Pteridophytes was at one time considered to be a simple affair. The occurrence of a single apical cell with two or more cutting faces was believed to be the rule. Lately with the appearance of more detailed and systematic studies we have begun to realize the complexities of the subject. Even in the living lycopods there is considerable variation and specialization (*see* Sifton, 1944; Wardlaw, 1950, 1952 and 1953). Although the problem of shoot apex organization in the living lycopods has attracted good-deal of attention, there are still certain points that can be opened up profitably. Besides, it may be worthwhile to see if there are any trends of specialization exhibited in these genera.

Material and Methods

The present study deals with fifteen species, of which six belong to *Selaginella* (*S. monospora* Spring; *S. involvens; S. chrysorrhizos; S. adunca; S. remotifolia* and *S. tamariscina*); six to *Lycopodium* (*L. lucidulum; L. serratum; L. squarrosum; L. clavatum; L. nikoense* and *L. complanatum*) and the remaining three to *Isoetes* (*I. coromandelina; L. engelmannii* and *L. japonica*). Most of the material comprising spical portions of growing shoots was fixed in F.A.A., but some apices of *I. coromandelina, L. serrtaum* and *L. squarrosum* were fixed in a mixture of absolute alcohol and acetic acid (3:1). The material was dehydrated and cleared with ethyl alcohol and tertiary butyl alcohol and embedded in paraffin. Sections 6 to 18 microns thick were cut and stained with Safranin-fast green, Haidanhain's haematoxylin and Delafield's haematoxylin-fast green, the last combination giving better results.

Observations

Selaginella Spring.—Vegetative stem apices of only mature plants were studied in the present investigation. They are in most of the

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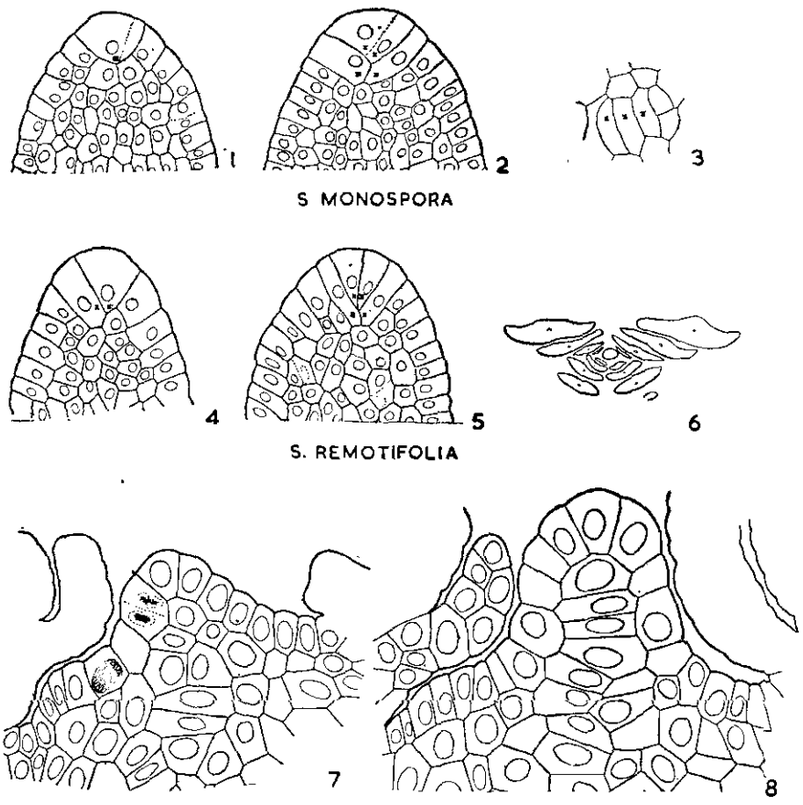
species dorsiventrally flattened except in *S. tamariscina* where the extreme terminal portions are radially symmetrical, the plant being erect. In most of the species, as the plant creeps on the ground, the growing tip bends upward in crozier fashion, a feature becoming more pronounced during dehydration. It is, therefore, always difficult to get a good median horizontal section or a vertical transverse section of the tip. Vertical longitudinal sections, however, are easy to obtain and several of them have to be co-ordinated together to have a complete picture of the apex.

The growing point in *Selaginella* is well protected by ligulate leaves that are much crowded. The apical protuberance is mostly conical in form and projects considerably above the youngest leaf primordium. In a vertical longitudinal median section the apex is slightly more conical than it is in a horizontal longitudinal section. At the tip there is generally one, rarely two (perhaps due to section being oblique), prominent, thin-walled, triangular apical cell (Text-Fig. 1). An examination of sections on either side of this median one reveals a few other similar cells which together form a horizontal plate. These cells, though larger in size, are somewhat less cytoplasmic than the cells occurring beneath them. Sometimes they appear prismatic in nature, *i.e.*, their cytoplasm is more or less transparent and the walls are shining in nature. The nuclei in all of them are, however, similar in shape and size. It seems probable that they divide rarely, or else the divisions in them are very quick and have eluded us (*cf.* Ball, 1960). These apical cells on their lower side are followed by a hump of meristematic cells which can be distinguished into an outer superficial layer on the flanks and an inner core (Text-Figs. 1 and 2). The former is composed of densely stained columnar cells which divide both anticlinally and periclinally. The underlying tissue consists of irregularly arranged, homogeneous isodiametric cells. These are slightly smaller than those of the superficial layer, thin-walled and densely cytoplasmic and divide irregularly in different directions, transverse divisions being more frequent.

In horizontal longitudinal sections the apex is somewhat oval in appearance and at the tip there is generally a plate of three or four large and somewhat rectangular cells (Text-Figs. 2 and 4). These are less cytoplasmic and bigger in size and correspond to those cut serially one after another in vertical longitudinal sections. Other cells of the superficial layer and inner tissue are as described above.

In a transverse section the stem apex is mostly oval in outline and is surrounded on all sides by young leaves and ligules (Text-Fig. 6). Herein the peripheral cells show a more regular arrangement than the inner ones. In extreme apical region there is seen a plate of three or four larger cells surrounded by some smaller ones that are obviously obliquely cut (Text-Fig. 3). It may be emphasized here that in none of the cases there was any indication of there being a single, three- or four-faced, apical cell. In *S. tamariscina* also where the apex is radially symmetrical there exists a group of cells rather than a single apical cell. Further work on this species, however, is still in progress.



FIGS. 1–8. Fig. 1. Median vertical longitudinal section of the stem apex of *Selaginella monospora* cut at right angles to the dorsiventral surface. Fig. 2. Median horizontal longitudinal section of the same cut parallel to the dorsiventral surface. Fig. 3. Transverse section of the same showing a plate of cells. Fig. 4. Median vertical longitudinal section of the stem apex of *Selaginella remotifolia*. Fig. 5. Median longitudinal section of the same. Fig. 6. Transverse section of the same showing dorsiventrally flattened apical tip. Figs. 7 and 8. Median longitudinal sections of the shoot apex of *Isoetes coromandelina*, of a very young plant and of a middle-sized plant respectively. All Figs. except Fig. 6, × 285. Fig. 6, × 28.

From the preceeding account of *Selaginella* species it is clear that the vegetative apices that are mostly dorsiventrally flattened, have a group of conspicuous apical cells at the top. These are arranged horizontally in a file and are generally less cytoplasmic and appear to divide rarely in comparison to cells on the lateral flanks down below.

Lycopodium L.—In the case of *Lycopodium* also stem apices of only mature plants have been studied here. In all the species the apical

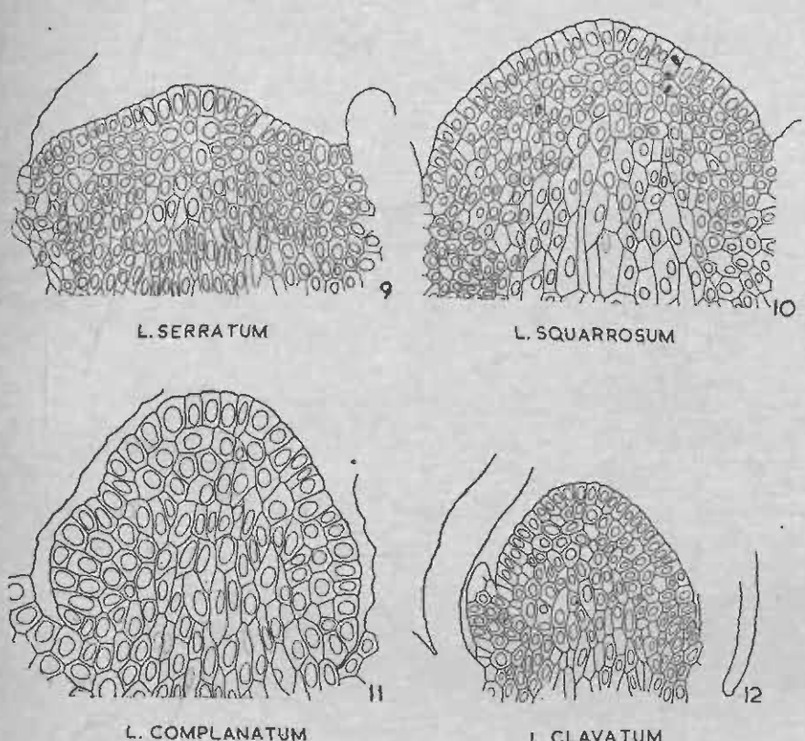
protuberance is protected by a rosette of acropetally developed leaves. The growing point shows some variation in form in different species. For instance, it may be mostly flat as in *L. lucidulum* or it may be a low mound about 630 microns above the youngest leaf primordium as in *L. serratum* and *L. squarossum* (Text-Figs. 9 and 10) or it may be conical projecting considerably above the youngest leaf primordium as in *L. nikoense*, *L. complanatum* and *L. clavatum* (Text-Figs. 11 and 12). In the last species it projects nearly 2,100 microns vertically up from the youngest leaf primordium. It is significant to note that most of the species having elongated apices belong to the sub-genus *Rhopalostachya* while the rest belong to the sub-genus *Urostachya* which are generally slow growers. It is, therefore, obvious that the form of the apex is correlated with the mode of its growth. Slow growers as a rule have flat and rapid growers, conical apices.

All the species studied here have a more or less discrete outer superficial layer covering an inner dome of irregularly arranged meristematic cells. This outermost layer is composed of somewhat prominent cells whose outer walls are somewhat thickened. In *L. serratum*, *L. squarassum* however, a few central cells of this layer appear to be more prominent than those present on the lateral flanks (Text-Figs. 9 and 10). In *L. nikoense*, *L. clavatum* and *L. complanatum*, although the division figures are more common on the sides, there is no apparent differentiation, all the cells of the superficial layer being almost equal in size (Text-Figs. 11 and 12). In general the inner region of the shoot apex is composed of cells that are irregularly arranged and somewhat smaller than those of the outer layer. They are thin-walled, less vacuolated and divide in different planes. Transverse divisions are, however, more numerous than longitudinal. On the lateral flanks, below the foliar buds, the cells are densely cytoplasmic and undergo frequent divisions.

Isoetes L.—In *Isoetes* also the stem apices of only mature plants have been studied. In case of *I. coromandelina*, however, axes of different ages have been studied as they were available locally. In very young axes, having about 6–15 leaves as well as in older ones with more than 100 leaves, the apices are somewhat flattened while in the prime youth of the plant it becomes dome-shaped or somewhat conical, a feature obviously connected with the mode of growth of the apex.

The apical protuberance in *Isoetes* is distinguishable into two well-demarcated regions (Text-Figs. 7 and 8). The outer layer is composed of columnar cells that are equal in size, thin-walled and somewhat poor in cytoplasm. The cells here divide most frequently by anticlinal divisions, but those on the sides may also show some periclinal divisions (Text-Fig. 7). No one of these cells looks like an apical cell. The underlying tissue is composed of irregularly arranged somewhat larger cells (Text-Figs. 7 & 8). These are thin-walled, sometimes vacuolated, and divide in different planes. Below a newly-formed foliar buttress, however, the divisions are more frequent than elsewhere. It should be pointed out here that the stratification and arrangement of these inner cells do not indicate any direct relationship with the cells of the super-

ficial layer and so it cannot be suggested that they have been derived directly from the superficial cells, rather it appears to be a self-perpetuating zone, like the superficial one.



FIGS. 9–12. Median longitudinal sections through the stem apicies of *L. serratum*, *L. squarrosum*, *L. camplanatum* and *L. clavatum* respectively.

All Figs. × 130.

DISCUSSION

It is generally seen that in the shoot apices of angiosperms and gymnosperms there is a marked tendency towards elimination of periclinal divisions in the superficial layer or layers that become somewhat distinct from the inner irregularly dividing zone. Such a differentiation of the apcial meristem is envisaged in the so-called tunica corpus concept. No such differentiation has been reported for any of the lower vascular plants.

In *Selaginella*, generally two conditions have been reported envisaging a single apical cell or a group of apical cells. The presence of a single two-sided apical cell was first described by Pfeffer (1872, quoted

from Sifton, 1944) in *S. martensii*. Barclay (1931) too reported a tetrahedral three-sided apical cell in *S. willdenovii*, and so did Hsu (1937) in *S. sinensis*. Russow (1872, quoted from Sifton, 1944), on the other hand, is said to have suggested the occurrence of a group of cells in the stem apex of *Salaginella*. De Bary (1884) also held that the apical constitution in *Selaginella* is so as to form a transitional series between a structure with a single apical cell and an apex with a well-differentiated meristem. Bruchmann (1897, 1909, 1910) reported in *S. poulteri* a three-sided apical cell which, according to him, changes into a group of apical initials as the apex enlarges. Wand (1914) suggested that there is in *Selaginella* every intergradation from a three- or four-sided apical cell to a group of apical cell—a viewpoint adopted by many text-book authors (*see* Bower, 1935; Smith, 1938 and 1955; Foster and Gifford, 1959; etc.). Strasburger (1873, quoted from Sachs, 1882) and Williams (1931) described two initial cells each in *Selaginella wallichii* and *S. grandis*. Wardlaw (1950, 1952 and 1953), in the same manner, recognizes the presence of a group of conspicuous apical cells and so does Cusik (1955) in *S. willdenovii*.

On the basis of observations recorded here, we are inclined to believe that in *Selaginella*, at the extreme tip there is a group of large conspicuous cells which appear to divide rarely in comparison to the cells that they cover. The tissue which is present on the lateral flanks and below these conspicuous cells can be distinguished into an outer superficial layer which divides both anticlinally and periclinally and an inner tissue which divides in various planes. In no case was the e seen a single apical cell. Individual vertical longitudinal sections, however, may show a single prominent cell that has sometimes (*see* Barclay, 1931) been confused in the past with an apical cell. As also brought out by Williams (1931) this is obviously the result of incomplete observations.

In *Lycopodium* also the shoot apex has been described to have a single apical cell or a group of cells. Nageli (1846, quoted from Turner, 1924) was perhaps the first to describe a definite apical cell in *L. clavatum*. Subsequently Hofmeister (1862) and van Tieghem (1891) supported this view. Strasburger (1872), on the other hand, is said to have applied the histogen hypothesis to the apex of *Lycopodium* (Turner, 1924; Foster, 1939). He believed that there are two superposed tiers of initials, the outer tier which has a single cell gives rise to dermatogen and periblem and the lower one which is two or three cells deep gives rise to plerome. De Bary (1884) confirmed this view although he reported a group of 2 to 4 cells in the upper tier and a single cell or group below it. Subsequently many other workers (*e.g.*, Jones, 1905; Campbell, 1913, 1940; Schuepp, 1926; Turner, 1924; Williams, 1932-33; Chowdhury, 1937; Bower, 1935) supported it. Sachs (1882) pointed out that the growing apex consists of small-celled primary meristem which is not distinguishable into dermatogen and periblem. Wigglesworth (1907) observed in *L. complanatum* several large cells of equal size that divide both anticlinally and periclinally. He did not, however, say anything about the cells formed by the activity of

this group. Spessard (1928) speaks of an apical meristem extending about 15 microns from the extreme tip and a sub-apcial group up to 70 microns below. The apical region according to him consists of actively dividing epidermis and inner fundamental tissue. Hartel (1938) also found a single terminal group of initials at the apex of a few species of *Lycopodium* and compared it with the corpus found in angiosperms. Wardlaw (1950, 1953) also speaks of a group of superficial inconspicuous cells, a situation regarded intermediate between the several conspicuous-celled condition in *Selaginella* and a weak zonal constitution in angiosperms On the basis of the present observations we are inclined to conclude that the apex in *Lycopodium* consists of a hump of meristematic cells distinguishable into a peripheral layer and an inner mass. The former divides anticlinally and periclinally like the outer layer of the lateral flanks of *Selaginella*. But divisions in the central region of this layer are very rare in such species as *L. lucidulum* and *L. serratum*. The cells of the inner mass, on the other hand, divide both transversely and longitudinally. No definite apical cell could ever be observed in any of the species examined nor does the cell arrangement in apical protuberance warrant the postulation of an apical cell.

In *Isoetes* also the growing apex is described to have either a single apical cell (Hofmeister, 1862; van Tieghem, 1891; Scott and Hill, 1900) or a group of meristematic cells (Bruchmann, 1874; Farmer, 1890; Smith, 1900; West and Takeda, 1915) (for details *see* Bhambie, 1957). In all the species studied here there is a group of meristematic cells distinguishable into two regions, an outer prominent superficial layer and an inner dome of irregularly arranged cells. In the former the cells undergo mostly anticlinal divisions while in the latter they divide irregularly.

From this brief analysis it will be seen that there is no uniformity in these three genera in so far as the organization and structure of their shoot apices are concerned. As in many other features, they are highly specialized in their own way in this respect as well. *Selaginella*, *Lycopodium* and *Isoetes* appear to form an ascending series in so far as the complexities of the shoot apex organization are concerned. We have, however, no intention of suggesting that they constitute a phylogenetic series. Ontogenetic studies in our understanding have little to contribute to phylogenetic speculation. Such an attitude in our opinion will avoid much confusion.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Dr. Y. Nozu, University of Tokyo, Japan; Dr. S. C. Gupta, Government College, Nainital; Mr. S. C. Verma, University of Punjab, Chandigarh, for fixing and sending some material used in this study.

REFERENCES

BALL, E. 1960. Cell divisions in living shoot apices. *Phytomorphology* **10**: 377–96.

BARCLAY, B. D. 1931. Origin and development of tissue in stem of *Selaginella willdenovii*. *Bot. Gaz.* **91**: 452-61.

BHAMBIE, S. 1957. Studies in Pteridophytes. I. The shoot apex of *I. coromandelina* L. *J. Indian bot. Soc.* **36**: 491-502.

BOWER, F. O. 1935. *Primitive Land Plants*, London.

*BRUCHMANN, H. 1897. Untersuchungen über *Selaginella spinulosa* A. Br. *Gotha* **64**.

———. 1909. Ven den vegetations organen der *Selaginella lyalli* Spring. *Flora* **99**: 436.

———. 1910. Uber *Selaginella preissiana* Spring. *Ibid* **100**: 288-95.

CAMPBELL, D. H. 1913. *The Structure and Development of Mosses and Ferns*. London.

———. 1940. *The Evolution of Land Plants*, London.

CHOWDHURY, N. 1937. Notes on some Indian species of *Lycopodium* with remark on the distribution of the genus in India. *Trans. nat. Inst. India* **1**: 187-226.

CUSIK, F. 1953. Expeirmental and analytical studies of Pteridophytes. XXII. Morphogenesis in *Selaginella willdenovii* Baker. I. Preliminary morphological analysis. *Ann. Bot., Lond.* **17**: 369-83.

DE BARY, A. 1884. *Comparative Anatomy of Phanerogams and Ferns*. English Transl. by F. O. Bower and D. H. Scott, London.

FARMER, J. B. 1890. On *Isoetes lacustris*. *Ann. Bot., Lond.* **5**: 37–62.

FOSTER, A. S. 1939. Problems of structure growth and evolution in the shoot apex of seed plants. *Bot. Rev.* **5**: 454–70.

—— AND GIFFORD, JR. E. M. 1959. *Comparative Morphology of Vascular Plants*. San Francisco, California.

HARTEL, K. 1938. Studien an Vegetationspunkten einheimischer Lycopodien. *Beitrage zur Biol. der Pflanzen.* **25**: 125–168.

*HOFMEISTER, W. 1862. *Germination, Development and Fructification of the Higher Cryptogamia*, London.

HSU. J. 1937. Anatomy, development and life-history of *Selaginella sinensii*. I Anatomy and development of the shoot. *Bull. Chinese bot. Soc.* **3**: 75–95.

JONES, C. E. 1905. The morphology and anatomy of the stem of the genus *Lycopodium*. *Trans. Linn. Soc. Lond. Bot.* **7** (Pt. II): 15–36.

SACHS, J. 1882. *Text-book of Botany*, Oxford, II Ed. (Transl. by S. H. Vines).

SCHUEPP, C. 1926. *Meristems, in Linsbeuer, Handb. Pflanzenanatomie*, Abt. 1., Teil. 2, Bd. 4, Lief. 16,114 pp., Berlin.

SCOTT, D. H. AND HILL, T. G. 1900. The structure of *Isoetes hystrix*. *Ann. Bot., Lond.* **14**: 413-54.

SIFTON, H. B. 1944. Developmental morphology of vascular plants. *New Phytol.* **43**: 87–129.

SMITH, R.W. 1900. The structure and development of the sporophylls and sporangia of *Isoetes*. *Bot. Gaz.* **29**: 225–58, 323–46.

SMITH, G. M. 1938, 1955. *Cryptogamic Botany*. **2**: I & II Edi., New York.

SPESSARD, E. A. 1928. Anatomy of *Lycopodium* sporeling. *Bot. Gaz.* **85**: 323-33.

TURNER, J. J. 1924. Origin and development of vascular system of *Lycopodium lucidulum*. *Ibid.*, **78**: 215-25.

VAN TIEGHEM, PH. 1891. *Traité de Botanique* II, Edi. Paris.

WAND, A. 1914. Beitrage zur Kenntnise des Scheitelwaschstums und der Verzweigung bei *Selaginella*. *Flora* **106**: 237-63.

WARDLAW, C. W. 1950. Comparative morphogenesis in Pteridophytes and Spermatophytes by experimental methods. *Rep. Proc. Intern. Bot. Congr. Stockholm* also in *New Phytol.* **50**: 127-34 (1951).

———. 1952. *Phylogeny and Morphogenesis*, London.

———. 1953. Comparative observations on the shoot apices of vascular plants. *New Phytol.* **52**: 195-209.

WEST, C. AND TAKEDA. H. 1915. On *Isoetes japonica* A. Br. *Trans. Linn. Soc. Lond. Bot.* **8**: 333-76.

WIGGLESWORTH, G. 1907. The young sporophyte of *Lycopodium complanatum* and *Lycopodium clavatum*. *Ann. Bot., Lond*, **21**: 211-34.

WILLIAMS, S. 1931. An analysis of the vegetative organs of *Selaginella grandis* Moore, together with some observations on abnormalities and experimental results. *Trans. roy. Soc. Edinb.* (Part I) **57**: 1-24.

———. 1932-33. A contribution to the experimental morphology of *Lycopodium selago* with special reference to the development of adventitious shoots *Ibid.* (Part III) **57**: 711-37.

* Not seen in original.

SYMPOSIUM II

CYTOGENETICAL EVOLUTION IN ANGIOSPERMS

CYTOGENETIC EVOLUTION IN ANGIOSPERMS—MAYDEAE

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Angiosperms form the dominant flora of the world and a hierarchy of groups like species, genera, families and still higher categories have been recognised on systematic grounds. If phylogeny of every group is represented by a diagram in the form of a branching tree, the various diagrams present a different shape and the evolution of each group may be regarded as a 'pattern'. To start with, evaluation of the progressive steps in evolution was largely based on work in traditional descriptive fields of systematics and morphology. In recent years there has been increasing co-operation and interchange of ideas among workers in the various fields of biological enquiry and consequently it has become possible to have a broader conception of the processes of evolution.

Evolution is visualised primarily as the resultant of interaction of environmental variation and the genetic variability occurring in a population. The chromosomes form the physical basis of heredity and therefore of the genetic variability and as such are regarded as forming the genetic system of the organisms. What changes effect these, also effect the evolution of the species for a proper study of which a reliable understanding of variations in the species is essential. The rise of genetics in recent years dealing with relationships between groups of organisms, between individuals in a species and species in a genus, has opened up new opportunities to the student of evolution and has brought to the fore the need for synthesizing and combining the results from cytology and genetics in any approach to solve evolutionary problems. Chromosomal apparatus, particularly the number of chromosomes and their behaviour at meiosis, is one of the primary elements of the genetic system. The amount of genetic recombination in any particular mating group is determined by the chromosome number, amount of crossing-over in each chromosome which in turn is determined by the chiasma frequency. Other factors that count in evolution are hybridization in nature, polyploidy and breakdown of sex mechanisms and apomixis and natural chromosomal aberrations.

In studying cytogenetic evolution the following provide valuable criteria:

(i) Karyotype in relation to alteration in basic number, form and size of chromosomes, number of satellites and secondary constrictions and the distribution of euchromatin and heterochromatin. The

latter is important for, when it is present in considerable amount in the chromosome complement, alterations in form, size and number of chromosomes can be accomplished (without sacrifice of euchromatic portions) by unequal translocations involving breaks which usually take place in the heterochromatic regions. Thus heterochromatin is desirable and is present in organisms showing dynamic evolution as a safety factor.

(ii) Morphology and behaviour of meiotic chromosomes including the morphology of pachytene chromosomes. This could only be studied so far in a few plants and prominently in maize and its occidental relatives. The size, the differentiation of the chromosomes, the symmetry or asymmetry of the arms and other details can be studied in the meiotic chromosomes particularly at the mid-prophase stage in a greater detail than in the somatic chromosomes. Through study of pairing relations at pachytene in hybrids one could learn about homologies of the chromosomes and see what amount of genetic recombination is possible.

(iii) Polyploidy, (iv) apomixis and (v) aberrations.

Applying the above criteria evolution has been studied in a few plant groups and it may now be considered as to how far these criteria could be applied to a study of interrelationships in Maydeae, possible origin of its members and generally gain an idea of the cytogenetic evolution in this group. Previously the interest in this group was largely centred round maize, which is not only one of the most important crop plants of the world but also has been one of the most favourable materials for elucidation of cytogenetical processes.

Maydeae, to which maize belongs, is one of the subtribes of the tribe Andropogoneae. It comprises eight genera, five of which are oriental and three American. The oriental Maydeae include *Coix*, *Sclerachne*, *Polytoca*, *Chionachne* and *Trilobachne* which are all native to the region extending from India and Burma through East Indies to Australia and the Polynesian Islands. The American genera are three, namely *Zea*, *Euchlaena* and *Tripsacum*. "Botanically the Maydeae are characterised by the presence of unisexual spikelets, the staminate and pistilate spikelets placed in separate portions of the same inflorescence, the staminate above; sometimes the spikelets are placed in totally separate inflorescences as in maize. The staminate spikelets are placed in pairs or in threes, they are two-flowered with the lower floret imperfect; the pistillate spikelets are placed usually single, also two-flowered, the lower floret sterile. They are embedded in hollows of a thickened articulated axis and fall together with the joints, sometimes they are enclosed in an osseous involucre. The genus *Zea* is anomalous, the pistillate spikelets are crowded on much thickened axis" (Henrad, 1941). Of the American Maydeae, maize has attained a pan-global distribution in cultivation while *Euchlaena* (*E. mexicana*, *E. perennis* and *E. floridanum*) is distributed in West Central Mexico and Southern Florida. *Tripsacum* is a native of Mexico.

Much attention has been paid to elucidate the origin of maize and many theories have been put forward and in this connection recourse to the study of the maize and its relatives was made. In all recent studies on the subject, cytogenetic evidence relating to the American relatives of maize has only been used and no such information to any satisfactory extent is available in respect of the oriental members of the tribe. The information so far available in Maydeae including observations made by us on the oriential members of the tribe during the course of last few years is presented below with a view to examine and consider what bearing it has on the cytogenetic evolution in Maydeae.

I. Chromosome Number

Significant differences of the chromosome numbers are found in Maydeae. Haploid chromosome numbers of 5, 10, 18 and 20 followed by high degrees of polyploidy are found (Mangelsdorf and Reeves, 1939; Janaki Ammal, 1939; Longley, 1937; Simmonds, 1954; and Avdulov, 1931; *cf. Chromosome Atlas of Flowering Plants*). Polyploidy has obviously played a significant role in the delimitation of the species and genera. The genus *Tripsacum* is characterised by a different basic number in the tribe and $n = 18$ is found in five species while $n = 36$ is found in four species. In addition varying numbers of B-type chromosomes have been located in *Zea mays* (Randolph, 1928), *Euchlaena* (Longley, 1937), *Tripsacum* (Maguire, 1952) and *Coix aquatica* (unpublished data from this laboratory).

Whether the basic number is 5 or 10 or both is not quite clear. The lowest number is found in the genus *Coix* ($n = 5$) in which also a significant series of chromosome numbers is found. A much better picture of the chromosome morphology is obtained by a study of the pachytene chromosomes in these genera which invariably present a more detailed structure of the chromosome than by a study of the somatic chromosomes.

II. Chromosome Morphology of the Pachytene Chromosomes

The pachytene chromosomes of the corn are not only the best known in Maydeae but in all plants and observations on the pachytene chromosomes in Maydeae are given below.

(*a*) *Length range.*—The length range within the set is from 80–40 μ in *Zea* and *Euchlaena* while in *Tripsacum* (*Tripsacum floridanam*) the average length of the chromosomes is about half that in corn. The longest four chromosomes of *T. floridanam* are only as long as the shortest chromosomes in corn (Longley, 1941). According to our own observations, in *Coix aquatica* chromosomes are the longest in Maydeae so far studied with a length range of 112–70 μ, while in *C. lachryma-jobi* the range is from 85–35 μ which is somewhat nearer to *Zea mays*. There seems to be a significant difference between *Coix*

lachryma-jobi and *C. aquatica* in this respect. Unfortunately no such data are yet available in other oriental members. The above length range is also reflected in the somatic chromosomes.

(*b*) *Position of the centromere and length of the arms.*—In maize except chromosome 5, all have submedian centromeres with unequal arms, chromosome 6 having the shortest short arm which has a nearly terminal nucleolus organizing body (Rhoades, 1950). The complements of *Zea* and *Euchlaena* show more submedian chromosomes while several of *Tripsacum* chromosomes bear sub-terminal centromeres (Longley, 1937). In *Coix aquatica* three of the chromosomes of the haploid set are about median and the rest of the two submedian while in *C. lachryma-jobi*, they are mostly submedian as in corn.

(*c*) *Nucleolus organizer.*—One of the short chromosomes bears a subterminal nucleolus organizer in maize and in *Euchlaena*. In *Tripsacum* the nucleolus organizer is median in position. In *C. aquatica*, however, the nucleolus organizer is nearly terminal in one of the median chromosomes. The nucleolar chromosome in *C. lachryma-jobi* resembles *Zea mays* in the position of the nucleolus organizing body and the position of the centromere (Text-Fig. 1).

(*d*) *Knobs.*—Knobs are found in twenty-two different positions in the corn pachytene chromosomes. Eighteen of these are intercalary while four are terminal (*see* Rhoades, 1955). In *Euchlaena mexicana*, which agrees most with maize among all its relatives, thirteen knob positions are located (Longley, 1937), six of which being much in the same positions as in maize. The knobs in *Tripsacum floridanum*, *T. zopilotens*, and *T. dactyloides* are essentially terminal or terminal while in two other species (*T. australe* and *T. maizar*) there are either none or few (Randolph and Hernandez-Xolocotzi, 1950). In *Coix aquatica* all chromosomes have terminal knobs though they are rather small while in *C. lachryma-jobi* conspicuous terminal knobs occur as in maize.

(*e*) *Euchromatic and heterochromatic segments.*—The chromosomes of maize are described to be undifferentiated in the sense that there are no prominent heterochromatin segments such as occur in the pachytene chromosomes of tomato (Brown, 1949; Barton, 1950) and several Solanaceae (Gottschalk, 1954), *Plantago* (Hyde, 1953), castor (Jakob, 1956) and a few others. This is also true of *Euchlaena* and races of *Coix lachryma-jobi* (Plate IV; while *Tripsacum* chromosomes are differentiated into heterochromatic and euchromatic segments, the deeply stained heterochromatic regions being situated on either side of the centromere, a situation also again prominently seen in *Coix aquatica* and some races of *C. lachryma-jobi* (Plate IV). The same type of structure is indicated for *C. poilaneii*, another five chromosomed species of *Coix* growing in Orissa, as seen in the chromosomes of tapetal cells by Nirodi (1955).

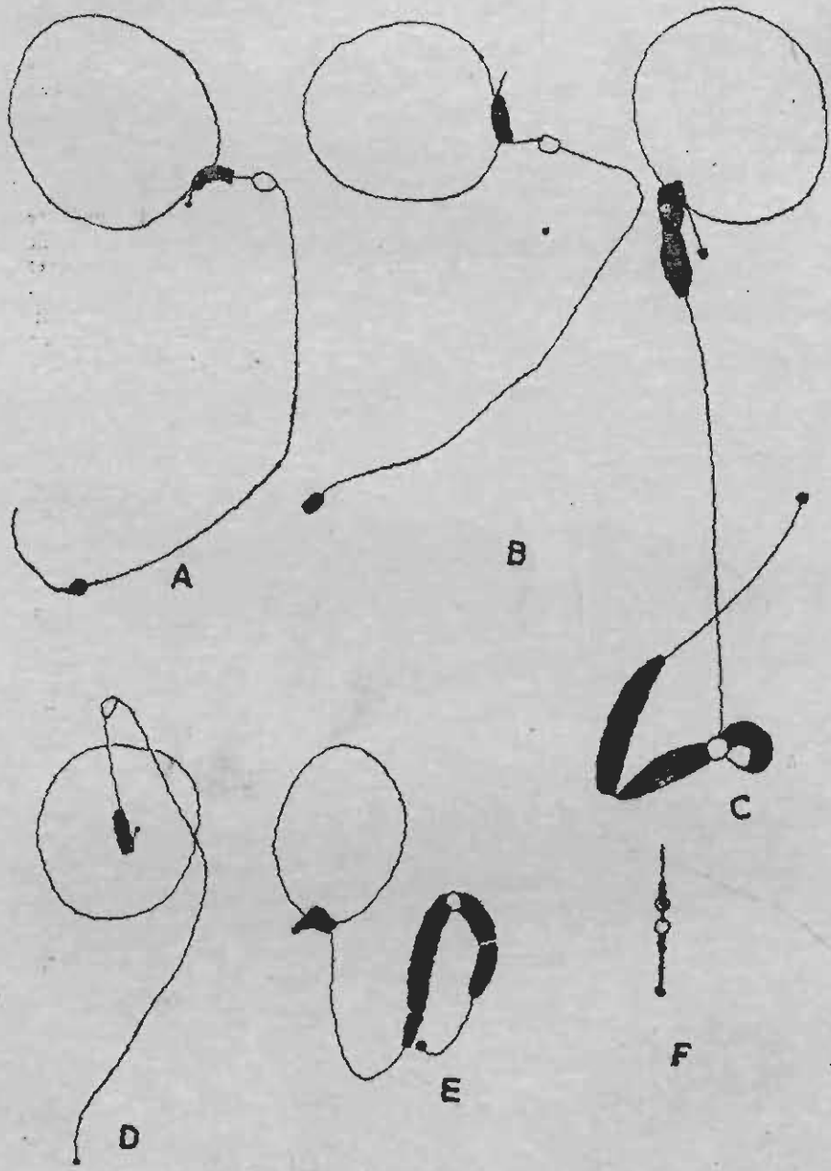
From the above characters it can be seen that cytologically corn and *Euchlaena* resemble each other very closely and that *C. lachryma-*

jobi (some races) also is nearer the above while *C. aquatica* and *Tripsacum* are much different. Meagre information available on *Polytoca* and *Chionachne* shows that they are unlike *Coix* (Nirodi, 1955). There is no information yet on the chromosome morphology in *Trilobachne* and *Sclerachne*.

III. Crossability

Corn readily crosses with *Euchlaena* and the hybrids obtained are highly fertile. The pairing of the chromosomes is regular and meictic behaviour is normal. Beadle (1932) reported frequent crossing over in these hybrids. Thus the chromosomes of these two genera are homologous to a great extent. Corn crosses with *Tripsacum* also easily when it is used as a male parent (Maguire, 1952), but when corn is used as female it requires special techniques. The hybrids of the cross are highly sterile and meiosis is irregular showing that the chromosomes are not completely homologous. In a trigeneric hybrid between *Zea*, *Euchlaena* and *Tripsacum* obtained by Mangelsdorf and Reeves (1945), the hybrid has thirty-eight chromosomes presumably containing the genomes of the three. In meiosis the maize chromosomes pair with those of *Euchlaena* while *Tripsacum* chromosomes remain unpaired and lag behind. Crossability experiments have been conducted at Waltair between *Coix aquatica* or *C. lachryma-jobi* as female and maize as male parent and although about twelve hundred pollinations have been made only twenty-eight seeds were obtained. Only three of these germinated and the seedlings turned out to be *Coix*. Probably no fertilization took place but apomictic development was induced. This phenomenon is quite common in grasses in general. Harada *et al.* (1954) has also reported having obtained seed from *Coix and* maize crosses but the seeds are yet to be germinated. Probably these are also apomictically produced.

From the cytological data obtained in our laboratories with special reference to a study of the pachytene chromosomes it is found that *Coix aquatica* resembles *Tripsacum* in possessing a differentiated chromosome structure more than it resembles *Euchlaena* (teosinte) while *Coix lachryma-jobi* shows several resemblances to maize and teosinte. However, some of the races of *C. lachryma-jobi* examined by us are chracterised by differentiated chromosomes. In a way it may be looked upon as occupying an intermediate position between the American and the Oriental members. From the available information which undoubtedly has to be augmented by more work on the oriental members it appears as though the two groups (Oriental and American) originated in different regions of the globe in the remote past and evolved on independent lines and that from among the American Maydeae, *Euchlaena* and *Zea* are very closely related. In fact some people have proposed the amalgamation of the two genera impressed by homologies of the chromosomes. Coming to *Coix aquatica*, a cytological analysis of populations made in our laboratories has uncovered small proportions of plants showing spontaneously occurring translocations, triploidy



TEXT-FIG. 1. Nucleolus organizing chromosomes of (A) *Zea*, (B) *Euchlaena*, (C) *Coix aquatica*, (D) *Coix lachryma-jobi* (undifferentiated), (E) *Coix lachryama-jobi* (differentiated) and (F) *Tripsacum* respectively, × 2,540 (*Tripsacum* drawn from Longley, 1937, ×2,000).

and tetraploidy. No doubt a detailed analysis will reveal the existence of duplications, deficiencies and inversions and other phenomena associated with organisms showing active evolution at the time. The genus is highly polymorphic and the presence of significant quantities of heterochromatin in *Coix aquatica* chromosomes and some races of *C. lachryma-jobi* affords a flexible system promoting breaks and other changes in the genetic system and bestowing adaptive significance.

In the end it may be mentioned that successful crossing has been achieved between sugarcane and maize by Janaki Ammal (1941); in some Andropogoneae like *Manisuris* there are nine chromosomes in the haploid set, that is, just half the number of 18 chromosomes making up the haploid set of *Tripsacum*. Further *Tripsacum* and *Manisuris* are very similar morphologically, the essential difference being in flowers. In *Tripsacum* the flowers are unisexual while in *Manisuris* they are perfect. Weatherwax (1935) and others have even suggested that both these genera should be placed in the same tribe. Thus a cytological study of the members of Andropogoneae, outside Maydeae, may throw some light on the interrelationships and our understanding the cytogenetical evolution of the Maydeae.

REFERENCES

BARTON, D. W. 1950. Pachytene morphology of the tomato chromosome complement. *Amer. J. Bot.* **36**: 374–81.

BEADLE, G. W. 1932. The relation of crossing over to chromosome association in *Zea-Euchlaena* hybrids. *Genetics* **17**: 481–501.

BROWN, S. W. 1949. The structure and meiotic behaviour of chromosomes of tomato. *Genetics* **34**: 437–61.

DARLINGTON, C. D. AND WYLIE, A. P. 1955. *Chromosome Atlas of Flowering Plants.* George Allen and Unwin Ltd., London.

GOTTSCHALK, W. 1954. Die chromosomen struktur der Solanaceen unter Berucksichtigung phylogenetischer Fragestellungen. *Chromosoma* **6**: 539–626.

HARADA, K., MURAKAMI, M., FUKUSHIMA, A. AND NAKAZIMA, M. 1954. Breeding study on the forage crops; studies on the intergeneric hybridization between the genus *Zea* and *Coix* (Maydeae). *Saiko. Univ. Fac. agr. Sci. Rpt.* **6**: 139–54.

HENRAD, J. T. 1941. Notes on the nomenclature of some grasses. *Blumea* **4**: 496–538.

HYDE, B. B. 1953. Differentiated chromosomes in *Plantago ovata*. *Amer. J. Bot.* **44**: 809–15.

JAKOB, K. M. 1956. The pachytene chromosomes of caster oil plant. *Cytologia* **21**: 76–80.

JANAKI AMMAL, E. K. 1941. Intergeneric hybrids of Saccharum—I, II and III. *J. Genetics* **41**: 217–53.

LONGLEY, A. E. 1937. Morphological characters of teosinte chromosomes. *J. agric. Res.* **54**: 835–62.

———. 1941. Chromosome morphology of maize and its relatives. *Bot. Rev.* **76**: 263–89.

MAGUIRE, M. P. 1952. Synopsis and crossing ons of *Tripsacum* and 2*ea* chromosomes derived from hybrids backcrossed to 2*ea*, Thesis, pp. 1–104, Cornell University, Ithaca, New York.

MANGELSDORF, P. C. AND REEVES, R. G. 1945. A trigeneric hybrid of *Zea*, *Tripsacum* and *Teosinte*. *J. Hered.* **26**: 129-40.

NIRODI, NALINI. 1955. Studies on the Asiatic relatives of maize. *Ann. Mo. Bot. Gard.* **42**: 103-30.

RANDOLPH, L F. 1928. Chromosome numbers in *Zea mays* L. *Cornell Univ. agric. exptl. Sta. Mem.* 117.

———. 1928. Types of supernumerary chromosomes in maize. *Anat. Record* **41**: 102.

—— AND HERNANDEZ-XOLOCOTZI. 1950. Cytotaxonomy of diverse *Tripsacum* in Mexico. *Genetics* **35**: 686.

RHOADES, M. M. 1950. Meiosis in maize., *J. Hered.* **41**: 58–67.

———. 1955. Cytogenetics of maize. In "*Corn and cron improvement*" Academic Press inc., New York."

WEATHERWAX, P. 1935. The phylogeny of *Zea mays*. *Amer. Midl. Nat.* **16**: 1–71.

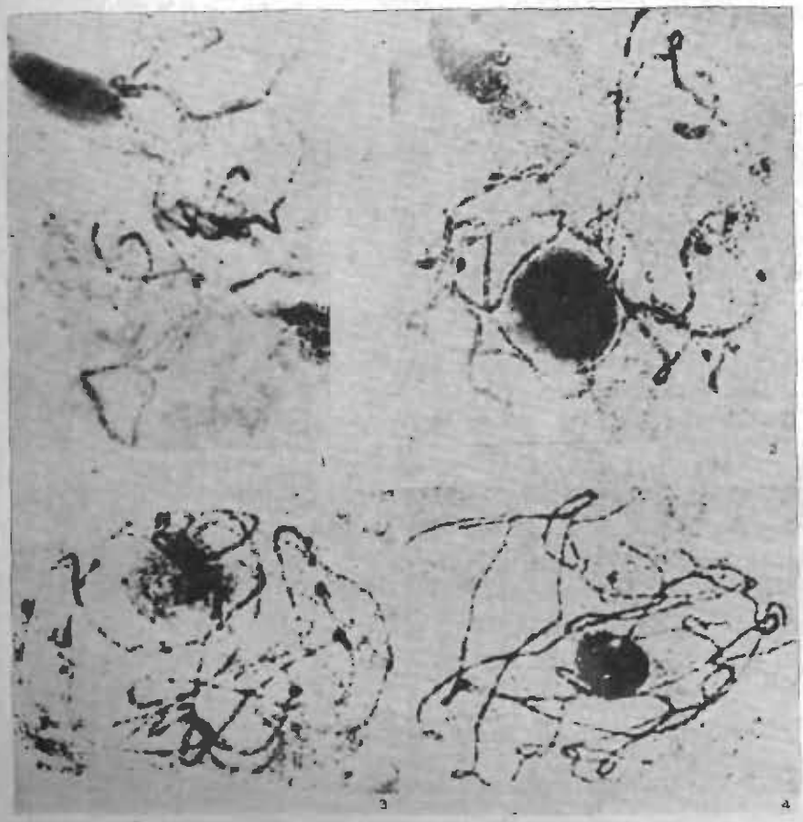
EXPLANATION OF PLATE IV

FIG. 1. Differentiated chromosomes of *Coix aquatica* ($n = 5$) at the pachytene stage.

FIG. 2. Differentiated chromosomes of a race of *Coix lachryma-jobi* ($n = 10$) from Assam wild.

FIG. 3. Differentiated chromosomes of *Coix lachryama-jobi* at the meiotic midprophase collected from Assam cultivated.

FIG. 4. Undifferentiated pachytene chromosomes of a race of *Coix lachryama-jobi*.



FIGS. 1–4

J. Venkateswarlu

CYTOGENETICAL MECHANISMS UNDERLYING SPECIES DIFFERENTIATION IN THE TUBER-BEARING *SOLANUMS*

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The evolutionary history of any large, widely distributed group of related organisms is apt to be long and difficult to trace. For, the origin of species is the result of the interaction of a complex of factors. In addition to a large number of external factors, different genetic factors or processes are usually involved. Many of these genetic processes, which provide new hereditary material, or set up new internal conditions which make differentiation possible, may often be going on simultaneously and it is not always possible to disentangle the role played by each of these factors.

The exact connotation to be attached to the term species has been a matter of controversy. Modern experimental taxonomy, however, taking advantage of the knowledge gained from Cytology, Genetics and Genecology, has succeeded in giving us more objective criteria by which to delimit the different taxonomic categories at the specific and infra-specific levels. The 'New Systematics' recognizes that in the final analysis the morphological, physiological and ecological or ethological differences between the species rest on differences in genetic constitution. When two populations, differing in their genetic constitution, have developed a certain degree of reproductive isolation, they can exist side by side in the same geographical region, and continuously diverging from each other, can adapt themselves to exploit different ecological habitats in the locality. Such a process has, perhaps, been responsible for the immense, and often bewildering, diversity of organic life met with even in relatively restricted habitats. The consensus of informed opinion, therefore, agrees in holding that the development of such barriers to free gene exchange is one of the most important steps in evolution.

Such reproductive isolation may be brought about by a variety of mechanisms acting at different stages in the life-history of the organism. Broadly, they may be classified into *External Factors*—such as spatial or seasonal isolation, which tend to prevent the occurrence of hetero-fertilization and *Internal factors*—which act after the gametes have united in fertilization, *i.e.*, during the development of the zygote to the stage of reproduction or even in later generations. The purpose of the present paper is to examine some of the internal mechanisms which may have been responsible for the development of reproductive isolation between the various tuber-bearing species of *Solanum*.

The tuber-bearing *Solanums* constitute a closely knit group of about 100 species with a natural distribution in a relatively restricted area in Central and South America. This group has been divided by taxonomists, on the basis of morphological criteria, into seventeen different, distinct series. Though these species differ markedly from each other, extensive cytogenetical studies have revealed the existence of certain peculiarities of behaviour, not commonly met with in other groups (*see* Magoon and Ramanujam, 1960, for a review).

There is much evidence in favour of the belief that structural alterations of chromosomes has been a very important source of hybrid sterility and consequent reproductive isolation of even sympatric populations. Perhaps the classical case in which the role of structural differences has been clearly worked out is *Crepis*. Unfortunately, however, the group under consideration at present—the tuberiferous *Solanums*—does not lend itself easily to such studies. First, the somatic chromosomes are comparatively small and it has been difficult till now to get satisfactory preparations in which the morphology of the chromosomes can be critically studied. Furthermore, the chromosomes are devoid of any morphological features which could serve as landmarks in the comparative evaluation of karyotypes. Nor has the study of meiosis in hybrids between different species led to any decisive conclusions. Analysis of the pachytene has proved quite difficult and little useful information bearing on the presence or absence of structural difference has been obtained so far from such studies. Reliance has, therefore, had to be placed on the data obtained from the later stages of meiosis which are more amenable to critical evaluation. Earlier workers (*see* Swaminathan and Howard, 1953) reported that there was a remarkable absence of meiotic disturbances due to lack of homology between the chromosomes, even in hybrids between taxonomically distinct species and suggested that this could be taken to show that there were no gross structural differences between the genomes involved. Recent studies on an extensive series of interspecific hybrids (Magoon and Ramanujam, 1960) have not fully substantiated such an assumption as various meiotic abnormalities, such as the occurrence of quadrivalents, belated separation of paired chromosomes, inversion bridges with and without fragments, T-chromosomes and various other meiotic aberrations, some of which clearly point to the existence of gross structural changes between the genomes involved, have been recorded. However, the exact role played by such gross structural changes in the differentiation of the species in this group is not quite clear. For one thing, structural differences and the resultant meiotic upsets, though definitely present, are not as extensive as one would expect them to be, especially in wide crosses such as those between species belonging to different taxonomic series. Nor does there seem to be any correlation between the occurrence of such meiotic abnormalities and the apparent taxonomic relationship of the species involved. Again, even hybrids between clones of the same species sometimes exhibit some of these irregularities, though to a markedly lesser extent. In view of the fact that these species are maintained predominantly through vegetative propagation, it is possible

that structural changes have accumulated even within the various clones of the same species (Magoon, Cooper and Hougas, 1958 *a*, *b*). It, therefore, becomes rather difficult to evaluate the role of such structural changes in species evolution in this group of plants.

Stebbins (1950) was the first to point out that structural differences between chromosomes could conceivably co-exist with high degree of regular meiotic pairing if it is assumed that the chromosome sets concerned differ by a large number of small structural differences. He also suggested that one way of detecting such 'cryptic' alterations, which cannot be recognized cytologically, would be to compare the frequency of multivalent formation in amphidiploids with that in autotetraploids of the two concerned parental taxa. A study on these lines was undertaken by Howard and Swaminathan (1952) to test the existence of such 'cryptic' structural differences between the genomes of various diploid species of *Solanum*. They found that the F_1 hybrids of all the seven interspecific crosses studied by them showed regular pairing and were fertile. Much fewer quadrivalents were formed by the amphidiploids obtained from five of these hybrids than were formed by the concerned autotetraploids. They interpreted this as showing the existence of 'cryptic' structural differences between the chromosomes of these five species. The other two amphidiploids showed a similar frequency of quadrivalent formation as the corresponding autotetraploids and as such were held to be free of such 'cryptic' structural differences. However, in one of these latter cases, when a different clone of the male parent was used, a highly significant lowering of the quadrivalent frequency in the amphidiploid was observed. This, as well as the fact that some species belonging to the same taxonomic series show evidence of 'cryptic' structural differences while amphidiploids of other intra-series hybrids behave more or less like autotetraploids, would appear to suggest that caution is necessary in using this test in tuber-bearing *Solanums*. It may be pointed out here that most of the interspecific hybrids between the species of *Solanum* at the same level, say between diploids, show fairly good fertility.

It has been assumed above that reduced homology between the chromosomes of the parental species is always due to structural differences. This, however, is by no means clear for chromosome homology is not a precise but only a relative concept. Cases of pairing between non-homologous regions, apparently as a haphazard response to the tendency of single parts to pair, has been recorded which, incidentally, could not be distinguished from homologous pairing, even when chromomere patterns were compared. It is also well known that pachytene pairing of even homologous regions is subject to genetic, environmental and mechanical interferences. It may well be possible, then, that specific pairing may vary not only from one locus to another but also between different alleles at the same locus. For, if the extrapolation commonly made from the observed local specificity of pachytene pairing to a specific pairing affinity between homologous loci is accepted, it follows that widely different alleles at the same locus may possibly

show lesser affinity than allelic pairs which differ only slightly. There appears, however, to be little evidence available in the literature bearing on this question of whether 'heterozygous genes' have as strong an affinity for each other as 'homozygous' ones have. Till evidence can be obtained on this point, therefore, it may not be fully justifiable on theoretical grounds, to attribute reduced homology solely and wholly to structural changes, 'cryptic' or patent, and the possibility of this phenomenon having a genic basis must also be taken into account.

It may also perhaps be desirable to briefly consider whether it is possible to distinguish operationally between genic and chromosomal basis for haplontic sterility. One criterion which has been sought to be used in such an operational distinction is the improvement in fertility which follows doubling of the chromosome number. However, as shown by Oka (1955) in rice, the increase in fertility on chromosome doubling could be explained as well by postulating a number of complementary genes determining gametophytic development. Therefore, this criterion does not help us to positively distinguish between genic and chromosomal haplontic sterility. As mentioned before, the test of preferential pairing in amphiploids cannot be considered a theoretically very satisfactory criterion. Nor, as Stebbins (1958) has shown, are genetic criteria depending on linkage with qualitative characters or the segregation ratios observed quite satisfactory in arriving at a clear demarcation between these two. Of course, the evidence is not incompatible with the existence of 'cryptic' structural changes involving as few as two genes or even one gene. It may, perhaps, be pointed out that at this level the distinction between a genic change and a chromosomal rearrangement becomes a rather academic one in the light of the recent ideas concerning the fine structure of the gene (Benzer, 1957). It is a fairly commonly accepted working hypothesis now that the coding of the genetic material may be in terms of triplets of nucleotide pairs and as suggested by Beadle (1957), gene mutations may be the result of structural changes within such triplets involving deletion, duplication or substitution of nucleotides. To distinguish operationally, therefore, between a duplication or inversion in what we may consider to be the functional genetic unit and one involving one, or at the most very few such functional units as suggested by Stebbins in the case of rice, is likely to present almost insuperable difficulties.

Polyploidy is an exceedingly effective process of reproductive isolation since hybrids between taxa with different chromosome numbers are bound to be highly unbalanced and sterile to a high degree. That a polyploid series occurs among the tuber-bearing *Solanums* is quite clear (Magoon and Cooper, 1959). The nature of the ploidy, *i.e.*, whether auto- or alloploidy is, however, a matter of controversy. At the hexaploid level, of course, many investigators agree that at least one of the genomes involved is partially differentiated from the other two (Magoon *et al.*, 1959 *b*). The position at the other levels is not quite so clear. Some authors consider the cultivated potato, *S. tuberosum*, to be an autotetraploid while others feel that it is more likely to be an allotetraploid or at least a segmental allotetraploid. Also, there

is considerable disagreement regarding the basic chromosome number of the genus and hence of the real status of the 'diploids' (Magoon, 1957; Magoon, Cooper and Hougas, 1958 *a*, *b*; Magoon and Ramanujam, 1960). So far as reproductive isolation is concerned, both auto- and allopolyploidy are likely to be equally effective in isolating the new taxon from the parents, though the latter is more likely to result in divergent evolution. It may be noted, however, that though a polyploid series occurs in the tuberiferous *Solanums*, effective internal barriers to crossing between species at various levels have apparently not been so well developed since crosses can often be effected between species at different levels of ploidy. The reason for this is not quite clear, though as will be suggested later, the predominantly vegetative method of reproduction may possibly have some bearing on the matter (Magoon, Hougas and Cooper, 1958 *a*, *b*, c, and 1959 *a* and *b*).

Gene mutations underlie the whole complex of morphological and physiological differences between species and in this respect, therefore, accumulation of gene mutation and species differentiation may be considered to be almost synonymous. There are a class of gene mutations, however, which have a more immediate part in bringing about reproductive isolation. Such are the genes which lead to hybrid inviability or breakdown or result in weak and unthrifty progeny in later generations. The immediate causes of hybrid inviability may be incompatibility between parental chromosomes and genes, interaction between the genes of one species and the cytoplasm of the other or incompatibility between the zygote and the maternal tissue. The last aspect of the problem has received comparatively little attention but the few investigations suggest that such diplontic sterility might have played some part in species differentiation within this group. Dwarf and unthrifty plants have been noted by a number of workers (Magoon and Ramanujam, 1960) in the F_2 generation of crosses between different diploid species. The occurrence of such aberrant plants may perhaps indicate that there are 'cryptic' structural differences between the chromosomes derived from the two parents. But, such unthrifty plants in the F_2 may also be the result of the random segregation of species-specific modifier complexes (Harland, 1936) or to the effects of the imposition of inbreeding on the self-incompatible and hence naturally cross-pollinated diploid species of *Solanum* (Swaminathan and Howard, 1953). The results obtained by Hawkes (1958), however, do not completely support the latter suggestion since large numbers of F_2 progenies have been raised (chiefly with *S. sparsipilium*) in which no unthriftiness occurs.

A probable example of hybrid breakdown at a somewhat later stage is the case recorded by Koopmans (*see* Magoon and Ramanujam, 1960) who found many floral abnormalities among the F_2 individuals of the cross, *S. rybinii* × *S. chacoense*. Though she attributes these to plasmatic effects, such breakdown of the hybrid progeny can perhaps be also due to disharmonious interaction between the combinations of parental genes resulting from their random assortment during meiosis

in the hybrid. However, Stephens (1950) prefers to explain such genetic imbalance as being due to the random distribution of deficiencies and duplications for small chromosomal segments, resulting from the existence of 'cryptic' structural differences between the two species concerned. It must be noted here, however, that such cases of hybrid inviability or breakdown or even sterility in the F_1 of crosses between species at the same chromosomal level are not very frequent in the genus *Solanum*, section *Tuberarium* and hence such diplontic genetic sterility cannot be held to have played a very important role in species differentiation in this group.

We have so far been considering only mechanisms involving nuclear determinants. It is well known, however, that cytoplasmic differences do exist between different taxa. Such cytoplasmic differences have been shown to exist between different species of *Solanum*. For instance, a number of workers have reported that the cytoplasm of *S. tuberosum* is not conducive to the normal meiotic behaviour of foreign genomes and leads to sterility while the reciprocal hybrids are quite fertile (*see* Magoon and Ramanujam, 1960, for review). Buck (1960) has recorded the occurrence of male sterility in certain interspecific hybrids involving *S. verrucosum* and has shown that this is due to the interaction of the *verrucosum* cytoplasm and a nuclear factor carried by the other diploid species involved. It may also be that the floral abnormalities noted by Koopmans in crosses between *S. chacoense* and *S. rybinii* may be due to a similar interaction between the ctyoplasm of one species and the genome of the other parent. Magoon, Cooper and Hougas (1958 *b*) have also adduced evidence which suggests that not only do such differences in plasmon properties exist but that they may be modified to different degrees in different species as a result of polyploidy induction.

However, the exact role played by such factors in species differentiation appears to be problematical. For, not only can gene exchange between the two taxa occur through the reciprocal hybrid but even clones of the same species often show differential behaviour. As such, it would appear that isolating mechanisms based on nuclear elements are likely to have been of much greater significance in divergent evolution than cytoplasmically determined factors.

One striking point, which emerges from a consideration of the crossability relationship of tuberiferous *Solanums* is that though most of the internal isolating mechanisms considered above are present, barriers to crossability between species appear to be rather feebly developed. This becomes very clear when we compare the situation with that obtaining in the non-tuber-bearing species belonging to the same genus, where they appear to be much more strongly developed (Magoon, Ramanujam and Cooper, 1962). The reason for such a difference in two closely related groups is not quite clear but it may perhaps lie in the fact that where highly efficient means of asexual propagation has been developed the advantage of securing variability through occasional crosses between differently adapted forms may outweigh

the disadvantage caused by the lowered reproductive efficiency of the hybrid or its progeny. Selection pressure favouring complete reproductive isolation may, therefore, be relatively weak in such a situation as compared to the intensity of such pressure in a situation where reproduction is wholly sexual. This may perhaps be one reason why comparatively wide crosses can be successfully made in the tuber-bearing *Solanums* unlike in the non-tuberiferous species of the genus.

Before concluding, it may also be pointed out that, thanks to the extensive vegetative propagation met with in this group, multitudes of 'microspecies' and intergrading forms, which confuse the taxonomic treatment of the group, could result through a process analogous to 'random drift' characteristic of small populations. In all studies on the interrelationship of the various members of this group, the role played by asexual propagation must, therefore, be always kept in mind.

REFERENCES

BEADLE, G. W. 1957. Role of the nucleus in heredity. *Chemical Basis of Heredity.* John Hopkins Press. Baltimore.

BENZER, S. 1957. The elementary units of heredity. *Ibid.* John Hopkins Press, Baltimore.

BUCK, R. W. 1960. Male sterility in interspecific hybrids of *Solanum*. *J. Hered.* **51**: 13-14.

HARLAND, S. C. 1936. The genetical conception of species. *Biol. Rev.* **11**: 83-112.

HAWKES, J. C. 1958. Taxonomy, cytology and crossability. *Handb. der Zuchtung* **3**: 1-39.

HOWARD, H. W. AND SWAMINATHAN, M. S. 1952. Species differentiation in the genus *Solanum* section. *Tuberarium* with particular reference to the use of interspecific hybridization in breeding. *Euphytica* **1**: 20-28.

MAGOON, M. L. 1957. Cytogenetic studies of some *Solanums*, section *Tuberarium*. *Univ. Wis. Publ.* **22**: 3390.

—— AND COOPER, D. C. 1959. Crossability among Mexican tetraploid *Solanum* species. *Phyton.* **12**: 39-41.

——, —— AND HOUGAS, R. W. 1958 *a*. Cytogenetic studies of some diploid *Solanums* section *Tuberarium*. *Amer. J. Bot.* **45**: 207-22.

————. 1958 *b*. Induced polyploids of *Solanums* and their crossability with *S. tuberosum*. *Bot. Gaz.* **119**: 224-33.

——————. 1958 *a*. Cytogenetic studies of South American diploid *Solanums* section *Tuberarium*. *Amer. Potato J.* **35**: 375-94.

——————. 1958 *b*. Cytological studies of diploid *Solanums*, section *Tuberarium*. *Ibid.* **35**: 723.

——————. 1958 *c*. Cytogenetical studies of tetraploid hybrids in the genus *Solanum*. *J. Hered.* **49**: 171-78.

——————. 1959 *a*. Cytogenetical studies of complex hybrids in the genus *Solanum*, sect. *Tuberarium*. *Ibid.*, **49**: 285-93.

——————. 1959 *b*. Chromosome pairing at different ploidy levels in the tuber-bearing *Solanums*. *J. Genet.* **57**: 279-298.

MAGOON, M. L. AND S. RAMANUJAM. 1960. Nature of chromosome pairing in the genus *Solanum*, section *Tuberarium*. *Indian J. Genet.* **20**: 22-41.

——, —— AND D. C. COOPER. 1962. Cytogenetical studies in relation to the origin and differentiation of species in the genus *Solanum* L, *Caryologia*. **15**: 151–252.

OKA, H. 1955. Studies on tetraploid rice. IV. Fertility variation and segregation ratios for several characters in tetraploid hybrids of rice, *Oryza sativa* L. *Cytologia* **20**: 258-66.

STEBBINS, G. L. 1950. *Variation and Evaluation in Plants*. Columbia Univ. Press, New York.

———. 1958. The inviability, weakness and sterility of interspecific hybrids. *Advanc. Genet.* **9**: 147–215.

STEPHENS, S. G. 1950. The internal mechanism of speciation in *Gossypium*. *Bot. Rev.* **16**: 115–19.

SWAMINATHAN, M. S. AND HOWARD, H. W. 1953. The cytology and genetics of the potato (*Solanum tuberosum*) and the related species. *Bibliogr. genet.* **16**: 11-192.

—— AND MAGOON, M. L. 1961. The origin and cytogenetics of the commericial potato. *Advanc. Genet.* **10**: 217–256.

APPLICATION OF PACHYTENE ANALYSIS TO EVOLUTIONARY PROBLEMS IN THE GENUS *ORYZA*

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Although cytological investigations in the genus *Oryza* date back to 1910 by Kuawada, present knowledge concerning the interrelationships between the 26 valid species, on the nature of genetic differentiation between the two subspecies of *O. sativa* and of the genome analysis is too fragmentary. Analysis of post-diakinetic stages of meiosis for the studies on chromosomal pairing and of somatic metaphases for the study of karyomorphology, both of which are of limited value in species with small (0·7–2·8 μ at metaphases in root-tips) chromosomes seem to be the major reasons for this situation. More recently, however, rice cytologists have been employing pachytene analysis for the study of karyotypes (Shastry, Rao and Misra, 1960; Shastry and Mohan Rao, 1961), and the analysis of intervarietal (Yao, Henderson and Jodon, 1958; Shastry and Misra, 1961 *a*, *b*) and interspecific hybrids (Shastry, Sharma and Rao, 1960, 1961). The present paper deals with the experimental results secured by the author and his collaborators at the Division of Botany, Indian Agricultural Research Institute, New Delhi, during the year 1959–60.

1. *Karyotypic evolution in the genus* Oryza.—In several plant genera, the karyotypic data revealed that the primitive taxa have more symmetric karyotypes than the more evolved ones. Stebbins (1958) recognized two forms of asymmetry, *viz.*, the variation in size between the smallest and the largest chromosomes and the predominance of sub-telocentric chromosomes. Based upon the two criteria, he suggested 12 groups under which the variation with regard to asymmetry can be classified. Karyotypic data thus far collected by pachytene analysis (Shastry and Mohan Rao, 1961; Misra and Das, unpublished) led to some interesting indications. The primitive species of the genus, *O. australiensis*, *O. perennis* and *O. stapfii* are characterized by more symmetric karyotypes (group 2-*b* of Stebbins, 1958) than those of *O. sativa* (group 3-*c* of Stebbins, 1958). Within the species, *O. sativa*, the *japonica* types have more asymmetric karyotypes than the *indica* types.

2. *Genetic differentiation between the subspecies,* japonica *and* indica.—Since the time Kato recognized two subspecies, *japonica* and *indica* in the species, *O. sativa*, the nature of genetic differentiation between these subspecies and the causes for sterility in the inter-subspecific hybrids have received the attention of several workers (Kuang,

1951; Mello-Sampayo, 1952; Sampath and Mohanty, 1954; Yao *et al.*, 1958; Henderson, Yeh and Exner, 1959, and Oka, 1957). All these investigators except Oka and his collaborators (*cf.* Oka, 1957) agreed upon the cryptic structural differences between the karyotypes of these subspecies. Oka, on the other hand, postulated that the sterility in these hybrids is due to the operation of complementary lethal genes (Gamete development, Duplicate fertility). This controversy is attenuated by the citation of evidence from M. I and A. I data alone and has not led to any dependable conclusion in favour of either of the views.

Shastry and Misra (1961, *a*, *b*) for the first time reported that the pachytene pairing in some of the *japonica-indica* hybrids is extremely abnormal (Fig. 1). In one hybrid, 31 per cent. of the total chromatin remained unpaired either as terminal or interstitial differential segments. They pointed out that these differential segments might represent cases of translocations for reasons that (1) typical inversion loops are most infrequent and (2) inversion heterozygosity can lead to sterility only as a consequence of bridge formation, which is infrequent, as pointed out by Henderson *et al.* (1959) and was apparent in their data. This investigation highlighted the fallacy of considering all cryptic structural hybridity in the form of inversions, as was done by Yao *et al.* (1958) and Henderson *et al.* (1959) and the absence of inversion bridges in the hybrids as a proof for genic sterility as was done by Oka (1957).

Another significant observation was that the percentage of chromatin length unpaired, designated by them as "Differential Index" bears a positive relationship to sterility. This may be cited as evidence for the major contribution of translocations in the origin and manifestation of differential segments. Recent evidence of Mizushima and Kondo (1960) that the *Sp* locus occupies non-allelic positions in *japonica* and *indica* varieties will be an additional support to such a view of the role of translocations.

Pachytene data in the *japonica-indica* hybrids not only account for sterility, but also the accompanying genetic effects, *viz.*, non-recovery of recombinant phenotypes which might be considered to arise from crossing over in the paired regions of the bivalents leading to origin of deletions (in a system balanced for viability by structural changes) and for the origin of abnormal (*albina*) and "mutant" plants in selfed progenies, which might owe their origin to small viable deletions arising from a complex cryptic structural hybrid.

3. *Genomic relationships between the sections Sativa and Officinalis.*— Morphology of the panicle, size of the spikelets and anthers and the shape of the caryopses are the recognized characters in distinguishing the sections, Sativa and Officinalis. The typical members of these sections, *O. sativa* and *O. officinalis*, hybridize but result in a hybrid which is completely sterile and which forms 24 univalents at metaphase I (Ramanujam, 1938; Gopalakrishnan, 1959). These observations led Richharia (1960) to consider that there is no homology between the genomes (*P* and *O*) of these species.

The study of meiosis of the hybrid *O. sativa* × *O. officinalis* by Shastry, Sharma and Rao (1960) revealed that the anaphase disjunction of univalents was regular (12–12) in 29·3 per cent. and nearly regular (11–13) in 30·5 per cent. of the total 82 PMCs studied at anaphase I. These observations led them to suspect pairing at prophase. At diakinesis, varying number of bivalents were recorded while at diplotene 2–12 bivalents per PMC were observed (Fig. 7). At pachytene, the pairing was exceedingly normal (Fig. 2). Complete pairing at pachytene and the drop in the associations with the progress of meiosis (Figs. 4, 5, 7) led them to postulate that there exist no restrictions to homology between the chromosome complements of *O. sativa* and *O officinalis* and that the high degree of univalent formation at M. I is due to desynapsis between the chromosomes which have enough structural homology to pair at pachytene, but which have been enough differentiated by gene-mutations to affect viability accompanying their substitution by independent assortment. It will be clear how misleading the metaphase pairing alone can be for elucidation of homology. Further, this offers another example that chromosome structural differentiation and isolation are unrelated in their origin and complexities of manifestation. The divergent morphological specialization between the sections, Sativa and Officinalis might owe its origin to a primary event, the development of isolation barrier by desynapasis. The non-complementation of the chromosomes of *O. officinalis* and *O. sativa*, which might be considered as the main reason for complete sterility of this hybrid, might be cited as an additional evidence in favour of functional diploid nature of the species of *Oryza* with $2n = 24$.

4. *Timing imbalance in meiosis of the F_1 hybrid*, O. sativa × O. australiensis.—The species, *O. australiensis*, which is endemic to Australia is similar to *O. perennis* in the rhizomatous stem and spikelet and anther lengths and to *O. officinalis* in its panicle morphology. Further, Gopalakrishnan (1959) recorded a maximum of 8 bivalents in the F_1 hybrid *O. sativa* (bearing the same genome as *O. perennis*) × *O. australiensis* and only 4 bivalents in the F_1 hybrid, *O. sativa* × *O. officinalis*. Morphological and cytological data cited above were considered enough to place this species in an intermediate position between the sections, Sativa and Officinalis (Richharia, 1960) and to consider that it has originated by hybridization between the species belonging to these two sections (Gopalakrishnan, 1959).

High degree of heterochromatinization of the pachytene bivalents, high symmetry in the karyotypes and the non-occurrence of *O. officinalis* in Australia appear to be the major objections to the hybrid origin of *O. australiensis*. Since the meiotic data contributed the critical evidence for the hypothesis of hybrid origin, this cross, *O. sativa* × *O. australiensis* was reinvestigated by Shastry and Rao (1961). Contrary to the observation of Gopalakrishnan, no true allosyndetic bivalents were observed. The most frequent associations were non-chiasmatic, end-to-end pseudobivalents (Fig. 3) followed by 1–2 autosyndetic bivalents of *O. sativa*. The size difference between the chromosomes of the constituent species of the cross rendered identification of the complements

possible at all stages of meiosis from diplotene to anaphase I. Further, at the early prophase (diplotene) only *australiensis* chromosomes were visible (enough condensed) while those of *sativa* were barely visible as ghosts and were secondarily associated. At diakinesis, the number of univalents was variable (the full complement of *O. australiensis* with variable number of *sativa* univalents). At M. I, the univalents of *australiensis* migrated to poles earlier than those of *sativa* (Fig. 6).

The above observations clearly point out the timing imbalance in the condensation and migration of univalents of *O. sativa* and *O. australiensis*. Further, at no stage, the true allosyndetic bivalents were recorded which might indicate either that no homology exists between the complements of the species or that the timing imbalance is responsible for the lack of pairing. In any case, there is enough reason to consider that *O. australiensis* (in comparison to *O. officinalis*) is more distantly related to *O. sativa*, an inference in total contradiction to that of Gopalakrishnan. Further, it is pointed out that *O. australiensis* might represent an isolate from the pre-Sativa and pre-Officinalis complex.

5. *Pairing as a criterion of homology.*—The basic theme of the theory of meiosis is that homology is a prerequisite for synapsis. Despite the great impact of this concept on evolutionary problems and the cell mechanics, our knowledge concerning the mechanism of pairing and causes for failure of pairing are exceedingly meagre. Further, it is paradoxical that inferences on homology are by and large drawn from the pairing data from diakinesis and M. I stages, although it is well recognized that pairing is initiated much earlier and that terminalization, desynapsis, etc., greatly obliterate the persistence of the asociations. An additional limitation in this concept is the recognition of homology and non-homology as two discontinuous steps in the evolution leading to pairing or failure of pairing respectively.

Distinct genome symbols thus far proposed in several plant genera (*Triticum*, *Gossypium*, *Brassica*, *Oryza*, etc.) were all based upon the data secured from M. I stages, with the implication that no pairing occurs between the genomes. Recent work in bread wheat (Riley and Chapman, 1958), however, showed that homologous segments do occur between which the pairing is suppressed by a superimposed genic mechanism. Evolutionary significance of such phenomena cannot be underestimated since these might be a part of the natural trend towards diploidization of polyploids. From an original genetic pool, the origin of two distinct genomes is best visualized by a series of chromosome structural changes leading to failure in pairing. The consequence of such a differentiation is mainly the preservation of the integrity of the taxa. If the term genome is applied for cases where no homologous segments occur between the complements of two taxa, there exists no example in literature where this is unequivocally demonstrated. Even in cases where it can be demonstrated that pachytene pairing does not take place as between *O. sativa* and *O. australiensis*, it has not been possible to decide whether the lack of pairing is due to

lack of homologous segments or due to timing imbalance. The genetic differentiation between *O. sativa* and *O. officinalis*, on the other hand, as judged by pachytene pairing appears to be extremely limited and hence there is no justification in considering them under different genomes. If it is considered that all taxa whose chromosome complements retain their integrity with no recombination as belonging to distinct genomes, the genome concept loses its distinctiveness from other isolation mechanisms such as diplontic sterility, cross-incompatibility, and balanced lethals. To consider all taxa differentiated by any one of these various mechanisms as belonging to distinct genomes will be to disrupt the very concept itself.

The next problem to be considered is whether in all cases synapsis at pachytene can be considered as a result of homology. While the concept appears to be largely true, as established by pairing in gross structural hybrids, several reports of not realizing the expected pairing behaviour (*see* Ting, 1958 and Maguire, 1960) do exist in literature. However, for lack of another line of approach to this problem, at present it is safe to assume that during meiosis, homologous pairing takes place right down to molecular level and that stages closest to zygotene, where pairing is initiated, are to be relied greater than the later stages of meiosis. In conclusion, the merits of pachytene analysis may be summarized as follows:

(1) This is the most stable stage during meiosis, the condensation differences being comparatively less if PMCs at mid-pachytene are chosen.

(2) This is the earliest stage in the meiosis where analysis of the *entire chromosome complement is possible.*

(3) The primary unit of evolutionary change being a chromosomal segment, microscopic examination of structural hybridity is greatly facilitated by the juxtaposition of the chromosomes to be compared.

(4) A low degree of condensation in comparison to somatic metaphases renders this method ideally suited for species with small chromosomes, which have thus far been less investigated.

(5) Serial differentiation of the chromosomes, (eu- and heterochromatin), which is difficult to demonstrate in somatic metaphases, can be readily demonstrated at pachytene.

SUMMARY

(1) The karyotypes of the wild species of *Oryza* are more symmetric than those of the cultivated species. Within the species, *O. sativa*, the karyotypes of *japonica* types are more asymmetric than those of *indica* types.

(2) The differentiation between the subspecies *japonica* and *indica* is probably achieved by a series of translocations. The hybrids between them exhibit a high degree of abnormalitty in pairing at pachytene,

(3) Complete univalent formation in a majority of PMCs of the F_1 hybrid, *O. sativa* × *O. officinalis*, is due to desynapsis rather than due to lack of homology between the complements of the species.

(4) No pairing was observed between the chromosome complements of *O. sativa* and *O. australiensis*. The meiosis of the hybrid between these species is characterized by a timing imbalance in condensation and migration of univalents.

(5) The concepts, genome and homology were discussed in relation to interspecific genetic differentiation in the genus *Oryza*.

ACKNOWLEDGEMENTS

The author is grateful to Dr. B. P. Pal, Director and Dr. A. B. Joshi, Dean, P. G. School, for providing facilities, to Dr. M. S. Swaminathan for valuable discussions and to his colleagues, Messrs. D. R. R. Rao, P. K. M. Rao, S. D. Sharma and R. N. Misra, for their collaboration during this study.

REFERENCES

GOPALAKRISHNAN, R. 1959. Cytogenetical studies on interspecific hybrids in the genus *Oryza*. *Thesis, Indian Agric. Res. Inst., New Delhi.*

HENDERSON, M. T., YEH, B. P. AND EXNER, B. 1959. Further evidence of structural differentiation in the chromosomes as a cause of sterility in intervarietal hybrids of rice, *Oryza sativa* L. *Cytologia* **24**: 415-22.

KUANG, H. H. 1951. Studies on rice cytology and genetics as well as breeding work in China. *Agron. J.* **43**: 387-97.

KUAWADA, Y. 1910. A cytological study of *Oryza sativa* L. *Bot. Mag., Tokyo* **24**: 267-81.

MAGUIRE, M. P. 1960. A study of pachytene chromosome pairing in a corn—*Tripsacum* hybrid derivatives. *Genetics* **45**: 651-64.

MIZUSHIMA, U. AND KONDO, A. 1960. Fundamental studies on rice breeding through hybridization between Japanese and Foreign varieties. II. Structural differences of chromosomes between a Japanese and Indian variety proved by anomalous mode of segregation in apiculus anthocyanin in their hybrid (*Eng. sum.*). *Japanese J. Breed.* **10**: 1-19.

MELLO-SAMPAYO, T. 1952. An inversion occurring in an F_1 hybrid between two strains of *Oryza sativa* L. *Genet. iber.* **4**: 43-46.

OKA, H. 1957. Gene analysis for the sterility of the hybrids between distantly related varieties of cultivated rice. *J. Genet.* **55**: 397-409.

RAMANUJAM, S. 1938. Cytogenetical studies in the *Oryza*. III. Cytogenetical behaviour of an interspecific hybrid in *Oryza*. *J. Genet.* **35**: 223-58.

RICHHARIA, R. H. 1960. Origins of cultivated rices. *Indian J. Genet.* **20**: 1-14.

RILEY, R. AND CHAPMAN, V. 1958. Genetic control of the cytogenetically diploid behaviour of hexaploid wheat. *Nature, Lond.* **182**: 713-15.

SAMPATH, S. AND MOHANTY, H. K. 1954. Cytology of semisterile rice hybrids. *Curr. Sci.* **23**: 182-83.

SHASTRY, S. V. S., RAO, D. R. R. AND MISRA, R. N. 1960. Pachytene analysis in *Oryza*. I. Chromosome morphology in *Oryza sativa*. *Indian J. Genet.* **20**: 15–21.

——, SHARMA, S. D. AND RAO, D. R. R. 1960. Cytology of an inter-sectional hybrid in *Oryza*. *Naturwissenschaften* **24**: 608–09.

——, SHARMA, S. D. AND RAO, D. R. R. 1961. Pachytene analysis in *Oryza*. III. Meiosis in an inter-sectional hybrid, *O. sativa* × *O. officinalis*. *The Nucleus* **14**: 67–80.

—— AND MOHAN RAO, P. K. M. 1961. Pachytene analysis in *Oryza*. IV. Chromosome morphology of *O. australiensis*, *O. glaberrima* and *O. stapfii*. *Proc. Indian Acad. Sci.* **54** B: 100–112.

—— AND MISRA, R. N. 1961 *a*. Pachytene analysis in *Japonica indica* rice hybrids. *Curr. Sci.* **30**: 70–71.

————. 1961 *b*. Pachytene analysis in *Oryza*. II. Sterility in the *japonica-indica* rice hybrids. *Chromosoma* **12**: 248–271.

SHASTRY, S. V. S. AND RAO, D. R. R. 1961. Timing imbalance in the F_1-hybrid, *Oryza sativa* × *O. australiensis*. *Genetical Res. (Camb.)* **2**: 373–383.

STEBBINS, G. L. JR. 1958. Longevity, habitat and release of variability in higher plants. *Cold Spr. Harb. Symp. quant. Biol.* **23**: 365–78.

TING, Y. C. 1958. Inversion and other characteristics of *Teosinte* chromosomes. *Cytologia* **23**: 239–50.

YAO, Y., HENDERSON, M. T. AND JODON, N. E. 1958. Cryptic structural hybridity as a possible cause for sterility in intervarietal rice hybrids. *Ibid.* **23**: 46–55.

EXPLANATION OF PLATE V

FIG. 1. Pachytene. F_1 hybrid, T. 21 (*indica*) × A-18 (*japonica*). Note several differential segments and heteromorphic ends of bivalents.

FIG. 2. Pachytene. F_1 hybrid, *O. sativa* × *O. officinalis*. Note the regular pairing.

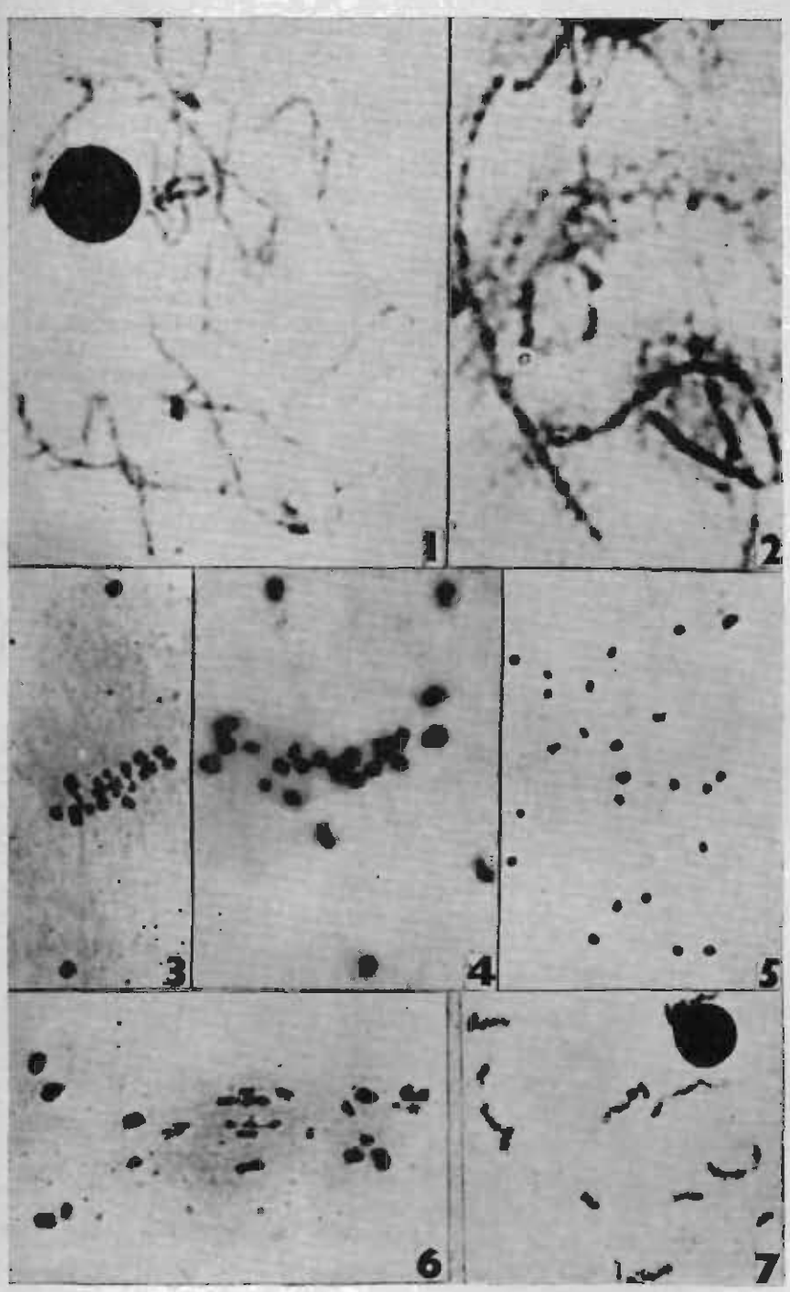
FIG. 3. Metaphase I. F_1 hybrid, *O. sativa* × *O. australiensis*. 2 univalents of *O. australiensis* at the poles. 4-end-to-end allosyndetic pseudobivalents.

FIG. 4. Metaphase I. F_1 hybrid, *O. sativa* × *O. officinalis* 24_I. Note the congression on the equatorial plate.

FIG. 5. Metaphase I. F_1 hybrid *O. sativa*, × *O. officinalis* 24_I. Note that the size difference between the univalents of the species is not clear.

FIG. 6. Meta-anaphase I. F_1 hybrid, *O. sativa* × *O. australiensis*. Note the large univalents of *O. australiensis* at the poles and the small univalents of *O. sativa* at equator.

FIG. 7. Diplotene. F_1 hybrid, *O. sativa* × *O. officinalis*. 6 bivalents with one chiasma each and 12 univalents.



FIGS. 1–7

S. V. S. Shastry

CYTOGENETICAL EVOLUTION IN *ANEILEMA SENSU LATO* IN* EASTERN INDIA

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Of late, the family Commelinaceae has been subjected to serious taxonomic and cytological investigations both in India and abroad. The recognition of *Aneilema* and *Murdannia* as two distinct genera and gradual transfer of the species of *Aneilema sensu lato* to *Murdannia*, raised interesting problems of nomenclature. Transfer of all but one East Indian species of the sections *Euaneilema* to *Murdannia* and retention of all but one species of the section Dictyospermum in *Aneilema* raised genuine doubt regarding the interspecific affinity of species included in different groups as proposed by Clarke (*cf.* Hooker, 1894). Further. the existence of various morphological forms within the polymorphic species *Murdannia* (= *Aneilema*) *nudiflora* including *A. nudiflora* var. *terminalis* Wight called for intensive studies in experimental taxonomy for their solution. Discovery of aneuploidy, polyploidy and other cytological aberrations in the family as a whole, coupled with their perennial habit and easy vegetative propagation necessitated the cytotaxonomic revision of the family Commelinaceae in India. While our observations on problems of nomenclature, with citation of literature, important morphological characters of taxonomic value and notes on the habitat and distribution, etc., and our findings on cytology of the 10 East Indian species are being published elsewhere, this paper summarises all available cytological data including our own on the genus *Murdannia* and *Aneilema sensu stricto* in a tabular form (Table I) and discusses the cytogenetical evolution in the genus *Aneilema sensu lato*.

Discussion

Study of the relevant literature suggests that basis of classification in the genus, *Aneilema sensu lato* is provided by the number of ovules and seeds in each cell of the tricarpellary ovary, all the Indian species of the genus being included in the subgenus *Tricarpellaria* Clarke. While the three sections of this subgenus are distinguished from each

* The Junior author (*viz.* K.V.K.) made the cotological preparations of the East Indian species cited in the paper; the senior author (*viz.* G. P.) is solely responsible for the preparations of the text and the conclusions embodied in this paper, which was written up and communicated in April 1962.

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TABLE I

Cytological data of Aneilema sensu lato *from India and abroad*

Subgenus 1. ***Tricarpellaria*** Clarke

	Mitosis	Meiosis	Locality	Investigators
SECTION 1				
EUANEILEMA:				
1. *Murdannia divergens*	..	30	Shillong	Panigrahi and Kamathy, 1961
2. *M. clata* ..	40	..	Rongo, India	Sharma and Sharma, 1958
(=*Aneilema herbaceum*)	42	21	Assam	Panigrahi and Kamathy, 1961
3. *M. spirata* ..	..	20	Shillong	Panigrahi and Kamathy, 1961
(=*A. spiratum*)	20	..	Darjeeling	Sharma and Sharma, 1958
	40	..	Plains of South India	Murthy, 1934
	..	9	Mysore	Raghavan and Seshagiri, Rao, 1961
4. *M. triquetra* ..	40	..	Dibrugarh	Panigrahi and Kamathy, 1961
5. *M. nudiflora*	..	10	Shillong	Panigrahi and Kamathy, 1961
(=*A. nudiflorum*)	20	..	South-East Asia	Simmonds, 1954
6. *M. nudiflora*	20	10	Plains of West Bengal	Sharma, 1955
var. *terminalis*	39	$18^{II}+1^{III}$	Margherita	Panigrahi and Kamathy, 1961
7. *M. simplex*	..	20	South India	Shetty and Subramanyam, 1961
(=*A. sinicum*)	..	30	Mysore	Raghavan and Seshagiri Rao, 1961
	..	40	Assam	Panigrahi and Kamathy, 1961
8. *M. gigantea* ..	..	11	Shillong	Panigrahi and Kamathy, 1961
				Seshagiri Rao *et al.*, 1960
SECTION 2				
DICTYOSPERMUM:				
9. *M. vaginata* (=*A. vaginatum*)	40	..	Plains of West Bengal	Sharma and Sharma, 1958
10. *Aneilema protensum*	..	59^{II}	Shillong	Panigrahi and Kamathy, 1961
11. *A. montanum* ..	..	14	South India	Shetty and Subramanyam, 1961
Excluded species				
12. *A. aequinoctiale* ..	..	24	Darjeeling	Panigrahi and Kamathy, 1961

other again on the number of ovules per cell of ovary (*viz.*, 2 ovules in EUANEILEMA, 4–20 ovules per cell in DICHAESPERMUM and 1 ovule per cell in DICTYOSPERMUM), further subdivisions within each section is based on the manner of disposition of leaves on the flowering axis. Recent authors, however, recognise two genera, *viz.*, *Aneilema* R. Br. *sensu stricto* and *Murdannia* Royle within the limits of *Aneilema sensu lato*, on the manner of disposition of the fertile stamens and sterile staminodes with respect to petals and in relation to anterior or posterior side of mother axis.

Accordingly, of the 16 species of *Aneilema sensu lato*, described by Hooker to occur in Eastern India 11 species belong to *Murdannia* Royle, of which 9 species are included in EUANEILEMA section whereas only one species belongs to each of the other two sections. The remaining 5 species, *viz.*, *Aneilema thomsonii*, *A. montanum*, *A. conspicuum*, *A. protensum* and *A. æquinoctiale* are treated under *Aneilema sensu stricto*.

Although no cytological investigation has yet been made on *M. hamiltoniana* of the DICHAESPERMUM section and only one chromosome number report, *viz.*, $2n = 40$ is available for *M. vaginata* of the Dictyospermum section, they are undoubtedly very different in their morphological features and ecological amplitude, not only from each other, but also from the members of the EUANEILEMA section.

Of the 9 species of the EUANEILEMA section studied here, *M. scapiflora* with its radical leaves and with the panicle borne on leafless scape and *M. triquetra* with 1–3 flowered, axillary cymes borne on semiprostrate leafy stems, are easily distinguished from the other 7 species in this section.

The four species, *M. divergens*, *M. elata*, *M. hookeri* and *M. spirata* all share panicled inflorescence borne on leafy stems, and 3 fertile stamens alternating with 3 sterile, trilobed staminodes (*cf. M. elata* has staminodes bilobed like those in typical *Aneilema*). Among these *M. divergens* and *M. elata* on the one hand and *M. hookeri* and *M. spirata* on the other appear more closely allied to each other both in general habit and in their preference for ecological habitats.

Although *M. nudiflora* (including var. *terminalis*), *M. simplex* and and *M. gigantea* share 2-ovuled ovary and 2-seeded capsule with each other, there are rather striking differences between them in their general habit. While *M. simplex* and *M. gigantea* each show great uniformity in morphological features and restricted nature of their distribution in Khasi Hills, *M. nudiflora* is rather a polymorphic species showing a great range of morphological variants spread almost all over India in varying ecological niches. *M. nudiflora* var. *terminalis* possesses intermediate morphological features between *M. nudiflora* complex and *M. simplex*.

Studies in cytology reveal interesting aspects of evolution in the group.

Discovery of $n = 10$ in the biotypes of *M. nudiflora* from Shillong (1500 m.) confirms the number $2n = 20$ reported earlier for the species by Sharma (1955) from the plains of West Bengal and by Simmonds (1954) from S.E. Asia. Therefore, *M. nudiflora* is a diploid species based on $x=10$. Whereas Sharma and Sharma (1958) report $2n = 20$ *in the biotypes of M. spirata from Darjeeling (1800 m.), our finding of* $n = 20$ from Shillong (1500 m.) is in agreement with that of $2n = 40$ in the South Indian biotypes of the same species (*cf.* Murthy, 1934). But the report of $n = 9$ in *M. spirata* from Mysore by Raghavan and Seshagiri Rao (1961) shows the prevalence of a different base number within the species. So, three cytological types, *viz.*, $n = 9$, 10, 20 characterise the population of *M. spirata*. These two species, therefore, confirm Sharma and Sharma's (1958) contention of the absence of any correlation between altitude and polyploidy in the genus *Murdannia*.

Again, the finding of $n = 30$ in *M. divergens* and $n = 40$ in *M. simplex* suggests their hexaploid and octoploid nature, respectively. The reports of $n = 20$ in *M. simplex* (*Aneilema sinicum*) by Shetty and Subramanyam (1961) and of $n = 30$ by Raghavan and Seshagiri Rao (1961), however, indicate that three cytological races, *viz.*, $4n$, $6n$ and $8n$ also occur in *M. simplex*. Therefore, the four species, *M. nudiflora* ($2n$), *M. spirata* ($2n$, $4n$), *M. divergens* ($6n$) and *M. simplex* ($4n$, $6n$ and $8n$), constitute a polyploid series based on $x = 10$. All these cytological races form regular bivalents at diakinesis, without any sign of multivalents or univalents, form good pollen grains stained red with warm acetocarmine, and are characterised by good seed-setting. It is, therefore, rather difficult to postulate the role of autopolyploidy in the speciation in this group, unless the very small size of the chromosomes met with in the genus *Murdannia*, may account for the failure *to form multivalents. Or, it may be that autopolyploid origin of these* species occurred long long ago so that, due to structural changes in chromosomes, the tendency to form multivalents is gradually lost. It is, therefore, essential to carry out both intraspecific hybridisation (*e.g.*, in *M. spirata* between $2n$ and $4n$) and interspecific hybridisation between these four species before hypothesising the process of evolution in the group.

This satisfying cytological picture of euploidy based on $x = 10$ in four species of the genus *Murdannia*, however, is complicated by the discovery of $n = 11$ in *M. gigantea* and of $n = 21$ in *M. elata*. *M. gigantea* forms 11 clear bivalents and *M. elata* shows distinctly 21 bivalents in a large number of cells at diakinesis. There are no univalents or multivalents, they form good pollen grains and yield good seeds profusely. Therefore, the existence of four different base numbers, *viz.*, $x = 10$ or 5, $x = 21$ or 7, $x = 11$ and $x = 9$ or 3 within a small group of Indian species cytologically investigated, is strongly suggested.

However, *M. gigantea*, with its striking morphological features, an odd base number together with the very large size of chromosomes can at once be set apart from all other species of *Murdannia* and, therefore, its evolutionary significance in the group can hardly be minimised.

Similarly, *M. elata* (*Aneilema herbaceum*) with its bilobed staminode characteristic of *Aneilema sensu stricto* shares its arrangements of stamens and staminodes in the flowers with that of *Murdannia* and is treated as *M. elata* by recent workers. But the discovery of $n = 14$ in *Aneilema montanum* (Shetty and Subramanyam, 1961) and our finding of $n = 21$ and $2n = 42$ in *M. elata* together with the possession of bilobed staminodes might suggest the retention of *M. elata* under *Aneilema sensu stricto*, the former serving as a connecting link between the two genera.

The clue to the origin of these different base numbers is yet shrouded in mystery and may only be conjectured from the occurrence of $2n = 39$ in *M. nudiflora* var. *terminalis* and $n = 59$ in *A. protensum* Wall. Whereas *M. nudiflora*, the diploid species, forms clearly 10 bivalents, its variety *terminalis* growing sympatrically shows 18 bivalents and 1 trivalent at diakinesis and 20–19 separation of chromosomes at anaphase I. This variety may, therefore, be looked upon as a tetraploid cytotype (*i.e.*, $4n - 1$), from which one chromosome is lost during the process of speciation from *M. nudiflora* with $n = 10$ or its derivatives. The formation of 2 types of pollen grains, one with 19 chromosomes and the other with 20 chromosomes, in *M. nudiflora* var. *terminalis* and the production of profuse viable seeds open out vast possibilities for microevolution and may explain the prevalence of a large number of morphological intergrades between *M. nudiflora* and its variety *terminalis*.

Similarly, *Aneilema protensum* with 59 bivalents at diakinesis forms good pollen grains and produces good seeds profusely. *A. protensum* with $n - 59$ or $(12n = 2)$ shows that even a typical species of *Aneilema sensu stricto* must have arisen from a base number $x = 10$ in the same way as the origin of $x = 9$ in *M. spirata* or of $2n = 39$ in *M. nudiflora* var. *terminalis*, discussed above.

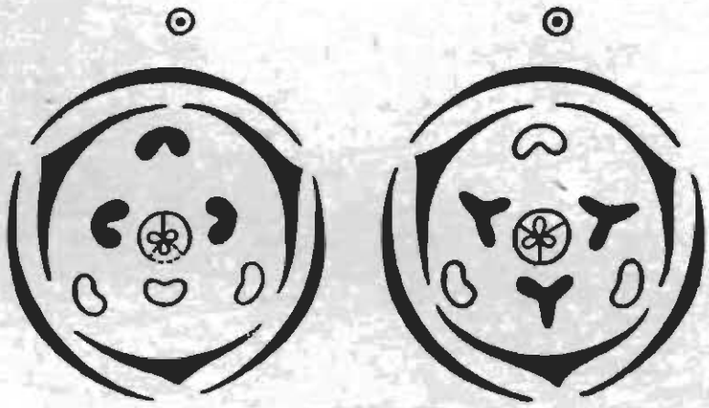
Aneilema aequinoctiale Kunth from Africa to which *Amelina wallichii* Clarke from Darjeeling is synonymous is treated as an excluded species from the scope of *Aneilema sensu lato* by Hooker (1894) in the *Flora of British India*. The root-stock producing 2 or 3 stout branches at the ground level, the hairy stems and pubescent leaves, older basal stems bearing dried up leaf-sheaths, the inconspicuous terminal inflorescence bearing only a few flowers and a few seeds and the Indian distribution of the species restricted to Darjeeling only, mark out the species as very distinctive amongst the Indian species of *Aneilema sensu lato*.

Does, then, cytology also provide any evidence in favour of Hooker's treatment as stated above? Presumably, it does. The discovery of $n = 24$ in *Amelina wallichii* Clarke from Darjeeling showing formation of a quadrivalent and 23 bivalents in one cell and of clear 24 bivalents in other cells together with the invariably end-to-end pairing of bivalents at diakinesis, present an altogether different cytological picture from that of all other Indian species of *Aneilema sensu lato*. The peculiar end-to-end pairing, despite its comparatively larger chromosomes (*cf.* the very small x-shaped or ring-shaped bivalents in *M. simplex*), suggests the lack of homology involving very large segments of

allelomorphic pairs. The formation of only terminal chiasmata involving 24 pairs of chromosomes (except a single tetravalent seen in one cell only) may suggest certain amount of genetic disharmony between the two genomes and may explain the low output of good pollen grains and viable seeds from the very few flowers produced during the season. Despite formation of 24 bivalents at diakinesis, therefore, *Aneilema aequinoctiale* Kunth or *Amelina wallichii* Clarke behaves as a partially sterile hybrid, which propagates itself largely by vegetative means.

Thus, the possession of $n = 24$ in this species provide a different base number $x = 24$ or 6 within the genus *Aneilema sensu lato*. Yet, this finding or the end-to-end pairing of chromosomes at diakinesis no more provide evidence for the exclusion of *Amelina wallichii* from *Aneilema sensu lato* (nor from *Aneilema sensu stricto*) than the discovery of $n = 59$ in *A. protensum* would justify the exclusion of the latter from *Aneilema sensu lato*.

Thus, meiotic studies involving a few of these species in Eastern India reveal a typical euploid series based on $x = 10$ or 5, *viz.*, *M. nudiflora* (2 *n*), *M. spirata* (2 *n*, 4 *n*), *M. vaginata* (4 *n*), *M. triquetra* (4 *n*), *M. divergens* (6 *n*), *M. simplex* (4 *n*, 6 *n*, 8 *n*); two euploid species based on $x = 14$ or 7, *viz.*, *Aneilema montanum* (4 *n*) and *M. elata* (6 *n*); one species based on $x = 11$ in *M. gigantea* (2 *n*); one cytotype based on $x = 9$ in *M. spirata*; one species based on $x = 24$ or 6 in *A. aequinoctiale* (2 *n* or 8 *n*) and two species/varieties characterised by aneuploidy, *viz.*, *A. protensum* ($n = 59$ or $12\,n - 2$) and *M. nudiflora* var. *terminalis* ($2\,n = 39$ or $4\,n - 1$). Although there are very strong reasons, both from morphological similarities and sympatric nature of distribution, to suspect autopolyploid origin for at least some of the intraspecific cytological races such as in *M. spirata* and *M. simplex*, complete absence of multivalents in them together with the discovery of taxa with aneuploid base numbers, *viz.*, $x = 9$ in *M. spirata* and $n = 59$ in *A. protensum* and of typical aneuploidy, *viz.*, $2\,n = 39$ in *M. nudiflora* var. *terminalis* establish *Aneilema sensu lato* as a genus of great evolutionary significance within the family Commelinaceae from the cytogenetical point of view. Considering the significance of the discovery of similar cytological picture both in *Aneilema* R.Br. *sensu stricto* and *Murdannia* Royle, both of which share the base number $x = 7$ in *A. montanum* and in *M. elata*, together with the presence of bilobed staminodes in *M. triquetra*, *M. elata* and *M. hamiltoniana* and the possession of beardless staminodes and the reduction of the third fertile stamen to a bearded filament with a sterile stump as in *M. nudiflora* var. *terminalis*, *M. simplex* and *M. gigantea* and absence of all the three staminodes from *M. hamiltoniana*, it appears that there are no fundamental differences, morphological or cytological, between *Aneilema* R.Br. and *Murdannia* Royle, at generic level, since they are connected by a range of morphological forms between the two extreme types illustrated in Text-Fig. 1. It is, therefore, best to treat the Indian species under the single genus *Aneilema sensu lato*, as was done by Clarke, Hooker, Gamble, Cook, Haines *et al.*



TEXT-FIG. 1. Floral diagrams

Finally, none of our preparations show any interbivalent connections or secondary associations in any of the species of *Aneilema sensu lato* including *A. nudiflorum*. Although the evidence of occurrence of different basic numbers, *viz.*, $x = 5, 6, 7, 9$ and 11 seems to be there, there is no evidence, direct or indirect, to postulate $x = 4$ for *Aneilema sensu lato*, despite Sharma's (1955) hypothesis of the common origin of *Aneilema*, *Commelina*, *Cyanotis* and "so-called *Cyanotis axillaris*" from an ancestral stock based on $x = 4$.

SUMMARY

1. Clarke's system and nomenclature of the genus *Aneilema* R.Br. includes all the Indian species under the subgenus EUANEILEMA. Yet, Bruckner's (1930) revival of the genus *Murdannia* Royle (1839) has necessitated the transfer of 23 of the 32 Indian species of *Aneilema sensu lato* to the genus *Murdannia*. This paper presents our observations on the cytology of 11 species of *Murdannia* and 4 species of *Aneilema* collected from Eastern India and attempts to visualise the cytogenetical evolution in *Aneilema sensu lato*.

2. Both mitotic and meiotic studies, including our own, involving only 13 taxa of *Aneilema sensu lato*, reveal a typical euploid series based on $x = 10$ or 5 in eight species, on $x = 14$ or 7 in two species, on $x = 11$ in one species, on $x = 24$ or 6 in one species, discovery of aneuploid base numbers, *viz.*, $n = 58$ in *A. protensum* Wall. and $n = 9$ in *M. spirata* in addition to the discovery of aneuploidy with $2n = 39$ in *Murdannia nudiflora* var. *terminalis*.

There is evidence for the occurrence of different basic numbers (*viz.*, $x = 5, 6, 7, 9$ and 11) for *Aneilema sensu lato*. But there is no

evidence, direct or indirect, to postulate $x = 4$ for *Aneilema sensu lato*, despite Sharma's (1955) hypothesis of common origin of *Aneilema*, *Commelina*, *Cyanotis* and *C. axillaris* from an ancestral stock based on $x = 4$.

3. Considering the significance of the discovery of similar cytological pictures both in *Aneilema* R.Br. *sensu stricto* and *Murdannia* Royle sharing common base number $x = 7$ together with presence of bilobed staminodes in *M. triquetra*, *M. elata* and *M. hamiltoniana* and the possession of beardless staminodes and the reduction of the third fertile stamen to a bearded filament with a sterile stump as *M. nudiflora* var. *terminalis*, *M. simplex* and *M. gigantea* and absence of all three staminodes from *M. hamiltoniana*, it appears that there are no fundamental generic differences, morphological or cytological, between *Aneilema* R.Br. and *Murdannia* Royle both of which are connected by a host of intergrading forms between the 2 extreme types illustrated in Text-Fig. 1. It is, therefore, suggested to treat all the 32 Indian species under *Aneilema* R.Br., a view shared by Clarke (1874, 1881 *a*, *b*), Hooker (1894), Fischer (1931) and Haines (1924).

ACKNOWLEDGEMENT

Grateful thanks are due to Rev. Father H. Santapau, S.J., Chief Botanist, Botanical Survey of India, Calcutta, for his keen interest and kind encouragement in pursuing the cytotaxonomic scheme of investigation in the Regional Circle of the Botanical Survey of India and to the authorities of the Council of Scientific and Industrial Research for the award of financial assistance including the grant of Junior Research Fellowship to the Junior author.

REFERENCES

BRUCKNER, G. 1926. Engl. *Bot. Jahrb.*, **61**, Beibl. **137**: 1–70.

———. 1930. In Engl. *et* Prantl. *Nat. Pflanzenf.*, ed. 2, 15 *a* (Account on *Murdannia* on p. 173).

CLARKE, C. B. 1874. *Commelinaceae et Cyrtandraceae Bengalenses* (*paucis allis ex terris adjacentibus additis*), Calcutta.

———. 1881 *a*. Notes on Commelinaceae. *J. Bot.* **19**: 193–202.

———. 1881 *b*. Commelinaceae in DC. *Monogr.* Phan. **3**.

FISCHER, C. E. C. 1931. *Flora of the Presidency of Madras*, London, Part 9, 1533–1552.

HAINES, H. H. 1924. *The Botany of Bihar and Orissa*, London **6**: 1074–82.

HOOKER, J. D. 1894. *The Flora of British India*, London, **6**.

MURTHY, K. L. 1934. Gametogenesis and embryogeny in some Commelinaceae. *Curr. Sci.* **3**: 258–59.

PANIGRAHI, G. AND KAMATHY, R. V. 1961. Studies in the cytomorphology of *Aneilema sensu lato* in E. India. *Proc. 48th Indian Sci. Congr.*, Part IV, 97–98.

RAGHAVAN AND SESHAGIRI RAO, R. 1961. *Curr. Sci.* **30**: 310–11.

ROYLE, J. F. 1839. In *Illustr. Bot. Himal.* 403, *t.* 95, Fig. 3.

SESHAGIRI RAO, R., PANIGRAHI, G. AND KAMATHY, R. V. 1960. Cytotaxonomic studies on Indian Commelinaceae. A preliminary note. *Proc. 47th Indian Sci. Congr.*, Part III: Abstracts, p. 366.

SHETTY, B. V. AND SUBRAMANYAM, K. 1961. Cytology of some taxa of Commelinaceae. *Proc. Indian Sci. Congr.*, Part III: Abstacts, p. 299.

SHARMA, A. K. 1955. Cytology of some of the members of Commelinaceae and its bearing on the interpretation of phylogeny. *Genetica* **27**: 323-93.

—— AND SHARMA, ARCHANA. 1958. Further investigations on cytology of members of Commelinaceae with special reference to the role of polyploidy and the origni of ecotypes. *J. Genet.* **56** (1): 1-21.

SIMMONDS, N. W. 1954. Chromosome behaviour in some tropical plants. *Heredity* **8**: 139-46.

INDUCTION OF "REDUCTIONAL GROUPINGS" (SOMATIC REDUCTION) BY CENTRIFUGATION FOLLOWING COLCHICINE TREATMENT

By D. Srinivasachar*

The role of polyploidy, with or without previous hybridization, is well recognised as a factor in evolution as more than half of the angiosperms are said to be polyploids. If polyploidisation constitutes an important process in evolution it is also reversible. Polyploidy and endomitosis on one hand appear to be compensated by the process of chromosome elimination on the other. If somatic reduction ("reductional groupings" in our terminology) occurs in a ploploid and a reduced tissue is formed in which sporogenous cells undergo ordinary meiosis some gametes will be formed which may approach the ancestral types. East's (1934) recovery of a 7-chromosome ancestral type gamete from a 42-chromosome strawberry and Kiellander's (1941) recovery of 18-chromosome *Poa* resembling *P. trivialis* from a 72-chromosome *P. pratensis* could be explained on the basis of somatic reduction followed by ordinary meiosis or gametophytic reduction following meiosis. Either of these types of double reduction could give rise to progeny resembling ancestral forms. Such cases of reversions have not been much emphasised because the possibility of contamination with foreign pollen or accidental admixture of seeds cannot be entirely ruled out.

Spindle abnormality in the megasporogenesis resulting in chromosome reduction from 118 to 86 has been reported by Parthasarathy (1951) in the case of the sugarcane variety C. 0·421 which is a complex hybrid between *Saccharum officinarum* and *S. spontaneum*. Such chromosome reductions might give rise to new secondarily balanced chromosome numbers which may form the basis of different lines of evolution.

Colchicine-induced autotetraploids of *Eruca sativa* were found by Rajan, Haridas and Parthasarathy (1950) to give rise to triploids and aneuploids under natural conditions. The breakdown is attributed to the formation of functional gametes with unbalanced chromosome numbers in both micro- and megasporogenesis, natural crossing of the tetraploids with their diploids and to disturbances in spindle mechanism leading to chromosome elimination.

Where fertility in new combinations, particularly in interspecific hybrids, is impaired by synaptic irregularities meiotic divisions often serve as regulating mechanism to produce balanced gametes. Similarly

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where sterility is due to chromosomal imbalance or genic incompatibility somatic elimination might serve to establish compatible complements.

Several instances of reduced cells occur in literature. High incidence of somatically reduced cells has been reported in *Ribes* (Varaama, 1949). Huskins and Chounard (1950) obtained from two tetraploid *Rhoeo discolor* plants 168 tetraploid, 16 triploid and 7 diploid main roots and one diploid shoot. Srinivasachar (1958) found in onion roots two prophase nuclei containing 7 chromosomes each in neighbouring cells with unbroken cell-walls. Brown (1947) has reported a case of reduced tissue in a complex polyploid hybrid of cotton. The cytologically unbalanced nature of the polyploid plant probably favoured development of a reduced tissue. Swaminathan and Singh (1958) found a haploid branch in an X-rayed diploid watermelon. Sampath (1950) has noticed variations in chromosome numbers in the root-tips of a hybrid *Oryza sativa* × *O. eichingeri*. In these cases "reductional groupings" are probably involved.

Huskins (1948) focussed our attention on the spontaneous occurrence of "reductional groupings". Besides sodium nucleate which has been shown by Huskins (1948) to increase the frequency of "reductional groupings", there are several chemicals such as sodium phosphate, vitamin K, chloral hydrate, sodium cacodylate, etc., which produce similar effects. The observation by Huskins and Cheng (1950) that cold shocks also increase the frequency of "reductional groupings" could not be confirmed by Srinivasachar and Patau (1958). Colchicine is also said to cause "reductional groupings". This was made use of by Franzke and Ross (1952) in practical plant breeding.

The author while working at the University of Wisconsin under the guidance of Late Dr. Leonard C. Huskins found that the proportion of metaphase "reductional groupings" could be greatly increased if colchicine treatment is combined with centrifugation. The preliminary observations made in this regard are reported herein.

Bulbs of one of the pickling varieties of onion were allowed to root on vials containing tap-water and when they were grown sufficiently they were transferred on to vials containing 0·5% aqueous colchicine. After half an hour of colchicine treatment the rooting bulbs were centrifuged for 5 minutes at 1,500 r.p.m. Roots picked at random were fixed in acetic alcohol, 1 : 3 and squashed after the Feulgen procedure. The slides were scored for metaphase "reductional groupings" only. Out of 1,830 mitoses scored 429 (23·4%) showed metaphase "reductional groupings". As compared to this only 3·9% of such groupings were found in the untreated controls. Cursory observations indicated that anaphase "reductional groupings" were not so preponderant. However, no systematic scoring was made either of anaphase or prophase "reductional groupings". It is yet to be seen how many of these metaphase "reductional groupings" could be realised as reduced cells.

Centrifugation probably helps groupings of the chromosomes which are floating free in the plasma due to the disturbances in the

spindle mechanism such as described by Darlington and Thomas (1937). If the spindle or spindles should be more or less parallel to the line determined by the centres of the two groups ordinarly cell-wall formation could isolate reduced nuclei in cells of their own. It is also conceivable that in some cases as in *Ribes* where there is a high frequency of somatically reduced cells a special genetic situation exists such as the presence of sub-lcthal genes which could be eliminated by somatic reduction. Thus the observed frequency of reduced cells may, in part, have been due to intercellular selection by means of superior division rate of certain types of reduced cells.

SYNOPSIS

The author while working at the University of Wisconsin found that the frequency of metaphase "reductional groupings" (somatic reduction) could be greatly increased if colchicine treatment was combined with centrifugation. In onion roots grown in 0·5% aqueous colchicine and subjected to centrifugation at 1,500 r.p.m. for five minutes 23·4% metaphase "reductional groupings" were obtained as against 3·9% in the untreated controls. Centrifugation probably helps grouping of chromosomes which are lying free in the plasma due to the paralysis of the spindle mechanism by colchicine.

"Reductional groupings" occur sporadically in nature. Their frequency can be greatly increased by sodium nucleate. Other chemicals such as chloral hydrate, sodium cacodilate, sodium phosphate, vitamin K and colchicine also increase their frequency. Reduction of chromosomes in mitosis has been reported in many cases including *Rhoeo*, onion, *Ribes*, cotton and water-melon. "Reductional groupings" are probably involved in these cases.

The role of polyploidy is well recognized as a factor, in evolution. Where fertility in new combinations is impaired by synaptic irregularities meiotic divisions often serve as a regulating mechanism to produce balanced gametes. Similarly where sterility may be due to chromosome unbalance or genic incompatibility somatic elimination might serve to establish a compatible complement. If polyploidy is important in evolution it is also reversible; autotetraploids may throw off diploid shoots and complex polyploids may revert to ancestral forms.

Polyploidy and endomitosis on the one hand appear to be compensated by chromosome elimination on the other hand.

REFERENCES

BROWN, M. S. 1947. A case of spontaneous reduction of chromosome number in somatic tissue of cotton. *Amer. J. Bot.* 34: 384–88.

DARLINGTON, C. D. AND THOMAS, P. T. 1937. The breakdown of cell division in a *Festuca-Lolium* derivative. *Ann. Bot., Lond.* N.S. 1: 747-61.

EAST, E. M. 1934. A novel type of hybridity in *Fragaria*. *Genetics* **19**: 167–74.

FRANZKE, C. J. AND ROSS, J. G. 1952. Colchicine-induced variants in *Sorghum*, *J. Hered.* **43**: 107–15.

HUSKINS, C. L. 1948. Segregation and reduction in somatic tissue. *Ibid.* **39**: 310–25.

—— AND CHENG, K. C. 1950. Segregation and reduction in somatic tissue. IV. Reductional groupings induced in *Allium cepa* by low temperature. *Ibid.* **41**: 13–18.

—— AND CHOUNARD, L. 1950. Somatic reduction: diploid and triploid roots and a diploid shoot from a tetraploid. *Rhoeo*. *Genetics*. **35**: 115.

KIELLANDER, C. L. 1941. Studies on apospory in *Poa pratensis* L. *Svensk Bot. Tidskr*. **35**: 321–32.

PARTHASARATHY, N. 1951. Chromosome elimination in *Saccharum*. *Nature, Lond.* **168**: 383–84.

RAJAN, S. S., HARIDAS, M. W. AND PARTHASARATHY, N. 1950. Breakdown of tetraploidy in colchicine-induced autotetraploid *Eruca sativa* Lam. *Indian J. Genet.* **10**: 43–55.

SAMPATH, S. 1950. Chromosome diminution in plant root. *Curr. Sci.* **19**: 185–86.

SRINIVASACHAR, D. 1958. A case of sub-haploid cells in mitosis. *Cytologia* **23**: 419–21.

—— AND PATAU, K. 1958. Reductional groupings in cold-treated onion roots. *Chromosoma* **9**: 229–37.

SWAMINATHAN, M. S. AND SINGH, M. P. 1958. X-ray induced somatic haploidy in water-melon. *Curr. Sci.* **27**: 63–64.

VARAAMA, A. 1949. Spindle abnormalities and variation in chromosome number in *Ribes nigrum*. *Hereditas* **35**: 136–62.

CUTTACK

SYMPOSIUM I

EVOLUTIONARY TRENDS IN GYMNOSPERMS

THE ARCHEGONIUM IN GYMNOSPERMS: A REVIEW

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Of the various fossil orders of gymnosperms (Pteridospermales, Caytoniales, Pentoxylales, Cycadeoidales and Cordaitales) only a few members of the Pteridospermales and Cordaitales have yielded well-preserved female gametophytes showing archegonia. All living members of the gymnosperms are characterized by the presence of archegonia excepting only *Welwitschia* and *Gnetum*. A comparative account of the more important forms is given in the following pages, laying special emphasis on the papers which have appeared since the publication of Chamberlain's (1935) book on gymnosperms.

Pteridospermales

In *Lagenostoma ovoides* (Long, 1944) the female gametophyte is differentiated into an outer layer of radially elongated cells and an inner part of polyhedral or rounded cells. The apical end of the prothallus is prolonged into the lower part of the cavity of the lagenostome and thus corresponds to the tentpole occurring in several other palaeozoic seeds and in *Ginkgo*. In one fortunate section Long saw three ovoid archegonia at the apical end of the gametophyte around the tentpole. Each consists of an egg cell, about 0·3 mm in length and surrounded by a definite jacket layer. The jacket cells are smaller than the surrounding prothallial cells and also appear to have thick walls, but there is no indication of the pitting seen in cycads.

The gametophyte of *Pachytesta hexangulata*, described by Stewart (1951), also shows a short tentpole with three archegonia around it. The carbonised cytoplasm of the egg, which measures $342\mu \times 525\mu$, is in the form of a network.

Cordaitales

As early as 1879 Renault figured archegonia in *Cycadinocarpus angustodunensis*. Since then Neely (1951) has described the gametophyte of *Taxospermum undulatum*. This is raised into a low tentpole, with one spherical or elliptical archegonium on either side of it. A tentpole is also seen in the gametophytes of *Cardiocarpus spinatus* (Andrews and Felix, 1952). One of these showed two jacketed archegonia having a maximum diameter of 500μ. Further details are not known so far.

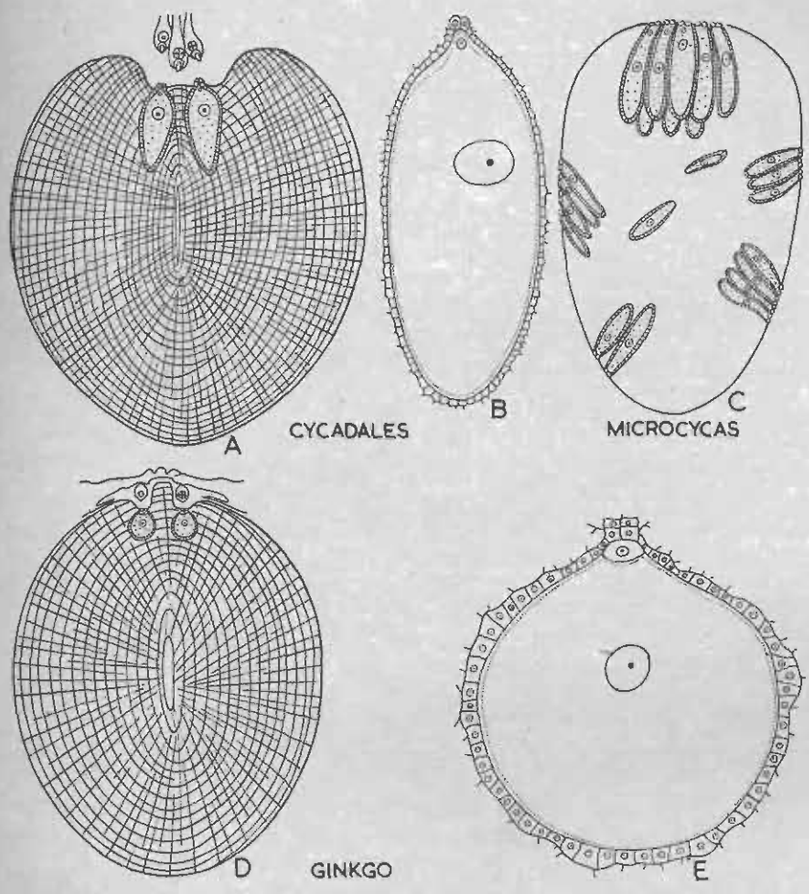
CYCADALES

There are usually 2-6 archegonia in an ovule. They occur singly and are restricted to the micropylar end of the prothallus (Text-Fig. 1 A). In *Macrozamia reidlei* (Baird, 1939) they are arranged in a circle around the apex. In some abnormal ovules two or three circles have been seen and many gametophytes belonging to a particular cone showed a lateral group of 12–15 archegonia. The genus *Microcycas* is exceptional in having a very large number of archegonia (Reynolds, 1924). Not only do they occur at the micropylar end but also on the lobes, sides and base of the gametophyte, and rarely even within the tissue of the prothallus so as to open into the middle cleft. However, the micropylar archegonia alone are functional.

The archegonium initial cell divides to form a primary neck cell and the central cell. The former divides anticlinally to form two neck cells. By the time the nucleus of the central cell is ready to divide, the neck cells become very large and turgid, and project into the archegonial chamber (Text-Fig. 1 A, B) except in *Microcycas*, in which they remain small. Exceptionally four neck cells may be seen in *Microcycas* (Reynolds, 1924), *Macrozamia* (Brough and Taylor, 1940) and *Cycas* (Swamy, 1948; De Silva and Tambiah, 1952), although in *C. circinalis* Rao (1961) reports only two. In *Zamia umbrosa* the presence of four neck cells seems to be a normal feature (Bryan and Evans, 1957), and in *Encephalartos* (Sedgwick, 1924) the original pair of neck cells may occasionally divide to give rise to several cells.

The central cell may grow for several months before its nucleus undergoes the first division. In *Zamia* the division spindle is intranuclear (Bryan and Evans, 1956). The egg of cycads is perhaps the largest in the plant kingdom, its nucleus alone having a diameter of 400–500 microns or more. The ventral canal nucleus moves up into the neck of the archegonium where it soon disorganises. Occasionally, however, it enlarges and simulates the egg nucleus. Sedgwick (1924) suggests that in *Encephalartos* such a ventral canal nucleus is capable of "fertilizing" the egg nucleus but this needs confirmation. In *Zamia umbrosa* (Bryan and Evans, 1957) sometimes the egg and ventral canal nuclei are of a similar size; the latter often becomes variously lobed and may even fragment into smaller segments. De Silva and Tambiah (1952) observed "protein vacuoles" in the mature archegonia of *Cycas rumphii* which become more numerous after fertilization.

Bryan and Evans (1956) report that in the egg nucleus of *Zamia* the chromosomes gradually contract into a globular mass which decreases in diameter from 15 microns to 6 microns. Another interesting feature observed by them is the modification of the nuclear membrane into a reticulate pattern of ridges and furrows, and simultaneously there is an extrusion into the cytoplasm of some globular bodies which are Feulgen negative, vacuolate and capable of limited growth. Such a phenomenon has not been reported previously for any other cycad but similar observations have been made on the large egg nuclei of certain lower animals.



TEXT-FIG. 1. A, B. Diagrammatic representation of the female gametophyte and achegonium in Cycadales. C. L.s. gametophyte of *Microcycas* showing irregularly arranged archegonia. D, E. Female gametophyte and archegonium of *Ginkgo*.

GINKGOALES

In *Ginkgo* there are two, rarely three or four, archegonia (Text-Fig. 1 D). The initials appear very early, even before the gametophyte is fully cellular (Lee, 1955; Favre-Duchartre, 1958). The primary neck cell divides to produce two and then four neck cells which become swollen and project above the surface. As the central cell enlarges, the adjacent cells form a well-organised jacket layer. According to Lee (1955) the dense cytoplasm and large nuclei of the neck cells suggest a secretory function. The central cell divides to form a small ventral canal cell and

the egg (Text-Fig. 1 E). Herzfeld (1927) occasionally found the ventral canal nucleus and egg nucleus lying side by side, and separated by only an incomplete wall. Favre-Duchartre (1958) observed both starch grains and lipoprotein granules in the vacuoles distributed in the egg protoplasm.

The nucleus of the central cell is extremely large and with the Feulgen stain it appears nearly colourless. In early stages the egg nucleus, formed from it, also reacts similarly except that the DNA appears confined within a small globule. The quantity of DNA seems to be similar to that in a jacket cell nucleus (Lee, 1955).

A few sterile archegonia have been observed in which the neck cells are present but the central cell fails to enlarge (Lee, 1955).

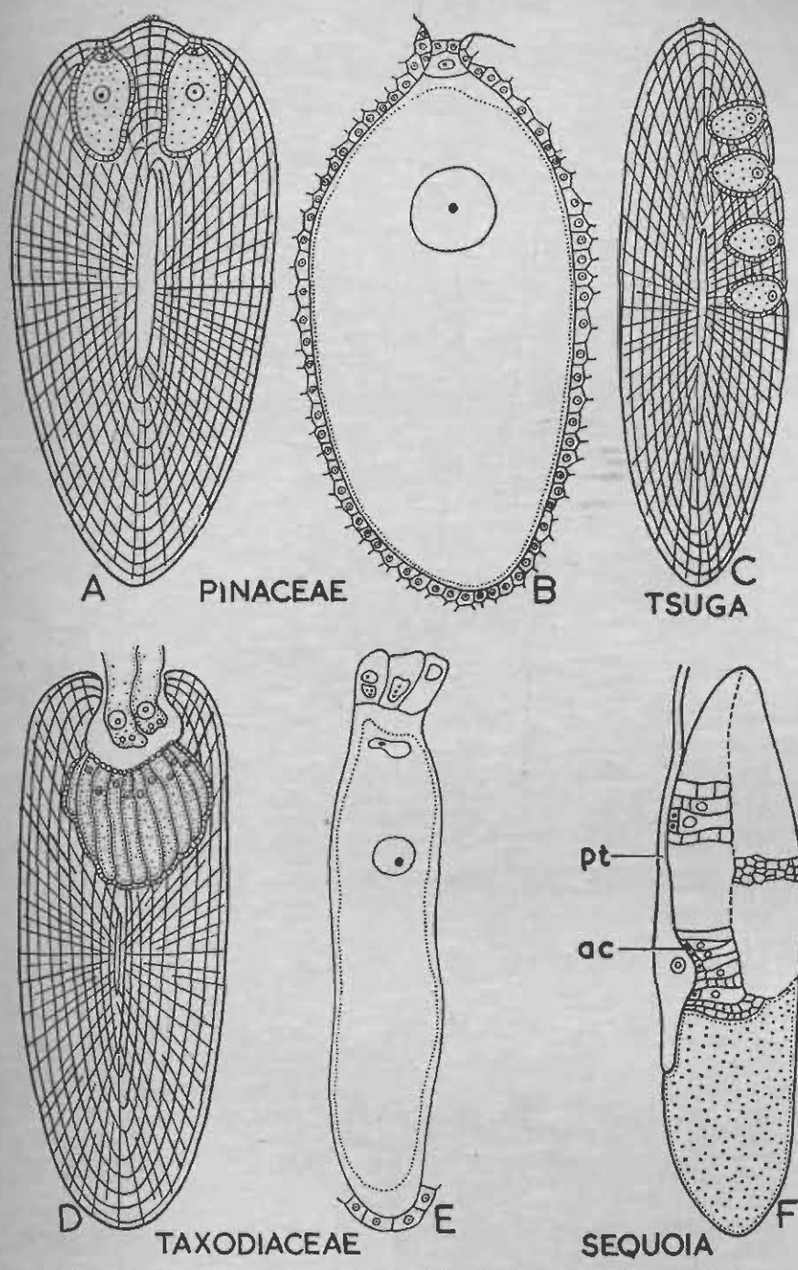
CONIFERALES

Pinaceae.—A large number of genera and species have been investigated (*see* Schnarf, 1933). The archegonia, one to seven in number, occur singly at the apical end of the gametophyte (Text-Fig. 2 A, B). Rarely lateral archegonia have been observed in *Pinus* (Konar, 1960) and *Tsuga* (Sterling, 1948 *b*) as shown in Text-Fig. 2 C. In *Pinus* and *Cedrus* archegonia may occur even at the chalazal end (Bell, 1925; Roy Chowdhury, 1961). In *Cedrus* two superposed archegonia occur sometimes but the lower is then devoid of a neck. One gametophyte showed archegonia all over its surface although most of these were only imperfectly developed.

The archegonial initials are superficial and the deep-seated appearance of the mature archegonia is due to the upward growth of the neighbouring cells of the prothallus. The neck consists of two to four tiers of four to eight cells each. In *Cedrus atlantica* ahe neck cells are arranged somewhat irregularly; according to Smith (1923) they may sometimes become multinucleate.

The nucleus of the central cell moves up and takes a position just below the neck. Its division is intranuclear and a definite ventral canal cell is cut off (Fig. 2 B). This may either degenerate or persist for some time after fertilization. In *Pinus roxburghii* (Sethi, 1929) the chromosomes at the upper pole may start degenerating even before they acquire a nuclear membrane. In *Cedrus atlantica* (Smith, 1923), *C. deodara* (Roy Chowdhury, 1961) and *Keteleeria davidiana* (Sugihara, 1943) the wall between the ventral canal nucleus and the egg may sometimes break down so that the two nuclei come to lie together. In some species a few supernumerary nuclei have been seen at the apical end of the archegonium, but their origin is not clear. As pointed out by Hutchinson (1915) in *Abies balsamea*, it is probable that in such cases the ventral canal nucleus is also fertilized and subsequently divides to form a few free nuclei.

The egg nucleus increases in volume and moves down to the centre of the cell. Many radiating fibres arise in the cytoplasm around it,



TEXT-FIG. 2. A, B. Female gametophyte and archegonium in Pinaceae. C. Abnormal prothallus of *Tsuga* with four lateral archegonia. D. Gametophyte of a typical member of the Taxodiaceae showing an apical archegonial complex with a common jacket. E. Single archegonim from the same showing a definite ventral canal nucleus. F. Gametophyte of *Seouoia*, the archegonial complexes (*ac*) are in contact with the lateral pollen tube (*pt*).

particularly on its upper side. The egg cytoplasm shows a number of "proteid vacuoles" which look like nuclei. Most authors agree that they have a nutritive function, and Ferguson (1904) calls them "nutritive spheres". Takao (1959), who made cytochemical studies of the proteid vacuoles in the egg of *Pinus thunbergii* finds that with the maturation of the egg they increase in number and their protein staining reaction reaches its peak just before fertilization. After fertilization their number declines and they disappear almost completely by the time the proembryos are formed. They contain not only proteins but also nucleoproteins and some polysaccharides.

Shimamura (1956) states that in *Pinus thunbergii* the newly formed egg and ventral canal nuclei are Feulgen positive, but with increase in size the former becomes Feulgen negative. In the nucleus of the fertilized egg the portions contributed by the male and female nuclei are both Feulgen negative and only the line of contact is Feulgen positive. In some fused nuclei, on the other hand, a positive Feulgen reaction was observed in both portions. This would indicate that the DNA disappears in the ventral canal nucleus as well as the male and female nuclei, but reappears in the zygote.

Vazart (1958) studied the effect of different fixatives on the localization of nucleic acids in the archegonia of *Pinus sylvestris* and *P. nigra*. Of the four fixatives employed only acetic alcohol permitted a fair detection of RNA, although all enabled the identification of DNA by the Feulgen method. The distribution of RNA was studied by staining with Unna's mixture.[1] During the growth of the archegonia there was a notable increase of the ribonucleoproteins which were abundant in the cytoplasm and the nucleoli of the egg as well as the jacket cells. No DNA could, however, be detected in the nucleus of the central cell until the time of its division. During the course of division the chromosomes were distinctly stainable. In telophase the chromosomes of the ventral canal nucleus retained their stainability but not those of the egg; the latter were again recognizable when the nuclear membrane became organized.

In *Pinus* a large circular space appears in the egg cytoplasm just below the neck. This is referred to as the "receptive cavity", because it receives the contents of the pollen tube.

Taxodiaceae.—The majority of the genera have an apical archegonial complex in a common jacket (Text-Fig. 2 D), but a few have lateral archegonia (Text-Fig. 2 F). The number of archegonia varies considerably, and rarely the archegonial complex occupies a basal position. In *Cunninghamia sinensis* (Miyake, 1910) there is a ring of 13–16 archegonia around an apical mass of sterile tissue. *Sciadopitys* (Tahara,

[1] This is a methyl green-pyronin stain for the detection and estimation of RNA. The various cell constituents stain from pink to red according to the concentration of RNA. The chromosomes stain light to dark green according to the concentration of the nucleic acid (Darlington and LaCour, 1960).

1940) differs from the other genera of the family in having no archegonial complex; the four or five archegonia are arranged singly.

In *Sequoia*, *Sequoiadendron* (Looby and Doyle, 1942) and *Athrotaxis selaginoides* (Brennan and Doyle, 1956) the pollen tubes grow along the side of the female gametophyte (Text-Fig. 2 F). The pollen tube swells after coming in contact with the prothallium and produces an invagination in the latter. The number of archegonial groups usually corresponds to the number of pollen tubes. The archegonial initials are definitely superficial and not deep-seated as reported previously by certain authors. As a rule they are produced only in contact with the pollen tube and their number varies from 16 to 36.

There are 2–16 neck cells in *Taxodium* ; 4 in *Cryptomeria*, *Sciadopitys*, *Cunninghamia* and *Sequoiadendron* ; and 2 in *Sequoia* and *Athrotaxis*. In *Sequoia* (Buchholz, 1939; Looby and Doyle, 1942) and *Athrotaxis* (Brennan and Doyle, 1956) the ventral canal nucleus is not produced regularly. In *Sequoiadendron* (Looby and Doyle, 1942) also its occurrence is probably not a regular feature. A definite ventral canal nucleus is observed in *Taxodium*, *Sciadopitys* and *Cunninghamia* (Text-Fig. 2 E); in *Cunninghamia* it is ephemeral, while in *Taxodium* and *Sciadopitys* it may divide to produce a few nuclei.

Cupressaceae.—The archegonia are found in complexes which are usually apical in the Cupressineae and lateral in the Callitrineae. Some forms also show gradations between the two types. Among comparatively recent records, *Thuja occidentalis* (Martin, 1950) shows an apical complex with 5–7 archegonia; in *C. funebris* (Banerjee, 1959) there are 10–17; in *Chamaecyparis pisifera* (Sugihara, 1938) 5–17; in *Biota orientalis* (Singh and Oberoi, 1962) 15–28; and in *Thujopsis dolabrata* (Sugihara, 1939) 7–9.

In *Callitris verrucosa* there are numerous archegonia forming a lateral complex. Two complexes organise when two pollen tubes are present (Looby and Doyle, 1940; Baird, 1953). In *Widdringtonia cupressoides*, Moseley (1943) found 25–100 archegonia. One group abuts on the swollen tip of the pollen tube but several additional groups occur near its upper part. The latter are said to be deep-seated and devoid of any neck cells. In *W. juniperoides* (Saxton, 1934 *b*) the archegonia sometimes extend along almost the whole length of the prothallus and their number goes up to 200.

In *Fitzroya cupressoides* (Doyle and Saxton, 1933), which seems to be intermediate between the Callitroideae and the Cupressoideae, there is usually an apical archegonial complex but there is a strong tendency towards the formation of lateral archegonia.

As a rule there is a 4-celled neck, but in *Actinostrobus* (Saxton, 1913 *a*) and *Fitzroya* (Doyle and Saxton, 1933) there are only 2 cells. In *Cupressus funebris* there are 4–8 cells in two tiers (Banerjee, 1959), while in *Thujopsis* there are 8 cells in a single tier (Sugihara, 1939). In *Callitris*, *Widdringtonia* and *Biota* the neck cells disappear just before or at the time of fertilization.

Shortly before the division of the central cell, peculiar aster-like bodies called "Strahlungszentren" and "asteroids" have been observed in the cytoplasm in *Juniperus communis* (Norén, 1907; Nichols, 1910; Mathews, 1939), *Fitzroya* (Doyle and Saxton, 1933), *Thuja* (Land, 1902) and *Tetraclinis* (Saxton, 1913 *b*). They may be two or more in number, and at least one of them is always associated with the nucleus of the central cell. Some investigators consider that the asteroid is responsible for producing the spindle fibres for the mitosis of the central cell nucleus (*see* Mathews, 1939). In *Juniperus virginiana* (Mathews, 1939) it is produced just three days before fertilization and is generally evanescent. At times it may persist as in *Tetraclinis* (Saxton, 1913 *b*), and in *Callitris* it may even divide (Looby and Doyle, 1940). In *Biota* (Singh and Oberoi, 1960), *Fitzroya* (Doyle and Saxton, 1933) and *Thujopsis* (Sugihara, 1939) the asteroid may sometimes occupy a lateral position.

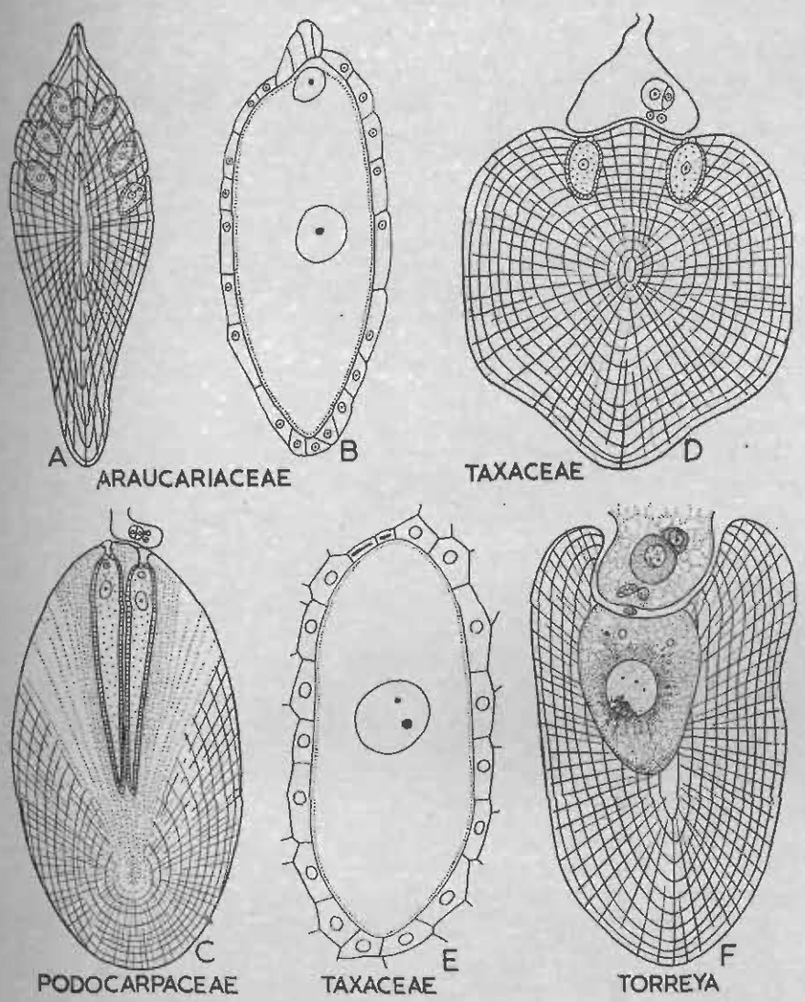
Land (1902) believes that in *Thuja occidentalis* occasionally both the ventral canal nucleus and the egg nucleus may become fertilized. The probability of such an occurrence is strengthened by his finding a group of four or eight nuclei at the upper end of the archegonium.

In *Juniperus* (Mathews, 1939) and *Thujopsis* (Sugihara, 1939) numerous proteid vacuoles begin to appear at the time of the division of the central cell but are never as conspicuous as in the Pinaceae. Several "paranuclei" have been seen in the egg of *Cupressus funebris* (Banerjee, 1959); one of them, of a rather large size, is invariably found associated with the egg nucleus.

In those members of the Cupressaceae which have lateral archegonia the pollen tube grows along the side of the gametophyte and travels down to about one-third or half of its length. The tube definitely lies outside the megaspore membrane and never penetrates the prothallus, although it may appear falsely so by the subsequent growth of the prothallial tissue.

Araucariaceae.—The archegonia are confined to the upper half or one-third of the gametophyte. Although apparently sunken they probably arise as usual from superficial initials which later come to lie at a lower level as a result of the upgrowth of the gametophytic cells (Text-Fig. 3 A). In *Araucaria* the archegonia are oriented in a ring around the apex of the prothallus while in *Agathis* they form three groups—a small terminal cluster and two uneven encircling crowns at short intervals. There is a tendency towards the formation of an archegonial complex for sometimes the archegonia are closely adjacent without intervening sterile cells. The number of archegonia varies from 3 to 25 but not all mature or become fertilized.

The neck is dome-shaped (Text-Fig. 3 A, B) and comprises about 12 wedge-shaped cells in a single tier. These are so compact and their walls so thick that the pollen tube enters from a side. Frequently the entire neck is shed. In *Araucaria* the neck cells leave a narrow passage in the centre.



TEXT-FIG. 3. A, B. Female gametophyte and archegonium in Araucariaceae. C. Female gametophyte of a member of the Podocarpaceae, the dotted area represents a cone of smaller cells. D.E. Female gametophyte and archegonium in Taxaceae. F. Gametophyte of *Torreya taxifolia* with a single apical archegonium.

An ephemeral ventral canal nucleus (Text-Fig. 3 B) has been seen in *Agathis* (Eames, 1913; Ghose, 1924) being always cut off towards one side. Burlingame (1914) failed to observe any ventral canal nucleus in *Araucaria*.

Podocarpaceae.—There are 2, rarely 3, archegonia in *Podocarpus andinus* (Looby and Doyle, 1944) and *P. nivalis* (Boyle and Doyle, 1953); 1–3 in *Dacrydium colensoi* (Stiles, 1911); 2–6 in *Pherosphaera hookeriana* (Elliott, 1950); 3 in *Saxegothaea* (Looby and Doyle, 1939); 2 in *Phyllocladus glaucus* (Holloway, 1937) and probably also *Acmopyle* (Sahni, 1920). Usually they lie singly but at times neighbouring archegonia have a common jacket layer and occasionally there is no jacket at all. The distribution of archegonia approaches that in *Cunninghamia* where they form a ring with a common jacket and surround a central mass of sterile tissue. In *Microcachrys tetragona* (Lawson, 1923 *b*) there is a complex of 5 or 6 archegonia with a common jacket layer.

The range in the number of neck cells is shown by the following examples: 4 in *Phyllocladus* (Holloway, 1937), 4 or 8 in *Pherosphaera* (Elliott, 1950); 6–10 in *Saxegothaea* (Looby and Doyle, 1939); 5 or 6 (rarely in two tiers) in *Podocarpus nivalis* (Boyle and Doyle, 1953); and 10–15 in *P. andinus* (Looby and Doyle, 1944).

The division spindle of the central cell is oblique in *Podocarpus nivalis* (Boyle and Doyle, 1953), and consequently the ventral canal nucleus lies on one side. Normally evanescent, it sometimes persists until fertilization and may even undergo some enlargement.

The mature archegonium is generally long and narrow with a tapering base (Text-Fig. 3 C). Three kinoplasmic masses were observed in the central cell of *Podocarpus nivalis*. Of these the upper becomes denser and the egg nucleus moves into it.

Taxaceae.—There are 4–8 archegonia in *Taxus canadensis* (Dupler, 1917); 6–25 in *T. cuspidata* (Sterling, 1948 *a*); 3–5 in *Austrotaxus spicata* (Saxton, 1934 *a*); 2–5 in *Torreya californica* (Robertson, 1904) and only 1 in *Torreya taxifolia* (Text-Fig. 3 F) (Coulter and Land, 1905). In *Taxus cuspidata* (Sterling, 1948 *a*) the archegonia are arranged in a ring around the invagination produced by the pollen tubes (Text-Fig. 3 D). When there are two or more gametophytes in the same ovule, as is sometimes observed in *Taxus* (Dupler, 1917) and *Austrotaxus* (Saxton, 1934 *a*), in the upper gametophyte the archegonia may develop towards either the chalazal or the micropylar end, while in the lower they are placed normally and abut directly on the pollen tube which grows between the two gametophytes. In *Taxus cuspidata* (Sterling, 1948 *a*) some of the archegonia are said to be hypodermal. A few are superposed and are presumed to be derived by the periclinal division of a single initial. The internal archegonia generally lack neck cells. Each archegonium has its own jacket but there is some tendency towards the formation of an archegonial complex.

The neck comprises 4–6 cells in *Torreya californica*; 2 in *T. taxifolia*; several in *Taxus canadensis*; 2–4 in *T. cuspidata*; and 16 in *Austrotaxus spicata*. In the mature archegonium the neck cells are flattened and inconspicuous.

In *Torreya taxifolia* (Coulter and Land, 1905) and *Taxus canadensis* (Dupler, 1917) the nucleus of the central cell has not been observed to

divide; probably the cell functions directly as the egg (Text-Fig. 3 E). In *Taxus cuspidata*, according to Sterling (1948 *a*), the ventral canal nucleus is "rarely if ever formed". In *Austrotaxus spicata* a ventral canal nucleus has not been seen, although Saxton (1934 *a*) believes that it is probably present as in other gymnosperms.

Cephalotaxaceae.—In *Cephalotaxus drupacea* there are 2–5 archegonia situated singly at the micropylar end of the gametophyte (Singh, 1961). The neck comprises 2–5 cells. In unfertilized ovules two equal nuclei are sometimes seen in the archegonia, in addition to the ventral canal nucleus. These are probably the products of a parthenogenetic division of the egg nucleus (Favre-Duchartre, 1957). In the cytoplasm of the archegonium, Singh (1961) found the usual "proteid granules" which are probably the same as the " sonderophilic granules " of Favre-Duchartre.

Ephedrales

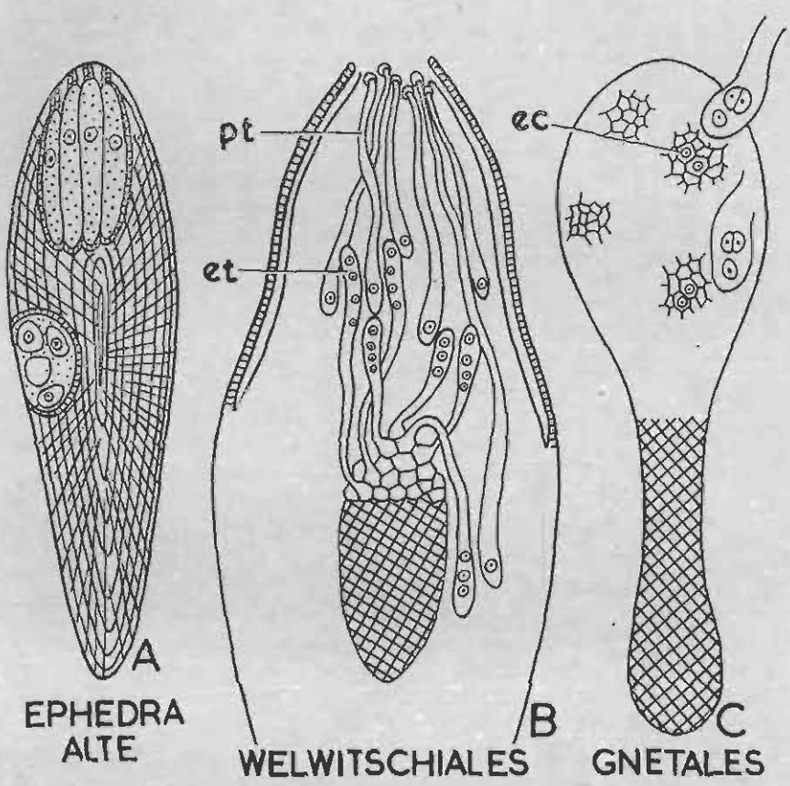
The number of archegonia varies from 1 to 6 and these are usually arranged singly at the micropylar end of the gametophyte. Maheshwari (1935) reported an example of the existence of two archegonia within a common jacket in *E. foliata* and Narang (1956) gave examples of the occurrence of archegonial complexes in *E. campylopoda, E. torreyana, E. alte, E. distachya, E. sinica* and *E. americana.* In *E. torreyana* and *E. distachya* there are 4–8 archegonia in a complex, and 2 or 3 in *E. americana.* In *E. alte* the occurrence of an archegonial complex is specially common and separate archegonia are less frequent (Text-Fig. 4 A). Rarely, lateral archegonia occur even in the lower half of the female gametophyte, either singly or in groups, in *E. campylopoda, E. sinica, E. foliata,* and *E. alte* (Text-Fig. 4 A).

It is difficult to count the exact number of neck cells since they merge into the adjacent prothallial tissue. Maheshwari (1935) reported 30–40 in *E. foliata.* Narang (1956) found a neck of 9–12 tiers of cells in *E. sinica*; 8–10 tiers in *E. americana, E. alata, E. torreyana,* and *E. foliata*; 6–8 tiers in *E. campylopoda* and *E. distachya*; and 4–5-tiers in *E. alte.*

The nucleus of the central cell divides as usual into the egg and ventral canal nuclei. Berridge and Sanday (1907) suspected an amitotic division of the ventral canal nucleus but this has not been confirmed by other workers. Normally the nucleus begins to degenerate soon after it is formed but Herzfeld (1922) and Khan (1943) report that in *E. campylopoda* and *E. foliata* it may even get fertilized and divide to produce a few ephemeral daughter nuclei which simulate the proembryonal nuelei but soon degenerate and disappear.

Welwitschiales

In *Welwitschia* (Pearson, 1929) the cells belonging to the micropylar region of the gametophyte contain several large nuclei. The



TEXT-FIG. 4. A. Apical and lateral archegonial complexes in the gametophyte of *Ephedra alte*. B. L.s. nucellus of *Welwitschia*; the embryo-sac tubes (*et*) are growing upwards, and the pollen tubes (*pt*) are growing downwards. C. Gametophyte of *Gnetum*; egg cells (*ec*) are differentiating under the stimulus of the pollen tube.

cells begin to grow up toward the micropyle in the form of slender tubes. The cytoplasm and nuclei also migrate in the same direction so that the lower parts of the cells become almost empty. These tubes grow out through the wall of the gametophyte into the nucellus, and have been designated by Pearson as "embryo-sac tubes" (Text-Fig. 4 B). Each tube grows independently of the rest and most of them continue to elongate until a pollen tube is encountered. The nuclei in an embryo-sac tube lie close together in early stages, but later they become arranged in a row. The nucleus which lies foremost is likely to become fertilized.

GNETALES

In *Gnetum* there is nothing comparable to the archegonia of other gymnosperms. Thompson (1916), Fagerlind (1941) and Sanwal

(1962) observed that in *Gnetum gnemon* after a pollen tube comes in contact with the embryo-sac one or two of the free nuclei in its vicinity become conspicuous by their larger size. These are the egg nuclei; the cytoplasm around them becomes denser and more striated. In *G. ula* (Vasil, 1959) there are a few groups consisting of half a dozen cells situated in the upper, nuclear part of the gametophyte. In each of these groups one or rarely two eggs differentiate after the entry of the pollen tube (Text-Fig. 4 C).

Conclusion

None of the gymnosperms has any neck canal cells in the archegonium, although a ventral canal cell and neck are present. There is a tendency towards shortening of the neck and elimination of the ventral canal cell. Many gymnosperms have no ventral canal *cell* but only a nucleus, and in a few conifers even the nucleus has not been seen.

The archegonia are usually situated towards the micropylar end of the gametophyte but this is not always so. In *Microcycas* their arrangement is irregular. In *Sequoia* and *Widdringtonia*, where the pollen tube follows a lateral course, the archegonia also show a lateral position. Exceptionally such lateral archegonia are found in many conifers and even in *Ephedra*.

In *Welwitschia* and *Gnetum* archegonia are eliminated completely. *Welwitschia* shows the formation of embryo sac tubes which grow upward and meet the pollen tubes. In *Gnetum* certain free nuclei become differentiated as eggs. *Ephedra* differs from both these genera in having normal archegonia with a large neck, and as in conifers lateral archegonia have also been reported in some prothalli.

On the basis of this study the view that *Ephedra* stands apart from *Welwitschia* and *Gnetum* and is closer to the conifers than to these genera appears to be well founded.

References

Andrews, H. N. and Felix, C. J. 1952. The gametophyte of *Cardiocatpus spinatus* Graham. *Ann. Mo. bot. Gdn.* **39**: 127–35.

Baird, A. M. 1939. A contribution to the life-history of *Macrozamia reidlei*. *J. roy. Soc. W. Australia* **25**: 153-75.

———. 1953. The life-history of *Callitris*. *Phytomorphology* **3**: 258-84.

Banerjee, S. K. 1959. The morphology, embryology and anatomy of *Cupressu-funebris* Endl. *M.Sc. Thesis*, Delhi Univ., Delhi.

Bell, A. M. 1925. Some anomalies in the seed of *Pinus*. *Proc. Indian Acad. Sci.* **35**: 189-96.

Berridge, E. M. and Sanday, E. 1907. Oogenesis and embryogeny in *Ephedra distachya*. *New Phytol.* **6**: 128–34, 167–74.

BOYLE, P. AND DOYLE, J. 1953. Development in *Podocarpus nivalis* in relation to other podocarps. I. Gametophytes and fertilization. *Sci. Proc. R. Dublin Soc.* **26**: 179-205.

BRENNAN, S. J. AND DOYLE, J. 1956. The gametophytes and embryogeny of *Athrotaxis. Ibid.* **27**: 193-252.

BROUGH, P. AND TAYLOR, M. H. 1940. An investigation of the life-cycle of *Macrozamia spiralis* Miq. *Proc. Linn. Soc. N.S.W.* **65**: 494-524.

BRYAN, G. S. AND EVANS, R. J. 1956. Chromatin behaviour in the development and maturation of the egg nucleus of *Zamia umbrosa. Amer. J. Bot.* **43**: 640-46.

———. 1957. Types of development from the central nucleus of *Zamia umbrosa. Ibid.* **44**: 404-15.

BUCHHOLZ, J. T. 1939. The embryogeny of *Sequoia sempervirens* with a comparison of the sequoias. *Ibid.* **26**: 248-57.

BURLINGAME, L. L. 1914. The morphology of *Araucaria brasiliensis*. II. The ovulate cone and the female gametophyte. *Bot. Gaz.* **57**: 490-508.

CHAMBERLAIN, C. J. 1935. *Gymnosperms, Structure and Evolution.* Chicago.

COULTER, J. M. AND LAND, W. J. G. 1905. Gametophytes and embryo of *Torreya taxifolia. Bot. Gaz.* **39**: 161-78.

DARLINGTON, C. D. AND LA COUR, L. F. 1960. *The Handling of Chromosomes.* 3rd Ed., London.

DE SILVA, B. L. T. AND TAMBIAH, M. S. 1952. A contribution to the life-history of *Cycas rumphii* Miq. *Ceylon J. Sci.* **12 A**: 1-22.

DOYLE, J. AND SAXTON, W. T. 1933. Contributions to the life-history of *Fitzroya. Proc. R. Irish Acad.* **41**: 191-217.

DUPLER, A. W. 1917. THE gametophytes of *Taxus canadensis* Marsh. *Bot. Gaz.* **64**: 115-36.

EAMES, A. J. 1913. The morphology of *Agathis australis. Ann. Bot., Lond.*, **27**: 1-38.

ELLIOTT, C. G. 1950. A further contribution on the life-history of *Pherosphaera Proc. Linn. Soc. N.S.W.* **75**: 320-33.

FAGERLIND, F. 1941. Bau und Entwicklung der *Gnetum*—Gametophyten. *K. svenska Vetensk Akad. Handl.* **19**: 1-55.

FAVRE-DUCHARTRE, M. 1957. Contribution á l' étude de la reproduction chez *Cephalotaxus drupacea. Rev. Cytol. Biol. Veg., Paris* **18**: 305-43.

———. 1958. Ginkgo, an oviparous plant. *Phytomorphology* **8**: 377-90.

FERGUSON, M. C. 1904. *Contributions to the* knowledge of the life-history of *Pinus* with special reference to sporogenesis of the gametophytes and fertilization. *Proc. Washington Acad. Sci.* **6**: 1-153.

GHOSE, S. L. 1924. A contribution to the morphology of *Agathis ovata* (Moore) Warb. *J. Indian bot. Soc.* **4**: 79-86.

HERZFELD, S. 1922. *Ephedra campylopoda* Mey. Morphologie der weiblichen Blüte und Befruchtungsvorgang. *Denkschr. Akad. Wiss. Wein. Math.-nat. Kl.* **98**: 243-68.

———. 1927. Beiträge zur Kenntnis von *Ginkgo. Jahrb. Wiss. Bot.* **66**: 814-62.

HOLLOWAY, J. T. 1937. Ovule anatomy and development and embryogeny in *Phyllocladus alpinus* (Hook.) and in *P. glaucus* (Carr.). *Trans. roy. Soc. N.Z.* **67**: 149-65.

HUTCHINSON, A. H. 1915. Fertilization in *Abies balsamea*. *Bot. Gaz.* **60**: 457–72.

KHAN, R. 1943. Contributions to the morphology of *Ephedra foliata* Boiss. II. Fertilization and embryogeny. *Proc. nat. Acad. Sci., India* **13 B**: 357–375.

KONAR, R. N. 1960. The morphology and embryology of *Pinus roxburghii* Sar. with a comparison with *Pinus wallichiana* Jack. *Phytomorphology* **10**: 305–19.

LAND, W. J. G. 1902. A morphological study of *Thuja*. *Bot. Gaz.* **34**: 249–59.

LAWSON, A. A. 1923. The life-history of *Pherosphaera*. *Proc. Linn. Soc. N. S. W.* **48**: 499–516.

LEE, C. L. 1955. Fertilization in *Ginkgo biloba*. *Bot. Gaz.* **117**: 79–100.

LONG, A. J. 1944. On the prothallus of *Lagenostoma ovoides* Will. *Ann. Bot. Lond. N. S.* **8**: 105–17.

LOOBY, W. J. AND DOYLE, J. 1939. The ovule, gametophyte and proembryo in *Saxegothaea*. *Sci. Proc. R. Dublin Soc.* **22**: 95–117.

———. 1940. New observations on the life-history of *Callitris*. *Ibid.* **22**: 241–55.

———. 1942. Formation of gynospore, female gametophyte, and archegonia in *Sequoia*. *Ibid.* **23**: 35–54.

———. 1944. The gametophytes of *Podocarpus andinus*. *Ibid.* **23**: 222–37.

MAHESHWARI, P. 1935. Contributions to the morphology of *Ephedra foliata*, Boiss. I. The development of the male and female gametophytes. *Proc. Indian Acad. Sci.* **1 B**: 586–606.

MARTIN, P. C. 1950. A morphological comparison of *Biota* and *Thuja*. *Proc. Pa. Acad. Sci.* **24**: 65–112.

MATHEWS, A. C. 1939. The morphological and cytological development of the sporophylls and seed of *Juniperus virginiana* L. *J. Elisha Mitchel sci. Soc.* **55**: 7–62.

MIYAKE, K. 1910. The development of the gametophytes and embryogeny in *Cunninghamia sinensis*. *Beih. bot. Centralbl.* **27**: 1–25.

MOSELEY, M. F. 1943. Contributions to the life-history, morphology and phylogeny of *Widdringtonia cupressoides*. *Lloydia* **6**: 109–32.

NARANG, NIRMAL. 1956. Morphological and embryological studies in Ephedrales. *Ph.D. Thesis*, Delhi Univ., Delhi.

NEELY, F. M. 1951. Small petrified seeds from the Pennsylvanian of Illinois. *Bot. Gaz.* **113**: 165–67.

NICHOLS, G. E. 1910. A morphological study of *Juniperus communis* var. *depressa*. *Beih. bot. Centralbl.* **25 A**: 201–41.

NORÉN, C. O. 1907. Zur Entwicklungsgeschichte des *Juniperus communis*. *Uppsala Univ. Arsskr. Mat-Nat.* **1**: 1–64.

PEARSON, H. H. W. 1929. *Gnetales*. Cambridge University Press.

RAO, L. N. 1961. Life-history of *Cycas circinalis* L. *J. Indian bot. Soc.* **40**: 601–19.

RENAULT, B. 1879. Structure comparee des quelques tiges de la flore carbonifre *Nouv. arch. Mus. hist. nat.* **2**: 213–48.

REYNOLDS, L. G. 1924. Female gametophyte of *Microcycas*. *Bot. Gaz.* **77**: 391–403.

ROBERTSON, A. 1904. Studies in the morphology of *Torreya californica*. I. Spore formation in *Torreya californica*. II. The sexual organs and fertilization. *New Phytol.* **3**: 133–48, 205–16.

ROY CHOWDHURY, C. 1961. The morphology and embryology of *Cedrus deodara* (Roxb.) Loud. *Phytomorphology* **11**: 283–304.

SAHNI, B. 1920. On the structure and affinities of *Acmopyle pancheri* Pilger. *Phil. Trans.* **210 B.**: 253–310.

SANWAL, M. 1962. Morphology and embryology of *Gnetum gnemon* L. *Phytomorphology* **12**: 243–64.

SAXTON, W. T. 1913 *a*. Contributions to the life-history of *Actinostrobus pyramidales* Miq. *Ann. Bot., Lond.* **27**: 321–45.

———. 1913 *b*. Contributions to the life-history of *Tetraclinis articulata*, with some notes on the phylogeny of the Cupressoideae and Callitroideae. *Ibid.* **27**: 577–605.

SAXTON, W. T. 1934 *a*. Notes on Conifers. VIII. The morphology of *Austrotaxus spicata* Compton. *Ibid.* **48**: 411–27.

———. 1934 *b*. Notes on Conifers. IX. The ovule and embryogeny of *Widdringtonia*. *Ibid.* **48**: 429–31.

SCHNARF, K. 1933. *Embryologie der Gymnospermen*. Berlin.

SEDGWICK, P. J. 1924. Life-history of *Encephalartos*. *Bot. Gaz.* **77**: 300–10.

SETHI, M. L. 1929. Contribution to the knowledge of life-hisotry of *Pinus longifolia*. *J. Indian bot. Soc.* **7**: 105–51.

SHIMAMURA, T. 1956. Cytochemical studies on the fertilization and proembryo of *Pinus thunbergii*. *Bot Mag.*, Tokyo **69**: 524–29.

SINGH, H. 1961. The life-history and systematic position of *Cephalotaxus drupacea* Sieb. *et* Zucc. *Phytomorphology* **11**: 153–97.

—— AND OBEROI, Y. P. 1962. Life-history of *Biota orientalis* Endl. *Ibid.* (In Press).

SMITH, R. W. 1923. Life-history of *Cedrus atlantica*. *Bot. Gaz.* **75**: 203–08.

STERLING, C. 1948*a*. Gametophyte development in *Taxus cuspidata*. *Bull. Torrey bot. Cl.* **75**: 147–65.

———. 1948 *b*. Abnormal prothallia in *Tsuga canadensis*. *Bot. Gaz.* **109**: 531–34.

STEWART, W. N. 1951. A new *Packytesta from the* Berryville locality of Southeastern Illinois. *Amer. Midl. Nat.* **46**: 717–42.

STILES, W. 1911. A note on the gametophytes of *Dacrydium*. *New Phytol.* **10**: 342–47.

SUGIHARA, Y. 1938. Fertilization and early embryogeny of *Chamaecyparis pisifera* S. *et* Z. *Sci. Rep. Tôhoku Univ.* **13**: 9–14.

———. 1939. Embryological observations on *Thujopsis dolabrata* var. *hondai* Makino. *Ibid.*, **14**: 291–303.

———. 1943. Embryological observations on *Keteleeria davidiana* Beissner var. *formosana* Hayata. *Ibid.* **17**: 215–22.

SWAMY, B. G. L. 1948. Contributions to the life-history of a *Cycas* from Mysore (India). *Amer. J. Bot.* **35**: 77–88.

TAHARA, M. 1940. The gametophytes, fertilization and proembryo of *Sciadopitys verticillata*. *Sci. Rep. Tohoku Univ.* **15**: 19–28.

TAKAO, A. 1959. A cytochemical study on the proteid vacuoles in the egg of *Pinus thunbergii* Parl. *Bot. Mag., Tôkyo* **72**: 289–97.

THOMPSON, W. P. 1916. The morphology and affinities of *Gnetum*. *Amer. J. Bot.* **3**: 135–84.

VASIL, V. 1959. Morphology and embryology of *Gnetum ula* Brogn. *Phytomorphology* **9**: 167–215.

VAZART, B. 1958. Cytologie vegetale. Influence des fixateurés au cours de la miée en évidence et de la localisation des acides ribo et désoxyribonucléique dans les archegones de *Pinus silvestris* var. *scotica* et du *Pinus nigra* var. *austriaca*. *C.R. Acad. Sci., Paris* **247**: 952–55.

ORIGIN OF GYMNOSPERMS—RECENT EVIDENCE

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Fossil history of gymnosperms dates back far into the geologic time, covering a span of more than 300 million years. Their origin still remains an unsolved riddle. The gymnosperms are supposed to have come by way of ferns and two fairly distinct major lines of evolution, Phyllospermae and Stachyospermae (some recognise more), have been recognised by many workers. These lines remain distinct as far as the fossil records go.

Pteridosperms and Cordaitales were undoubtedly the first among the gymnosperms to appear on the face of the earth. The first sure records of seeds are from the Lower Carboniferous. This was perhaps the age when gymnosperms came into existence. However, by this time the seed structure was so much advanced that it suggests a Devonian origin for the gymnosperms.

During the later part of the Devonian, heterospory was either established or was in the process of being established in a number of diverse phyla. It appears that the seed habit was also developed sometime during this period; distinguishing the higher plants from the spore-producing members. Thus it follows that the plants of this age are likely to give us some indication about the origin of gymnosperms.

The Devonian period is usually divided into the Lower, the Middle and the Upper. The plants of the Lower Devonian (and perhaps also of the Silurian) were small and simple. The landscape must have been covered with small straggling vegetation with no trees or shrubs. The plants consisted simply of dichotomising vascularized axes with their terminal branches carrying sporangia. The branching of the axes was accomplished in different ways. With varying differentiation of the different parts of the sporophytes, there resulted a diverse assemblage of plants. The well-known examples are *Psilophyton, Cooksonia, Taeniocrada, Trimerophyton, Hedeia. Zosterophyllum, Protobarinophyton Pectinophyton, Gosslingia* and others. Some forms are more evolved than the others and show rudiments of leaves. However, we are not concerned with these at the moment.

As we pass on to the Middle and the Upper Devonian, a marked change is evident in the plant life. Among the small herbaceous plants there appear on the landscape some arborescent forms with well-developed secondary wood having gymnospermous characters. No doubt,

our knowledge of the reproductive structures of many of these is very scanty, but at least some have come to be known better in recent years and have for the first time given us a glimpse of rather unusual plants combining both gymnospermous and pteridophytic characters. They are evidently important from the point of origin of gymnosperms.

The plant *Eospermatopteris* bearing Aneurophyton type of leaves is one such case in point. The plant is fairly well known from the Middle and the Upper Devonian rocks of Germany and New York. It is a tree of about 40 feet height with a loose crown of fronds, 6 to 9 feet long. The stump with numerous slender roots radiating out is bulbous in shape, up to 3 feet in diameter, and quickly tapers to a more slender trunk. The stumps have been found as casts and nothing is, therefore, known of their internal structure, except a coarse reticulate pattern on the stem casts suggesting the characteristic cortical fibre strands found in the later pteridosperms.

The foliage attributed to *Eospermatopteris* has been shown to be that of *Aneurophyton* (Kraüsel and Weyland, 1935). It is a thrice pinnate frond with ultimate ramification that is little more than a dichotomising termination of the branch system. This type of frond or the branch system appears to be derived from the psilopsid type of dichotomising axes. However, the rachis consists of a central triangular core of primary wood surrounded by secondary wood composed of tracheids with numerous series of circular bordered pits and tall uniseriate rays. It has been shown that the larger stem and its small branches are similar in structure.

Some of the dichotomously forking ultimate branches terminate in ovoid bodies, 6 mm. long, which were originally thought to be seeds, but have now been shown to be sporangiate organs. The sporangia, like the leaves, are indicative of psilophytic origin. However, the solid primary xylem surrounded by compact secondary wood indicates pteridospermous affinities.

So here we have a plant showing unmistakable gymnospermous characters in internal structure but at the same time, according to the evidence available at present, reproduced by cryptogamic method.

We can take another Upper Devonian plant, *Tetraxylopteris schmidtii*, described by Beck in 1957. The plant attained a height of about 9 feet, and consisted of a stem to which were attached spirally arranged fronds or branch systems. The sterile frond had a monopodial axis consisting of a rachis and three lesser orders of axes with bilobed ultimate divisions. The branching was opposite or subopposite and decussate. The fertile fronds bore large terminal exannulate sporangia in their apical portion, again suggestive of psilophytic origin. They have no specialized means of dehiscence.

The primary xylem is tetrarch with mesarch protoxylem. Both secondary xylem and secondary phloem occur in the stem. The secondary xylem consists of tracheids and numerous uniseriate or multiseriate rays of varying heights. The pits on the tracheidal walls are

circular, bordered and alternate, usually hexagonal and crowded like the araucarian pits. The presence of extensive amount of true secondary xylem is a strong pteridospermic character in this plant. However, the reproduction is still by cryptogamic method.

Beck (1960 *a*) made another astonishing discovery of a specimen showing *Archaeopteris* and *Callixylon* in organic connection. These two are among the most widespread and well-known Devonian genera. *Archaeopteris* is based on large vegetative and fertile bipinnate compound leaves, and is commonly believed to be a fern. *Callixylon* (Arnold, 1930) is based on large petrified stems and roots with compact secondary xylem and is, therefore, placed in Cordaitales.

In accordance with the rules of priority the whole plant is now known as *Archaeopteris* (Beck, 1960 *b*). It was a large tree, about 60 feet tall, with lateral branches bearing large spirally arranged bipinnate leaves. The axis discovered by Beck in organic connection belongs to the species *Callixylon zalesskyi*. The pith is small, septate and surrounded by primary mesarch bundles. The secondary wood is, for the most part, of the coniferous type and consists of tracheids and uni- or biseriate rays. The tracheids have multiseriate bordered pits, segregated in groups on the radial walls. The wood is thus similar in organization to that of *Cordaites*.

The fronds are large bipinnate with a strong striated central rachis having primary branches bearing wedge-shaped pinnules. Sporangia are attached to the pinnules and have no special mechanism of dehiscence. The spores have a remarkable similarity to the pteridosperm genus, *Crossotheca*, and in fact the leaves as such are comparable to those of the pteridosperms in their general morphological features.

It may be pointed out that in the attached specimen only one kind of spores have been found; nevertheless, some of the species of *Archaeopteris* have been known to be heterosporous (Arnold, 1947).

A tree closely related to *Callixylon* is *Pitys* and is known from the Lower Carboniferous. Another is *Archaeopitys* with a single species known from the Upper Devonian of Kentucky. It has a secondary wood like that of *Callixylon* with multiseriate pitted tracheids and rays. Yet another is *Palaeopitys* (Kidston and Lang, 1923) from the Middle Devonian. It has a secondary wood very similar to that of *Protopitys*. Walton (1957) has recently described a heterosporous fertile specimen of *Protopitys—P. scotica*. This genus has a stem of gymnospermous type, but the fertile specimen shows sporangia borne terminally on the ultimate divisions of the frond. There is no distinct annulus. Some sporangia bear large spores (147 μ), some bear small (82 μ) ones and still others have spores of intermediate size. Here, it seems heterospory was in the offing.

In addition to the above, there existed other plants with secondary wood of gymnospermous type. Beyond that we do not know anything about them as yet.

From the foregoing account it becomes fairly evident that during the Devonian time, prior to the emergence of true gymnosperms, there were present several distinct and highly evolved phyla of plants of arborescent habit with gymnospermous internal structure but with cryptogamic reproduction. They perhaps became extinct before the Upper Carboniferous. Are these the ancestors of gymnosperms? As stated earlier, the available evidence suggests that heterospory was being established in a number of phyla during the later part of the Devonian. Perhaps the seed habit was also being evolved about the same period. This must have given a distinct advantage to some of the arborescent plants over the other small, herbaceous, spore-producing members.

That brings us to the question, how must have the seed originated? There is the telomic concept in which it is supposed that one sporangium (fertile telome) became surrounded by sterile telomes which fused together to form the integument.

Professor Andrews (1961) has recently given another concept which he terms 'nucellar modification concept'. He starts from the megasporangium of *Stauropteris burntislandica* described by me (Surange, 1952). This megasporangium is rather unusual. It has an elongate body and a tapering apex with a minute opening. More than half of its lower portion is sterile, while the upper portion is hollow and contains two megaspores. A slender vascular strand runs through the sterile portion, indicating the most extensive vascularization of any fern or fern-like sporangium. It obviously is not an ordinary sporangium, but it is not a seed either. Andrews derives the seed from such a sporangium through the following stages: (1) reduction of two megaspores to one and an increase in size of the latter, (2) sinking of the megaspore towards the basal part of the sporangium, and (3) the displacement of the vascular strand and its division into two or more branches which grow up around the megaspore.

From this point two possible lines are postulated by Andrews. With slight modification of sporangium wall and apex as well as the vascularization of the nucellus, the medullosan type of seed is reached. With somewhat extensive modification or specialization of tissue in the peripheral wall of the megasporangium, a seed of lyginopterid type results.

While this scheme does offer an explanation for the vascularization of the nucellus in the Medullosan seeds and the apparent fusion of integument and nucellus of the lyginopterid seed, there still remains a big gap between heterospory and the seed habit. We still have to find evidences for the intermediate stages in the evolution of a seed in the fossil record.

Coming back to the groups of plants showing both gymnospermous as well as pteridophytic characters, it appears very likely that they comprise the ancestral complex from which major groups of gymnosperms had evolved (*see* Beck, 1960 *b*).

Beck (1960 *b*) has in fact proposed a new class—Progymnospermopsida—for grouping such plants together. Under this he includes Aneurophytales (*Aneurophyton*, *Eospermatopteris*, *Tetraxylopteris*), Protopityales (*Protopitys*), and Pityales (*Pitys*, *Archaeopteris*, *Callixylon*, *Archaeopitys*). I am sure, as our knowledge progresses, some more plants will be added to this class.

The class Progymnospermopsida includes plants which appear as likely ancestors of gymnosperms. In a number of morphological characters they are remarkably similar to the two groups of gymnosperms, *viz.*, Pteridospermales and Cordaitales which most probably represent the Cycadophyte and Coniferophyte lines of gymnospermous evolution. Also, certain primitive features of these groups suggest that Progymnospermopsida are descended directly from some Psilophyte-like ancestors.

It follows that there is no necessity to consider any group of ferns as ancestors of gymnosperms. The true ferns appeared much later, probably in the Lower or the Upper Carboniferous time. It is true that stratigraphic sequence is often of limited use, yet its importance cannot be minimised. A true fern is yet to be discovered from the Devonian, although it is probably correct that the fern line was also in the process of being evolved perhaps from simple Psilophytalean type of plants or from some other stock at the same time when gymnosperms were also evolving.

REFERENCES

ANDREWS, H. N. Jr. 1961. *Studies in Palaeobotany.* New York and London.

ARNOLD, C. A. 1930. The genus *Callixylon* from the Upper Devonian of Central and Western New York. *Pap. Mich. Acad. Sci.* **11**: 1–50.

———. 1947. *An Introduction to Palaeobotany.* New York.

BECK, C. B. 1957. *Tetraxylopteris schmidtii* gen. et sp. nov. A probable pteridosperm precursor from the Devonian of New York. *Amer. J. Bot.* **44**: 350–67.

———. 1960 *a*. Connection between *Archaeopteris* and *Callixylon*. *Science* **131**: 1524–25.

———. 1960 *b*. The identity of *Archaeopteris* and *Callixylon*. *Brittonia* 12: 351–68.

KIDSTON, R. AND LANG, W. H. 1923. On *Palaeopitys milleri* M'Nab. *Trans. roy. Soc. Edinb.* **53**: 409–17.

KRÄUSEL, R. AND WEYLAND, H. 1935. Pflanzenreste aus dem Devon. IX. Ein Stamm von *Eospermatopteris*—Bau aus dem Mitteldevon des Kirberges, Elberfeld. *Senckenbergiana* **17**: 9–20.

SURANGE, K. R. 1952. The morphology of *Stauropteris burntislandica* P. Bertrand and its megasporangium *Bensonites fusiformis* R. Scott. *Phil. Trans.* **237B**: 73–91.

WALTON, J. 1957. On *Protopitys* (Göppert) with a description of a fertile specimen *Protopitys scottica* sp. nov. from the calciferous sandstone series of Dunbartonshire. *Trans. roy. Soc. Edinb.* **63**: 333–40.

POLLEN GRAINS OF *EPHEDRA* AND *WELWITSCHIA* AND THEIR PROBABLE FOSSIL RELATIVES

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Introduction

Among the living gymnosperms, the order Gnetales with its 3 genera—*Ephedra*, *Welwitschia* and *Gnetum*—has been of considerable academic interest in the past. The morphological individualities of these genera have given rise to phylogenetic speculations as well as controversies and taxonomically they have taxed the imaginations of many systematists who have suggested various methods of subgrouping them. The last in this line has been the suggestion to put each of these genera into a separate order, *viz.*, Ephedrales, Welwitschiales and Gnetales (Eames, 1952, *sensu stricto*).

An important factor contributing to the confusion in the systematics and phylogeny of Gnetales (*sensu lato*) has been the lack of their significant fossil history. As opined by Seward (1919), the paucity of fossil Gnetales is " the result of the imperfection of the geological record and the difficulty of distinguishing between fragmentary remains of Gnetalean genera and vegetative or reproductive organs of similar external form belonging to other plants". Seward (1919) believed that "in view of the morphological features characteristic of the present members of the Gnetales and the geographical distribution of the species of *Ephedra*, *Gnetum* and *Welwitschia*, it would seem safe to conclude that the absence of fossil forms is not explicable on the hypothesis of a recent origin of the group".

In recent years, fossil pollen grains closely comparable to those of *Ephedra* and *Welwitschia* have been observed in sedimentary rocks of various ages extending to Permian. This lends support to the above-mentioned observations of Seward.

The pollen grains in the three living genera are fairly distinguishable from each other. However, in *Ephedra* and *Welwitschia*, the longitudinally striated, oval, non-saccate pollen grains are very characteristic and are unlike all others (excepting *Spathiohyllum*—Araceae) known from any living or fossil plant. In *Gnetum* the circular, finely spiculate pollen grains, without any germinal mark, are not so distinctive because comparable forms also occur in a number of conifers.

DESCRIPTION

Ephedra foliata Boiss.—Mature pollen grains broadly oval, ends pointed with a rounded contour, exine thick; structureless, thickest at the ends, bearing 9–18 psilate, longitudinal ridges separated from each other by a groove. Ridges mostly with well-defined wavy to straight vertex and grooves straight but faintly apparent (Plate VI, Figs. 1, 2). Ridges normally meeting at the pointed ends but occasionally also meeting before reaching the ends (Text-Fig. 1). Transverse section of nearly mature pollen grain more or less circular with 9–18 tooth-like projections (the ridges), each with an acute apex and a broad base, contiguously arranged over a thin, glistening layer of exine overlying a darker lining layer (Plate VI, Figs. 2, 3). Ridge exine in fully mature pollen grains seen with a cavity inside due to separation of sexine from nexine (Plate VI, Fig. 2; Text-Fig. 2).

Immature pollen grains oval and ridged like the mature pollen grains but smaller in size, ridges rounded at apex without a defined apex, and grooves more prominent optically. In a transverse section immature pollen grains showing a hemispherical outline with one side of the grain straight or even depressed a little (Plate VI, Figs. 3, 4).

Acetolysed, mature pollen grains oval and ridged, sexine always closely appressed to the nexine, sexine without structure (Plate VI, Figs. 5–13). Ridge vertex straight (Plate VI, Figs. 5, 7) or wavy (Plate VI, Figs. 6, 8–10, 12). Certain grains so folded as to appear monocolpate (Plate VI, Figs. 8–13).

Tetrads tetragonal (Plate VI, Fig. 14), the four pollen grains being vertically grouped together.

Other species of Ephedra.—Steeves and Barghoorn (1959) have studied the pollen grains of most of the living species of *Ephedra*. In general, the pollen morphology of *E. foliata* described here agrees with that of the other species of *Ephedra*. However, Steeves and Barghoorn (1959) find the pollen morphology in the genus slightly varied, falling into four categories based upon variation in the nature of the ridges. According to these authors, their Ephedra-pollen-types A, B and C possess mostly distinct colpi (germinating grooves) at the base of each furrow between the ridges but those of type D do not show any distinct colpi; the sexine being extremely thin in the furrows.

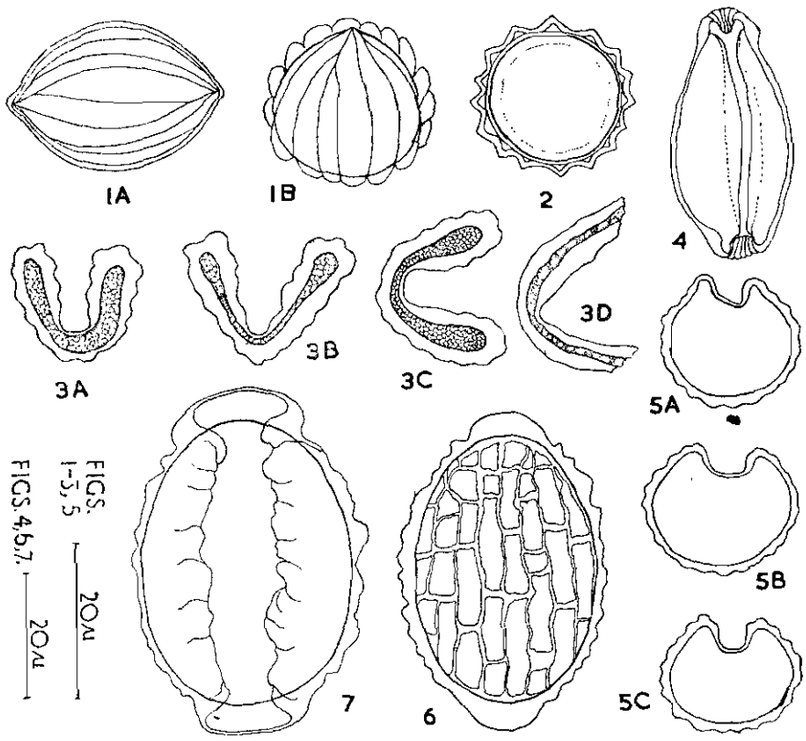
Fossil Ephedra-like pollen grains.—The earliest recognition of a fossil pollen of Ephedralean affinity was done by Wodehouse (1933) from the Eocene of Green River Formation. The resemblance of this dispersed grain was so complete with *Ephedra* pollen that it was referred to as *Ephedra eocenipites* by Wodehouse. Subsequently Wodehouse (1935) recognised *Ephedra* pollen in Pleistocene of Kashmir. Thiergart (1938) described a Gnetalean pollen grain from Miocene as *Gnetaceaepollenites ellipticus* creating a new genus for dispersed ephedroid fossil pollen grains. Kirchheimer (1950) has described ephedroid pollen grains from Oligocene. Thiergart (1944) has likened

some pollen grains recovered from Upper Cretaceous (Paleocene strata) to *Ephedra* and Cookson (1956) reports *Ephedra* type of pollen grains from Paleocene to Eocene of Tasmania and Australia. Bolkhowitina (1953) has described, from a cretaceous formation, ribbed pollen grains similar to those of *Ephedra* or *Welwitschia*, but has named them as *Welwitschiapites*. Kuyl, Muller and Waterbolk (1955) describe *Ephedra strobilacea* type of pollen grains from Upper Cretaceous of Venezuela as well as Nigeria. Steeves and Barghoorn (1959) describe an ephedroid pollen grain from Cretaceous. Scott (1960) considers striated, longish-oval pollen grains from the Upper Triassic of Arizona as closely similar to those of *Ephedra*. Jansonius (1961) has described a number of striated non-saccate pollen grains comparable to ephedroid organisation from the Lower Triassic of W. Canada and has assigned them to *Gnetaceaepollenites* (including *Ephedripites* Bolkhow.). Wilson (1959) has illustrated a number of non-saccate, striated pollen grains from Middle Permian of U.S.A. and has referred them to *Ephedripites*. Recently, I (Bharadwaj, 1962) have described some striated non-saccate pollen grains (comparable to those of Wilson, 1959) from the Upper Permian of Raniganj Coalfield (Plate VI, Figs. 15–18). These grains are broadly oval with structured and longitudinally striated exine, the striations sometimes bifurcating and joining adjacent ones. Between the striations the ridges are low and rounded without any distinct vertex or a crest. The characters of these grains answer best the circumscription of the dispersed spore genus *Welwitschiapites* Bolkhowit.

Welwitschia mirabilis Hook.—Mature pollen grains longish oval with rounded ends (Plate VII, Figs. 19, 20) in polar view but with pointed ends in lateral view (Plate VII, Figs. 21, 22). In polar view, colpus distinctly seen, slightly biconvex in shape and extending to full length of the grains. The lips of the colpus thickened and dense. Exine thick, structureless, bearing 16–25 longitudinal striations, demarcating round-topped ridges. In transverse section (Text-Fig. 3; Plate VII, Figs. 21–23) the grain variously cup-shaped with two halves. The free end of each half fairly thick and longitudinally striated on both faces as well as frequently bent away from that of the other half. Approaching the middle of the grain closer to the sulcus floor, each flap longitudinally striated only on the outside. sexine as thick as around the free edge but on the inner side it being non-striated and thinner (Text-Fig. 3). Between the walls the cytoplasmic contents scanty and alveolar.

Immature pollen grains longish-oval in polar view with broadly pointed, rounded or truncate ends (Plate VII. Figs. 24–26). In lateral view (Plate VII, Fig. 27) the grains spindle-shaped with colpus-bearing side more buldging and the exine drawn out like a spout at the ends. Longitudinal striations on the exine crossing over the edge of the spout and converging to meet a little backwards in the colpus (Text-Fig. 4). Besides longitudinal striations, a large number of closely spaced striations present and appearing to run across the length of the grain (Plate VII, Figs. 31-33). These cross-striations tending to become

faint and even obliterated at some places in mature grains. As seen in a transverse section, colpus made up of two, small, raised up flanks with a broad floor where the sexine is very thin, almost invisible (Text-Fig. 5; Plate VII, Figs. 23, 28-30). The inner cavity of the grain filled copiously with granular cytoplasm. Exine structureless with only two distinguishable layers: the outer hyaline, and the inner opaque.



TEXT-FIGS. 1–7. Figs. 1–2. *Ephedra foliata* Boiss. Fig. 1. A, B, Branched ridges in polar and slightly oblique views respectively, Maheshwari slide (1). Fig. 2. T.s. mature pollen grain; Maheshwari slide (1). Figs. 3–5. *Welwitschia mirabilis* Hook. Fig 3. A–D. T.s. mature pollen grains Maheshwari slide (*a*). Fig. 4. Polar view showing the extent of colpus and the converging striations on the spout. Maheshwari slide (*b*). Fig. 5. A–C. T.s. immature pollen grains with bigger cell cavities, Maheshwari slide (*b*). Figs. 6–7. *Welwitschia mirabilis* Hook. Acetolysed grains in polar view. Fig. 6. Striations with vertical connecting striations. Fig. 7. Wrinkled sexine and the colpus.

Acetolysed grains broadly oval, usually with the sexine irregularly loosened from the pollen grain to a varying degree (Plate VII, Figs. 34–38). Sexine bearing longitudinal striations as well as a few to many vertical striations irregularly connecting the longitudinal striations (Text-Fig. 6; Plate VII, Fig. 36). Colpus usually not to be made out but for

the occasional folds of the loose, inflated sexine (Text-Fig. 7; Plate VII, Figs. 37, 38).

Fossil pollen grains related to Welwitschia.—Monocolpate, longitudinally and cross-striated pollen grains as described here for *Welwitschia* have not been frequently reported in fossil condition. Kuyl, Muller and Waterbolk (1955) describe a grain from Middle Cretaceous of Iraq as of *Ephedra trobilacea* type but it shows a distinct longitudinal colpus and could more reasonably be a *Welwitschia* type. *Decus satisporites* Leschik (1955) is monocolpate but the exine is closely striated only at right angles to the length of the grain (Plate VII, Fig. 35) as has been described here in immature grains of *Welwitschia*. *Decussatisporites* is described from Triassic horizon. Recently Bharadwaj and Salujha (under preparation) have discovered some longitudinally as well as cross-striated monocolpate pollen grains (Plate VII, Figs. 39, 40) from the Upper Permian of Raniganj Coalfield. Morphographically, the closest approach of these pollen grains is to *Welwitschia*. Balme and Hennelly (1956) have described longish-oval, monocolpate, longitudinally striated pollen grains from the Permian of Australia as *Marsupipollenites sinuosus* (Plate VII, Fig. 41), now transferred by me to *Gnetaceaepollenites* (Bharadwaj, 1962). This corresponds, in general morphology, to the pollen grains of *Welwitschia*. Some other monocolpate grains with strongly structured exine (Plate VII, Figs. 42, 43), and *Vittatina* as well as some saccate pollen grains having body exines bearing horizontal and vertically connecting striations from Upper Permian of Raniganj Coalfield (Bharadwaj, 1962), may also be probable relatives of *Welwitschia*.

Discussion

The pollen grain in *Ephedra* is organizationally a polycolpate grain, as every groove between the ridges is structurally a potential germinal furrow or colpus. As is apparent from a study of these pollen grains in various stages of maturity, polycolpate organization is differentiated early. Normally the germination of the pollen grain in *Ephedra* occurs by a split along only one of the colpi as is known in the case of *E. altissima* (Berridge, 1909) or *E. foliata* as well as *E. gerardiana* (Mehra, 1938) where the split extends a little on the other side also. Structurally all colpi are alike so that prior to germination it is not possible to tell which colpus will split. However, among acetolysed pollen grains of *E. foliata*, some acquire monocolpate appearance, apparently due to the collapse of the wall following loss of inner contents rather than the presence of any organizational characteristic suggestive of a monocolpate condition. On the other hand, the pollen grain in *Welwitschia* possesses only one colpus differentiated early during its development. The thinner exine at the base of the colpus in *Welwitschia* suggests, as also observed by Strasburger (1892), that the colpus is functional and for that reason the rest of the pollen wall converges to protect this germinal furrow. This monocolpate condition in *Welwitschia* is also a primitive feature shared by Cycadopsida. It is thus apparent that

the polycolpate and monocolpate condition present a difference, between *Ephedra* and *Welwitschia*, of a fundamental nature, connected with the important process of their germination. This organisational difference in the pollen grains of these genera has also been marked by Wodehouse (1935), Cookson (1956) and Wilson (1959).

The pollen grains in *Ephedra* as well as *Welwitschia* have longitudinally striated exine. In the former genus the ridges as well as the furrows are mostly well defined, with characteristic undulating or zigzag patterns and the grooves in furrows are the colpi. On the other hand, in *Welwitschia* the ridges are low and the furrows are mere notches between two adjacent ridges and being not connected with germination of the pollen grain, are only of ornamentational significance.

Striated exine similar to that of *Welwitschia* pollen also occurs in the central bodies of the saccate pollen grains of Late Palaeozoic to Middle Mesozoic ages (Bharadwaj. 1962). This similarity and the occurrence of reduced or vestigial sacci in some specimens of the latter, led Tchigouriaeva (1954) to postulate the origin of *Welwitschia* type of pollen grain from the horizontally striated saccate pollen grains of the Permian. However, he failed to comprehend the importance of the difference in the direction of the colpus with reference to the direction of the striations between the two. Thus, a direct derivation of one from the other seems improbable. On the other hand, the similarity in the nature of striations between them indicates some phylogenetic nearness, as if due to common ancestry.

It is apparent that on grounds of pollen morphography *Ephedra* and *Welwitschia* are unrelated to each other, the similarity in the striated nature of the exine between these genera being of a superficial nature. This fact is also supported by differences exhibited by these genera in floral morphology, male and female gametophytes, ovule and seed structures as well as stomatal apparatus and the cuticle. Whereas in respect of these features *Ephedra* shows nearness to primitive conifers and Cordaitales (Pearson, 1929; Eames, 1962), *Welwitschia* points to Bennettitales as its close relatives (Pearson, 1929; Florin, 1931). In this regard the monocolpate nature of the pollen grains, common to *Welwitschia* and Bennettitales, is an additional evidence.

SUMMARY

The pollen grain of *Ephedra foliata* Boiss is described in detail. Its exine consists of a number of ridges with intervening furrows, each with a groove which is supposed to be a potential colpus. Fossil pollen grains comparable morphographically wtih those of *Ephedra* are traced to Permian strata.

The pollen grains of *Welwitschia mirabilis* Hook., having striated exine, are described in detail and shown to be monocolpate with a structurally defined colpus. Fossil pollen grains resembling those of *Welwitschia* are traced back to Permian age.

It has been shown that pollen grains of *Ephedra* and *Welwitschia* are polycolpate and monocolpate respectively and thus fundamentally different from each other. The similarity in the longitudinally striated nature of the exine of the two genera is of a superficial nature. In pollen morphology, *Welwitschia* is close to Bennettitales.

I am extremely thankful to Prof. P. Maheshwari for the loan of slides containing sections of pollen grains of *Ephedra* and *Welwitschia* and to Prof. G. Erdtman for the slide containing acetolysed pollen grains of *Welwitschia*.

REFERENCES

BALME, B. E. AND HENNELLY, J. P. F. 1956. Monolete, monocolpate, and alete sporomorphs from Australian Permian sediments. *Aust. J. Bot.* **4**: 34–64.

BERRIDGE, E. M. 1909. Fertilization in *Ephedra altissima*. *Ann. Bot., Lond.* **23**: 509–12.

BHARADWAJ, D. C. 1962. The Miospore genera in the coals of Raniganj Stage (Upper Permian), India. *The Palaeobotanist* **9**: 68–106.

—— AND SALUJHA, S. K. 1962. The Palynological study of Jambad-Bowlah Seam of Raniganj Coalfield (in press).

BOLKHOVITINA, N. A. 1953. Characteristics of the spores and poller of Cretaceous strata of the central basin of U.S.S.R. *Acad. Sci. U.S.S.R. Geol. Ser.* **61**: 1–187.

CHOKSON, I. C. 1956. Pollen grains of the *Ephedra* type in Australian Tertiärs. deposits. *Nature*, Lond. **7**: 47–48.

EAMES, A. J. 1952. Relationships of the Ephedrales. *Phytomorphology* **2**: 79–100.

FLORIN, R. 1931. Untersuchungen zur Stammesgeschichte der Coniferales and Cordaitales. *Kungl. svenska Vetensk Akad. Handl.* **10**: 515–20.

JANSONIUS, J. 1962. Palynology of Permian and Triassic sediments, Peace River Area, Western Canada. *Palæontegraphica* **110 B**: 35–90.

KIRCHHEIMER, F. 1950. Mikrofossilien aus Salzablagerungen des Teritars. *Palaeontographica* **90A**: 127–60.

KUYL, O. S., MULLER, J. AND WATERBOLK, H. TH. 1955. The application of Palynology to Oil Geology, with special reference to Western Venezuela. *Geol. en Mijnbour.* N.S. **17**: 49–76.

LESCHIK, G. 1955. Die Keuperflora von Neuewelt bei Basel. II. Iso-und Mikrosporen. *Schweiz. palaeont. Abh.* **72**: 1–70.

MEHRA, P. N. 1938. The germination of pollen grains in artificial cultures in *Ephedra foliata* Boiss and *Ephedra gerardiana* Wall. *Proc. Indian Acad. Sci.* **8B**: 218–30.

PEARSON, H. H. W. 1929. *Gnetales*. Cambridge.

POTONIÉ, R 1958. Synopsis der Gattungen der *Sporae dispersae* pt. II. *Beih. Geol. Jb.* **31**: 1–114.

SCOTT, R. A. 1960. Pollen of *Ephedra* from the Chinle formation (Upper Triassic) and the genus *Equisetosporites*. *Micropaleontologist* **6**: 261–76.

SEWARD, A. C. 1919. *Fossil Plants* IV, Cambridge.

STEEVES, M. W. AND BARGHOORN, E. S. 1959. The pollen of *Ephedra*. *J. Arnold Arbor.* **40**: 221–55.

STRASBURGER, E. 1892. Über das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. *Histol. Beitr.* **4**: 1–158.

TCHIGOURIAEVA, 1954. Structure du pollen des Gnetales. *Grana Palynol.* **1**: 95–98.

THIERGART, F. 1938. Die Pollenflora der Niederlausitzer Braunkohle, besonders in Profil der Grube Marga bei Senftenberg. *Jb. preuss. geol. Landesanst.* **58**: 282–351.

———. 1944. Die Pflanzenreste des Posidonienschiefers. *Archiv Lagerst. forsch.* **77**: 45–48.

WILSON, L. R. 1959. Geological history of the Gnetales. *Oklahoma Geol. Notes* **19**: 35–40.

WODEHOUSE, R. P. 1933. The oil shales of the Eocene Green River formation. *Bull. Torrey bot. Cl.* **60**: 479–524.

———. 1935. The Pleistocene pollen of Kashmir. *Mem. Conn. Acad. Arts Sci.* **9**: 1–18.

EXPLANATION OF PLATES VI & VII

PLATE VI

FIGS. 1–14. *Ephedra foliata* Boiss. FIGS. 15–18. *Ephedra*-like pollen grains. All figures, × 500.

FIG. 1. Pollen grains in various views (microtome sections) ph. No. 246/32.

FIG. 2. Pollen grains in various positions (microtome sections) Ph. No. 246/35.

FIG. 3. T.s. immature pollen grain. Ph. No. 246/29.

FIG. 4. Immature pollen grains. Ph. No. 246/34.

FIG. 5. Acetolysed large pollen grain with straight ridges and grooves. Ph. No. 246/25.

FIG. 6. Acetolysed small pollen grain with wavy ridge-crest. Ph. No. 246/19.

FIG. 7. Two acetolysed pollen grains in different foci. Ph. No. 246/22.

FIGS. 8–10. Acetolysed pollen grains in polar and lateral views and in successive foci. Ph. Nos. 246/11–13.

FIG. 11. A pollen grain in lateral, optical section. Ph. No. 246/20.

FIGS. 12, 13. Two acetolysed pollen grains in polar and lateral views, the latter simulating a monocolpate appearance in successive foci. Ph. Nos. 246/15, 16.

FIG. 14. A pollen tetrad and an adjoining pollen grain with monocolpate appearance. Ph. No. 246/37.

FIGS. 15–18. *Ephedra*-like pollen grains from Upper Permian of Raniganj Coalfield. Ph. Nos. 135/29, 106, 110/7, 168/18.

PLATE VII

FIGS. 19–33, 35–38. *Welwitschia mirabilis* Hook. Figs. 34, 39–43. Pollen grains related to *Welwitschia*. Fig. 35, × 365; all others, × 500.

FIGS. 19–20. Mature pollen grains in polar view. Ph. No. 246/6, 7.

FIG. 21. Mature pollen grain in lateral view and in t.s. Ph. No. 246/8.

FIG. 22. Mature pollen grains in lateral and oblique transverse views. Ph. No. 239/20.

FIG. 23. T.s. mature pollen grains. Ph. No. 246/10.

FIGS. 24–27. Immature pollen grains in various views. Ph. Nos. 245/5, 245/3, 245/3, 239/2.

FIG. 28. Immature pollen grains in lateral and transverse views. Ph. No. 245/4.

FIGS. 29, 30. T.s. immature pollen grains. Ph. Nos. 245/10, 245/9.

FIGS. 31–33. Immature pollen grains showing horizontal striations at successive foci. Ph. Nos. 239/31, 30, 29.

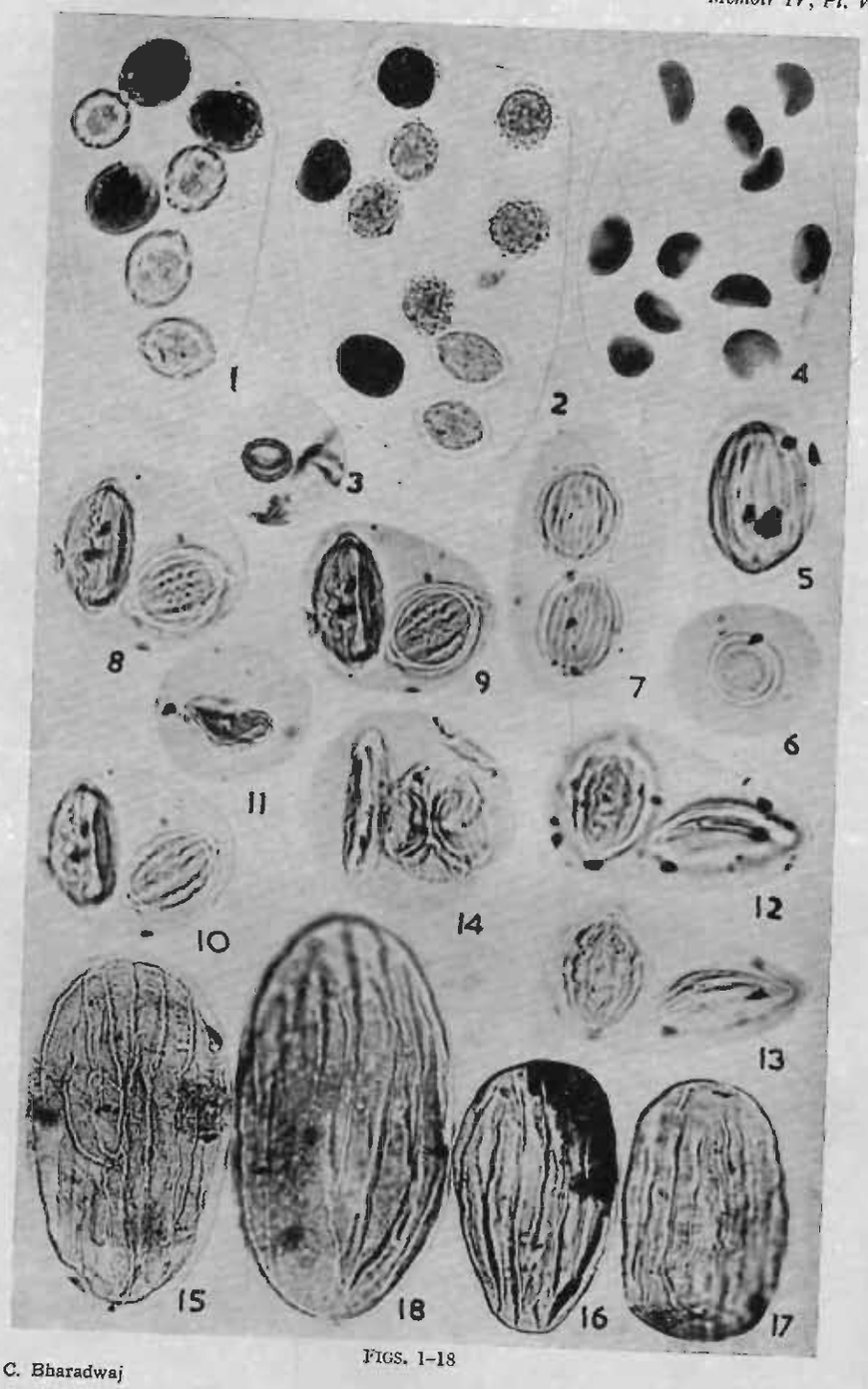
FIGS. 35–38. Acetolysed pollen grains showing striations and colpus in various views. Ph. Nos. 245/11, 247/19, 246/3, 247/19.

FIG. 34. *Decussatisporties*, Genotype sketch after Potoriè, 1958.

FIGS. 39, 40. Pollen grains possibly related to *Decussatisporites* from Raniganj Stage, India, Ph. Nos 180/161.

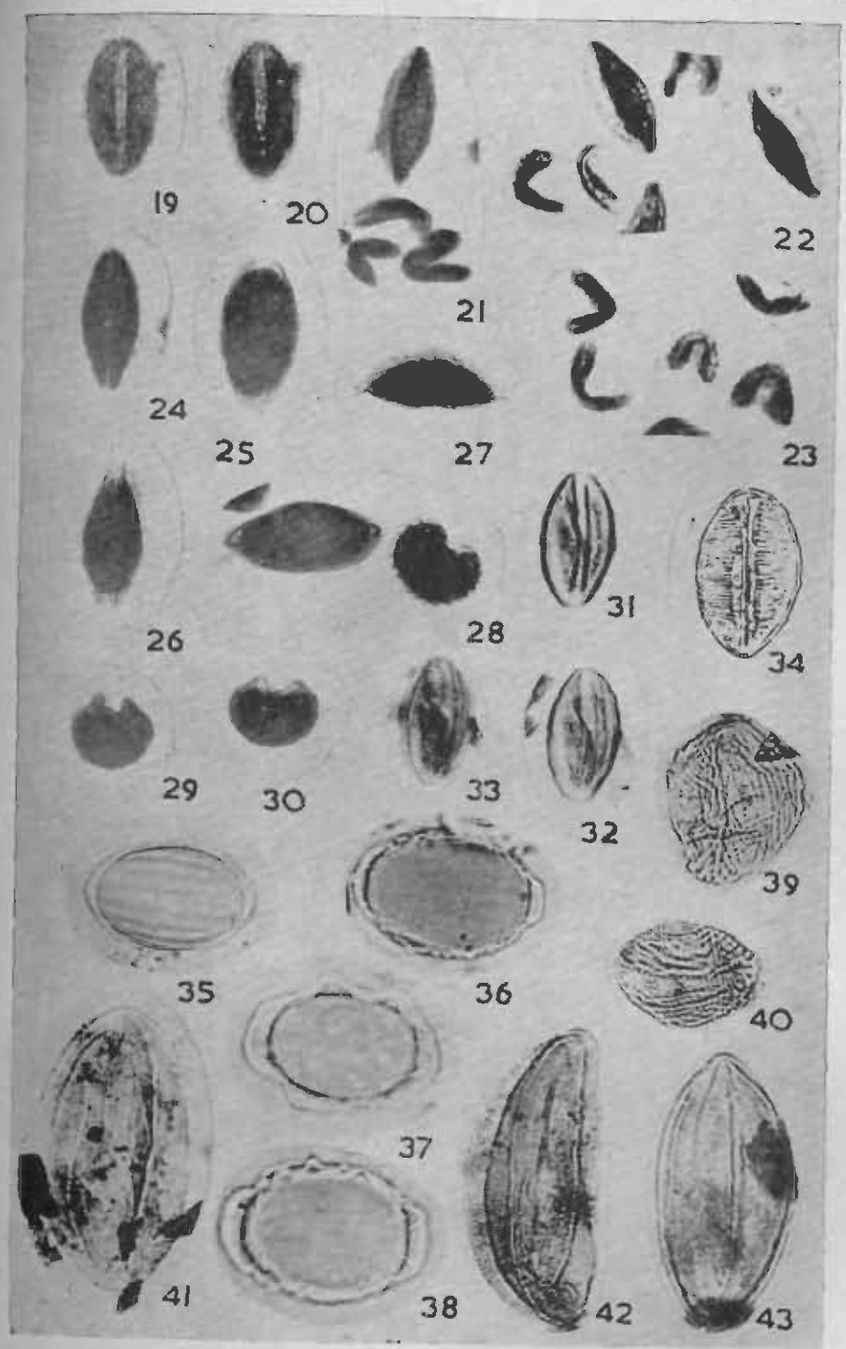
FIG. 41. *Gnetaceaepollenites sinuosus* (B. & H.) Bharad. from Raniganj Stage (Uppder Permian), India. Ph. No. 147/22.

FIGS. 42, 43. Pollen grains possibly related to *Welwitschia* from Ranigarj Stage (Upper Permian), Ph. Nos. 158/1, 165/35.



FIGS. 1–18

D. C. Bharadwaj



D. C. Bharadwaj

FIGS. 19–43

CYTOGENETICAL EVOLUTION IN THE GYMNOSPERMS

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The basic cytogenetical processes of evolution in the gymnosperms are gene mutations, hybridization, karyotypic alterations, polyploidy and apomixis. Out of these, the most apparent, but not necessarily the most important, type of evolutionary process is the karyotypic alteration. This will be dealt with first.

Karyotypic Alterations

A detailed survey of karyotypic alterations as evolutionary factors in this group has already been presented elsewhere (Khoshoo, 1960, 1961). Only the important conclusions will be presented here.

Except for Cycadaceae ($x = 8$, 9, 11, 13) and Podocarpaceae ($x = 9$–13, 15, 17–19) a single basic number can be recognised in the remaining 11 living families of the gymnosperms: Ginkgoaceae 12, Araucariaceae 13, Pinaceae 12, Taxodiaceae 11, Sciadopityaceae 10, Cupressaceae 11, Cephalotaxaceae 12, Taxaceae 12, Ephedraceae 7, Welwitschiaceae 21 and Gnetaceae 22. If, however, the karyotypic evolution in Podocarpaceae has been unidirectional, then most of the karyotypes in the family can be derived from 10 metacentric, or 20 telo- or sub-telocentric chromosomes, depending upon whether fragmentation or fusion has been the physical basis of evolution. The inference can, therefore, be drawn that Podocarpaceae is also monobasic. The multibasic nature of Cycadaceae is understandable because it is an antique family and has seen a lot of vicissitudes during the course of its evolution. The present-day genera are regarded as the terminal points of a long and a continuous series. The links have perished and it is therefore not possible to interrelate the various numbers.

Usually the familial distinction is based on gain or loss of a chromosome. This is clear by taking the example of conifers. If it is true that the basic number of the Coniferales is 12, then Araucariaceae ($x = 13$) arose by the addition of a chromosome, Taxodiaceae and Cupressaceae ($x = 11$) arose by the loss of a chromosome, and Sciadopityaceae ($x = 10$) also arose by further loss of a chromosome.

The genera within a family (except for Cycadaceae and Podocarpaceae) have the same chromosome number and are at a homoploid level. The notable exceptions within Pinaceae ($x = 12$) are *Pseudotsuga*

($x = 13$) and *Pseudolarix* ($x = 22$), and in Taxaceae ($x = 12$) are *Torreya* and *Amentotaxus* (in both, $x = 11$). Out of these, the high number in *Pseudolarix* can be relegated to 12 metacentrics because the composition of its karyotype is 2 metacentrics + 20 telo- or subtelocentrics. If we view karyotypic evolution within Podocarpaceae in terms of the number of chromosomal arms (Robertson's law), then most of the genera can also be relegated to the same basic number.

Basikaryotype is generally constant within genera, except in *Podocarpus* and *Dacrydium*. In these genera basikaryotype is constant within some sections, but if we apply the revised interpretation, then the basikaryotype is constant in the entire genera.

Groups of genera within a family resemble in karyotype and often on morphological and also genetical grounds these genera are very close and form compact taxonomical alliances. Examples are: *Ceratozamia-Zamia* (Cycadaceae), *Picea-Tsuga* (Pinaceae) and *Cupressus-Chamaecyparis* (Cupressaceae).

Species within a genus differ in absolute chromosome length and/or number, nature and location of secondary constrictions and satellites. Some interspecific and probable intraspecific hybrids show that the species are differentiated by segmental interchanges and cryptic structural hybridity.

Intraspecific differences, though nearly of the same nature as the interspecific differences, are relatively of a minor character. However, both inter- and the intraspecific differentiations show that the rigidity in similarity of karyotypes, ascribed to the gymnosperms earlier, is not present in the absolute sense.

Decrease in chromosome number and absolute chromosome size in conifers appears to be correlated with the phylogenetic advancement. This is clear from the comparison of Pinaceae with Taxodiaceae and Cupressaceae. The former has 12 long chromosomes, while the latter two families have 11 smaller chromosomes. Furthermore they are phylogenetically more advanced than the former.

Increase in karyotypic asymmetry is often characteristic of genera that show specialization in morphology in their respective evolutionary series. This is true to a varying extent in the case of *Cycas*, *Microcycas*, *Ginkgo*, *Pseudolarix*, *Welwitschia*, etc. In the last genus probably all the chromosomes appear to be telocentric (Khoshoo and Ahuja, 1962) which fact goes very well with its specialized morphology.

The various factors responsible for the karyotypic evolution are: unequal translocation, segmental interchanges, peri- and paracentric inversion and cryptic structural hybridity. The karyotypic repatterning exercises its genetic effects because it leads to reshuffling in the linkage groups. Out of the new gene sequences, those with an adaptive value are preserved and further moulded. It is in this manner that the karyotypic repatterning exercises its evolutionary effects.

Gene Mutations and Hybridization

In the ultimate analysis all changes revolve round changes in gene arrangement and structure. The karyotypic alterations are one way by which new gene arrangements can be brought about. The point becomes clear from the fact that Taxodiaceae ($x = 11$) has not evolved from Pinaceous ancestors ($x = 12$) merely by the loss of a chromosome, but that the differences are more fundamental in character and extend to their very genes. This is how the role of gene mutations comes in. There are, however, no data to show that gene mutations contribute to evolution as soon as they arise, but initially they add to the "gene pool". Then the new mutations are recombined in various ways with the already existing variation to produce new adaptive systems on which natural selection acts. For this reason the role of hybridization also comes in, and the two processes (gene mutations and hybridization) are not mutually exclusive.

Cross-pollination coupled with the lack of double fertilization in gymnosperms increase the chances for hybridization. Recent work on conifers, in particular on *Pinus* (Duffield, 1952), shows that within a genus, hybridization is very easy among members of a series and increasingly difficult between members of two series or sections. It is almost impossible between two subgenera.

Cytological studies on hybrids have shown that meiosis is nearly *regular resulting in* 50-60% *normal pollen and the seed fertility is even* higher (*cf.* Sax, 1960, etc.). The hybrids are usually vigorous. This renders the task of both breeder and nature very easy. From such hybrids new adaptive systems can arise by recombination. Such systems in gymnosperms, particularly in conifers, can get stabilized principally through two ways. These are: selection from amongst the segregating progeny and introgression.

There are now enough data for angiosperms to support the fact that new stabilized taxa can arise out of the segregating progeny from interspecific hybrids in response to particular selective factors. In gymnosperms, we have a fine example in the genus *Abies* (Mattfeld, 1930; Stebbins, 1950). *Abies borisii-regis* has arisen as a result of ancient hybridization between *A. alba* and *A. cephalonica*. The former grows in northern and western part of the Balkan Peninsula and extends southward to northern boundary of Greece. *Abies cephalonica* grows in the mountains of Central and Southern Greece. Both these species are present in the purest form in these regions. However, the intermediate populations constituted by *A. borisii-regis* are found in North-Eastern Greece and parts of Bulgaria. A logical explanation supported by the fossil evidence put forward by Mattfeld is that *A. borisii-regis* arose sometimes in Pleistocene when *A. alba* was pushed southward and came in contact with *A. cephalonica*. The two hybridized and the stabilized hybrid segregates have persisted since then.

The second way in which the products of hybridization can be stabilized is introgression. An excellent example of allopatric intro-

gression is available in the genus *Juniperus* (Hall, 1952). In this case pure and typical *J. virginiana* occupies the central core (Kentucky and Tennessee) of its distributional range. This region has remained stable both geologically and floristically. On its periphery, the species has come in contact with 4 different species namely, *J. barbadense* in south-east, *J. ashei* in south-west, *J. scopulorum* in north-west and *J. horizontalis* in north. Furthermore, *J. virginiana* has given rise to four different races or subspecies at the points of contact, which have been shown by Hall to be the result of introgression of *J. virginiana* into four species surronnding it. Introgression has also been reported among the species of *Pinus*.

Due to introgression, a species becomes polytypic as has happened in *J. virginiana*. The taxonomic work on conifers is replete with such polytypic species. Furthermore, there are several cases in which natural species hybrids have been recognised by taxonomists as good species. It is, therefore, reasonably clear that hybridization has played an important role in the evolution of the gymnosperms.

Polyploidy

The role of polyploidy in evolution of gymnosperms has been evaluated by Khoshoo (1958, 1959). Only the most pertinent points will be brought out here. Polyploidy has been encountered in isolated seedlings and trees in otherwise strictly diploid species. There are very few species and genera which are of polyploid constitution.

Polyploid seedlings and trees are always found in nurseries, farms and estates. All these habitats are characterized by the fact that there is no competition and in fact of late breeders have been focusing their attention on these aberrants. We have no such cases reported in the wild populations.

Regarding the second category of polyploids, we have 12 cases (*Larix gmelinii*, *Sequoia sempervirens*, *Juniperus chinensis-pfitzeriana*, *J. squamata-meyeri*, and eight species of *Ephedra*). It is evident that polyploids are so far totally unknown in Cycadales, Ginkgoales, Welwitschiales and Gnetales. In Coniferales about 1·8% and in Ephedrales 44·4% of the species are polyploid. On the whole, gymnosperms have 4·5% polyploids. These figures are very low in comparison to angiosperms and pteridophytes. The reasons for the lack of polyploidy have been discussed earlier by Khoshoo, and here only principal facts will be brought out.

There are reasons to believe that the ratio between the nuclear size and the cell-size has reached an "upper limit" (Darlington, 1937) in gymnosperms. With polyploidy the number of chromosomes multiplies but cell-size does not increase proportionately. Therefore, change in the ratio is detrimental to growth processes which cannot proceed normally in polyploids.

A proof for this contention is available. All the seedlings and trees of natural and artificial polyploid origin in gymnosperms are short,

stumpy and very slow growing. It is unlikely that seedlings and trees with such characteristics can be a success in nature, where for any niche opened for colonization there are hundreds of competitors. As such, only fast growing individuals would be at a selective advantage.

The other reason advanced by Khoshoo (1958, 1959) was that in gymnosperms there is evidence of an ecospecific differentiation between the compatible taxa. As such polyploids resulting from the hybrids involving even morphologically distinct species will be largely auto- or segmental alloploid in constitution. That such polyploids have been unable to diverge into perfect alloploids is clear from the fact that even though polyploid seedlings constantly arise in nature, yet polyploidy is rare in this group. Polyploids with such cytogenetical properties will have very small chance of survival, particularly because of their being poor competitors and lack of vegetative reproduction and agamospermy. It is well known that the latter features are very useful in averting the bottle neck of the initial sterility.

Apomixis

Following the classification of Gustafsson (1946), apomixis is distinguished into vegetative reproduction and agamospermy. Strictly speaking, vegetative reproduction is known only in some cycads and in *Ephedra*. Regarding agamospermy there are doubtful reports in two species namely, *Pinus pinaster* (Saxton, 1909) and *Pseudotsuga menziesii* (Allen, 1942; Orr-Ewing, 1957). It has been suspected to occur only in unpollinated cones. The probable mechanism for agamospermy, as believed by these workers, is either adventive embryony or, what appears to be more plausible, the fertilization of the egg nucleus by the ventral canal nucleus. In literature there are several suggestions for the latter process. The gymnosperms are well worked out embryologically, and it is strikingly clear that agamospermy has not been found so far as a normal feature in any species. This is an interesting point and needs some explanation which will be put forward elsewhere.

Conclusion

The foregoing short survey reveals that in gymnosperms much of the cytogenetical evolution has taken place through gene mutations, hybridization and karyotypic changes. Polyploidy and apomixis have played negligible or no role at all.

A more detailed discussion of the cytogenetical evolution in gymnosperms will shortly appear elsewhere.

References

* Allen, G. S. 1942. Parthenocarpy, parthenogenesis and self-sterility of Douglas-fir. *J. For.* **40**: 642-44.

Darlington, C. D. 1937. *Recent Advances in Cytology*. London.

DUFFIELD, J. W. 1952. Relationships and species hybridization in genus *Pinus*. *Z. Forstgenetic* v. *Forstpflanzenzüchtung*. **1**: 93–97.

GUSTAFSSON, A. 1946. Apomixis in higher plants. I. The mechanism of apomixis. *Lunds Arssker. N.F. Avd.* II, **42**: 1–67.

HALL, M. T. 1952. Variation and hybridization in *Juniperus*. *Ann. Mo. bot. Gdn.* **39**: 1–64.

KHOSHOO, T. N. 1958. Cytological investigations on some conifers and natural polyploids in some angisoperms. Doctoral Thesis, Panjab Univ., Chandigarh.

———. 1959. Polyploidy in gymnosperms. *Evolution* **13**: 24–39.

———. 1960. Cytogenetical evolution in the gymnosperms—karyotype. *Proc. Summer School Bot. India* (Darjeeling), 119–35.

———. 1961. Chromosome numbers in gymnosperms. *Silvae Genetica* **10**: 1–9.

—— AND AHUJA, M. R. 1962. The karyotype in *Welwitschia mirabilis*. *Nature, Lond.* (In press).

* MATTFELD, J. 1930. Über hybridogene Sippen der Tannen. *Biblicgr. bot.*, Stuttgart **25**: 1–84.

ORR-EWING, A. L. 1957. Possible occurrence of viable unfertilized seeds in Douglas-fir. *For. Sci.* **3**: 243–48.

SAX, K. 1960. Meiosis in interspecific Pine hybrids. *For. Sci.* **6**: 135–38.

SAXTON, W. T. 1909. Parthenogenesis in *Pinus pinaster*. *Bot. Gaz.* **47**: 406–09.

STEBBINS, G. L. 1950. *Variation and Evolution in Plants*. New York.

* Not consulted in original.

XYLOTOMY OF THE GYMNOSPERMS

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In his classification of the gymnosperms Arnold (1948) has discarded the traditional taxon Gymnospermae and instead, clubbed the various orders of gymnosperms into three divisions of equal rank, *viz.*, Cycadophyta—including the pteridosperms, Cycadeoidales and Cycadales; Coniferophyta—including the Cordaitales, Coniferales and Ginkgoales; and Chlamydospermophyta represented by Gnetales. Johansen (1951) and Andrews (1961) while agreeing in principle with Arnold, have replaced the taxon Gymnospermae by an even greater number of phyla or divisions, *viz.*, the Pteridospermophyta, Cycadophyta, Ginkgophyta, Coniferophyta and the Ephedrophyta (Gnetophyta). For xylotomical purposes it would perhaps be more convenient to retain Ginkgoales under Coniferophyta and then deal with four major groups of equal rank, *viz.*, Pteridospermophyta, Cycadophyta, Coniferophyta and Chlamydospermophyta.

It is proposed to study the structural specialization of xylem of the above four groups, one after the other. Because of the lack of truly comprehensive data regarding the structural variability of the wood structure and also due to instances of convergent or parallel evolution, one should be rather cautious in utilizing the xylotomical characters in the discussions pertaining to the classification and phylogeny of the diverse gymnosperms.

Pteridospermophyta.—A very uniform feature of the stems of these primitive gymnosperms is that the volume of the wood produced is quite meagre because of the sluggish activity of the vascular cambium. It is obvious that in these archaic plants the woody tissue is inconspicuous and concerned mainly with the function of conduction. In these plants the cambial activity thus seems to have been something of a 'new improvement' (Andrews, 1961).

The primary wood of these plants is mesarch and in well-preserved specimens shows scalariform, bordered and transitional type of pitting. The secondary wood is manoxylic and does not show any seasonal growth. Xylem parenchyma is absent as a rule. The only elements of the wood then, are, the vertical tracheids and the horizontal xylem rays.

The tracheids are quite large (72 μ in *Lyginopteris oldhamia*, 128 μ in *Medullosa* sp.) and probably the largest in gymnosperms (Andrews, 1940). They are further unique in being extremely long (6–24 mm.) (Bailey and Tupper, 1918). Both the radial and the tangential facets

of the tracheids are often profusely pitted. It is interesting to note that the tangential pitting is commonly present in such primitive forms as *Tetrastichia* and species of *Heterangium* and sporadic in the highly advanced types like *Medullosa* and *Sutcliffia*. Jeffrey (1917) was of the opinion that the tangential pitting in the gymnospermous woods developed only in the Mesozoic era and that there was a definite correlation between its origin and the appearance of growth rings. However, the occurrence of distinct tangential pitting in the Palaeozoic pteridosperms, which did not possess any seasonal growth, indicates that this type of pitting was not new to the Palaeozoic gymnosperms and that its origin was not necessitated by the appearance of the growth rings.

The highly characteristic radial pitting is of an unspecialized type. The pits are small, numerous, multiseriate and crowded, covering the entire radial facets of the tracheids. They are angular, variable in size and shape and irregularly seriated in contradistinction to the regularly aligned vertical rows in the cordaitean and other woods. Palingenetically, this reticulate-bordered pitting is considered to be the primitive type for the metaxylem and the secondary tracheids of the pteridosperms. No scalariform pitting has been observed in the secondary xylem of this group. However, since we do not know the xylotomy of all the diverse pteridosperms discovered so far, the possibility of finding such a type of pitting in the future xylotomical studies of these plants cannot be ruled out.

The xylem rays constitute the most important elements of the pteridosperm woods. In general, the rays are very high (well over 1·5 cm.) and broad (4 to 12 or more seriate) and heterogeneous (Andrews, 1940). As seen tangentially the sides are nearly parallel. Low uniseriate rays are very rare. The multiseriate rays often anastomose with the tracheides in a zig-zag manner to form large systems of composite or aggregate rays. The ray cells are thin-walled, smooth, unpitted and angular.

When all the significant features of xylotomy of these plants are taken into consideration, it would appear that it is of a unique and very unspecialized type among the gymnosperms. This is, as it should be, in conformity with the extremely low and unspecialized organization (both exomorphic and endomorphic) of the vegetative and reproductive organs of this group of plants.

The wood structure of the pteridosperms differs very much from that of Cordaitales, their palaeozoic contemporaries. One may safely conclude that xylotomically they show little or no relationship with the latter.

Cycadophyta.--The stems of these plants possess a large pith, wide cortex and a meagre amount of xylem. Except in the species of *Bucklandia* and *Sahnioxylon* (Bose, 1953; Bose and Sah, 1954) seasonal growth is usually absent.

The primary wood of the stems is always endarch and the transitional zone between the primary and early secondary tracheids is quite

broad (Bierhost, 1960). The primary wood shows scalariform pitting in the transitional zone. Subsequently the wood comes to have numerous, multiseriate, circular bordered pits in vertical rows.

The secondary wood is manoxylic but in *Bucklandia* and *Sahnioxylon* it is dense and pycnoxylic. The tracheidal elements, although longer than those of the conifers and Gnetales, are shorter and narrower than those of the pteridosperms. The radial pits in most of the extant Cycadales are large, multiseriate, circular and alternate or opposite. In the Mesozoic Cycadeoidales, however, the tracheids are mostly scalariformly pitted. It may be of some significance to note that *Sahnioxylon*, a recently created genus of cycadeoidean woods (Bose and Sah, 1954), shows both scalariform and circular, multiseriate radial pits.

The xylem rays are either small and extremely narrow (1–2 seriate) as in most of the extinct cycadeoids, or fairly long and broad (3–6 seriate) as in many extant Cycadales (Greguss, 1955). The rays are parenchymatous, homogeneous with rounded to oval cells, the horizontal and tangential walls of which are thin, smooth and unpitted. According to Greguss (1955) this condition represents the lowest step in the evolutionary ladder of the xylem ray development. At this juncture, it may be mentioned that in pteridosperms too, the rays are primitive *i.e.*, they are thin-walled, smooth and unpitted.

Some of the Cycadophyta, *viz.*, the Cycadales, show a fair amount of xylem parenchyma. This tissue, however, does not represent a conspicuous element of the secondary xylem of the Cycadeoidales.

The xylem of Cycadophyta shows many similarities with that of pteridosperms, but can be distinguished from the latter in some important details of its tracheidal elements (both primary and secondary) and xylem rays. With Coniferophyta and Chlamydospermophyta, however, there seems to be little or no xylotomical relationship, barring a few exceptions.

Coniferophyta.—The Coniferophyta including the palaeozoic Cordaitales, Lebachias (Voltziales), Coniferales and Ginkgoales possess a very characteristic type of wood structure. The indefinite cambial activity in Coniferophyta seems to have been a character that was acquired and established early in the evolution of this group.

With the exception of a few Cordaitales, the primary xylem is endarch. The transitional zone between the helical elements of the primary wood and the first pitted elements of the secondary wood is fairly broad in the Cordaitales and Ginkgoales (Bailey, 1925; Bierhost, 1960). Because of the complete elimination of the scalariform type of pitting and the early appearance of the circular bordered pits in the developmental sequence, the primary wood of Coniferales and Ginkgoales is usually considered to be of a highly specialized type (Bailey, 1925). In the Palaeozoic Cordaitales, however, scalariform pitting has been reported to be present in the primary wood (Seward, 1917).

It is generally contended that the circular bordered pits of the metaxylem and of the secondary tracheids of the Coniferales and Ginkgoales originated phylogenetically from the scalariform pits by the 'breaking up' process. Bierhost (1960), however, suggests that the circular bordered pits in the conifers and *Ginkgo* developed independently of the scalariform bordered pits.

In Coniferophyta the tracheids are long in Cordaitales and short in Coniferales (Bailey and Tupper, 1918). No scalariform pitting is present in the secondary xylem. The radial pitting, although exhibiting a good deal of variation, may be broadly grouped into two more or less distinct types, the cordaite-araucarian and the abietinean. The former is characteristic of the palaeozoic Cordaitales, Lebachias and the modern Araucariaceae (Seward, 1919; Florin, 1951; Greguss, 1955). The latter, though very well represented in Pinaceae, is also met with in various other families of Coniferales and in *Ginkgo*.

Among the myriad types of pitting in gymnosperms, Boureau (1949) has distinguished three principal stages in their evolutionary development, *viz.*, (1) the primitive multiseriate type of Palaeozoic era (cordaitean pitting), (2) the uniseriate type appearing early in the Mesozoic era, and (3) a lately developed, over-evolved multiseriate pitting now represented in the Araucariaceae. The relative evolutionary specialization of the araucarian and abietinean types of pitting and their bearing on the phylogeny of Coniferales has been and is still a very much debated problem (Grambast, 1960). Jeffrey (1917) and his associates regarded the abietinean type of wood (hence Abietineae) as the most ancient and primitive type and the araucarian type of wood as representing the highly advanced modern type derived from the former. Gothan (1905), Thomson (1913), and more recently Greguss (1955), on the contrary, considered the araucarian wood to be the most primitive among the Coniferales, as it is closely related to the Palaeozoic cordaitean and lebachian woods. Among the fossil conifers, the araucarian type of wood is the older one (Kräusel, 1932). We may as well extend this to the entire gamut of the Coniferophyta in view of the available palaeobotanical evidence (*e.g.*, the Devonian *Pitys* and its allies). The cordaite-araucarian type of wood structure goes back to the Devonian, while the abietinean type of wood did not become widespread until the Triassic period.

Xylem parenchyma is represented in some of the Coniferophyta, *e.g.*, Cupressaceae, Taxodiaceae, Podocarpaceae, Pinaceae, etc., as either diffuse resinous cells or groups of epithelial cells surrounding the resin canals (Greguss, 1955; Kräusel, 1949; Sahni, 1931; Ramanujam, 1953, 1954). Xylem parenchyma is absent in the Palaeozoic Cordaitales, Voltziales, the modern Araucariaceae, Taxaceae and Ginkgoaceae. The solitary, smooth-walled parenchyma cells probably represent a simpler type than the pitted ones, particularly where the latter have developed into vertical resin ducts. According to Jeffrey (1925) and Takhtajan (1959), wood parenchyma originated during the Jurassic period and was correlated with the appearance of the annual

rings. However, there are many Palaeozoic forms, *viz.*, *Callixylon erianum* (Arnold, 1947), *Megalomyelon myriodesmon* (Cribbs, 1940) and *Dadoxylon* sp. (Arnold, 1947), which do not possess any xylem parenchyma despite exhibiting distinct seasonal growth.

Xylem rays are very small, mostly uniseriate but occasionally 2 or 3 seriate, fusiform and often with resin ducts. The linear rays among the Coniferophyta are believed to be more primitive than the larger ones. The rays, as a rule, are entirely parenchymatous and simple, but in some of the Coniferales like Pinaceae, they are highly complicated, possessing ray parenchyma, ray-tracheids, and resin ducts. When entirely parenchymatous, the ray cells are either thin-walled, smooth and unpitted (primitive) as in Cordaitales, Ginkgoales, Araucariaceae and Podocarpaceae, or more or less thick-walled and variously pitted as in Pinaceae, Taxodiaceae, etc. (Greguss, 1950, 1955).

The ray-tracheids, which were absent in the Palaeozoic Coniferophyta, probably made their appearance for the first time during the Cretaceous period at the expense of the ray parenchyma. Gothan (1905), Bannan (1936) and others considered the occurrence and relative abundance of these elements as a definitely advanced feature.

The cross-field pits among the Coniferophyta are of diverse types, *viz.*, podocarpoid, pinoid, taxodioid, cupressoid, etc. When taken in conjunction with other characters, they provide a fairly reliable criterion for the indentification and classification of the fossil and modern woods of Coniferophyta.

Lastly, resin canals which are absent in the secondary xylem of the Palaeozoic Coniferophyta, seem to have appeared and established during the Mesozoic era. The well-developed system of resin canals in the fossil and modern Pinaceae indicates that they have reached their developmental climax in this family (Bannan, 1936).

Chlamydospermophyta.—The xylem of Gnetales is the most highly speciailzed among the gymnosperms. The primary wood, where the scalariform or transitional type of pitting is completely eliminated, is of a highly modified type. The outstanding feature of the Gnetales is the presence of true vessels. However, here the vessels have originated by the dissolution of pit membranes and tori of the circular bordered pits, in contradistinction to those of the angiosperms which are formed from tracheids with scalariform bordered pits (Thompson, 1918). This is a feature of fundamental sinificance. The vessels of *Gnetum* and *Welwitschia* are more advanced than those of *Ephedra*, where they are longer and narrower, possessing circular bordered pits, crassulae, tertiary spirals and trabeculae (Florin, 1955). The simple perforations of the vessels of *Gnetum* and *Welwitschia*, according to Jeffrey (1917), Thompson (1918) and Bailey (1925) have originated from the foraminate type by the fusion of several smaller pores. Bierhost (1960), on the other hand, suggests that the fusion interpretation cannot be applied to *Welwitschia*, where the simple perforations seem to have originated by a reduction in the number of pores. In

Epherdra, however, the perforations of the vessels are typcially foraminate.

Among the Gnetales, the genus *Ephedra* shows some resemblances with the conifers in its tracheidal pitting and in the possession of distinct bars of sanio. Xylem parenchyma is of the diffuse type and is fairly abundant. The xylem rays of Gnetales are uniformly broad and high. In *Gnetum*, they are homogeneous while in *Ephedra* the rays are composite or aggregate and heterogeneous. The ray cells are thick-walled and pitted.

The xylotomy of *Ephedra*, in its various features, is relatively simpler than that of the other two members. However, when all the significant features are taken into consideration, there can be no doubt that xylotomically *Ephedra*, *Welwitschia* and *Gnetum* constitute a compact and natural group, more closely related to each other than to any other gymnosperm.

It may not be a digression if it is mentioned that the absence of the scalariform pitting in the primary wood and the fundamental difference in the ontogeny of the vessels negates any possibility of deriving the angiosperm wood from that of Gnetales (Bailey, 1953).

A critical chronological survey of the developmental history of gymnosperms indicates in no uncertain a manner that they have evolved quite progressively and rapidly during the Carboniferous, Permian, Triassic and Jurassic periods, while from Cretaceous onwards they have been characterized by relative stability. In this connection it should be pointed out that the great majority of the Upper Cretaceous and Lower Tertiary petrified woods exhibit complete similarity with the woods of diverse members of the families like Araucariaceae, Podocarpaceae, Pinaceae, Taxodiaceae, Cupressaceae, etc. (Kräusel, 1949; Andrews, 1961). One may then safely conclude that very little change has taken place in the structural specialization of the gymnosperm xylem during the last 60–80 million years.

I am thankful to Prof. J. Venkateswarlu, President of the Botany Section, for kindly inviting me to take part in this symposium.

References

ANDREWS, H. N. 1940. On the stelar anatomy of the pteridosperms with particular reference to the secondary wood. *Ann. Mo. bot. Gdn.* **27**: 51–118.

———. 1961. *Studies in Palaeobotany*. New York.

ARNOLD, C. A. 1947. *Introduction to Palaeobotany*. New York.

———. 1948. Classification of the gymnosperms from the viewpoint of palaeobotany. *Bot. Gaz.* **110**: 2–12.

BAILEY, I. W. 1925. Some salient lines of specialization in the tracheary pitting. I. Gymnosperms. *Ann. Bot., Lond.* **39**: 587–98.

———. 1953. Evolution of the tracheary tissue of land plants. *Amer. J. Bot.* **40**: 1–8.

BAILEY. I. W. AND TUPPER, W. W. 1918. Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. *Proc. Amer. Acad. Arts Sci.* **54**: 149–204.

BANNAN, M. W. 1936. Vertical resin ducts in the secondary wood of Abietineae. *New. Phytol.* **35**: 11–46.

BIERHOST, D. W. 1960. Observations of tracheary elements. *Phytomorphology* **10**: 249–304.

BOSE, M. N. 1953. *Bucklandia sahnii* sp. nov. from the Jarassic of Rajmahal hills, Bihar. *The Palaeobotanist* **2**: 41–50.

—— AND SAH, S. C. D. 1954. On *Sahnioxylon rajmahalense*, a new name for *Homoxylon rajmahalense* Sahni, and *S. andrewsi* a new species of *Sahnioxylon* from Amrapara in the Rajmahal hills, Bihar. *Ibid.* **3**: 1–8.

BOUREAU, E. 1949. Sur l' évolution de la ponctuation araucariénae dans les bois vivants et fossiles. *Bull. Soc. bot. Fr.* **96**: 94–97.

CRIBBS, J. E. 1940. Structure of fossil stem of pityean affinity from the Reed springs formation of Missouri. *Bot. Gaz.* **101**: 582–97.

FLORIN, R. 1951. Evolution of Cordaitales and conifers. *Acta Hort. berg.* **15**: 285–388.

———. 1955. The systematics of the gymnosperms. In *A Century of Progress in the Natural Sciences*, 1853–1953, San Francisco, 323–403.

GOTHAN, A. 1905. Zur Anatomie lebender and fossiler gymnospermen Hölzer. *Abh. preuss. geol. Landesanst.* **44**: 1–108.

GRAMBAST, L. 1960. Evolution des structures ligneuses chez les coniferophytes, *Bull. Soc. bot. Fr.* **107**: 30–41.

GREGUSS, P. 1955. *Identification of Living Cymnosperms on the Basis of Xylotomy.* Budapest.

———. 1960. Xylotomisscher Bestimmungsschlüssel der heute lebenden Koniferen-Gattungen. *Acta Biol. Acad. Sci. Hungaricae* **1**: 207–327.

JEFFREY. E. C. 1917. *The Anatomy of Woody Plants.* Chicago.

———. 1925. The origin of parenchyma in geological time. *Proc. nat. Acad. Sci., Wash.* **11**: 106–10.

JOHANSEN, D. A. 1951. A modern classification of the plant kingdom. *Chron. Bot.* **12**: 188–204.

KRÄUSEL, R. 1932. Relation of *Pinoxylon dakotense* Knowlton to *Protopiceoxylon* Gothan. *Bot. Gaz.* **94**: 419–20.

———. 1949. Die fossilen Koniferenhölzer (unter Ausschluss von *Araucarixoylon* Kräus). II. Kritsche Untersuchungen zuur Diagnostik lebender and fossiler Koniferen-Hölzer. *Palaeontographica* **89 B**: 83–203.

RAMANUJAM, C. G. K. 1953. On two new species of *Mesembrioxylon* from the vicinity of Pondicherry, S. India. *The Palaeobotanist* **2**: 101–06.

———. 1954. On some silicified woods from near Pondicherry, South India. *Ibid.* **3**: 40–50.

SAHNI, B. 1931. Revisions of Indian fossil plants. II. Coniferales (b. petrifactions). *Mem. Palaeont. Indica* **11**: 51–124.

SEWARD, A. C. 1917. *Fossil Plants* **3**: Cambridge Univ. Press.

———. 1919. *Ibid.* **4**: Cambridge Univ. Press.

TAKHTAJAN, A. L. 1959. *Essays on the Evolutionary Morphology of Plants.* Washington.

THOMPSON, W. P. 1918. Independent evolution of vessels in Gnetales and angiosperms. *Bot. Gaz.* **65**: 83–90.

THOMSON, R. B. 1913. On the comparative anatomy and affinities of the Araucarineae. *Phil. Trans.* **204 B**: 1–50.

THE PODOCARPACEAE IN INDIA

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IN this Symposium dealing with the "Evolutionary trends in Gymnosperms", I will confine my observations to just one group of Coniferales, namely, the Podocarpaceae and will discuss its stratigraphical sequence in India and the light it throws on the evolution of the family.

According to Dallimore and Jackson (1948), the eight living genera (number of species indicated in brackets) of this group are distributed as follows:

Acmopyle pancheri (1): New Caledonia.

Dacrydium (16): Mostly New Zealand, but also Malay Peninsula, Borneo, Australia, Tasmania, New Caledonia and Chile.

Phyllocladus (5): Tasmania, New Zealand, Philippine Islands and Borneo.

Phaerosphaera (2); Tasmania and New South Wales.

Podocarpus (65): Mountainous forests of warm temperate and subtropical regions of the southern hemisphere; some occur in Japan, China, India, Malay States and Philippines.

Saxegothaea (1): S. America.

Microcachrys (1): Tasmania.

It thus appears that all the genera are confined to the southern hemisphere except the genus *Podocarpus* which is the only one to cross the equator.

The distribution of the fossil Podocarpaceae is also equally interesting in that they too were confined mostly to the southern hemisphere.

Prof. Florin (1940) studying the tertiary fossil conifers of South Chile found that the assemblage of fossil conifers there was distinctly southern and free from northern elements. In this connection he also reviewed in detail the knowledge about conifers known from Mesozoic and Tertiary deposits of the southern hemisphere and noticed that these strata are rich in podocarpaceous remains. He also expressed the opinion that *Podocarpus* was essentially a southern genus.

Dealing with the Indian conifer flora he pointed out that the following species could all be podocarpaceous:

Mesembrioxylon malerianum Sahni, *M. godaverianum* Sahni, *M. parthasarathyi* Sahni, *Retinosporites indica* (Old and Morr.) R. Holden, *Elatocladus tenerrima*, *E. jabalpurensis*, *E. plana*, *E. conferta*, *Cupressinoxylon coromandelinum* Sahni. Other more recently discovered petrified woods referred to the Podocarpaceae are *Mesembrioxylon sahnii* Ramanujam, *M. tiruvaikkarainum* Ramanujam (1953) both from Tiruvaikkerai area; *M. speciosum* Ramanujam (1954) from the Pondicherry area; *M. tirumangalense* Suryanarayana (1953) from Sripermatur and *M. indicum* Bharadwaj (1953) from the Rajmahal hills, Bihar.

The above list includes only the vegetative parts of suspected Podocarps. Prof. Sahni (1931), who too had already suggested a possible podocarpaceous affinity for some of these woods, had even prophesied that "undoubted Podocarpineae may be discovered in the Mesozoic rocks of India". It was, therefore, very gratifying when after a few years there came to light in the Indian Rajmahal flora (Jurassic) a number of reproductive shoots and microspores which could be referred only to the Podocarpaceae (Rao, 1953). A very brief reference to these and some subsequently disocvered, undoubted podocarpaceous remains may not be out of place here; particularly because they are of some importance in tracing the evolution of the Podocarpaceae, and have been found only in India.

Nipaniostrobus sahnii Rao (Rao, 1943) is a petrified megastrobilus bearing single-seeded scales arranged in a lax spiral on a slender axis. The slightly dorsiventral ovule was partly buried inside the scale. It had a two to three layered integument and a ventrally curved micropyle. The ovules were probably erect at first and later became inverted along with the scale. The cone in all its features offers comparison with the genus *Dacrydium*.

Nipanioruha granthia Rao (Rao, 1947), a pycnoxylic petrified shoot with intercalary thickenings and spirally disposed, needle-like decurrent leaves, bore cones exactly like *Nipaniostrobus sahnii* and appears to be again podocarpaceous. The cones in this case are more compact.

Microspores with three inflated wings grouped on the ventral side occur scattered in the silicified blocks from Nipania, and have been designated *Podosporites tripakshi* (Rao, 1943 *a*). These could very well be compared with the microspores of *Microcachrys*, *Phaerosphaera* or *Podocarpus dacrydioides*. Two winged microspores found scattered in the matrix and known as *Pityosporites nipanica* Rao, *Pityosporites* sp. may also be podocarpaceous. *Masculostrobus rajmahalensis* Rao (Rao, 1943 *a*) is a part of a male cone bearing microsporophylls inside one of which were found three winged microspores This cone, according to Mittre (1956), may be the microstrobilus of *Nipanioruha granthia* which is regarded as podocarpaceous on the basis of the characters of its foliage and cone. *M. sahnii* Mittre (Mittre, 1956), a part of another male cone may also turn out to be podocarpaceous. *M. podocarpoides* Mittre (Mittre, 1957) is yet another microstrobilus referred to Podocarpaceae.

A solitary specimen of *Stachytotaxus* with Elatocladus conferta type of leaves found in the Rajmahal Hills has also been referred to Podocarpaceae by Rao (1950). *Strobilites sewardi*, whose foliage was probably *Elatocladus jabalpurensis*, was also regarded by Prof. Sahni as possibly podocarpaceous.

Mittre (1957) added some more structural details to our knowledge of the above conifers and described several new finds from the Rajmahal hills in Bihar. *Nipaniostrobus pagiophylloides* Mittre resembles *N. sahni* Rao in general features but is supposed to be borne on shoots of a different type with foliage like that of *Pagiophyllum*. A silicified female cone similar to the above but much smaller in size and borne on shoots with acicular leaves is described under the name *N. aciculifolia* Mittre. *Mehtaia* is the new name given to some pertified cones which differ from *Nipaniostrobus* in having seed scales with erect ovules and in being devoid of sterile parts. *M. rajmahalensis*, *M. nipaniensis* and *M. santalensis* are three new species of cones described under this genus. *Sitholeya rajmahalensis* represents another type of shoot with a single terminal inverted seed like that of modern *Podocarpus*. Mittre (1957) has also been able to add considerably to our knowledge of *Nipanioruha granthia* Rao and has described two sterile shoots *Nipanioruha lanceolata* and *Nipanioruha curvifolia*. *Indophyllum sahni*, *I. raoi* and *I. nipanica* are some more sterile shoots described from the Rajmahal hills. The last one has also attached microstrobili whose microsporangia contain two-winged pollen grains comparable to those of the Podocarpaceae. The sterile shoots cannot be satisfactorily compared to definite genera of the Podocarpaceae, but according to Mittre (1957) their reference to this family is beyond doubt.

Nipaniostrobus is very much like *Dacrydium* in structure. *Sitholeya* is closely comparable to *Podocarpus*. *Mehtaia*, on the other hand, is not fully comparable to any podocarpaceous genus on the basis of the available data. But at the same time there is no other family of conifers in which this coniferous genus can be accommodated. Thus, although a generic comparison of *Mehtaia* must await further data, its attribution to the Podocarpaceae is quite evident. The fact that several species of each one of these genera have been found in the Rajmahal flora is significant. The rich podocarpaceous remains in the Indian Jurassic flora are summarized in Table I.

The existence of this rich podocarpaceous flora in the Indian Jurassic strata in particular, and to a lesser extent in some of the contemporaneous floras of the other Gondwana components suggests that the vertical range of the Podocarpaceae probably starts from the Rhaetic. Their present and past distribution shows that this group evolved in the southern hemisphere. Florin (1950) has already suggested that "*Dacrydium* presumably developed in the Upper Mesozoic from some centre in the East Australian Antarctic region. *Acmopyle* appears to have spread from an original centre of distribution in the Indo-Australian region to Antarctica and South America. *Phyllocladus* may also be looked upon as a genuine southern genus. The remaining genera,

Saxegothaea, *Pherosphaera*, and *Microcachrys* are hardly known in the fossil state but there is no reason to doubt their southern origin". In view of the *Dacrydium*-like aspect of some of the above-mentioned petrified strobili from Rajmahal, it is not unlikely that *Dacrydium* evolved in the Indo-Australian region.

Buchholz and Gray (1948), however, opine that the genus *Podocarpus* could have originated in "the area from Southern Japan, China, Nepal, Sumatra to Australia including Tasmania and New Zealand and thence northwards to the Fiji islands and the Philippines". They are led to this conclusion because this area includes seven out of the eight sections into which this genus is divided. Judging from fossil evidence also, the region defined by Buchholz and Gray covers the area in which according to Florin, the Podocarpaceae had originated. They, however, also include Southern Japan, China and Nepal in this area.

Prof. Florin, from his extensive knowledge of living and extinct conifers, has suggested that from Permian onwards the "conifers divided into two groups one of which has its roots in the northern hemisphere and the other was a markedly southern group". This southern group could only be the Podocarpaceae, which had spread itself all over Gondwana land. To this day, some of the genera that evolved during the Jurassic period continue to live in their original homes.

As already pointed out (Rao, *1953*), the Podocarpaceae were well represented in peninsular India during the Jurassic, but today this family is represented by only one genus, *Podocarpus* which grows wild only in some parts of peninsular India where it has also been found in a fossil state in the Tertiary period. On the contrary, Australia, a Permo-carboniferous neighbour of India, shows podocarpaceous remains in the present flora as well as in the Jurassic and Tertiary periods. Indeed in the latter period they seem to be quite abundant. We do not fully know the fossil conifer flora of the other components of Gondwana land. Its testimony would indeed be very interesting.

As already stated, in Australia the podocarpaceous remains continue from Triassic onwards (*see* Florin, 1940). The conifer flora of Antarctica, South America, New Zealand, Tasmania, and Australia, also shows some podocarpaceous remains in the Mesozoic although not to the same extent as that of India or Australia. However, there are many living Podocarpaceae in most of these lands. The fact that podocarpaceous genera are distributed in the Mesozoic strata of all these lands shows that this family was widely spread all over Gondwana land where it certainly evolved during the early Mesozoic times. It is definitely a southern conifer family. While the rich podocarpaceous flora continued in the dismembered countries of the Gondwana land till Tertiary and recent times, it seems to have dropped off only from India by the end of the Jurassic period. This is probably because peninsular India was the only mass of land that moved into the northern hemisphere when Gondwana land broke up into its component parts. The other separated units remained within the southern hemisphere,

TABLE I

Name	Locality	Stage	Nature of fossil
Triassic or Lower Jurassic			
Mesembrioxylon malerianum Sahni ..	Rewah	Maleri	Petrified wood
Mesembrioxylon godaverianum Sahni ..	Bogapalmilla, Godaveri area	,,	,,
Lower Jurassic			
Elatocladus conferta ..	Nellore Kavalli Talud	Rajmahal	Shoot
Mesembrioxylon indicum Bharadwaj ..	Rajmahal Hills, Bihar	,,	Petrified wood
Retinosporites indica (Old and Morr.) R. Holden ..	,, and also Golapili	,,	Shoot
Elatocladus tenerrima (Fst.) Sahni ..	Kotah, Jabalpur and Cutch	Kotah, Jabalpur and Umia	,,
Nipaniostrobus sahnii Rao ..	Rajmahal Hills	Rajmahal	Megastrobilus
Nipaniostrobus prgiophylloides Mittre ..	,,	,,	,,
Nipaniostrobus aciculifolia Mittre ..	,,	,,	,,
Nipanioruha granthia Rao ..	,,	,,	Shoot and megastrobilus
Nipanioruha lanceolata Mittre ..	,,	,,	Shoot
Nipanioruha curvifolia Mittre ..	,,	,,	,,
Stachyotaxus sp. ..	,,	,,	Shoot and strobilus
Mehtaia rajmaholensis Mittre ..	,,	,,	Shoot and megastrobilis
Mehtaia nipaniensis Mittre ..	,,	,,	,,
Mehtaia santalensis Mittre ..	,,	,,	,,
Sitholeya rajmahalensis Mittre ..	,,	,,	Fertile shoot (cf. *Podocarpus*)
Indophyllum curvifolia Mittre ..	,,	,,	Shoot

Indophyllum sahnii Mittre ..	"	"	"
Indophyllum raoi Mittre ..	"	"	"
Indophyllum nipanica Mittre ..	"	"	Shoot cf. *Podocarpus*
Elatocladus sahnii Mittre ..	"	"	Shoot
Masculostrobus rajmahlensis Rao ..	"	"	Microstrobilus
Masculostrobus sahnii Mittre ..	"	"	"
Masculostrobus podocarpoides Mittre ..	"	"	"
Podosporites tripakshi Rao ..	"	"	Microspores
Pityosporites sp. ..	"	"	"
Middle Jurassic			
Mesembrioxylon parthosarthyi Sahni ..	Vellum, near Sripermatur	Kotah	Petrified wood
Elatocladus plana (Fst.) Seward ..	Rajmahal and Sripermatur	Kotah and Jabalpur	"
Cupressinoxylon coromandelinum Sahni ..	Near Madras	Kotah	"
Elatocladus jabalpurensis (Fst.) ..	Vemavaram and Jabalpur	Kotah and Jabalpur	Shoot
Athrotaxites feistamanteli Sahni ..	Naogaon, West of Maleri	Maleri	"
Upper Jurassic			
Elatocladus conferta ..	Rajmahal Hills and Chirakunt	Rajmahal, Kotah and Jabalpur	"
E. jabalapurensis (Fst.) ..	Jabalpur, Vemavaram	Kotah and Jabalpur	"
Retinosporites indica ..	Rajmahal Hills, Cutch	Kotah, Jabalpur and Umia	Shoot cf. *Acmopyle*
Tertiary			
Mesembrioxylon schmidianum Schleiden Sahni	Tiruvaikkarai	Exact stage not known	Petrified wood
Mesembrioxylon sahnii Ramanujam ..	"	"	"
M. tiruvaikkarianum Ramanujam ..	"	"	"
M. speciosum Ramanujam ..	Pondicherry area	"	"
M. tirumangalense Suryanarayana ..	Sripermatur, Madras coast	Sripermatur	"

enjoyed their earlier temperate climate and continued to support the indigenous podocarpaceous vegetation. The genus *Podocarpus* which had spread itself far and wide in Gondwana land is probably now in a position to migrate northwards and perhaps that explains the existence of this essentially southern genus in a living state in lands above the equator. The genus *Elatocladus* is represented by rather closely approximating forms in the Mesozoic flora of almost all the Gondwana constituents. It is not unlikely that this genus is the precursor of *Podocarpus*. The latter genus is evidently the most successful amongst the Podocarpaceae not only in the evolution of its different species but also from the point of view of wide distribution, occurring as it does in both the hemispheres.

The living Podocarpaceae show three main types of organization of the female strobilus, (*a*) a compact cone, as in *Dacrydium*, (*b*) a lax strobilus as in *Podocarpus spicata* and (*c*) a much reduced receptacle bearing a single seed, as in *Podocarpus blumei*. The petrified Rajmahal strobili referred to above suggest that the compact cone and the lax strobilus were more in evidence during the Jurassic period. We have, however, some evidence in the genus *Sitholeya* that a receptacle bearing a single seed was also present in the Indian Jurassic conifer flora.

It thus appears that *Podocarpus* is the only surviving member of the indigenous conifer vegetation that India supported during the Mesozoic period. The various genera of conifers like *Pinus*, *Cedrus*, *Abies*, *Taxus*, *Cephalotaxus*, *Cryptomeria*, etc., that now inhabit the Himalayan regions are all northern genera that perhaps migrated southwards when the Tethys Sea floor was elevated into the Himalayan ranges in the Pleistocene epoch, thus providing a hospitable climate for the growth of these conifers.

REFERENCES

BHARADWAJ, D. C. 1953. Jurassic woods from the Rajmahal Hills, Bihar. *The Palaeobotanist* **2**: 59–70.

BUCHHOLZ, T. AND GRAY, N. E. 1948. A taxonomic revision of *Podocarpus* 1. The sections of the genus and their subdivisions with special reference to leaf anatomy. *J. Arnold Arbor.* **29**: 49–63.

DALLIMORE, W. AND JACKSON, A. B. 1948. *A Handbook of Coniferae*. London.

FLORIN, R. 1940. The Tertiary fossil conifers of South Chile and their phytogeographical significance, with a review of the fossil conifers of Southern Lands. *K.svenska Vetensk Akad. Handl.* **19**: 1–104.

———. 1950. On two conifers from the Jurassic of South Eastern Australia. *The Palaeobotanist* (The Birbal Sahni Commemoration Voloume): 177–182.

MITTRE, V. 1956. *Masculostrobus sahnii* sp. nov., a petrified conifer male cone producing three-winged and one and four-winged, abnormal pollen grains from the Jurassic of Bihar. *Grana Palynol.* **1**: 99–107.

———. 1957. Studies on the fossil flora of Nipania (Rajmahal Series), Bihar. Coniferales. *The Palaeobotanist* **6**: 82–112.

RAMANUJAM, C. G. K. 1953. On two new species of *Mesembrioxylon* from the vicinity of Pondicherry, South India. *Ibid.* **2**: 101–06.

———. 1954. On some silicified woods from near Pondicherry, South. India. *Ibid.* **3**: 40–50

RAO, A. R. 1943. *Nipaniostrobus*, a new genus of *Dacrydium*-like seed-bearing cones and other silicified plants from the Rajmahal series. *Proc. nat. Acad. Sci., India* **13**: **1** 113–133.

———. 1943 *a*. Jurassic spores and sporangia from the Rajmahal Hills, Bihar. *Ibid.* **13**: 181–197.

———. 1946. *Nipanioruha granthia* gen *et* sp. nov.—a new petrified coniferous shoot from the Rajmahal Hills. Bihar, *J. Indian bot. Soc.* (M.O.P. Iyengar Commemoration Volume): 389–397.

———. 1949. The megastrobilus of *Nipanioruha granthia* Rao. *J. Indian bot. Soc.* **18**: 447–448.

———. 1950. Two hitherto unreported plant fossils from the Rajmahal Hills, Bihar. *Ibid.* **19**: 378–380.

———. 1953. Some observations on the Rajmahal flora. *The Palaeobotanist* **2**: 25–28.

SAHNI, B. 1931. Revisions of Indian fossil plants. II. Coniferales (b. Petrifactions). *Palaeont. indica*, N.S. **11**: 51–124.

SURYANARAYANA, K. 1953. *Mesembrioxylon tirumangalense*, a new species from the Sripermatur group near Madras. *J. Indian bot. Soc.* **32**: 159–164.

SYMPOSIUM II

PARASITISM AND SYMBIOSIS

PARASITISM AND SYMBIOSIS

By T. S. Sadasivan

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I have pleasure in opening this symposium with an expression of thanks to Professor J. Venkateswarlu, President of the Botany Section of this 49th Indian Science Congress at Cuttack, for inviting me to take part in it.

I propose dwelling here on some aspects of the vast question of fungal parasitism in plants, a subject that has attracted my attention for more than twenty years. I shall also mention in a passing way about the phenomenon of symbiosis. The term parasitism in a mundane sense means sycophancy; a servile complaisance. In biology, however, it means in a strict, or restricted sense, the condition of being a parasite, and in pathology, it signifies disease caused by a parasite. It is also variously defined as antagonistic symbiosis or a close internal and external partnership between two organisms, detrimental to the host and beneficial to the parasite—the parasite obtaining its nourishment at the expense of the host. It follows that any agent capable of interfering with the life processes of a plant is a pathogen regardless of whether it invades one or more plant tissues. Thus *Cassytha*, *Dendrophthoe* or *Santalum* which have numerous hosts on which they grow are to be considered to parasitize them albeit ability to synthesize their own food-stuffs remains unimpaired. This is so because they do create a diseased condition on their host and consequently interfere with normal synthesis of the host by deranging vascular movement of minerals and the phloem translocatory stream, and perhaps, also inject metabolic inhibitors into the host tissues. Other speakers in this symposium would, doubtless, draw our attention to many types of parasitic as well as commensal associations as in the case of the mycorrhizal fungus which is known to be parasitic on the roots of trees, in some instances, and in others, to be beneficial by increasing root absorption and uptake of minerals from soils (Burges, 1936; Harley, 1952; Harley and Brierley, 1955). There would also be mention during this session of bacterial symbiosis as in the case of root nodules and a contrasting account on plant-virus relationships. Despite this, my task is not any lighter, as fungal parasitism of the obligate type and symbiosis as a general phenomenon are sufficiently complex to warrant cautious interpretation.

Generally speaking, the more vigorous and destructive a parasite, the less the chances of its survival. As a matter of fact, the more tolerant the host is to a parasite, the more chronic the disease, and the greater the chances of survival of the parasite. Taking the case of the

rust, let us examine host-parasite balance in pathogenesis. If there is a loss in virulence in the pathogen, there would be greater survival of susceptible hosts and, therefore, the host-parasite balance would be at a lower level of parasitism. The converse is also true. However, resistance and virulence constantly get readjusted and reach invariably a balanced state and this might be termed in nature, 'the law of survival in pathogenesis' (McNew, 1960). In the United States, breeding of wheat for resistance to *Puccinia graminis* is a classical example of matching man's ingenuity with that of nature and, indeed, the germ plasms of *Puccinia* and *Triticum* and their interactions have been excellently studied and understood. Taking breeding for resistance programmes for the past fifty years, there has been a quick and dramatic succession of breeding of resistant varieties, to stem emergent situations arising out of the breaking of resistance periodically with the appearance of new races of rust. The stem rust susceptible varieties, Bluestem and Fife, were replaced by the rust escaping Marquis in 1910 only to be soon followed by another replacement with the Durum wheats in 1916 after a severe rust epidemic. Rust race 11 subsequently made the growing of Durum varieties impossible and soon another replacement followed by introducing variety Ceres which continued to show immunity to the races of black stem rust from 1926 to 1934. In 1935, race 56 became a menace to variety Ceres and, indeed, it was a tragic year for wheat growing in the U.S.A. The onslaught of race 56 was halted by replacing Ceres with Thatcher and later both varieties Thatcher and Hope and their hybrids with Durum parentage held fort. However, rust race 15 B upset the balance in favour of the pathogen in 1939 and in course of time produced a biotype 15 B2 which broke Durum resistance with devastating ease. Since 1953 the U.S. plant breeders started looking for resistant parents from elsewhere, particularly from Kenya varieties of wheat. It has become evident that as soon as a new set of resistant genes was incorporated in the breeding programme, new virulence was seen to emerge from new races of parasites that are in constant process of evolution progressively from non-parasitic through facultative parasitism to obligate parasitism.

It seems unfortunate that plants do not seem to have the mechanism for antibody formation so common in higher animals and, therefore, our sole dependence is on breeding for resistance and adopting cultural methods that would minimize chances of infection. However, with the discovery of a defense reaction in orchid bulbs by the production of a substance called orchinol, which is present in large quantity in bulbs as compared with roots consequent on infection by micro-organisms (many fungi and bacteria have been tested), a new chapter in immunology appears to have been opened (Gäumann and Kern, 1959 *a, b*; Gäumann and Hohl, 1960). Not only are bulbs of *Orchis militaris* capable of producing orchinol in response to infection but stems and roots also are capable of synthesizing this substance. The activity spectrum of orchinol is relatively wide and almost aspecific. The first incitant of orchinol following primary infection protects tissues against homologous reinfections and further produces a polyvalent protection against secondary parasites and saprophytes. This

is regarded by these authors as a genuine induced antibody formation *in vivo*. This is, indeed, a milestone in antibody production in plants.

A disease outbreak depends on the inherent susceptibility of a host, the inoculum potential of the parasitic organism, and the effect of environment on parasitism leading to pathogenesis. It should be understood, however, that pathogenesis is only the result of a parasitic establishment in most diseases. As far as evidences go, the direct effects of parasitism is not so severe and it is the subsequent formation of abnormal metabolites or toxins *in vivo* consequent on infection that aggravate the situation. Once infection is established, the parasite generally enters into a specific intercellular relationship with the host cell. Ultimately parasitism develops, in some cases like rusts, from an intercellular invasion (aided by chemical degradation of the wall) into a haustorial relationship or as in the case of Erysiphaceae, from an external habit with haustoria developed in the host cell.

Generally, there is little sign of damage in tissues colonized by obligate parasites for some time, although the cells may be penetrated by haustoria and heavy intercellular growth of hyphae is obvious. It has been suggested that the metabolism of host tissue in the proximity of lesion development is stimulated by metabolites produced by the invading pathogen and/or the host cell as evidenced by accumulation of C^{14} compounds in cereal leaves with lesions by *Puccinia graminis* and *Erysiphe graminis* (Shaw and Samborski, 1956). If we assume that no stimulation and accumulation of nutrients by the pathogen is operative, then pathogen would grow only with energy from the invaded cell. Indeed, there is evidence that increase in metabolic rate of host cells takes place in most cases and this might well be a factor enabling establishment of parasitism. Indeed, the barrier to infection by plants resistant to fungal obligate parasites seems to be susceptibility of the host cell cytoplasm to products of metabolism of the fungal cell living in intimate association, and in extreme cases of obligate parasitism like in *Plasmodiophora brassicae* this association is in the cytoplasm itself (Wood, 1960).

There are many types of barriers in plants that profoundly affect parasitism, such as, absence of essential nutrients, presence of inhibitory materials *in vivo*, physical conditions obtaining in the cell like pH and osmotic pressure, general metabolic condition of the host, and cell-wall barriers. Experiments with mutant strains of bacteria and fungi, deficient for certain amino-acids and other nutrilites, were less virulent compared to the wild types but it must be mentioned that notwithstanding these findings, a pathogen can effectively parasitize only in tissues that contain materials for its growth and proliferation. Among inhibitory substances in host cells, phenolic compounds have been studied in detail and, particularly, increased resistance of potato tuber to infection by *Streptomyces scabies* has been ascribed to the presence of chlorogenic acid. However, there is little clinching evidence on whether these *in vitro* inhibitions in the activity of these organisms could indicate its possible role *in vivo*. Physical conditions like pH

and osmotic pressure *in vivo* have also been considered in this general question of induction of parasitism. While it is true that by inducing a change in pH in the invaded tissue the permeability of the pathogen's cell to toxins would be reduced, it has another side to the question, namely that the pathogen's products of metabolism may react with toxins to form innocuous substances. Osmotic pressure, likewise, is regarded as a factor in the establishment of parasitism but its exact role in pathogenicity is not fully understood. In the obligate fungal parasites degradation of cell-wall barriers does not appear to be important as in the case of the non-obligate type.

With obligate parasites resistance is generally understood to be a hypersensitivity of the cell to the onslaught of the parasite resulting in the death of cells consequent to invasion and, therefore, becoming unsuitable substrates. The exact role of the invading pathogen resulting in the death of the cell still appears controversial, whether this is due to production of fungal toxins or due to rapid depletion of host cell nutritive reserves. The successful micro-organisms, therefore, to be able to parasitize must possess the ability to utilize nutrients of the host cells without secreting toxic substances *in vivo*. How well these attributes to an ideal cell for infection could be proved in genetic susceptible material and equally, how well the causes that lead to resistance in tissues of resistant plants to the parasite together with analytical data on differences in biochemical status is still open for experimentation and, indeed, would be a most rewarding field of enquiry as far as I am aware.

Let us now turn to a general consideration of symbiosis. The best known and often quoted examples are the lichens and nodule bacteria. Much has been said about them in the past and, in fact, we have more than one speaker here who will unravel the many aspects of the role of rhizobia in symbiotic nitrogen fixation and lichen commensalism. I shall, however, attempt to review some of the salient features of another promising line of symbiosis between the soil algae and the higher plant. The algae appear to have in common with most other micro-organisms the property of excreting their cellular products of metabolism (Fogg, 1960). Many workers have reported that *Chlamydomonas* and blue-green algae in cultures leave behind varying amounts of organic matter, mostly composed of nitrogen in a soluble form, also galactose, arabinose (Lewin, 1956) and polypeptide-like substances (Fogg and Boalch, 1958). However, little free amino-nitrogen has been found in cultures of *Calothrix brevissima* although appreciable quantities of aspartic acid, glutamic acid and alanine were liberated (Watanabe, 1951). Many workers have detected liberation of antibiotics by algae especially fatty acids with antibacterial activity. The liberation of extracellular metabolic products has undoubtedly ecological importance as, indeed, apart from their utilization by other soil micro-organisms there is the larger question of their availability to the higher plant. Capacity to fix atmospheric nitrogen by blue-green algae, particularly *Cylindrospermum sphaerica* (Venkataraman *et al.*, 1959), an endophytic *Nostoc* strain isolated from the root nodules

of Trifolium alexandrinum (Venkataraman, 1960) and *Anabaena cylindrica* (Fogg, 1960) has been unequivocally established. There are other cases of such association of blue-green algae, as for instance, in the coralloid roots of *Cycas* or in many orchids and these will be invaluable material for further investigation on this commensal association. The tropical soils and, more particularly, fresh water lakes and paddy field soils which abound in algae could be studied, if for nothing else, at least for supporting fundamental investigations on the role of algae in the rhizosphere of plants.

REFERENCES

BURGES, A. 1936. On the significance of Mycorrhiza. *New Phytol.* **35**: 117–31.

FOGG, G. E. 1960. Recent advances in our knowledge of nitrogen fixation by blue-green algae, In *Proceedings of the Symposium on Algology* (Ed. P. Kachroo). Indian Council of Agricultural Research, New Delhi, pp. 115–18.

———. 1960. The extracellular products of Algae, *Ibid. Symposium on Algology* (Ed. P. Kachroo). pp. 138-43.

—— AND BOALCH, G. T. 1958. Extracellular products in pure cultures of a brown alga. *Nature, Lond.* **181**: 789–90.

GÄUMANN, E. AND HOHL, H. R. 1960. Weitere Untersuchungen über die chemischen Abwehrreaktionen der Orchideen. *Phytopath. Z.* **38**: 93–104.

—— AND KERN, H. 1959 *a*. Über chemische Abwehrreaktionen bei Orchideen. *Ibid.* **36**: 1–26.

———. 1959 *b*. Über die Isolierung und den chemischen Nachweis des Orchinols. *Ibid.* **35**: 347–56.

HARLEY, J. L. 1952. Associations between micro-organisms and higher plants (Mycorrhiza). *Annu. Rev. Microbiol.* **6**: 367–86.

—— AND BRIERLEY, J. K. 1955. The uptake of phosphate by excised mycorrhizal roots of the beech. VII. Active transport of ^{32}P from fungus to host during uptake of phosphate from solution. *New Phytol.* **54**: 296–301.

LEWIN, R. A. 1956. Extracellular polysaccharides of green algae. *Canad. J. Microbiol.* **2**: 665–72.

MCNEW, G. L. 1960. The nature, origin, and evolution of parasitism. In *Plant Pathology* (Eds. J. G. Horsfall and A. E. Dimond). Academic Press, N.Y. **2**: 19–60.

SHAW, M. AND SAMBORSKI, D. J. 1956. The physiology of host-parasite relations I. The accumulation of radioactive substances at infections of facultative and obligte parasites including tobacco mosaic virus. *Canad. J. Bot.* **34**: 389–405.

VENKATARAMAN, G. S. 1960. Nitrogen fixation and production of extracellular nitrogenous substances by an endophytic *Nostoc* strain isolated from the root nodules of Egyptian Clover (*Trifolium alexandricum*). In *Proceedings of the Symposium on Algology* (Ed. P. Kachroo). Indian Council of Agricultural Research, New Delhi, pp. 119–27.

——DUTTA, N. AND NATARAJAN, K. V. 1959. Studies on nitrogen fixation by blue-green algae. I. Nitrogen fixation by *Cylindrosporium sphaerica* Prasad. *J. Indian bot. Soc.* **38**: 114–19.

WATANABE, A. 1951. Production in cultural solution of some amino-acids by atmospheric nitrogen fixing blue-green algae. *Arch. Biochem. Biophys.* **34**: 50–55.

WOOD, R. K. S. 1960. Chemical ability to breach the host barriers. In *Plant Pathology* (Eds. J. G. Horsfall and A. E. Dimond). Academic Press, N.Y., **2**: 233–72.

GENERAL ASPECTS OF PARASITISM

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"WITHDRAWAL into a unique ecological niche, such as the chemolithotrophic nitrifying bacteria do, is one highly effective means of meeting the biological challenge of competition." There is another method that has been adopted by a large number of organisms, particularly microorganisms, to meet this challenge, namely, by adapting to existence in continued close association with some other form of life. This is the biological phenomenon known as *Symbiosis*, in the broadest sense of the term. "Symbiotic relationships can be classified into two groups: (1) *mutualistic*, from which both partners derive ecological advantages; (2) *parasitic*, from which one participant, the parasite, benefits while the second, the host, gains nothing and may even suffer more or less severe disadvantages (*see* Stanier *et al.*, 1958).

Parasitism is, by definition, living at another's expense, and is essentially dualistic and antagonistic. There is always a physiological association between the partners in it. "No doubt, the original basis of parasitism was the nutrition of the parasite, and although this is not the only basis in some instances, it has probably remained the main physiological feature of parasitism" (Lapage, 1956).

A parasite is not necessarily a pathogen. For instance, man is relatively unaffected by the large number of parasitic microorganisms that make up the normal flora of his body. Nevertheless, since nutrition of the parasite is the chief characteristic of parasitism, in many instances the host is damaged, and hence the rather indiscriminate usage of the terms 'parasitism' and 'pathogenicity', though both are not synonymous. The injury suffered by the host may be relatively slight, or may be sufficient to cause what we call disease. This results chiefly from the methods used by the parasite to obtain food and to the reaction of the host's tissues against the parasite, either before or after penetration, or both. However slight the effects of the parasite on the host, they are always there. The injured or diseased condition can take many forms, such as structural modifications, poisoning and necrosis due to toxic substances released by the parasite or the parasite-host complex, and a host of other physiological disturbances leading to deranged metabolism.

Parasitic relationship may be established between one animal and another, one plant and another, a plant and an animal, a bacterium and a plant or animal, a virus and a plant or animal, and finally, between a virus and a bacterium. It can occur in many graded conditions—from the almost free-living fleas, through facultative parasitism, to

purely obligatory association, such as that of *Plasmodium* or viruses to their hosts.

In this special mode of life that is parasitism, the peculiar fact is that there is another living organism which forms a major part of the parasite's environment, and the differences that exist between free-living and parasitic organisms may be regarded as due to adaptations necessary for the conquest of this new environment. In what way this conquest has been made is debatable. The two possibilities are: (1) The direct path from a free-living to a parasitic condition, and (2) the indirect path, through commensalism and symbiosis (in its conventional sense) and thence to one-sided association of parasite and host. The bulk of evidence seems to support the former (*see* also Vines and Rees, 1959).

The major adjustments, from the free-living condition, shown by parasites in general have been summarized as follows (Vines and Rees, 1959):

(1) The parasite has been able to find all its nutritional requirements in the materials of the host cell or tissue, or in the food which the host acquires for itself.

(2) The parasite is able to form a more or less permanent attachment to the host from which it cannot be dislodged easily. In the extreme case of intracellular parasites, a constant physical attachment is achieved, but these run the risk of being exposed to physiological resistance reactions offered by the host.

(3) The parasite has to be able to ensure that succeeding generations can reach new hosts, particularly where there is host specificity.

In achieving these major adjustments, to life in association with another organism, parasites have evolved many variations—structural, physiological and reproductive—from their free-living counterparts. Structurally, associated with their mode of nutrition and attachment, we find the development of highly specialized attachment and absorbing organs, such as the mouth parts of aphids, haustorial and appressorial structures such as the hyphopodia and stomatopodia of the Perisporiaceae, Erysiphaceae and *Cuscuta*, the sinkers of the mistletoe, etc.

Parasites as a whole show a tendency towards degeneracy in many directions; structurally, as we go higher towards the highly specialized type, namely, intracellular parasitism, there is a great reduction in structure, of which the virus nucleoproteins represent perhaps the extreme.

Associated with increased chances of successful dissemination among new hosts, we find production of enormous numbers of disseminative units [fecundity, as called by Gäumann (1950)], high degree of resistance of reproductive bodies, such as thick-walled resting spores, zygotes, sclerotia, etc., outside the host, employment of specialized reproductive phases in the life-cycle, such as polyembryony or parthenogenesis, and, use of alternate or alternative hosts.

Physiologically, associated with penetration, establishment and nutrition, we find the development of extracellular metabolites by which the host tissues are penetrated and digested. Cytolytic and cell-wall dissolving enzymes are known to be produced by a large number of parasites, such as *Cuscuta*, many fungi, bacteria, and even bacterial viruses (*see* Trager, 1960). Pectolytic and cellulolytic enzymes are produced by many plant parasites, particularly of the facultative group. Although cuticular penetration is considered to be mechanical, because as yet no cutin-dissolving enzyme is known to be produced by any fungus (*see* Lilly and Barnett, 1951), the recent evidence for the utilization of the inner layer of the cuticle as a food source by the olive leaf spot fungus (Tenerini and Loprieno, 1960) points to a need for revision of this view.

Associated with the particular nutritional requirements of the parasites, a variety of metabolites are found to be produced by the so-called primitive or unspecialized up to the specialized parasites. Among plant parasites themselves, starting with parasites which obtain nutriment by damaging living but dormant tissues, such as fruits and storage organs, we have a whole range of parasites attacking active but tender tissues, such as seedlings and ground tissues, then more mature and active tissues of the roots, stem and leaves, and finally, the vascular parasites colonizing specifically the vascular elements. All these parasites are destructive, inducing necrosis in host with the help of extracellular metabolites that have an action in advance. The production of these extracellular substances seems to depend to a great extent on the nature of the host tissue attacked. Strong enzyme action alone may be sufficient in order to obtain food from dormant storage organs, or even tender tissues such as has been found in the case of parasites like *Botrytis*, *Pythium*, etc., where presence of a separate toxin responsible for killing of cells has not yet been established (Brown, 1955). In such debility parasites we find the minimum of host specificity. Some of these primitive parasites can and do attack more mature tissues and cause extensive damage, as for example in the root and foot rots of plants. Perhaps there is a difference in degree of their enzymic activities. But in many such diseases, the parasite being confronted with more active cells is perhaps forced to secrete toxic metabolites. In the case of *Armillaria* root-rot, the fungus is considered to produce a toxic substance killing the tissues in advance of colonization (*see* Gäumann, 1950).

Coming to the more advanced vascular parasites, production of extracellular toxins is known in quite a few instances, as for example, the vivotoxin fusaric acid produced by many species of *Fusarium*. The nature of these parasites is to rapidly proceed from the points of entry, whether a root-hair or a wound in a branch, towards the vascular strands. Although the toxins implicated in these diseases are generally considered to contribute to the final disease syndrome in pathogenesis, there is evidence to show that they may play a role, more significant than hitherto recognized, in the initial establishment of the parasitic pathogen. The 'wilt toxin' fusaric acid has been shown to be produced

in the rhizosphere of tomato plants 48 to 72 hours after inoculation with *Fusarium lycopersici* (Kalyanasundaram, 1958). The parasite is essentially a necrobiont, colonizing chiefly the xylem vessels and permeating beyond into the ground tissues only in the advanced stages of the disease, after the plant is killed. But what happens up to its establishment in the vascular tissues, and how does it travel from the root-hair to the xylem through the living ground tissue which intervenes? Here, the small amounts of toxin produced in advance of the invading hyphae would undoubtedly be of importance in making this invasion, by killing the cells immediately ahead.

As against these destructive, necrotrophic parasites, we have the more highly specialized, balanced or biotrophic, parasites, depending on actively living cells and tissues for growth and development. Naturally, the relationship is very different from that existing with the predator-like parasites, which cytolyze and kill the host tissues by powerful enzymes and toxins. Of necessity, they do as little damage to the host as not to jeopardize their own development, at least till the time of reproduction. Some of the smuts are excellent examples of such balanced parasitism. The parasite seems so well adapted in its demands upon the host as to the ability of the latter to supply these needs and to continue to live and grow that both the parasite and the host develop together until reproduction of the parasite.

In balanced parasitism, the nutritional requirements of parasites which lie extracellularly, but derive their nutriments from within the cell, may not be very different from that of strictly intracellular parasites. When we consider the type of nutritional requirements of the parasites and the nature of host tissue from which these are to be obtained, we can well comprehend the basis for host specificity and parasitic specialization, even to the extent of physiological races and biotypes. The obligate parasites would seem to require some as yet unidentified, perhaps labile, constituents of the active host cell. Despite this fastidiousness, obligate parasitism is by no means rare; among parasitic plants alone, about one-fourth are obligate parasites (Yarwood, 1956).

Even in these instances of balanced parasitism, as for example in the bacterial viruses, viruses of plants and animals, rust fungi, etc., the association finally leads to death and dissolution of the host cells. Exactly how the parasites bring about this dissolution is not clearly known.

Perhaps the most critical step in parasitism, and the most difficult to understand, is the initial establishment of the parasite. An elucidation of this may give us an explanation of parasitic specialization and host specificity.

Just how a parasite obtains its food from its host is not clear in all instances. Many parasites are known to have osmotic or suction pressures greater than that of their hosts (*see* Thatcher, 1942; Gäumann, 1950). It may also be by the action of some secretion from the parasite's cells or haustoria on the plasma membrane of the host cells making

it more permeable to the contained solutes so that they diffuse out and are absorbed by the parasite. Indeed, increased permeability has been noticed in many instances of parasitic attack, even in bacteriophages (*see* Trager, 1960). Evidence for lysis of host protoplasm during infection even by the so-called obligate parasites is presented by the recent work of Moore and McAlear (1961).

In some cases of parasitism, we have the obvious effects of hypertrophy, hyperplasia and neoplasia. These aspects are being dealt with in detail elsewhere in this symposium. Why these parasites should induce this overdevelopment of their hosts is a matter for conjecture. It is significant that in many cases, the stimulus to hypertrophy travels in advance of the invading organism. It is quite possible that, just as the necrobionts need previously killed tissues, these biotrophs require products of enhanced host metabolism. In many cases of obligate parasitism, a close association of the parasite's cell or haustorium with the host nucleus is noticed, and in extreme cases the parasite may be completely intranuclear, as the polyhedral viruses of insects. It is only reasonable to conclude that this association directs the further metabolism of the host cell to the parasite's advantage. The extreme such redirection of host activity is seen in the case of crown gall, where once the TIP is formed in one cell, the presence of the parasite is no longer needed. The recent report about the possible role of nucleic acids in crown gall tumour induction (Manigault and Stall, 1958) is indeed interesting. This induced yet uncontrolled host activity reminds one very much of the similar activity of man-made nuclear weapons !

It is common knowledge that the relationships of parasites to their hosts vary greatly. The same species may be innocuous in one but highly pathogenic in another; again, may be mutualistic under some circumstances but destructive under others. For instance, we do not normally think of the relationship between leguminous plants and root nodule bacteria as a parasitic one for the obvious reason that the plant gains much from the same. In recent years, however, it has been found that the association does not necessarily benefit the plant. With some strains of bacteria, the symbiosis established is *ineffective*, *i.e.*, does not permit active nitrogen fixation. As a result, the plant receives little or no benefit from the association, though the bacteria that have established themselves in its roots still profit, because they are able to develop at the expense of materials produced by their host plant. In other words, a symbiosis that is normally mutualistic becomes parasitic in this case. "It is true that the parasitism is a mild one; nevertheless, the situation differs only in degree and not in kind from the one that occurs when a frankly pathogenic becterium invades a plant and establishes itself within the plant tissues" (Stanier *et al.*, 1958). This is a good example of the fact that the dividing line between mutualism and parasitism is 'a hazy shifting one that can be crossed in either direction' (Stanier *et al.*, 1958).

An enticing problem is the origin of the parasitic habit. A factor in evolution of parasitism has been opportunity. Most of the evidence

would indicate that parasites arose from free-living organisms. The most primitive type of parasitism, in which microbes feed on secretions and detritus of the surface tissues, would be only a small change from a purely saprophytic mode of existence. Life on the body surface of a host is highly competitive and any organism that can invade host tissues has a tremendous advantage, since it brings it into direct contact with the rich supply of nutrients in the host. This invasion has brought with it the various types of advanced parasitic abilities, ranging all the way from facultative to obligate parasitism. The obligate, intracellular parasites, such as *Plasmodiophora* and the viruses, represent an extreme of ecological specialization. In other words, parasitism is certainly a positive capacity, as suggested by Skene (1952), and 'not a consequence of diminished capacity for independent existence, nor of reduction of vegetative structure; these have followed'.

In this connection it is most interesting to ponder over Trager's (1960) suggestion that 'many of the present-day cellular organelles originated as infectious microorganisms which finally entered into wholly stable association with the host cell'. After all, in nature there is the inevitable balancing out of effects. In the words of Trager: "As the size of the intracellular bodies becomes smaller, and as we have less knowledge about them and fewer criteria for their identification, the problem of differentiating between a parasitic microorganism and a self-duplicating cellular inclusion becomes progressively more difficult. Eventual success, in cultivation of intracellular parasites or symbionts apart from the living host cell, is not in itself proof of their microorganismal nature, because there is no reason why conditions could not be found which would support the multiplication *in vitro* of any self-duplicating cellular entity."

It is indeed a pleasure to thank Professors J. Venkateswarlu, Sectional President, and T. S. Sadasivan, Chairman of this Symposium, for having given me the opportunity to participate in these deliberations.

References

BROWN, W. 1955. On the physiology of parasitism in plants. *Ann. appl. Biol.* **43**: 325-41.

GÄUMANN, E. 1950. *Principles of Plant Infection.* (Translation by W. B. Brierly). Crosby Lockwood, London, 543 pp.

KALYANASUNDARAM, R. 1958. Production of fusaric acid by *Fusarium lycopersici* Sacc. in the rhizosphere of tomato plants. *Phytopath. Z.* **32**: 25-34.

LAPAGE, G. 1956. *Mönnig's Veterinary Helminthology and Entomology*, 4th edn. Bailliere, Tindall and Cox, London, 511 pp.

LILLY, V. G. AND BARNETT, H. L. 1951. *Physiology of the Fungi.* McGraw-Hill Book Co., Inc., New York, Toronto and London, 464 pp.

MANIGAULT, P. AND STOLL, C. H. 1958. The role of nucleic acids in crown-gall tumour induction. *Experientia* **14**: 409.

MOORE, R. T. AND MCALEAR, J. H. 1961. Fine structure of mycota 8. On the aecidial stage of *Uromyces caladii*. *Phytopath. Z.* **42**: 297-304.

SKENE, M. 1952. *The Biology of Flowering Plants*, 6th impression, Sidgwick and Jackson Ltd., London.

STANIER, R. Y., DOUDOROFF, M. AND ADELBERG, E. A. 1958. *General Microbiology*. Macmillan and Co. Ltd., London, 682 pp.

TENERINI, I. AND LOPRIENO, N. 1960. Ricerche istologische sulle zone di insediamento del *Cycloconium oleaginum* Cast., agente dell' "occhio di pavone" dell' olivo. *Phytopath. Z.* **39**: 101–19.

THATCHER, F. S. 1942. Further studies of osmotic permeability relations in parasitism. *Canad. J. Res.*, **20 C**: 283–311.

TRAGER, W. 1960. Intracellular parasitism and symbiosis. In *THE CELL, Biochemistry, Physiology, Morphology*, Vol. IV (Jean Brachet and A.E. Mirsky, Eds.), Academic Press Inc., New York, 511 pp.

VINES, A. E. AND REES, N. 1959. *Plant and Animal Biology*, Vol. I, Sir Isaac Pitman and Sons Ltd., London, 1306 pp.

YARWOOD, C. E. 1956. Obligate parasitism. *Annu. Rev. Pl. Physiol.* **7**: 115–42.

PHYSIOLOGY OF NODULE FUNCTION

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In nature the capacity of root nodule bacteria to fix nitrogen in association with a host species varies widely. Based on this variation effective, ineffective and parasitic responses could be defined in relation to specific host and rhizobial strains. There are two important facets to the problem—one pertains to the physiological influence of the host plant in determining nodule number, volume, function and decadence, while the other to biological or chemical factors in the soil.

The purpose of this article is to refer to factors of host physiology which have a bearing on the nitrogen fixing capacity of the effective root nodules. These complex factors involving the distribution and functional behaviour of effective, ineffective and parasitic strains of rhizobia are largely speculative.

Virtanen *et al.* (1947) has shown that one determinant of effectiveness in symbiosis is dependent on the capacity of the root nodule to synthesize haemoglobin. Therefore spectrophotometric determination of the chlorophyll content in leaves and the haem of nodules were undertaken in relation to nodulation in *Arachis hypogaea.*

Table I presents a pattern of nodule formation in *Arachis* in terms of the development of an effective symbiosis, a result of a detailed study of four varieties of *Arachis hypogaea.* The tabular representation throws light on the following points: (1) The influence of chlorophyll development in leaves on nodule appearance, function and senescence; (2) The increase in haemoglobin content of nodules at every flush of flowering; and (3) The repeated formation of nodules during the time of active vegetative growth and flowering. It would therefore seem probable that the host more than the rhizobial symbiont plays a predominant role in nodule formation.

An additional support to demonstrate the influence of host factors becomes all the more evident from the data presented in Table II. They pertain to the relationship between the haem content in the nodules and chlorophyll content in leaves in *Arachis hypogaea* in determining the number, volume and weight of nodules.

It is apparent that the chlorophyll content of leaves and the haem content of nodules of the TMV 3 variety of *A. hypogaea* was higher than that of the variety TMV 2. Correspondingly the number, weight and volume of nodules were also proportionately higher in TMV 3

TABLE I

Pattern of symbiosis in Arachis hypogaea

			Days from sowing
	Rhizobia in the Rhizosphere	Sowing	0
		Germination	4–5
	Infection through points of lateral root emergence	..	..
	Nodule formation commences	Appearance of 3rd pair of compound leaves	18–20* 25–28†
Rapid accumulation of chlorophyll in leaves	Nodules on primary and lateral roots	Flowering commence	28–30
	Haemoglobin is detectable	..	..
	Fluctuations in haemoglobin levels during the entire period of flowering	Minimal flushes in flowering	..
Maximum chlorophyll in leaves	Maximum haemoglobin in root nodules	Maximum flowering of host	48–50
Decline in chlorophyll	Haemoglobin degeneration	Peg formation; flowering declines	60
	Haemoglobin changes to the green pigment	..	65–70
Yellowing of leaves ..	Plant senescence; nodule decay	Maturation of pods	100

* Under field conditions. † Under greenhouse conditions.

TABLE II

Nodule number, weight and volume, haemoglobin in nodules and chlorophyll in leaves in Arachis hypogaea*

Variety	Nodules†			Haemoglobin µg./gram of nodules	Total chlorophyll µg./l. in leaves
	Number	Wt. in grams	Volume c.c.		
TMV 2 ..	782	4·05	5·0	295	3259
TMV 3 ..	1045	5·70	6·7	340	3506

* Plants grown under field conditions for 60 days. An effective rhizobial strain (R_4) was applied as seed inoculum.

† Total number of nodules per three plants.

than in TMV 2 indicating a direct relationship between haem content and the volume, size and number of nodules. Richmond and Salomon (1955) observed the labelling of haem by 2^{-14}C-glycine and 1^{-14}C and 2^{-14}C-acetate in homogenates of field-grown soya nodules. The author has further shown that glycine and acetate could be precursors for porphyrin synthesis in the formation of the haem protein in root nodules of leguminosae. Besides, glycine and acetate have been shown to be utilized by green plants in the synthesis of chlorophyll (Della Rosa *et al.*, 1953). In the light of these findings it is possible to envisage that chlorophyll levels in leaves might regulate nodule number, weight and volume as well as the haemoglobin content of nodules. Still another aspect of effectiveness in symbiosis concerns the formation of the pigment haemoglobin in root nodules. It has been shown by Virtanen and co-workers (1947) that there is a positive correlation between nodule haemoglobin concentration and nitrogen fixation. Recently, Bergersen (1961) provides evidence that the haemoglobin content is correlated with the volume of nitrogen fixing tissue in the nodules. Current work done on *Arachis* in our laboratory has shown an increase in haemoglobin content of nodules corresponding with every flush of flowering and a direct relationship between the haemoglobin concentration and the nodule volume.

A point of considerable interest in nodule formation in *Arachis hypogaea* is the observation in the repeated flushes of formation of nodules in the four varieties studied. This could be explained on the basis of root excretions and their role in the rhizosphere. The excretion of various organic substances by roots of higher plants into the rhizosphere has now become an accomplished fact (Sadasivan and Subramanian, 1960). Tam and Schendel (1954) and Virtanen and Laine (1939) have also reported the excretion of amino-acids by nodulated legumes. In the light of these reports it becomes suggestive that such excretions into the rhizosphere might provide suitable substrates for renewed activity of rhizobia stimulating newer focii of infections and repeated nodule formation.

Thus the importance of rhizosphere studies in relation to nodule formation appears to be one of the promising lines of approach, since excretion especially of nucleotides, flavones and growth-promoting substances either stimulatory or toxic to symbiotic micro-organisms have been reported extensively (Lundegardh and Stenlid, 1944; Brown, Robinson and Johnson, 1949; Nutman, 1951; Katznelson *et al.*, 1954; Rovira, 1956).

A great deal of evidence exists at present to show that rhizobial strains which are effective on one host may be ineffective on closely related species or even varieties of the same host plant (Erdman and Mears, 1954; Gregory and Allen, 1953; Parker and Allen, 1952). This and related questions on the complexity of 'rhizobia-legume' relationships are difficult to answer. From what we know it may be stated that the cultural aud physiological characterisiics of rhizobia (Burton and Lochhead, 1952; Jordan and Garrard, 1952 *b*) their antigenic structure

(Kleczkowski and Thornton, 1944; Vincent, 1944), colonial mutation (Jordan and Garrard 1952 *a*; Kleczkowska, 1950), phage sensitivity (Bruch and Allen, 1955) or sensitivity to antibiotic action (Abdel-Ghaffar and Allen, 1952) cannot be correlated with strain effectivity. The problem of strain specificity appears to be a multifaced one and it is perhaps a result of several undefined factors that play a cumulative role in the symbiosis between rhizobia and legumes.

However, the idea that the host plant is a dominant participant in the symbiotic reaction is gradually gaining ground. For factors which govern the physiology of the host such as length of day (Sironval, 1958), solar radiation (Diener, 1950), availability of nitrogen, seed variation (Wilson, 1946), pollination character (Wilson, 1939), chromosome numbers (Wipf, 1935) and genetical factors (Nutman, 1949; Williams and Lynch, 1954) should obviously influence the extent and the efficiency of symbiosis.

REFERENCES

ABDEL-GHAFFAR, A. S. AND ALLEN, O. N. 1952. The effects of certain micro-organisms on the growth and function of rhizobia. *Int. Congr. Soil Sci.* **3**: 93-96.

BERGERSEN, F. J. 1961. Haemoglobin content of legume root nodules. *Biochim. Biophys. Acta* **50**: 576-78.

BROWN, R., ROBINSON, E. AND JOHNSON, A. W. 1949. The effects of D-xylotose and certain root exudates on extension growth. *Proc. roy. Soc.* (London) **136 B**: 577-91.

BRUCH, C. W. AND ALLEN, O. N. 1955. Description of two bachenophages active against Lotus rhizobia. *Proc. Soil. Scl. Soc. Amer.* **19**: 175-179.

BURTON, J. C. MARGARET, O. AND LOCHHEAD, A. G. 1952. Production of vitamin B_{12} by *Rhizobium* species. *Canad. J. Bot.* **30**: 521-24.

DELLA ROSA, R. J., ALTMAN, K. I. AND SALOMON, K. 1953. The biosynthesis of chlorophyll as studied with labelled glycine and acetic acid. *J. biol. Chem.* **202**: 771-79.

DIENER, T. 1950. Uber die Bedingungen der Wurzelknollchenbildung bc-*pisum sativum* L. *Phytopath. Z.* **16**: 129-70.

ERDMAN, L. W. AND URA MAE MEARS. 1954. Strains of *Rhizobium* effective on *Trifolium ambiguum*. *Agron. Abstr. Annu. Meetings Amer. Soc. Agron.* **22**:

GREGORY, K. F. AND ALLEN, O. N. 1952. Physiological variations and host plant specificities of rhizobia isolated from *Caranga arborescens* L. *Canad. J. Bot.* **31**: 730-38.

JORDAN, D. C. AND GARRARD, E. H. 1952 *a*. Studies on the legume root nodule bacteria. II. The production and behaviour of colonial mutants produced by X-ray irradiation. *Ibid.* **30**: 103-25.

———. 1952 *b*. Growth factor requirements for effective, ineffective and parasitic strains. *Ibid.*, **30**: 693-700.

KATZNELSON, H. ROUATT, J. W. AND PAYNE, T. M. 1954. Liberation of amino-acids by plant roots in relation to desiccation. *Nature, Lond.* **174**: 1110-11.

KLECZKOWSKA, J. 1950. A study of phage resistant mutants of *Rhizobium trifolii*. *J. gen. Microbiol.* **4**: 298–310.

KLECZKOWSKI, A AND THORNTON, H. G. 1944. A serological study of root nodule bacteria from pea and clover inoculation groups. *J. Bacteriol.* **48**: 661–72.

LUNDEGARDH, H. AND STENLID, G. 1944. On the exudation of nucleotides and flavones from living roots. *Ark. Botanik.* **31 A**: 1.

NUTMAN, P. S. 1949. Nuclear and cytoplasmic inheritance of resistance to infection by nodule bacteria in red clover. *Heredity* **3**: 263–91.

———. 1951. Colour reactions between clay minerals and root secretions. *Nature, Lond.* **167**: 288.

PARKER, P. J. AND ALLEN, O. N. 1952. The nodulation status of *Trifolium ambiguum*. *Proc. Soil Sci. Soc. Amer.* **16**: 350–53.

RICHMOND, J. E. AND SALOMON, K. 1955. Quoted from: The nature, function and biosynthesis of the haem compounds and porphyrins of legume root nodules, in *Utilization of Nitrogen and its Compounds by Plants*, Symposia of the Society for experimental biology. No. 13, Cambridge University Press.

ROVIRA, A. D. 1956. Plant root excretions in relation to the rhizosphere effect. *Plant & Soil* **7**: 178.

SIRONVAL, C. 1958. Relation between chlorophyll metabolism and nodule formation in soya bean. *Nature, Lond.* **181**: 1272.

SADASIVAN, T. S. AND SUBRAMANIAN, C. V. 1960. Interaction of Pathogen, soil, other micro-organisms in the soil and host. *Plant Pathology* **2**: 273–313.

TAM, E. AND SCHENDEL. U. 1954. Loss of nitrogen from legume roots. *Z. Pflanzenernahr. Dung. U. Bodenk.* **64**: 147.

VINCENT, J. M. 1944. Variation in the nitrogen fixing property of *Rhizobium trifolii*. *Nature, Lond.* **153**: 496–97.

VIRTANEN, A. I. AND LAINE, T. 1939. The excretion products of root nodules. The mechanism of nitrogen fixation. *Biochem. J.* **33**: 412–27.

———, ERKĀMA, J. AND LINKOLA, H. 1947. On the relation between nitrogen fixation and leghaemoglobin content of leguminous root nodules. *Acta. Chem Scand.* **1**: 861–70.

WILLIAMS, L. F. AND LYNCH, D. L. 1954. Inheritance of a non-nodulating character in the soybean. *Agron. J.* **46**: 28–29.

WILSON, J. K. 1939. A relationship between pollination and nodulation of the Leguminosae. *J. Amer. Soc. Agron.* **31**: 159–70.

———. 1946. Variation in seed as shown by symbiosis. *Mem. Cornell. agric. Exp. Sta.* **272**: 21.

WIPF, L. 1939. Chromosome numbers in root nodules and root-tip of certain Leguminosae. *Bot. Gaz.* **101**: 51–67.

VIRUSES AND PARASITISM

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Plant viruses have a peculiar capacity of *in vivo* multiplication with ability to thrive and reproduce in living cells of host plants and also affect such cells to which they are not intrinsic. This, in fact, is the basis of their relationship. A brief consideration of closely interdependent relationship of some of the following aspects as: virus and its constituents; the virus-host complex and virus-vector relationship, would reveal itself that in addition to obligate parasitism, there exist several degrees of pathogenicity ranging from ready expression of disease to masked symptoms now referred to as commensalism.

Virus and Its Constituents

All plant viruses hitherto purified are known to be ribonucleoproteins. In these, the central core of nucleic acid seems essential for infectivity while the protective protein covering confers not only stability but carries the serological specificity of the virus.

In plants infected with Turnip yellow mosaic virus, two types of particles are found, both superficially alike, but on purification from infected plant sap one could recognise: a non-infectious "top" component devoid of nucleic acid, while the infectious "bottom" component contained the nucleic acid. Both these components exist in the ratio of 1:2 *in vivo*.

Reports of activation of one tobacco necrosis virus by another introduces a new factor in relationship of serologically unrelated particles (Kassanis and Nixon, 1961). The smaller and larger particles are associated in a unique fashion, for the former will multiply only in presence of latter particles (found in the preparations of the Rothamsted culture of tobacco necrosis virus), both being present in the plant.

Virus-Host Complex

Plant virus diseases are considered to be essentially disturbances in the host's nucleoprotein metabolism (Bawden and Pirie, 1952). The presence of highly deformed, blister-like areas, filiform and cup-like outgrowths (enation) in Dolichos enation mosaic virus or in tobacco leaf-curl, shoe-strings in yellow cucumber mosaic virus on *Nicotiana glutinosa* and strains of tobacco mosaic virus and also in many other virus-infected leaves, are indeed suggestive of violent disturbances in host cell metabolism. In our studies with complex virus disease of *Solanum jasminoides* Paxt., leaf abscission was seen in *Physalis floridana* Rydb.

infected with potato virus Y isolate, which was one of the components of disease (Badami and Kassanis, 1959). There was no such abscission in healthy leaves of similar plants. Similar abscissions are known to be caused in other virus-infected plants, which are shown to be due to low auxin content in virus-diseased plants (Pavillard, 1955; Chessin and Dyson, 1961), or an upset in the auxin metabolism.

Another characteristic feature of many virus infections is pronounced stunting, mainly due to retardation of growth in such plants Where stunting is the major effect on growth. auxin may not be involved, and recent work with the gibberellins suggest that these compounds may also be important for stem elongation in higher plants (Brain and Hemming, 1955). The effect of gibberellic acid as foliar spray on stunted plants of *Nicotiana tabacum* and *N. glutinosa* infected by severe etch virus was studied by Chessin (1958). He reported that initially, the stunting was completely overcome by the treatment but this effect ultimately wore off, while there was no response in height growth when sprayed with indole acetic acid.

Among other morphological changes noticed are: poor root development accompanied by considerable reduction in number of nodules (John, 1959). However, changes brought about in the nodules consequent on virus infection are still obscure. Another issue which centres around this is, whether the nitrogen fixed in nodules could be utilized for synthesis of virus protein.

The concept of virus disease as disturbances of the host nucleoprotein metabolism almost necessitates the conclusion that the ability of viruses to infect, multiply and cause symptoms will vary greatly with changes in the physiological condition of the host. Almost every change in the growing condition that affects plant growth would also affect the behaviour of viruses. The increase in susceptibility to infection by viruses when plants are pretreated to a period of darkness or an abundant supply of water, or nitrogen and exposing plants to high temperatures (around 36° C.) seems to support this view. However, responses to same treatments in post-inoculation period were not so spectacular. The response to pre-inoculation treatments is much pronounced than post-inoculation treatments. The latter fact is borne out by studies with cucumber mosaic virus and its strains in relation to a high temperature of 36° C. and darkness treatments (Kassanis, 1954; Bhargava, 1951; Badami, 1959).

Inhibitors of virus multiplication, like thiouracil and 8-Azaguanine are known to act on viruses chiefly through the host system, as revealed by the pronounced yellowing and curling noticed in the treated plants. Thiouracil inhibited the multiplication of tobacco mosaic virus (TMV) but had little effect against cucumber mosaic virus in tobacco, however the latter is inhibited by 8-Azaguanine (Badami, 1959).

It is of common knowledge that plant viruses are pre-eminently parasites causing visible symptoms ranging from 'Mosaic' to 'Yellows'

besides producing several imperceptible changes such as occur in masked and latent virus diseases. The last two characteristics are seen in latent infections with the masked strain of tobacco mosaic virus, carnation latent virus, tobacco ringspot virus (with the well-known phenomena of apparent recovery from the virus infection), Dandelion latent mosaic virus, and paracrinkle virus. In all such virus infections 'hidden' or 'disguised' have been used as synonyms for latency and masking.

Bawden (1958) in the symposium on "Latency and masking in viral and Rickettsial infections" has put forth a new idea of 'COMMENSALISM'. He defines commensalism, as applied to plant virus as "the freedom of an infected plant from visible lesions". He argues this concept to be more "appropriate since it conveys the idea of existing together in harmony, or harmlessness towards a given plant, without at the same time suggesting, as do latency and masking, that the lack of virulence depends on some change in the state of the virus, a change that makes it no longer identifiable by methods applicable when the virus is obviously pathogenic". In fact, Cayley (1928) suggested that the "broken" condition in the tulips can hardly be considered a disease in strict sense, but the relation of virus to host as symbiotic. Paradoxically enough, commensalism between virus and host in the Tulip break disease (oldest of virus disease 1637) has led to extensive trade of tulips in The Netherlands. In earlier days Dutch bulb-growers knew how to transmit the symptoms of broken tulips to uncoloured breeder tulip by means of grafting a diseased tulip on a healthy one. But presently this practice of using virus-diseased bulbs has nearly outdated. However it would be possible to obtain or continue to grow Rembrandt-tulips and other beautiful broken tulips under special care without risking the health of other stocks. There was a time when fortunes were exchanged for a single broken bulb.

Virus-Vector Relationship

In the absence of visible pathogens, proof of evidence of transmissibility of such abnormal conditions as due to a virus has to be established. The chief criterion of a plant virus disease is its transmissibility which could be accomplished through mechanical or/and biological means. It is the latter that stands out unique in virus-vector relationship. This could range from specificity to non-specificity, to loss or gain or change in transmissibility and even pathogenicity to vectors. The first two are well known to need elaboration.

Cucumber mosaic virus has a very wide host range and is known to be transmitted by 34 different species of aphids (Day and Bennetts, 1954). The strains of cucumber mosaic virus have also been found to differ in the ease with which they are transmitted by aphids (Bhargava, 1951). The Spinach strain of cucumber mosaic virus isolated in 1946 by Bhargava was readily transmitted by *Myzus persicae* Sulz. until 1955, when it lost this property during propagation, while other strains remained transmissible. *Aphis gossypii* Glov., *Myzus ascalonicus* Don. and *Myzus circumflexus* Buckt. transmitted this strain but not

M. persicae (Badami, 1958). A number of instances have been reported on loss/gain in transmissive ability, while an isolate was under observation (Black, 1953; Hollings, 1955; Watson, 1956; Swenson, 1957; Badami and Kassanis, 1959).

The high degree of specificity in leaf-hopper transmitted viruses may be correlated with their ability to multiply inside the vectors, for example aster yellows in *Macrosteles fascifrons* Stal. (Kunkel, 1927; Maramorosch, 1952), clover club-leaf virus in its vector *Agalliopsis novella* Say. (Black, 1950), corn stunt virus in *Dalbulus maidis* Del and Wol. (Maramorosch, 1951), European wheat striate mosaic in *Delphacodes pellucida* Fab. (Slykhuis and Watson, 1958; Watson and Sinha, 1959) and potato leaf-roll in the aphid *M. persicae* (Day, 1955; Stegwee, 1960). Thus all these exhibit much closer relationship with their vector hosts. Maramorosch (1956) suggested that the fat-body cells rather than the salivary gland is the site of virus multiplication, possible because of the damage caused to fat-body cells.

A very striking and interesting relation was noticed in the aforementioned virus-vector partnership. The evidence that plant viruses cause actual disease in the insects they invade was lacking till 1954 (Black). However, recently three instances have been recorded of presumed adverse effects on insect hosts. Littau and Maramorosch (1956, 1960) found that fat-body cells in plant hoppers (*M. fascifrons*) carrying aster yellows virus appeared abnormal and underwent progressive deterioration. Jensen (1958, 1959) reported that peach yellow leaf-roll virus strain of western X-disease affects the longevity of its vector (*Calladonus montanus* Van Duzee.), for the viruliferous insect (= virus-bearing) survives to an average of 24 days as opposed to non-viruliferous ones surviving up to 51 days. Recently, Sinha (1960) reported of the pathogenic effects observed only in eggs of mothers that had fed on diseased plants as nymphs and had themselves infected plants; mothers that fed as nymphs on diseased plants, but did not themselves become infective, laid healthy eggs. Egg mortality was, therefore, independent of the nutrition of the mother, something that provides further evidence for the view (Watson and Sinha, 1959) that wheat striate mosaic virus is pathogenic to the eggs of *Delphacodes pellucida* F. Authors noted that egg survival and the production were poor in infected leaf-hoppers. They also found that the embryos did not survive in the eggs of plant hoppers. Instances such as these suggest that viruses are true pathogens of the insect as well as of the plant.

Apart from virus multiplication in leaf-hoppers, potato leaf-roll virus multiplies in the aphid *M. persicae* (Day, 1955; Stegwee and Ponsen, 1958). A consideration of the relationship between potato leaf-roll virus and the natural transmitter aphid—*M. persicae*—revealed a surprising factor of relationship between virus multiplication and the intensity of respiration in the insect (Stegwee, 1960). He found from 8 hours onwards a significant decrease of the oxygen consumption in the viruliferous aphids. The lowest respiration rate was reached after about 30 hours which was little longer than the incubation period of

the virus in the aphid. But virus was not detected after 8 hours in the insect blood.

In Japan, Yoshii and Kiso (1957) reported that in some, but not all respects, the virus causing dwarf disease of satusma orange has a metabolic effect on the plant hopper vector, *Geisha distinctissima* Wal. similar to that found in infected orange leaves. Both oxygen consumption and total phosphorus were reduced in a similar manner in host plant and vector. The authors do not indicate whether or not viruliferous insects have reduced longevity or other evidences of adverse effects due to the virus. However, the decreased utilization of oxygen would suggest that infective vectors are more likely to be suffering than benefiting from the presence of this virus in their bodies.

In virus host relation almost every possible observation was made on effects of tobacco mosaic virus infection on respiration such as increase, decrease or no change. However, among these the most striking effect is that observed in inoculated leaves, where respiration is increased by 10% within an hour after inoculation (Owen, 1955), maintained this increased rate for some days during which the virus content is increased and culminated in a fall in respiration rates much below the rate of uninfected leaves. A similar situation exists in systemically infected leaves when they have high virus content. The increased respiration rate of tobacco leaves inoculated with tobacco mosaic virus is unique, among virus-host combinations. This is not the case with TMV in *N. glutinosa* or potato virus X and tobacco etch virus in tobacco. All three latter viruses have greater effect on respiratory rates which increase from 20 to 40%.

Virus Diseases of Insects

The role of insects as vectors in disseminating plant viruses are too well known to be discussed here. There are over 200 insect viruses known that kill their hosts, but plant viruses and vertebrate viruses transmitted by insects have been considered harmless to their vectors. However, another relationship where the insect is itself parasitized through a virus disease, proving lethal to the host, affords possibilities of biological control of insect pests. Some virus diseases of insects (polyhedral disease, granulosis) have proved lethal to their hosts. Mostly larval forms of insects belonging to Lepidoptera (butterflies and moth), Hymenoptera (ants, bees) and Diptera are susceptible to virus diseases. But methods of spread of viruses in these insects are obscure. So far destruction of Spruce forests in Central Europe by larvae of Nun moth (*Lymantria monacha*) pine saw-fly and common clothes moth have been effectively controlled (Smith and Markham, 1954). Similarly the menace of hordes of locusts (Orthopterous member gregarious in habit) or threat of aphids (the natural transmitter of plant viruses, clustering so many of farm and garden crops) could be reduced if only a virus epidemic could be started in these potential enemies. In India, white-flies are the principal agents for transmission of plant

viruses and any means of biological control of these vectors through virus disease warrants attention.

The widely divergent relationship ranging from lethal parasitism to commensalism amongst viruses and its constituents, virus-host complex, virus-vector partnership and the role of insect viruses in biological control of injurious pests are presented.

I wish to place on record my grateful thanks to Professor J. Venkateswarlu, and to the Indian Science Congress Association for giving me this opportunity to take part in this symposium.

REFERENCES

BADAMI, R. S. 1958. Changes in the transmissibility by aphids of a strain of a cucumber mosaic virus. *Ann. appl. Biol.* **46**: 554–62.

———. 1959. Some effects of changing temperature and of virus inhibitors of infection by cucumber mosaic virus. *Ibid.* **47**: 78–89.

—— AND KASSANIS, B. 1959. Some properties of three viruses isolated from a diseased plant of *Solanum jasminoides* Paxt., from India, *Ibid.* **47**: 90–97.

BAWDEN, F. C. 1958. Gradations and transitions between pathogenicity and commensalism in infections with plant viruses'. *Symp. Latency and Masking in Viral and Rickettsial Infections*. pp. 80–87. Minneapolis, U.S.A. Burgess Publishing Co.

—— AND KASSANIS, B. 1954. Some effects of thiouracil on virus-infected plants. *J. gen. Microbiol.* **10**: 160–73.

—— AND PIRIE, N. W. 1952. The nature of virus multiplication. *Second Symp. Soc. gen. Microbiol.* Cambridge University Press.

BHARGAVA, K. S. 1951. Some properties of four strains of cucumber mosaic virus. *Ann. appl. Biol.* **38**: 377–88.

BLACK, L. M. 1950. A plant virus that multiplies in its insect vector. *Nature, Lond.* **166**: 852.

———. 1953. Loss of vector transmissibility by viruses normally insect transmitted. *Phytopathology* **43**: 466.

BRAIN, P. W. AND HEMMING, H. G. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. *Physiol. Plant.* **8**: 669–81.

CAYLEY, D. M. 1928. "Breaking" in tulips. *Ann. appl. Biol.* **15**: 529–39.

CHESSIN, M. 1958. Growth substances and stunting in virus-infected plants. *Proc. 3rd Conf. Potato Virus Diseases.* Lisse-Wageningen, pp. 80–84.

—— AND DYSON, J. G. 1961. Effect of auxius on virus-induced leaf abscission. *Phytopathology* **51**: 195.

DAY, M. F. 1955. The mechanism of the transmission of potato leaf-roll virus by aphids. *Aust. J. biol. Sci.* **8**: 498–513.

—— AND BENNETTS, M. J. 1954. A review of problems of specificity in arthropod vectors of plant and animal viruses. pp 172 (mimeographed), C.S.I.R.O. Canberra, A.C.T., Australia.

HOLLINGS, M. 1955. Investigations of chrysanthemum viruses. I. Aspermy flower distortion. *Ann. appl. Biol.* **43**: 86–102.

JENSEN, D. D. 1958. Reduction in longevity of leaf-hoppers carrying peach yellow leaf-roll virus. *Phytopathology* **48**: 394.

———. 1959. A plant virus lethal to its insect vector. *Virology* **8**: 164–75.

JOHN, V. T. 1959. Root and nodule development in *Dolichos lablab* L. infected with Dolichos enation mosaic virus. *Curr. Sci.* **28**: 211.

KASSANIS, B. 1957. Effect of changing temperature on plant virus disease. *Adv. anc. Virus Res.* **4**: 221–41.

—— AND NIXON, H. L. 1961. Activation of one tobacco necrosis virus by another. *J. gen. Microbiol.* **25**: 459–71.

KUNKEL, L. O. 1926. Studies on aster yellows. *Amer. J. Bot.* **13**: 646.

LITTAU, V. C. AND MARAMOROSCH, K. 1956. Cytological effects of aster yellows virus on its insect vector. *Virology* **2**: 128–30.

———. 1960. Cytological effects of aster yellows virus on its insect vector *Ibid.* **10**: 483–500.

MARAMOROSCH, K. 1951. Mechanical transmission of corn stunt virus to an insect vector. *Phytopathology* **41**: 833–38.

———. 1952. Direct evidence for the multiplication of aster yellows virus in its insect vector. *Ibid.* **42**: 59.

MARKHAM, R. AND SMITH, K. M. 1949. Studies on the virus of turnip yellow mosaic. *Parasitology* **39**: 330–42.

OWEN, P. C. 1955. The respiration of tobacco leaves after systemic infection with tobacco mosaic virus. *Ann. appl. Biol.* **43**: 114–21.

PAVILLARD, J. 1955. La teneur en auxine des pommes de terre virosees. *Proc. 2nd Conf. Potato Virus Diseases.* pp. 178–84, Lisse-Wageningen.

SINHA, R. C. 1960. Comparison of the ability of nymph and adult *Delphacodes pellucida* to transmit European wheat striate mosaic virus. *Virology* **10**: 344–52.

SLYKHUIS, J. T. AND WATSON, M. A. 1958. The transmission of a virus disease of cereals resembling wheat striate mosaic by *D. pellucida* Fab. *Ann. appl. Biol.* **46**: 537–40.

SMITH, K. M. AND MARKHAM, R. 1954. *Mumps, Measles and Mosaics.* The New Naturalist Series, Collins, St. James Place, London.

STEGWEE, D. 1960. Further investigations on the relation between *M. persicae* and potato leaf-roll virus. *Proc. 4th Conf. Potato Virus Diseases. Braunschweig.* 106–10.

—— AND PONSEN, M. B. 1958. Multiplication of potato leaf-roll virus in the aphid *M. persicae. Ent. exp. and appl.* **50**: 291–300.

SWENSON, K. N. 1957. Transmission of bean yellow mosaic virus by aphids. *J. eco. Ent.* **50**: 727–731.

WATSON, M. A. 1956. The effect of different host plants of potato virus C in determining its transmission by aphids. *Ann. appl. Biol.* **44**: 599–607.

—— AND SINHA, R. C. 1959. Studies on the transmission of European wheat striate mosaic virus by *Delphacodes pellucida* Fab. *Virology* **8**: 139–63.

YOSHII, H. AHD KISO, A. 1957. Studies on the nature of insect transmission in plant viruses. II. Some researches on the unhealthy metabolism in viruliferous plant hopper *Geisha distinctissima* Wal. which is the insect vector of the dwarf satusma orange. *Virus (Osaka)* **7**: 315–20.

ECTOTROPHIC FUNGAL ASSOCIATIONS WITH ROOTS*

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The region of soil immediately surrounding living roots provide a micro-habitat characterized by active liberation of metabolites and a rise in the population of micro-organisms as a result of the enriched substrate in the root environs (Sadasivan and Subramanian, 1960); such a micro-habitat has been defined as the rhizosphere (Hiltner, 1904) and the observed difference between the numbers and activity of the microflora of the rhizosphere and that of the microflora of the soil away from roots is referred to as the rhizosphere-effect. The rhizosphere-effect may then be considered as one of the demonstrable interactions between roots and soil microflora. The innermost stratum of rhizosphere is known as the rhizoplane which comprises the root surface with the attendant microflora, discernible only after removal of the rhizosphere soil and subjecting the roots to repeated washing. In the following paragraphs. it is my intention to confine to the root-fungus interactions in angiosperms and elucidate the physiological significance of ectotrophic fungal associations with plant roots. The initial focus of such an association is the rhizoplane, and considerations of the biology of fungal associations in the rhizoplane have received little attention while emphasis has no doubt been placed on typical mycorrhiza formations. Indeed, Jahn (1934) had suggested the usage of the term peritrophic mycorrhiza for rhizoplane fungi to distinguish them from strict mycorrhiza formers. Since cortical penetration of the fungal hyphae is characteristic of mycorrhiza, the usage of the term peritrophic mycorrhiza was discontinued and instead, the terms root surface or rhizoplane microflora have come into vogue. Harley and Waid (1955) and Subba-Rao and Bailey (1961) have demonstrated methods by which active mycelia on the root surface could be examined and isolated by serial washing of root segments and plating them on suitable agar media. These fungi exist as mycelia on the surface of roots and the possible physiologic relationship between the fungi and the plant is not yet clearly understood although we have evidence to show that certain rhizosphere bacteria diminish or augment the supply of essential nutrients to plants from the soil. The manganese deficiency disease of oats, referred to as 'Grey speck' has been ascribed to the competition of certain rhizosphere bacteria with the host for the supply

* Summary of paper presented in the symposium entitled 'Parasitism and symbiosis' under the Botany Section of the 49th Session of the Indian Science Congress held in Cuttack in January, 1962.

of manganese (Gerretsen, 1937). The intensity of symptoms depended on the manganese content of the host. Seedling mortality by necroses of the leaf-tissue resulted when the manganese content was below minimum. However, at manganese content of the host ranging from 5–35 p.p.m., the plants were free of symptoms. Addition of 5–10% of the original bacteria-infested soil to sterilized soil caused the grey-speck symptoms to reappear. These results indicate that a lowered resistance of roots to the invading organism is dependent on manganese deficiency. In a similar study (Gerretsen. 1948), the part played by bacteria in the intake of phsophorus by plants has been shown in pot cultures of several plants which were grown on sterile and bacteria-infested soil after addition of insoluble phosphates. In many instances the plants growing in bacteria-infested and phosphate-enriched soil registered a rise in the overall weight of the plants and the phosphorous content as well. When pure cultures of bacteria were isolated from the soil and infected into sterile cultures of oats amended with phosphate. the dry weight of the host plants increased and the phosphorous content rose from 16 to 54%. The increased intake of phosphorus was accounted for by burying glass plates smeared with phosphorus containing agar into unsterilized soil when clear solubilization zones were visible as distinct spots, especially under the root-tips of mustard seedling indicating thereby that the bound phosphate was made available to the plant in the vicinity of roots for absorption. More recently, Krassilnikov (1959) has shown the influence of soil bacteria on the accumulation of vitamins and amino-acids in plants grown in soil infested by these bacteria.

While the foregoing studies have demonstrated the role of bacteria associated with roots of higher plants, we have no information concerning the part played by fungi of the root region in the physiology of the plant. A study in this direction was initiated employing techniques to achieve interaction between roots of seedlings grown under aseptic conditions and a particular fungus dominating the rhizoplane of the same species growing naturally in garden soil. In this investigation (Subba-Rao *et al.*, 1961) two species of fungi dominant in the rhizoplane of tomato plants were used. The fungi were *Fusarium* sp. and *Trichoderma* sp., the former predominating the root surface of a variety of tomato, Bonny Best, while the latter was dominant on the root surface of the variety, Loran Blood. Plants were grown aseptically and the roots were infested with the particular fungus by pouring conidial/mycelial suspensions of the fungus into the container in which the roots were growing and allowing the inoculum to establish on the roots for 48 hours. The infested and the uninfested plants were then transferred to solutions containing radioactive sulphur, phosphorus, bicarbonate and glucose. When appreciable radioactivity was detected in the shoot system, the tops of the plants were scissored off and the total radioactivity measured. In addition, in glucose-treated plants, alcoholic extracts of shoots were chromatographed in two dimensions and radio-autographed to determine the relative distributions of C^{14} in the uninfested and infested plants. To a certain extent, both

Fusarium as well as *Trichoderma* suppressed the uptake of sulphur and phosphorus. This tendency was not seen so far as glucose was concerned; while *Fusarium* considerably retarted the uptake of glucose, *Trichoderma* accelerated it. Infestation of roots of the variety Bonny Best with *Fusarium* considerably reduced the amount of radioactivity entering amino-acids and increased the radioactivity of sugars. While in *Fusarium*-infested Bonny Best plants, radioactivity was traced in glucose, fructose, sucrose and faintly in organic acids, none was found in amino-acids. On the other hand, in plants free of *Fusarium*, amino-acids such as aspartic acid, glutamic acid, asparagine, glutamine and alanine became radioactive and only traces of radioactivity were seen in fructose and organic acids. Infestation of roots of the variety Loran Blood with *Trichoderma* had much less effect on the distribution of radioactivity in shoots. In this instance, the amount of radioactivity entering amino-acids was almost unaffected, but infestation with the fungus resulted in a reduction of radioactivity in sucrose. A notable inference from the foregoing results was that the presence of the fungus on the root surface not only affected the total amount of material entering shoots but also affected the subsequent metabolism of at least glucose. The cause for reduction in the uptake does not appear to be a simple case of physical interference with the uptake mechanism or a simple competition between the root and the fungus for the common substrate. The following physiological explanation may be offered to account for the observed differences in the further transformation of glucose entering roots in infested and uninfested states: In the absence of *Fusarium*, the roots converted glucose to amino-acids for translocation up to shoots (Martin *et al.*, 1961), but the presence of the fungus on roots resulted in a decrease in the metabolization of glucose in roots and it was, instead, transported to shoots where conversion to other sugars took place. In other words, infestation with *Fusarium* altered the ability of roots to metabolize glucose. The results appear interesting and although no general conclusion could be drawn from them, it is apparent that fungi in the rhizoplane influence the fate of substances absorbed by the roots. Hence, these studies should be viewed as forerunners to a more intensive study with regard to the physiological role of root surface flora in the metabolism of the higher plant.

The two-way transport in ectotrophic mycorrhiza.—Many earlier workers on ectotrophic mycorrhiza have observed a correlation between the nutrient level of soil and the intensity of mycorrhizal association. In soil deficient in nutrients greater mycorrhizal development takes place than in soil enriched with available nutrients. This observation naturally suggests the role of the lower symbiont in the absorption of nutrients. The work of Melin and Nilsson (1950, 1952) on intact seedlings have demonstrated that the external hyphae of ectotrophic mycorrhiza of conifers act in a manner analogous to root hairs in absorbing and transporting labelled phosphorus and inorganic and organic nitrogen into roots. Harley and his associates (*see* Harley, 1959) showed the transport of labelled phosphate from the fungus to the host in excised roots of beech and reported that about 90% of the phosphorus

absorbed was retained in the fungal sheath. Employing an elegant technique, Melin and Nilsson (1952) have shown that *Boletus variegatus* is able to transfer nitrogen from a solution of ammonium salt to the tissues of the roots of pine. In fact, Kramer and Wilbur (1949) were the first to produce autoradiographs of excised roots of loblolly pine after exposure to P^{32} phosphate. These autoradiographs showed clearly a greater accumulation of phosphorus in mycorrhizal roots than in uninfected roots.

The results of the foregoing studies have established conclusively that the fungal symbiont functions as nutrient absorbing structure. Is there a reverse flow of organic materials from the higher plant to the fungal associate? This question has been tackled by several investigators. Bjorkman (1942, 1944, 1949) established a correlation between the amounts of soluble carbohydrates in the roots and the frequency of mycorrhiza. His conclusions point out that excess of sugars in roots results in heavy mycorrhizal infection and conversely a deficiency results in poor infection. In other words, the establishment of the lower symbiont on the roots depends upon the availability of the photosynthate in the root system. This hypothesis was extended by Melin and Nilsson (1957) who demonstrated the transport of C^{14} labelled photosynthate to the fungal associate from pine seedlings. The radioactivity values of the fungal mantles were somewhat higher than those of uninfected roots indicating a higher concentration of labelled photosynthate in the fungal sheath than in roots.

Growth substances produced by the lower symbiont.—The majority of fungi producing ectotrophic mycorrhiza belong to the basidiomycetes, particularly to the families Agaricaceae and Boletaceae. Most mycorrhizal fungi are heterotrophic to thiamine or one of its constituent moieties, pyrimidine or thiazole (Melin, 1954), although deficiency for other B-vitamins such as pantothenic acid, nicotinic acid, biotin and inositol have been reported occasionally. Besides these vitamins, Melin (1953) discovered that exudates from excised roots of pine stimulate the growth of mycorrhizal fungi in media supplemented with B-vitamins and amino-acids; Melin called this 'M factor'. Later Melin and Das (1954) showed that this factor was not specific to pine roots but were exuded by roots of several angiosperms.

The root system of pine trees have long roots on which short laterals are borne. These lateral roots could broadly be classified morphologically as follows: (1) dichotomously branched and swollen roots, which under extreme conditions may become coralloid or tubercular, and (2) unbranched laterals which are often infected by non-mycorrhizal fungi; some of these were called 'Pseudomycorrhiza' by Melin. The typical morphogenesis of the root system has been attributed to the fungal associate of the mycorrhiza. Excised roots of pine were grown in crude culture filtrates of *Boletus* spp. and forking and swelling of short laterals observed (Slankis, 1958). Addition of indole acetic acid (IAA) to synthetic media on which excised roots were raised brought about similar changes in the morphology

of the root system (Slankis, 1958). When white and red pine seedlings were raised under aseptic conditions without change of nutrients and proper aeration, the short laterals got forked and the ends of long roots became swollen. Restoration of such seedlings to congenial conditions resulted in the elongation of the ends of swollen long roots and the forked short laterals (Subba-Rao, observation). Forking of pond pine roots were caused by such compounds as kinetin, sulphanilamide, pyridoxine and several amino-acids which are chemically unrelated to one another (Barnes and Naylor, 1959). IAA was chromatographically detected in the swollen and forked mycorrhizal short laterals of white and red pine but not in culture filtrate of *Boletus* sp. grown on tryptophane-free medium supplemented with pine root exudates collected from plants grown aseptically (Subba-Rao and Slankis, 1959). Sugar pine seedlings grown aseptically demonstrated typical dichotomy of short laterals even in the absence of the fungus and additions of IAA at concentrations of 10^{-6} M–10^{-11} M to aseptically growing excised roots of sugar pine did not induce dichotomy of short laterals (Ulrich, 1960). The culture filtrates of several mycorrhiza-forming fungi have been screened for indole compounds. All species of *Boletus* except *B. badius* produced IAA and the latter and other slow-growing ones needed tryptophane in the medium. *Coprinus comatus* has been shown to have IAA oxidase system and it readily converted the IAA into several other compounds (Ulrich, 1960).

The aforesaid results suggest that the factors involved in the characteristic morphology of mycorrhizal short roots are manifold and a greater understanding of the physiology of interaction between the two symbionts is essential for a proper elucidation of the cause of forking in pine roots. The observation of forking and repeated dichotomy of short lateral roots of pine in sterile cultures need further investigation. As indicated earlier, forking of short laterals in aseptically growing white and red pine seedlings takes place under adverse cultural conditions. Similarly, it is quite conceivable that forking and repeated dichotomy of short laterals in naturally growing plants is a means to increase the absorptive area of the root and in order to accomplish this, the plants have exploited the seasonal changes in soil climate. In temperate soils, during winter the soil is frozen and aeration is inadequate, a condition analogous to prolonged aseptic culture of pine seedlings in an environment of unchanged nutrient and insufficient aeration. This adverse condition may probably favour the accumulation of certain metabolites (growth substances) in the roots responsible for the initial dichotomy of short laterals during the first year of seedling growth. With the advent of spring when adequate aeration is feasible for the normal activity of both the symbionts, the swollen ends of long roots grow vigorously, the fungus grows over the root surface and the Hartig net gets established with an ectotrophic fungal mantle. As the root system grows further, the interaction products of both the symbionts such as growth substances are translocated and again accumulate during subsequent winter and cause forking of the short laterals followed by repeated dichotomy. The foregoing account is a hypo-

thesis and therefore the possibility of influence of seasonal changes in the soil climate on the symbiotic phenomenon and the consequent morphogenesis of the root system merit investigation.

Production of antibiotics in soils, their uptake by plants and the importance of systemic fungicides in the control of plant diseases have now been well recognized (Brian, 1957). In view of the occurrence of a specialized flora in the rhizoplane, an intensive study of the substances produced on the root surface by these micro-organisms should be undertaken. I am referring to the possibility of existence of a physiological activity on the root surface akin to the role of root hairs in absorption, whereby the organic substances produced by the micro-organisms in the rhizoplane are being constantly absorbed and utilized by the plant. Explorations in these directions need elegant techniques by which we could establish an interaction between a particular micro-organism or a group of dominant micro-organisms and the plant in the rhizoplane which will approximate conditions obtained on roots growing naturally in soil. In other words, soil microbiologists and root disease pathologists must pay more attention to the energy relationship on the root surface, especially since root surface constitutes the primary locus of infection. Since rhizosphere population could materially be altered by foliar sprays (Ramachandra-Reddy, 1959), it would be possible to alter the rhizoplane flora and induce the establishment of the most beneficial microbial community on the root surface and thus indirectly contribute to the well-being of the plant. Some of these and related problems need attention by soil microbiologists in future.

REFERENCES

BARNES, R. L. AND NAYLOR, A. W. 1959. Effect of various nitrogen sources on growth of isolated roots of *Pinus serotina*. *Physiol. Plant* **12**, 82–89.

BJORKMAN, E. 1942. Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. *Symb. bot. upsaliens* **6**: 191.

———. 1944. The effect of strangulation on the formation of mycorrhiza in pine. *Svensk bot. Tidskr.* **38**: 1–14.

———. 1949. The ecological significance of ectotrophic mycorrhizal association in forest trees. *Ibid.* **43**: 223–62.

BRIAN, P. W. 1957. Effects of antibiotics on plants. *Annu. Rev. Pl. Physiol.* **8**: 413–26.

GARRETT, S. D. 1956. *Biology of Root-Infecting Fungi.* Cambridge University Press, pp. 293.

GERRETSEN, F. C. 1937. Manganese deficiency of oats and its relation to soils bacteria. *Ann. Bot., Lond. N.S.* **1**: 207–30.

———. 1948. The influence of micro-organisms on phosphate intake by the plant. *Plant & Soil* **1**: 51–81.

HARLEY, J. L. 1959. *The Biology of Mycorrhiza.* Leonard Hill (Books), Lond. pp. 233.

HARLEY, J. L. AND WAID, J. S. 1955. A method of studying active mycelia on living roots and other surfaces in the soil. *Trans. Brit. mycol. Soc.* **38**: 104–18.

HILTNER, L. 1904. Uber neuer Erfahrungen und Probleme auf dem Gebeit der Boden bacteriologie und unter besonderer Beruchsichtigung der Grundungung und Brache. *Arb. dtsch. Landw Ges.* **98**: 59–78.

JAHN, E. 1934. Die peritrophe Mykorrhiza. *Ber. dtsch. bot. Ges.* **52**: 463.

KRAMER, P. J. AND WILBUR, K. M. 1949. Absorption of radioactive phosphoru by mycorrhizal roots of pine. *Science* **110**: 8–9.

KRASSILNIKOV, N. A. 1959. Influence of certain bacteria on the accumulation of vitamins and amino-acids in plant tissues. *Proc. int. bot. Congr.*, Montreal (Abstract) **2**: 206.

MARTIN, P., MORTIMER, D. C. AND GORHAM, P. R. 1961. The translocation of assimilated carbon to and from the roots of young soybean plants. *Proc. Canad. Soc. Pl. Physiol.*

MELIN, E. 1953. Physiology of mycorrhizal relations in plants. *Annu. Rev. Pl. Physiol.* **4**: 325–46.

———. 1954. Growth factor requirements of mycorrhizal fungi of forest trees. *Svensk bot. Tidskr.* **48**: 86–94.

—— AND DAS, V. S. R. 1954. The influence of root-metabolites on the growth of tree mycorrhizal fungi. *Physiol. Plant.* **7**: 851–58.

—— AND NILSSON, H. 1950. Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. *Ibid.* **3**: 88–92.

———. 1952. Transfer of labelled nitrogen from an ammonium source to pine seedlings through mycorrhizal mycelium. *Svensk bot. Tidskr.* **46**: 281–85.

———. 1957. Transport of C^{14} labelled photosynthate to the fungal associate of pine mycorrhiza. *Ibid.* **51**: 166–86.

RAMACHANDRA-REDDY, T. K. 1959. Foliar spray of urea and rhizosphere microflora of rice (*Oryza sativa* L.). *Phytopath. Z.* **36**: 286–89.

SADASIVAN, T. S. AND SUBRAMANIAN, C. V. 1960. Interactions of pathogen, soil, other micro-organisms in the soil, and host. In *Plant Pathology* (Eds. J. G. Horsfall and A. E. Dimond), Academic Press, New York **2**: 273–313.

SLANKIS, V. 1958. The role of auxin and other exudates in mycorrhizal symbiosis of forest trees—In *Physiology of Forest* Trees, (Ed., K. V. Thimann.) Ronald Press, New York. pp. 427–43.

SUBBA-RAO, N. S. AND BAILEY, D. L. 1961. Rhizosphere studies in relation to varietal resistance or susceptibility of tomato to *Verticillium* wilt. *Canad. J. Bot.* **39**: 1747–58.

——, BIDWELL, R. G. S. AND BAILEY, D. L. 1961. The effect of rhizoplane fungi on the uptake and metabolism of nutrients by tomato plants. *Ibid.* **39**: 1759–64.

—— AND SLANKIS, V. 1959. Indole compounds in pine-mycorrhizae. *Proc. int. bot. Congr.* Montreal (Abstract) **2**: 386.

ULRICH, J. M. 1960. Effect of mycorrhizal fungi and auxins on root development of sugar pine seedlings (*Pinus lambertiana*, Dougl.). *Physiol. Plant.* **13**: 493–504.

GENERAL ASPECTS OF SYMBIOSIS

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SYMBIOTIC associations between micro-organisms and other micro-organisms or plants or animals are as varied as they are widespread. Associations so interpreted in the past include those of algae with fungi to form lichens, of algae (Zoochlorella, Zooxanthella) with freshwater and marine animals; of fungi with plant roots to form mycorrhiza, of yeasts and yeast-like fungi in the rumen of sheep and other animals and also associated with wood-feeding and plant-sap-sucking insects, of *Septobasidium* spp. with scale insects, of fungi and ambrosia beetles; of *Rhizobium* spp. with roots of legumes to form nodules, and of bacteria forming an essential intestinal microflora in many insects and animals. We shall try to summarise here the significant progress that has been made in understanding some of these relationships.

The plant root-fungus associations seen in the ectotrophic and ectendotrophic mycorrhizae of forest trees have been investigated critically by many workers (Melin, 1953; Harley, 1959; Slankis, 1958, 1961). From a study of mycorrhizae of pines, it has become obvious that a deep intracellular infection can be found in the cortical cells in the initial stage of the symbiotic relationship. Cytological studies have shown that, with increasing resistance of the host plant, most of the intracellular hyphae are digested by the host cells. The fungal hyphae thence remain intercellular and form "Hartig nets". Melin considers this phase one of mutual adjustment. According to Melin (1953), "since the mycorrhizal fungus invades the host, it may be considered primarily as a parasite. Contrary to many real parasites it does not seem, as a rule, to be producing substances toxic to the host". The uniqueness of the association is that the fungus is parasitic but not pathogenic, and the host, in turn, is tolerant although infected. Nay, there is much more that is unique in this association, as may be seen from the physiological interactions between the two partners. The majority of the mycorrhiza-formers referred to belong to the Basidiomycetes (Hymenomycetes and Gasteromycetes) and sporophore development takes place only in association with tree roots. Contribution of certain unknown factors favouring reproduction of the fungus by the plant roots is thus implied. Indeed, the rhizosphere provides the fungus with sugars, amino acids, vitamins and possibly other organic

substances required by the fungus. The obligately mycorrhizal Basidiomycetes are incapable of producing cellulase except rarely and then possibly adaptively. The saprophytic activity of these organisms is thus severely curtailed and their requirement of sugar for growth has led to the suggestion that the main factor in mycorrhiza formation is the presence of soluble carbohydrates in the roots (Björkman, 1944). However, isolated pieces of mycorrhizal pine roots survive and live for several seasons simulating chlorophyll-less plants. New normal-appearing mycorrhiza are formed by these segments. It is clear that the carbohydrates accumulated in these roots are derived from the soil through the fungus hyphae. Thus it appears that the explanation may not be as simple as proposed by Björkman. It has been pointed out that, when in mycorrhizal association, fungus partners with cellulose-decomposing ability may not produce cellulase as long as soluble carbohydrates are produced by the roots, but that the cell wall and its constituent cellulose may form the substrate for their activity when the roots cease to offer sugars: a possible explanation of the ectendotrophic mycorrhizae. Yet another interesting fact is that the fungi forming ecto- and ectendotrophic mycorrhiza enter the roots by producing pectolytic enzymes, but not all soil fungi which show this pectolytic ability form mycorrhizae. It is fitting to postulate that tree roots may excrete substances toxic to certain fungi, but not others; such a postulate appears inescapable in order to explain further the ability of several mycorrhiza-forming Hymenomycetes to associate with only one or a few genera of trees. There is thus no doubt that the rhizosphere of these trees not only functions as a locale for the development of chosen mycorrhizal partners but also provides them with substrates required for their development, particularly growth factors for which they are heterotrophic and amino acids. On the other hand, what does the higher partner (the host) get in return? Much evidence is now available indicating that the ecto- and ectendotrophic mycorrhizae absorb nutrients and a mycorrhizal relationship is invariably beneficial and often essential. Mycorrhiza-bearing plants have been shown to accumulate considerably more nitrogen, phosphorus and potassium than plants without mycorrhizae. Using labelled phosphorus, several workers have demonstrated accumulation of this ion in the roots *via* the fungal hyphae. Similar results have been obtained for labelled inorganic nitrogen and organic nitrogen in the form of glutamic acid. The morphological features such as repeated dichotomous branching of the coralloid mycorrhiza of pine are now believed to be induced by supra-optimal levels of auxin (? IAA) produced by the fungus. Increased auxin levels for the mycorrhizal roots as compared to the non-mycorrhizal roots have also been demonstrated. Apart from causing all the morphological abnormalities which are essential to the establishment of ecto- and ectendotrophic mycorrhizae of pine, the auxin exuded by the fungus affects the entire root system when translocated from the short roots to the long roots. The sequence and the frequency of short roots would be governed by the auxin concentration. The morphological abnormalities so produced would add to the absorbing surface of the root system of the tree and to the benefit the tree derives from the association.

We shall now consider the root nodules of legumes on which much outstanding work has been done (Thornton, 1954; Thimann, 1955; Allen and Allen, 1958; Hallsworth, 1958; Raggio and Raggio, 1962). Infection of root systems by *Rhizobium* is accomplished *via* root hair tips and this event is stated to be preceded by exudation of organic substances from the root hair tips. It has been suggested that infection is correlated with production of polygalacturonase. Following infection, the bacteria multiply rapidly and form a thread of numerous rods enclosed in a tubular cellulose sheath which is contributed by the host. Cell-to-cell penetration follows. The occasional tetraploid cortical cells usually met with in legumes appear to be the primary foci of infection. It is significant that colchicine treatment has been shown to improve nodulation. In the cortical cells of the root the bacteria multiply very rapidly and stimulation of cell division in advance of the infection such as is commonly observed suggests some diffusible substance being formed. The hyperplasia and the abnormally large and later degenerating nuclei of the cells of the infected tissue could well be classified as pathological effects brought in by infection. There is as yet apparently no benefit to the legume from these internal changes except that the bacterium contributes auxin for nodule growth and development. The bacteria multiply in the new tissues laid down which form the nodule and are found as "bacteroids". Nodule development is obviously governed by initial nutritional supply to the area of bacterial activity. Indeed, in "effective" nodules a well-developed vascular supply connects the bacteroid zone of the nodule with the main vascular supply of the host root. It is the effective nodules which partake in symbiotic nitrogen fixation and an understanding of these in comparison with the "ineffective" nodules is a prerequisite for any discussion of the nature of the relationship. The accumulation of polysaccharide (dextrin ?) between protoplast and cell wall of infected and adjacent uninfected cells of ineffective nodules of some species of *Trifolium*, but not in effective nodules, indicates perhaps that carbohydrate accumulation in the nodule may lead to ineffectiveness. In discussing effectiveness and ineffectiveness and the genesis of the root nodule, Thornton (1954) emphasized the balance of three processes which proceed distally: growth of meristem, progressive invasion of the inner layers by the bacterium, and the disintegration beginning at the base of the bacterial tissue. Both poor meristematic activity near the tip and rapid disintegration of the bacterial tissue from the base would contribute to limited volume of bacterial tissue and so to ineffectiveness. The substance of effectiveness, therefore, would be a balance between these two processes. The effective nodule is characterised by the presence of leghemoglobin which neither partner is known to produce individually. The ability of the effective nodule to fix nitrogen is also not shared by the two partners individually. A direct correlation between the nitrogen fixing capacity of a nodule and its hemoglobin concentration has been demonstrated. While isolated effective nodules continue to fix nitrogen only for a few hours, it is remarkable that in association with the host the nodules remain active in the process until senescence brings in decay of the nodule tissue. It is therefore obvious that the host contributes some-

thing significant to this process. The host plant also supplies the nutrients for the rhizobia which alone would permit the sequence of events just outlined. Ultimately the nodule disintegrates possibly due to cutting off of nutrients o· due to the production of some toxic substance. The rhizobia supply auxin required for nodule development. It must be admitted, however, that mere hyperauxiny would only contribute to simple abnormal growths devoid of hemoglobin and other pigments which are characteristic of these nodules. The available evidence indicates that fixation of nitrogen in nodules starts only after multiplication of rhizobia and host cells ceases. The ability to fix nitrogen finally attained by the nodule is the consummation of a mutual association of give-and-take by the two partners.

While the early stages of the association may be interpreted as being unilaterally beneficial to the bacterium, it would be interesting to know what the host receives in return, besides the unique consummation referred to above. There is little doubt that the plant is better supplied with nitrogen and grows better than its counterpart without the nodules. Evidence for definite transport of certain amino acids through the xylem of plants inoculated with effective strains is available and, indeed, the pattern of amino acid translocation is different for inoculated and uninoculated plants; in the case of peas, aspartic acid, asparagine and glutamine were detected in the sap from xylem of inoculated plants. It is pertinent to cite here the work of Virtanen, Moisio and Burris (1955) who have shown that photosynthetic products are necessary for nitrogen fixation in nodules. $C^{14}O_2$ supplied to illuminated soybean leaves was translocated speedily to the nodules where the C^{14} appeared in amino acids. Indeed, Bergersen (1960) suggests that the host supplies " carbon compounds which are partially oxidized by the bacteroids and serve as the source of electrons for the reduction of the activated N. The products of the incomplete oxidation of the substrates then serve as acceptors of ammonia in the production of amino acids by the bacteroids. These amino acids diffuse away and become available to the host plant." Further, comparative data on nitrogen accumulation in the foliage, the roots and the nodules show that well over 50% of the N accumulates in the tops and much less in the roots and the nodules, although the figures vary for different host species (*see* Allen and Allen, 1958).

Numerous examples of absence of nodulation in legumes are known. While these may be ascribed to the absence of suitable rhizobium strains, the possibility that host-governed limiting factors may be involved is obvious from the work of Manil (*see* Hallsworth, 1958) who demonstrated nodulation in *Trifolium ambiguum* following grafting of *T. repens*. *T. ambiguum* normally did not form nodules. On the other hand, by grafting susceptible red clover scions on to resistant stock and *vice versa*, it has been shown that host factors which may be involved, in resistance or susceptibility were not transferred across graft unions (*see* Allen and Allen, 1958).

A relationship somewhat akin to that in leguminous nodules is seen in the root nodules of non-legumes such as *Alnus*, *Eleagnus*, *Hippophae*, *Shepherdia*, *Casuarina* and *Myrica* (*see* Bond in Hallsworth, 1958; Bond, 1959; Allen and Allen, 1958). Non-leguminous nodules fix nitrogen better than or as well as leguminous nodules. The capacity to fix nitrogen is retained longer by excised nodules of non-legumes than of legumes. Hemoglobin has been found in nodules of *Casuarina* and possibly occurs also in *Alnus* and *Myrica*. Using N^{15} as an indicator, it has been shown that nitrogen compounds are translocated through the xylem in nodulated alder plants (Bond, 1956). The nature of the organisms partaking in the relationship and the physiology of the association need further elucidation.

The symbionts of insects which feed on restricted diets such as wood, blood or plant sap will now be briefly considered (*see* Trager, 1960; Richards and Brooks, 1958; Toth, 1959). Bacteria and yeast-like fungi are associated with many insects and are restricted to specialised cells (mycetocytes) or special organs (mycetomes). That some at least of these associations confer mutual benefits on the partners seems clear from recent experimental work. One of the major steps in the elucidation of the problem has been the use of techniques to obtain aposymbiotic insects for comparison with symbiotic individuals or asymbiotic species. The work on the symbionts of the beetles *Stegobium paniceum* and *Lasioderma serricorne* has shown that these, when infected by their natural symbionts, grew normally on vitamin-deficient synthetic diets which would not support growth of aposymbiotic individuals or other asymbiotic flour beetles. The symbiotic yeasts were shown to supply the insects with biotin, pteroylglutamic acid, thiamine, riboflavine, choline, pyridoxine, pantothenate, nicotinic acid and an essential sterol. The symbiotic yeasts obtained from *Stegobium* and *Lasioderma* are specifically distinct but are not host-specific since they can be interchanged. Similar work has been carried out on roaches. From long and close association with the insect host, the bacteroids of roaches appear to have become part and parcel of a system. It is debatable whether these bacteroids may not really be mitochondria or other organelles, for attempts towards *in vitro* culture of these have failed. However, the fact that these bacteroids are stimulated by metallic ions (Ca, Zn) known to be activators of enzymes strengthens the belief that they are micro-organisms (Brooks, 1960). While the earlier methods of obtaining aposymbiotic individuals cannot be considered perfectly satisfactory, the more recent study of aposymbiotic *Blatella germanica* raised from symbiont-free eggs by feeding parent roaches on a diet of ground dog biscuit and 0·1% aureomycin, has convincingly demonstrated that the aposymbionts would not do well on ground dog biscuit whereas normal roaches would. The symbotic bacteria in these cases not only supply accessory growth factors which can be more or less replaced by a diet high in yeast, but are also probably concerned in the removal of urate which was seen to accumulate only in the aposymbiotic individuals. Insects which feed throughout their life-cycle on blood invariably harbour symbiotic bacteria: a

natural corollary of the fact that blood is low in concentrations of vitamins. The blood-sucking lice (*Pediculus corporis*), the tsetse fly (*Glossina*) and bugs such as *Cimex* and *Rhodnius* are examples. On the other hand, insects having a diet in which microbes are abundant during their larval stage but feeding on blood only as adults (*e.g.*, mosquitoes) lack symbionts. Fixation of atmospheric nitrogen *in vitro* has been claimed for some symbionts of aphids, but studies using N^{15} do not confirm uptake by normal symbiotic aphids. There is evidence that symbionts of insects may be involved in digestion. For instance, the intestinal flora is thought to be responsible for digestion of wax by wax moth larvae (*Galleria*).

Indeed, it is now generally agreed that the digestion of substances such as wax, cellulose and chitin by animals requires the participation of intestinal microflora. In the wood-eating termites intestinal protozoa and sometimes bacteria too are believed to contribute to the association by the secretion of cellulase, although the chemical mechanisms underlying this association need further elucidation. The same may be said of the role of the intestinal microflora and microfauna of ruminants.

The symbiotic nature of lichens has been repeatedly mentioned (*see* Quispel, 1959). The algal symbiont carries on photosynthesis and forms carbohydrates and other organic substances, some of which would be available to the fungus partner. Where one of the symbionts is a blue green alga, fixation of atmospheric nitrogen is also a possibility demonstrated in some cases. There is some evidence that the fungus partner may be supplied with certain growth factors by the alga. On the other hand, the fungus may supply its partner with inorganic salts absorbed by the former. Supply of certain organic substances by the fungus is a possibility which needs more detailed study. There is little doubt that the algae are protected against desiccation by the fungus. Work carried out with N^{15} has shown that lichens in which blue green algae are involved fix atmospheric nitrogen, but not those in which green algae are involved (Bond and Scott, 1955; Scott, 1956). This is quite in keeping with the fact that several blue green algae are now known to fix atmospheric nitrogen (*see* Desikachary, 1959).

We shall now consider the general picture of symbiosis which emerges from the examples covered. Although in most cases the symbionts may be inseparable and interpretation may be impossible for this reason, the negative attribute of one or both partners being unable to survive or complete their full life-cycle in the absence of a relationship is often stressed in defining symbiosis. This is undoubtedly true for some relationships in varying degree. Thus, in the case of the ectotrophic mycorrhizae, the mycorrhizal fungi are unable to produce sporophores in the absence of such a relationship. In the case of certain orchids which lead a partly or wholly saprophytic life, a suitable mycorrhizal association ensures survival; the fungus partner utilises complex and varied forms of carbohydrates from the substrate and makes them

available to the orchid. In the same way, the aposymbiotic insects already referred to are able to survive only when supplied with complex growth factors or through the re-establishment of the symbiont within the insects. The survival of the chlorophyll-less *Monotropa* is achieved through a mycorrhizal relationship and it is likely that the fungus can survive effectively only in association with the roots of the host. In the case of the lichens, attempts have been made to study the symbionts in isolation, but without success. While it is desirable to know more about the independence of the symbionts in isolation, it may be stressed that this need not be the sole criterion for assessment of symbiotic relationships.

The rationale for this approach becomes evident when we consider examples such as the legume-rhizobium symbiosis. The rhizobium appears to be highly specialised in the choice of legumes as its effective partner; on the other hand, its nutritional requirements are neither exacting nor extraordinary. While it is known to survive in soils effectively, it is nevertheless subject to microbial antagonisms, especially from *Streptomyces* spp., and to phage action in soil and even within the nodule. It has been suggested that the legume rhizosphere, but not the rhizosphere of non-legumes, may be a suitable locale for the development and multiplication of rhizobia. This perhaps is not true since there are reports of their occurrence in the rhizosphere of Polygonaceae, Malvaceae, Gramineae, etc. (*see* Manil in Hallsworth, 1958). On the other hand, there have been indications of the presence of factors inhibiting development of rhizobia in legume rhizosphere. Notwithstanding these observations, it is clear that the rhizobia do not show heterotrophy. Likewise, asymbiotic legumes can grow to maturity when supplied with nitrogen and other normal requirements. However, this symbiotic association which is therefore not indispensable for the two partners is essential for symbiotic nitrogen fixation, and indeed for the events which lead to this consummation and are therefore significant. The essence of symbiosis in this case is the new abilities for nitrogen fixation which the "symbiotic system" as a whole has developed, but is not seen in the partners in isolation.

It is also clear that the relationship should be of mutual advantage to the partners. We have already noted that the degree to which each partner within a symbiotic system may benefit would vary. This raises the question of relative benefits derived by each of the partners in a system and whether one may not consider the partner receiving more benefit as a pathogen or at least the more aggressive of the partners. This question appears simple, but it may not be easy to state precisely which partner receives more. In some cases such as the ectotrophic mycorrhiza of pines, the orchid mycorrhizae and the insect symbioses, there is obviously considerable mutual advantage. The price of this mutual advantage is mutual tolerance of individual idiosyncrasies or whims or requirements. It is possible to interpret one of the symbionts (*e.g.*, the rhizobium in root nodules, the fungus in the lichen thallus) as parasitic in that it infects the other partner, but yet normally the symbionts establish between themselves a live-and-let-live association.

When this association has evolved to a stage when it may be considered obligatory, the partners are tied down to the disadvantages that normally flow from it, apart from the advantages already stressed. Thus, the distribution of *Monotropa* may be governed by the distribution of its fungus partner and *vice versa*. The distribution of some species of pines or of *Casuarina* may be restricted by the distribution of their mycorrhizal fungi and *vice versa*. Each partner in symbiosis has to sacrifice something to receive something in return. Indeed, there is no doubt that the ability of both partners to live together in balance without destroying the other or being destroyed is the essence of symbiosis.

That this balance is subtle and delicate becomes clear from examples which are known wherein anything that interferes with this balance changes the relationship altogether from one of symbiosis to parasitism leading to pathogenicity. In the case of the insect *Pediculus* it has been reported that blood containing overdoses of B vitamins or yeast extract is injurious, more so for normal lice than for aposymbiotic ones. The relation of salt composition of diets of cockroach to symbiosis is equally interesting. Mn, Zn and the Ca/Zn ratio of the diets appear to affect the host or symbiont or both in such a way that the association may prove deleterious. The bacteroids in this case could be interpreted to have become pathogenic (*see* Richards and Brooks, 1958). Similar observations have been reported for legume-rhizobium symbioses also. One such well-known observation is that of Brenchley and Thornton (1925) who observed that boron deficiency leads to the absence or weak development of vascular strands in the nodule and the rhizobia then tend to become pathogenic, attacking the protoplasm of the host cell. It was suggested by them that this change was concerned with the loss or reduced supply of carbohydrates normally brought into the nodule by the vascular strands. Thornton (1930) has pictured a defective vascular system in alfalfa due to boron deficiency, and lack of adequate photosynthesis eventually leading to pathogenicity of the rhizobia. Moreover, during nodule senescence and degeneration, which come up as an anticlimax to past events of nodulation and N-fixation, the rhizobia are known to invade the intercellular spaces and the middle lamellae of cell walls and multiply there (*see* Allen and Allen, 1958). During this phase, increased phage activity has been recorded which may partly explain nodule decadence, but we certainly need more information about the causes for nodule decadence and more especially what at this stage may be interpreted as the pathogenic phase of the rhizobium.

Diener (1950) has made the interesting observation that at low levels of phosphate, penetration by rhizobia may take place, but the infection remains latent and no nodules are produced. Thus, infection does not imply symbiosis. It appears pertinent here to consider infectiveness *vis-a-vis* effectiveness of rhizobium strains. An inverse correlation has been claimed for this and appears significant in any consideration of symbiosis *versus* pathogenicity. For, it is possible to interpret extreme ineffectiveness as parasitism leading to pathogenicity or some-

thing akin to it. Evidence is available that truly pathogenic rhizobial strains exist (Garrard and Jordan, 1951; Jordan and Garrard, 1951; van Schreven in Hallsworth, 1958) and it has been suggested that a pathogenic strain may be characterised by either a loss of an essential enzyme system or the ability to elaborate a phytotoxin. Further work may show that effective pectolytic activity may be involved. On the other hand, clipping of clover and other legumes resulted in the shedding of nodules which were replaced only when the plants put forth new growth (Wilson, 1942); there was no evidence of interim pathogenicity. The main point which emerges from considering these facts is that the distinction between pathogenicity and symbiosis is very subtle and is governed not only by the innate nature of the host and the innate nature of the symbiont but also the conditions under which a symbiotic system functions.

Mention should be made of specificity in symbioses. This is a problem yet unsolved. One of the main difficulties is that, in the majority of cases, attempts at separating the symbionts and cross-inoculation have not succeeded. We have similar problems relating to various plant pathogens which show a greater or less extent of specialisation to hosts. The conclusion would perhaps be justified that similar variation in specificity can be seen in symbioses also. The establishment of cross-inoculation groups in the rhizobia is necessitated by such variation. In certain organisms we even come across not only the ability to infect and become pathogenic, but also the tendency to enter into symbiotic relationships. Thus, the ubiquitous fungus, *Corticium solani*, besides being pathogenic to various cultivated plants, is known to be a potential mycorrhizal fungus (*see* Harley, 1959), although differences must exist between the strains involved. *Armillaria mellea* belongs in the same category. Both these fungi show the ability for free spread in soil, the former by ordinary hyphae and the latter by means of rhizomorphs. And yet, pathogenic and "symbiotic" strains exist.

It is therefore tempting to speculate on the evolution of symbiosis. It has been pointed out (*see* Garrett, 1956) that an evolutionary series from the obligate soil saprophytes to the "ecologically obligate parasites" whose relationship with the host is one of symbiosis can be visualised. The mycorrhizal habit is pictured as the end product of specialized parasitism. The truth of this thesis is apparent in several cases, but it may be difficult to state precisely whether the evolution has been in this direction or in the opposite direction. In the case of *Rhizobium* the possibility of its having developed from organisms originally capable of non-symbiotic nitrogen fixation has been indicated by Derx (*see* Jensen in Hallsworth, 1958). Due to the morphological and cultural resemblance of *Rhizobium* to *Beijerinckia*, the hypothesis has been put forward that rhizobia are evolved from *Beijerinckia* by the twin processes of acquirement of root-infecting power and the loss of nitrogen-fixing ability *in vitro*. The idea of a primitive group of rhizobia with wide "promiscuity" is not new and has found favour with several students (*see* Jensen and *see* Norris in Hallsworth, 1958).

Norris considers the cowpea type of rhizobium as known today to be the ancestral and typical form of the organism, the *Pisum-Trifolium* type with its narrow symbiotic range and its special requirements of high soil pH and available calcium being the "advanced-degenerate" type. In the case of mycorrhizae, it has been suggested that the mycorrhizal habit might allow the survival of auxotrophic forms and that the host plant growing in nature would then become obligately mycorrhizal. In some orchids, the spontaneous appearance of albinos has been noted; the albino seedlings soon exhaust the food reserve in the seeds and eventually die. Plants associated with mycorrhiza may survive despite permanent loss of photosynthetic ability. It is not unlikely therefore that saprophytic orchids might have originated in this manner (Hutner, 1953) and, what is more pertinent to this discussion, that the mycorrhizal habit might have originated in this manner. The participants in the symbiosis obviously are thrown together with perhaps loss of certain independent functions, and mutual alignment becomes a necessity for survival. According to Norris (in Hallsworth, 1958), bacterial symbiosis in legumes must have arisen under wet tropical conditions of low availability of essential soil nutrients. In the case of the leguminous tree, *Gilbertiodendron*, the interesting situation has been reported that throughout the virgin part of the forest in the central Congo Basin, only mycorrhiza but no nodules are seen on the roots; on river banks and beside roads, on the other hand, nodules were seen. These observations are considered to indicate that rhizobial symbiosis is an adaptation by the Leguminosae to a shortage of nitrogen (*see Hallsworth, 1958, p. 212*). In the case of lichens, attempts at studying the algal and fungal partners in isolation have failed. Attempts at synthesis by bringing together forms similar to these symbionts have also failed. The similarity of the free-living members of these two groups and their morphological counterparts in lichen thalli is thus more apparent than real. The possible lines of evolution of the symbiotic relationship in these cases can be suggested and evidence obtained for heterotrophic tendencies in the partners. It is hardly necessary, however, to consider the stages in evolution leading to heterotrophy of the partners as a downhill process since loss of certain obvious functions may be accompanied by new synthetic abilities as well. For example, with reference to the utilization of exogenous glucose by heterotrophs, Hutner (1953) has outlined the following sequence of changes: "First, the phosphorylating enzyme for glucose, hexokinase, must be elaborated; second, an investment of energy must be made when phosphate is transferred to glucose from adenosine triphosphate. As hexokinase is not concerned with the endogenous synthesis of glucose from triose precursors, it may be a newly elaborated enzyme in heterotrophs. In the end, teleologically speaking, the investment of energy is repaid with interest, else there would be no advantage in heterotrophy. It seems likely that even simple substrates such as acetate require a preliminary phosphorylation if they are to be swept into the stream of metabolism and the steep diffusion gradients necessary for their efficient absorption maintained. By this view, autotrophy may be obligate in some plants which lack phosphorylases for external substrates."

Although no definition of symbiosis has been attempted by us and it has been held by some that true symbiosis does not exist, we may take the unconventional view that symbiosis is one of the commonest phenomena in nature. Past studies on nutritional requirements of the "autotrophic" higher plants, for instance, are deficient in the use of aseptic cultures; and evidence is on hand indicating mutual beneficial interactions between plant root systems, for instance, and their rhizosphere microflora. Again, one might concur with Hutner (1953) that "the coupling of photosynthesis with *complete* autotrophy is a historical accident: the first studied higher plants and algae happened to grow in mineral media. The degree of truth in the text-book statements that higher plants need only minerals, light, water, and CO_2 cannot yet be estimated". Thus, it becomes obvious that numerous truly symbiotic phenomena can be counted. Pathogenicity may then be interpreted as the outcome of events interfering with the balance in any symbiotic system. Indeed, the shift from symbiosis to pathogenicity is essentially reversible and it is perhaps futile to speculate which of these is the primitive condition and formulate a general hypothesis.

In concluding, let us state that these thoughts are purely speculative and what appears important for the future is to focus attention on the many little known symbiotic phenomena with a view to elucidate the physiology of the symbiotic system. With the aid of the many tools and techniques which are at the command of the biologist today, considerable progress in the elucidation of this fascinating problem can be expected in the coming decade.

We are grateful to Professor J. Venkateswarlu and Professor T. S. Sadasivan for giving us the opportunity to participate in this symposium.

REFERENCES

ALLEN, E. K. AND O. N. 1958. Biological aspects of symbiotic nitrogen fixation. In W. Ruhland (Ed.), *Encyclopaedia of Plant Physiology* **8**: 48–118.

BERGERSEN, F. J. 1960. Biochemical pathways in legume root nodule nitrogen fixation. *Bact. Rev.* **24**: 246–50.

BJÖRKMAN, E. 1944. The effect of strangulation on the formation of mycorrhiza in pine. *Svensk bot. Tidskr.* **38**: 1–14.

BOND, G. 1956. Some aspects of translocation in root nodule plants. *J. exp. Bot.* **7**: 387–94.

———. 1959. Fixation of nitrogen in non-legume root nodule plants. *Symp. Soc. exp. Biol.* **13**: 59–72.

—— AND SCOTT, G. D. 1955. An examination of some symbiotic systems for fixation of nitrogen. *Ann. Bot, Lond.*, N.S. **19**: 67–77.

BRENCHLEY, W. E. AND THORNTON, H. G. 1925. The relation between the development, structure and functioning of the nodules on *Vicia faba*, as influenced by the presence or absence of boron in the nutrient medium. *Proc. roy. Soc., Lond.* **98 B**: 373–99.

BROOKS, M. A. 1960. Some dietary factors that affect ovarial transmission of symbiotes. *Proc. helminth. Soc. Wash.* **27**: 212–20.

DESIKACHARY, T. V. 1959. *Cyanophyta.* Indian Council of Agricultural Research, New Delhi, 686 pp.

DIENER, T. 1950. Ueber die Bedingungen der Wurzelknöllchenbildung bei *Pisum sativum L. Phytopath. Z.* **16**: 129–70.

GARRARD, E. H. AND JORDAN, D. C. 1951. Does parasitism exist among the legume nodule bacteria? *Agric. Inst. Rev.* **1951**: 1–3.

GARRETT, S. D. 1956. *Biology of Root-infecting Fungi.* Cambridge University Press, London, 293 pp.

HALLSWORTH, E. G. (Ed.). 1958. *Nutrition of the Legumes.* Butterworth Scientific Publications, London, 359 pp.

HARLEY, J. L. 1959. *The Biology of Mycorrhiza.* Leonard Hill (Books) Ltd., London, 233 pp.

HUTNER, S. H. 1953. Comparative physiology of heterotrophic growth in plants. In W. E. Loomis (Ed.), *Growth and Differentiation in Plants,* The Iowa State College Press, Ames., pp. 417–46.

JORDAN, D. C. AND GARRARD, E. H. 1951. Studies on the legume root nodule bacteria. I. Detection of effective and ineffective strains. *Canad. J. Bot.* **29**: 360–72.

MELIN, E. 1953. Physiology of mycorrhizal relations in plants. *Annu. Rev. Pl. Physiol.* **4**: 325–46.

QUISPEL, A. 1959. Lichens. In W. Ruhland (Ed.), *Encyclopaedia of Plant Physiology* **11**: 577–604.

RAGGIO, M. AND N. 1962. Root nodules. *Annu. Rev. Pl. Physiol.* **13**: 109–28.

RICHARDS, A. G. AND BROOKS, M. A. 1958. Internal symbiosis in insects. *Annu. Rev. Entomol.* **3**: 37–56.

SCOTT, G. D. 1956. Further investigations of some lichens for fixation of nitrogen. *New Phytol.* **55**: 111–16.

SLANKIS, V. 1958. The role of auxin and other exudates in mycorrhizal symbiosis of forest trees. In *The Physiology of Forest Trees,* by K. V. Thimann (Ed.), Ronald Press Co., N.Y., pp. 427–43.

———. 1961. On the factors determining the establishment of ectotrophic mycorrhiza of forest trees. *Recent Advances in Botany,* Univ. Toronto Press, pp. 1738–42.

THIMANN, K. V. 1955. *The Life of Bacteria.* The Macmillan Co., N.Y., 775 pp.

THORNTON, H. G. 1930. The early development of the root nodule of lucerne (*Medicago sativa* L.). *Ann. Bot., Lond.* **44**: 385–92.

———. 1954. The nodule bacteria and their host legumes: some problems that they still present. *Sci. Progr.* **166**: 185–204.

TOTH, L. 1959. Plant-animal symbioses. In W. Ruhland (Ed.), *Encyclopaedia of Plant Physiology* **11**: 639–54.

TRAGER, W. 1960. Intracellular parasitism and symbiosis. In J. Brachet and A. E. Mirsky (Ed.), *The Cell.* **4**(1): 151–213.

VIRTANEN, A. I., MOISIO, T. AND BURRIS, R. H. 1955. Fixation of nitrogen by nodules excised from illuminated and darkened pea plants. *Acta. chem. Scand.* **9**: 184–86.

WILSON, J. K. 1942. The loss of nodules from legume roots and its significance *J. Amer. Soc. Agron.* **34**: 460–71.

ABNORMAL GROWTH AND PARASITISM

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Parasitism

Parasitism by certain organisms causes disease in plants and animals. Besides the commonly occurring symptoms on the host, *viz.*, necrosis, canker, wilt, rot, atrophy, etc., the host tissues may show an abnormal growth, forming galls, tumours and witches brooms. Also caused by the parasite there are sometimes some profound secondary effects on the host which result in the transformation of various organs especially the floral parts into curious structures.

Many higher plants are dependent on mycorrhiza and the plants may not survive in the absence of the fungal partner. The fungus acts as an intermediary, assisting in the absorption of food substances from the soil, mainly for the benefit of the host. The Indian Pipe plant—*Monotropa* (Pyrolaceae)—is regarded as a saprophyte, existing on decomposing organic matter. In fact, *Monotropa* is mycorrhizal and parasitic on a root-inhabiting fungus. Probably this is the case with other mycorrhizal plants also. It presents, therefore, an unusual situation where a higher plant is parasitic on the fungus.

Calluna vulgaris Salisb. (Ericaceae) is mycorrhizal. The fungus grows profusely in and upon the roots and it may even penetrate the other parts of the plant. The fungus has an ability to fix nitrogen and the plant does not survive in its absence. Nevertheless, in the presence of an abundance of soil nitrogen, the fungus becomes parasitic and kills the host plant (Rayner, 1929). Such an association, therefore, points to the complexity of the relationship and the delicacy of the balance which exists between the two associates—higher plant and the fungus.

Interesting examples of dependent and parasitic relationship exist between the gametophytic and sporophytic generations of plants. In the bryophyta, the sporophyte is parasitic on the gametophyte by means of a conspicuous haustorium—the foot. There is some evidence, however, that the sporophyte can assimilate food materials and may even pass them to the gametophyte (Fulford, 1948). In any case, the sporophyte is always parasitic on the gametophyte for water and minerals. The dominant phase is the sporophyte in pteridophytes and it depends upon the independent, rather inconspicuous gametophyte, at least in early stages. In seed plants, the gametophyte is parasitic on the sporophyte. The relationship of the two generations, therefore, is

comparable to that of a parasite and its host. Hence, it may be worthwhile to seek answers of some of the questions involving parasitism in this field of inter-generation relationship.

Besides these, parasitism occurs in the algae, fungi and flowering plants. *Cephaleuros* is an algal parasite causing red rust and canker on tea and other hosts. The parasitic mode of life, however, is more prominent and well established in fungi. That this dependent mode of life is not harmful in any way to the fungi is revealed in the progress and prosperity of this group from an evolutionary standpoint as well as in number of species. Turning to the flowering parasitic plants, the chlorophyllous parasites are dependent on their host chiefly for water and minerals. Others depend completely on the host for their requirements.

The vegetative multiplication of *Aeginetia indica* (Orobanchaceae) recalls the highly efficient, polycentric mode of development and reproduction in *Physoderma*. Rhizomycelium of *Physoderma* forms storage cells which give rise to sporangia and rhizomycelium. Rhizomycelium, in turn, repeatedly gives rise to storage cells, sporangia and rhizomycelium. Similarly, a seed of *Aeginetia*, germinating on the host root gives rise to a flowering scape and a large number of fibrous roots. The fibrous roots, upon contacting a host root, repeatedly give rise to flowering scape and the fibrous roots.

Dodders or *Cuscuta* spp. have a serpent like coiled embryo. Upon germination it becomes erect, assumes the attitude of a serpent in a striking position. Its free end moves and coils around the host. It falls down, if a host is not encountered but tries again and again till it can infect a suitable plant. Further, the embryo has the capacity to elongate itself at the cost of the material stored in its hinder part. On the woody plants, dodder's haustoria perennate, regenerating at the approach of favourable season. Dodders are loose parasites and grow on a wide variety of hosts. It has not to face, therefore, the consequences of over-specialization, *viz.*, an ultimate extermination. To a great length, all this reveals the urge and determination of the parasitic plants to live successfully.

Abnormal Growths

Agencies causing the abnormal growth in plants are insects, nematodes, fungi, bacteria, viruses and various non-parasitic factors. Galls or tumours are localised outgrowths in which the cells are stimulated to excessive growth by the parasite. Enlargement of cells-hypertrophy or cell division-hyperplasy, or both, may result in galls. Galls may be simple anatomically or may show abnormal structures, very different from those found in normal tissues.

Galls are storage tissues for the use of the alien invader the parasite. Many galls are also used as food by man. *Albugo* galls on *Ipomoea reptans*; young *Ustilago* galls on maize and wild rice; *Sorosporium*

galls on sorghum and tuberous aecidial galls on *Urtica parviflora* are used as food by man.

Galls have been classified variously, depending upon the agent causing the gall, their pattern of development, morphology, and histology. Braun (1954) recognises two types of abnormal growths: self-limiting, possessing a definite pattern of development and non-self-limiting tumours in which cells proliferate irregularly and are transplantable. The diseased cells acquire autonomous development which permits their growth indefinitely. The nature of this acquired autonomy is interesting since its study may be helpful in an understanding of similar cancerous growths in man and animals. Crown gall caused by *Agrobacterium tumefaciens*, on a large number of wild and cultivated plants are of this nature. On the other hand, the self-limiting galls include root nodules, nematodes root-knots, insect, bacterial viral and fungal galls. Butler and Jones (1949) classify abnormal growths as histoid and organoid. The former, due to a local action of the parasite while the latter result from systemic infections and induce changes in habit and symmetry of the host. Under the influence of *Puccinia butleri*, *Launaea asplenifolia* develops an elongated many branched leafy stem. Similarly, the house-leek becomes an erect leafy plant under the influence of *Endophyllum*. Gaümann (1950) recognises four types of abnormal growths: gall, tumour, witches brooms and the activated rudimentary sexual organs.

Gall forming fungi: (Bessey, 1950; Brocks, 1953; Butler and Jones, 1949; Fischer and Holton, 1957; Gaümann, 1950, 1952; Horsfall and Dimond, 1959; Mundkur, 1959; Subramanian, 1959).—*Plasmodiophora brassicae* causes the well-known clubroot or finger and toe disease on the roots of Crucifers. There are reports of its occurrence in India also. *Sorodiscus radicicola* forms convoluted coral-like galls on the roots of *Gynandropsis gynandra* (Karling, 1942). Observations made at Jodhpur reveal that the affected plants apparently do not differ in any way from the healthy ones. The galls originate in the cortex. Their vasculature, however, is connected with the central stele of the root. *Sorosphaera veronicae* incites galls on *Veronica chamaedrys*. The galls, however, are restricted to the vascular region of the host. Swellings develop at the apex in the procambial region of the shoot, extending backwards in xylem and outwards to the young leaf bundles.

Many species of *Physoderma* are gall-forming ones. *P. alfalfae* causes crown wart of lucerne, *P. limnanthemi* (Thirumalachar, 1949) forms tuberous galls on the leaf, stem and flowers of *Limnanthemum indicum*. The profuse vascular supply of the gall originates from a cortical bundle of the host. The gall is filled with a large number of irregular sori containing sporangia (Rao, 1962). The galls on *Aeschynomene indica* caused by *P. aeschynomenis* appear to be very similar to those on *Limnanthemum* (Thirumalachar and Whitehead, 1951). *P. corchori* attacks the jute plant, *Corchorus olitorus* and pro-

duces hemispherical dark-brown galls on the stem and leaf midribs (Lingappa, 1955).

Synchytrium occurs on a large number of hosts. Some of the simplest galls which may be confined to a single hypertrophied cell are due to this fungus (Karling, 1955). *S. endobioticum*, now also known from Darjeeling, India, is a gall and witches broom-forming fungus on potato tuber.

Albugo candidus is responsible for very pronounced hypertrophies and abnormal growth on the stem and flowers of Crucifers. Many other species of *Albugo* have similar effects on their hosts. *Peronospora* causes abnormal growths on the effected plant parts. *Exobasidium* resembles *Albugo* and *Peronospora* in its biological effects on the host-producing galls and witches brooms on some hosts. *Protomyces* is a well-known gall-forming fungus. It is found on the stem, leaf and flowers of Umbelliferae and Compositae. *Protomycopsis* converts the leaflets of *Sesbaenia* into swollen sac-like structures full of sporangia of the parasite.

Many smuts cause galls on their host plants. Large tumours due to *Ustilago maydis* on maize are well known. Mundkur (1938) described root galls caused by *Urocystis brassicae*. *Tilletia tumefaciens* is another remarkable gall-forming smut on *Panicum antidotale* (Mundkur, 1944). *Melanotaenium lamii* produces tuberous galls on the base of the stem and upper roots of *Lamium album*. *Ustilago emodensis* forms large woody galls on stem of *Polygonum*. *Doassansia* causes galls on the leaves of *Sagittaria*. *Melanopsichium* galls are of hard charcoal-like consistency on the soybean pods (Whitehead and Thirumalachar, 1960).

Many rusts are also responsible for inciting galls on their hosts. *Gymnosporangium-juniperi virginianae* is a well-known example. The perennial telial mycelium in the shoots of the red cedar produces large galls—the cedar apples. *Uromyces hobsoni* attacks the leaf, stem and flowers of *Jasminum* and forms orange-coloured irregular cushion-shaped growths resulting in distortion and curling of the shoots (Thirumalachar, 1939). On *Urtica parviflora*, *Aecidium urticae* forms large tuberous galls. *Hapalophragmiopsis* causes woody galls on *Acacia*.

A number of fungi cause certain abnormal growths known as witches brooms. Under the influence of the parasite, dormant buds are incited to develop into a large number of closely grouped more or less parallel clusters of slender branches. *Pericladium* causes witches brooms on many species of *Grewia* (Mundkur and Thirumalachar, 1952). *Sphaeropsis tumefaciens* forms knots and brooms on lime in Rajasthan (Prasad and Bhatnagar, 1960). *Taphrina* produces witches brooms and plum pockets of the fruit on some temperate fruit trees. Witches brooms of 'deodar' are caused by *Peridermium cedri*. Brooms are also quite common on mango and bamboo inflorescences.

Profound secondary effects are produced on the host by certain fungi. *Ustilago violacae* induces growth in the staminodes in female

flowers of certain Caryophyllaceae and fills them with smut spores. Similarly, *U. vaillantii* causes the neuter flowers of grape hyacinth to develop stamens, full of smut spores. *Sphacelotheca reiliana* transforms the stamens and ovaries of Sorghum into leafy structures. *Sorosporium reilianum* also transforms the whole staminate head of maize into leafy structures. 'Bajra' attacked by *Sclerospora graminicola* converts the solid spicate ear into a loose green head composed of a mass of twisted green leaves.

I am thankful to Professor C. V. Subramanian and Professor T. S. Sadasivan for encouragement; Professor J. Venkateswarlu for inviting me to participate in this symposium.

REFERENCES

BESSEY, E. A. 1950. *Morphology and Taxonomy of the Fungi*, Philad.

BRAUN, A. C. 1954. Abnormal and pathological plant growth. *Brookhaven Symp. in Biol.* New York, No. 6, 115–27.

BROOKS, F. T. 1953. *Plant Diseases.* Oxford.

BUTLER, E. J. AND JONES, S. G. 1949. *Plant Pathology*, London.

FISCHER, G. W. AND HOLTON, C. S. 1957. *Biology and Control of the Smut Fungi*, New York.

FULFORD, M., 1948. Recent interpretations of the relationships of the Hepaticae. *Bot. Rev.* **14**: 127–73.

GAÜMANN, E. 1950. *Principles of Plant Infection*, London.

———. 1952. *The Fungi*, New York.

HORSFALL, J. G. AND DIMOND, A. E. 1959. *Plant Pathology—An Advanced Treatise*, Vol. I, New York.

KARLING, J. S. 1942. *Plasmodiophorales*, New York.

———. 1955. *Synchytrium ranunculi* Cook. *Mycologia* **47**: 130–39.

LINGAPPA, B. T. 1955. Two new species of *Physoderma* from India. *Ibid.*, **47**: 109–21.

MUNDKUR, B. B. 1938. Host range and identity of the smut causing root galls in the genus *Brassica*. *Phytopathology* **28**: 134–42.

———. 1944. *Tilletia tumefaciens*—a remarkable gall-forming smut from India *Ibid.* **34**: 143–46.

—— AND THIRUMALACHAR, M. J. 1952. *Ustilaginales of India*, Kew.

MUNDKUR, B. B. 1959. *Fungi and Plant Disease*, London.

PRASAD, N. AND BHATNAGAR, G. C. 1960, *Sphaeropsis* knots on lime (*Citrus medica* var. *acida* Linn.) in Rajasthan. *Curr. Sci.* **30**: 110–11.

RAO, C. G. P. 1962. Anatomical studies on fungal galls. I. Preliminary observations on *Phydoderma* on *Limnanthemum indicum*. *Phytomorphology* **12**: 201–04.

RAYNER, M. C. 1929. Seedling development in *Calluna vulgaris*. *New Phytol.* **28**: 377.

SUBRAMANIAN, C. V. 1959. Physiology of disease in plants. *J. Madras Univ.* **29 B**: 9–44.

THIRUMALACHAR, M. J. 1939. Rust on *Jasminum grandiflorum*. *Phytopathology* **39**: 783–92.

———. 1949. A chytridiaceous parasite of *Limnanthemum indicum*. *Indian Phytopathology* **2**: 128–30.

—— AND WHITEHEAD, M. D. 1951. An undescribed species of *Physoderma* on *Aeschynomene indica*. *Mycologia* **43**: 430–36.

WHITEHEAD, M. D. AND THIRUMALACHAR, M. J. 1960. An undescribed smut disease of soybeans. *Ibid.* **52**: 189–92.

MORPHOLOGY AND ANATOMY OF THE ROOT NODULES IN ANGIOSPERMS

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In conformity with the subject of the symposium it is consistent to dwell on the morphology and anatomy of a structure produced by the symbiotic life of micro-organisms and the root nodules in angiosperms provide an ideal material for such a consideration. The nodule is a desirable hypertrophy produced by mutual interaction of a micro-organism and a host on leaves, stems and roots of higher plants. It is the seat of biological fixation of vast stores of atmospheric nitrogen in contrast to the expensive, artificial fixation through several chemical processes. The nodules not only promote the vigorous growth of the host by providing nitrogen but also add to the soil nitrogen.

Although root nodules are frequently associated with the legumes, they are not uncommon in certain genera of unrelated families of the Archichlamydeae, such as the Betulaceae, Myricaceae, Casuarinaceae, Elaeagnaceae. Coriariaceae and Rhamnaceae. In the Leguminosae, out of 438 genera and 10,000 species only 1,112 species of 213 genera bear nodules while 116 species of 30 genera are without them (Allen and Allen, 1959). Amongst the non-legumes 190 out of 258 species bear nodules (Bond, 1958).

On the basis of the amount of nitrogen fixed, two types of nodules are recognized: (1) the *effective* nodule, fixing more nitrogen; and (2) the *ineffective* nodule, fixing much less nitrogen or none. Further, the nodules may be endogenous or exogenous, depending on the participation of stelar tissue in their formation. Among these investigated, the nodules of *Arachis hypogaea* (Allen and Allen, 1940) and *Aeschynomene indica* (Arora, 1954) are endogenous. In *Vigna catjang* and *Dolichos biflorus* (Narayana, unpublished) although the effective nodules are restricted to the primary and secondary roots and the ineffective nodules to the secondary and tertiary roots; the former are not infrequent on tertiary and quaternary roots. Effective nodules are pink, large, spherical, cylindrical, lobed or frequently branched into fan-shaped structures, whereas ineffective ones are quite colourless, small and never lobed or branched.

Six species of *Rhizobium* are recognised as causing nodulation in legumes. However, the identity of the organisms causing nodulation in non-legumes is debatable and has been variously held to be bacteria, filamentous fungi, Plasmodiophorales, or different species of Actinomycetes.

The Genesis of the Leguminous Root Nodule

Entry of rhizobia is mostly through the root hairs which are curved (Thornton, 1930; Bond, 1948; Harris, Allen and Allen, 1949; Arora, 1956 *b* and *c*; Gothwal, 1962; Narayana, 1963) or less frequently through the ruptured cortex at the vicinity of rootlet emergence as in *Arachis hypogaea* (Allen and Allen, 1940) and *Aeschynomene indica* (Arora, 1954), or through the epidermal cells alone as in *Neptunia oleracea* (Schaede, 1940). Entry through root hairs has been seen by me in *Vigna catjang* and *Dolichos biflorus* (Figs. 1, 4). Two or more infected root hairs may initiate a nodule as in *Medicago sativa* (Thornton, 1930), *Cicer arietinum* (Arora, 1956 *c*), *Phaseolus mungo* (Gothwal, 1962) and in *Vigna catjang* (Narayana, unpublished).

The number of the infection threads and their behaviour in root hairs are variable. Usually, the single thread is unbranched in the root hair, but branches after it enters the cortex. However, in *Medicago sativa* (Thornton, 1930) and *Canavalia gladiata* (Gothwal, 1962) the single infection thread branches in the root hair itself. In *Phaseolus vulgaris* (McCoy, 1929), *Glycine max* (Bieberdorf, 1938) and *Phaseolus mungo* (Gothwal, 1962), and *Vigna catjang* (Fig. 1) and *Dolichos biflorus* (Narayana, unpublished) a cluster of two or more infection threads are seen in a root hair.

The infected root hair may enlarge three to five times its original size and its wall may become thickened as in *Canavalia gladiata, Phaseolus mungo* (Gothwal, 1962), *Vigna catjang* and *Dolichos biflorus* (Narayana, unpublished). However, in *Trigonella foenum-graecum* (Gothwal, 1962) the hypodermal cell enlarges greatly, but not the root hair. The cells of the root cortex through which the thread traverses have been reported to be generally larger than the adjacent cells, and this is confirmed by our work on *Trigonella foenum-graecum* and *Cyamopsis tetragonolobus* (Gothwal, 1962; Narayana, 1963). The precise cause for this needs study.

The penetration of the cortex by the thread is intracellular and the depth of penetration may or may not have a correlation with the structure of the root. In *Glycine max* (Bieberdorf, 1938), *Trigonella foenum-graecum* and *Canavalia gladiata* (Gothwal, 1962) the infection thread penetrates three to four cell layers of the thick cortex of the tap root, but in the thin cortex of the lateral root it penetrates down to the layer adjacent to the endodermis, thus traversing five to six layers. A reverse condition is met with in *Cicer arietinum* (Arora, 1956 *c*). On the other hand, in *Pisum sativum* (Bond, 1948) and *Phaseolus mungo* (Gothwal, 1962) the thread penetrates up to the middle cortex irrespective of its thickness both in the thick tap root and in the thin lateral roots.

The infection thread bears blister-like swellings along its side. In *Medicago sativa* (Thornton, 1930) and *Cicer arietinum* (Arora, 1956 *c*) it is enclosed by a cellulose sheath only in the cortical cells and not in the root hair. This sheath is considered to have been deposited by the host cytoplasm as a defensive mechanism against the invading rhizobia,

and not by the rhizobium itself since it is absent in the region of middle lamella and its chemical composition is similar to that of the host cell wall (McCoy, 1932). The thread moves towards the nucleus of the cell, often enclosing the latter and apparently without causing any damage.

In most cases the infection thread forms a funnel-shaped swelling close to the cell wall through which the rhizobia pass into the adjacent cell. Several explanations such as physical effects (McCoy, 1929), shrinkage of the 'zoogleal mass' (Thornton, 1930) and accumulation of rhizobia (Harris, *et al.*, 1949) have been put forward to account for this swelling. However, the indistinctness of the funnel-shaped swellings in the newly infected cells and their distinctness close to the wall in the enlarged infected cells appear to suggest that the swellings of the infection thread are the consequence of emaciation of the thread in the middle as a result of enlargement of the infected cells (Narayana, 1963).

Rhizobial spread in host tissues may be due to invasion of the newly produced cells (Harris, *et al.*, 1949; Arora, 1956 *c*; Gothwal, 1962; Narayana, 1963; Narayana, unpublished) or to the division of infected cells (McCoy, 1929; Allen and Allen, 1940; Arora, 1954), or both (Bond 1948; Arora, 1956 *a*). The rhizobia are released into the cell by the bursting of the blister-like swellings of the threads. The nucleus shows signs of hypertrophy thereafter.

On the basis of the tetraploid nature of the infected cells in legumes, Wipf and Cooper (1938, 1940) have hypothesized that disomatic cells are essential for release of rhizobia, tissue proliferation and nodule formation. Arora (1956 *a* and *c*) has observed both tetraploid and diploid cells composing the nodule meristem in *Crotalaria juncea* and *Cicer arietinum*. However, McCoy (1929) and Thornton (1930) consider that the rhizobia elaborate some diffusible substance which stimulates much hypertrophy and cell division.

The mature nodule consists of four regions: the nodule meristem, the nodule cortex, the vascular system and the bacteroid zone. It does not emerge through the root cortex as the rootlet does for the root cortex that surrounds the nodule sloughs off and exposes the nodule. However, in *Aeschynomene indica* (Arora, 1954) the root cortex persists around the nodule.

The nodule meristem may be distally prominent as in *Vicia villosa* (Bieberdorf, 1938), *Medicago sativa* (Thornton, 1930), *Pisum sativum* (Bond, 1948), *Caragana arborescens* (Allen, Gregory and Allen, 1955), *Crotalaria juncea* and *Cicer arietinum* (Arora, 1956 *a* and *c*), *Trigonella foenum-graecum* (Gothwal, 1962), and *Cyamopsis tetragonolobus* (Narayana, 1963) or may be peripherally diffused as in *Phaseolus vulgaris* (McCoy, 1929), *Vigna sinensis* and *Arachis hypogaea* (Bieberdorf, 1938), *Aeschy-nomene indica* (Arora, 1954), *Phaseolus mungo* (Gothwal, 1962) and *Vigna catjang* (Narayana, unpublished). In *Dolichos biflorus* the meristem is diffused in the beginning (Fig. 5) and the nodule is spherical

(Narayana, unpublished). Within 10 to 15 days of inception of the nodule, the meristem becomes prominent at the distal end leading to the development of a cylindrical nodule. Thus, the relative position and activity of the meristem determine the shape of the nodule (spherical, cylindrical or irregularly branched), a distally prominent meristem producing a cylindrical nodule and a peripherally diffused meristem giving rise to a somewhat spherical nodule. When the nodules are branched, the original meristem is split into as many bits as there are branches and usually appears to be distally prominent.

The nodule cortex may be uniformly parenchymatous as in *Vigna catjang* and *Dolichos biflorus* (Narayana, unpublished) or may be heterogeneous with the middle sclerenchyma sandwiched between the inner and the outer parenchyma as in *Sesbania grandiflora* (Harris, *et al.*, 1949) and *Canavalia gladiata* (Gothwal, 1962). There may be tannin- or resin-filled cells as in *Dolichos biflorus* (Figs. 4, 5); the periderm may be continuous or discontinuous with that of the root. These protect the inner lying cells of the nodule from the invasion of extraneous micro-organisms.

The vascular supply of the nodule appears as the procambial strands formed by the divisions of the cortical cells of the root below the region of infection. The procambial strands either extend towards the root stele (Figs. 2, 4) or are connected with the root stele by the radial elongation of cells produced by the periclinal divisions of the pericyclic cells opposite the xylem and phloem of the root (Bond, 1948).

The vascular bundle is composed of xylem tracheids, phloem fibres, sieve tubes and companion cells surrounded by a parenchymatous sheath and endodermis.

There is variation in the number and orientation of vascular bundles that supply the nodule. One vascular bundle supplies the nodule in *Glycine max* (Bieberdorf, 1938), *Sesbania grandiflora* (Harris, *et al.*, 1949) and *Aeschynomene indica* (Arora, 1954), and *Phaseolus mungo* (Gothwal, 1962); two in *Vicia villosa* (Bieberdorf, 1938), *Pisum sativum* (Bond, 1948), *Cajanus indicus* (Arora, 1956 *a*), *Canavalia gladiata* (Gothwal, 1962) and *Vigna catjang* and *Dolichos biflorus* (Fig. 5) (Narayana, unpublished); two to five in *Crotalaria juncea* (Arora, 1956 *a*); three to five in *Cicer arietinum* (Arora, 1956 *c*) and four in *Trigonella foenum-graecum* (Gothwal, 1962) at different levels originating from more than one protoxylem point. Further, the vascular strand branches as it extends towards the distal end of the nodule and forms a skeletal mantle around the bacteroid tissue and this process keeps pace with the growth of the nodule. The number of the strands vary at different levels of the nodule: 6 in *Aeschynomene indica* (Arora, 1954), 7–8 in *Phaseolus mungo*, 10–11 in *Canavalia gladiata* (Gothwal, 1962), 7–18 in *Dolichos biflorus* and 12 in *Vigna catjang* (Fig. 3), 20 in *Cicer arietinum* (Arora, 1956 *c*), *Caragana arborescens* (Gregory, 1949; cited by Allen and Allen, 1954), 20–24 in *Trigonella foenum-graecum* (Gothwal, 1962) and as many as 126 in a one-year-old nodule

of *Sesbania grandiflora* (Harris, *et al.*, 1949). Differences in orientation have also been recorded: the strands are inversely collateral in *Pisum sativum* (Bond, 1948) and *Trigonella foenum-graecum* (Gothwal, 1962); amphicribral in *Glycine max* (Bieberdorf, 1938), *Sesbania grandiflora* (Harris, *et al.*, 1949) and *Canavalia gladiata* (Gothwal, 1962); both collateral and amphicribral in *Aeschynomene indica* (Arora, 1954); amphicribral or bicollateral at the base and inversely collateral higher up in *Crotalaria juncea, Cicer arietinum* (Arora, 1956 *a* and *c*); amphicribral at the base and amphicribral or bicollateral higher up in *Phaseolus mungo* (Gothwal, 1962), *Vigna catjang* (Fig. 3) and *Dolichos biflorus* (Fig. 5) (Narayana, unpublished) and diarch in *Caragana arborescens* (Allen, *et al.*, 1955). Wherever there is distal meristem in the nodule, the vascular bundles do not fuse at the tip. In *Phaseolus vulgaris* (McCoy, 1929), *Vigna sinensis* (Bieberdorf, 1938), *Phaseolus mungo* and *Canavalia gladiata* (Gothwal, 1962), *Dolichos biflorus* and *Vigna catjang* (Narayana, unpublished) where there is no distal meristem, the vascular bundles exhibit distal anastomosis.

In some species (Bond, 1948) a layer of cells with Casparian strips delimits the nodule cortex besides the one that surrounds the xylem and phloem. This layer of "nodule endodermis" is considered to restrict exchange of gases and nutrients within the bacteroid area.

The extent of infected cells in the bacteroid tissue is variable: about 100% in *Vigna sinensis, Arachis hypogaea* (Allen and Allen, 1954) and *Aeschynomene indica* (Arora, 1954), 50–70% in *Vicia, Sesbania,* and *Caragana* (Allen and Allen, 1954) and *Canavalia gladiata, Trigonella foenum-graecum,* and *Phaseolus mungo* (Gothwal, 1962) and 20–60% in *Cajanus indicus, Cicer arietinum* (Arora, 1956 *b* and *c*). In *Dolichos biflorus* and *Vigna catjang* the infected cells are 25% and 55% respectively while it has been suggested (Thornton, 1954) that the effectiveness of nodules is proportional to the volume of bacteroid tissue, no attempts appear to have been made towards a correlation between percentage of cells infected and nitrogen fixation by various genera.

In some arborescent members (Harris, *et al.*, 1949; Allen, *et al.*, 1955) it has been found that certain large nodules bear rootlets each of which bears abundant root-hairs. In several cases these hairs showed curling and contained infection threads.

The degeneration of the nodule is marked externally by change in colour of the nodule from pink to dark brown, by wrinkling of the surface and by loss of turgidity. Internally, the rhizobia clump together and lose stainability. The hypertrophied and darkly staining nucleus degenerates before the ultimate disappearance of the bacterial and other contents of the host cell. Many degenerating spots appear scattered in the bacteroid zone. These changes proceed either from the base upwards or centrifugally. The nodule cortex persists for some time as an empty shell. In annuals the nodules are short-lived, but in perennials they persist for as many as six years (Jimbo, 1927) by virtue of an active meristem, extensive vascular system with secondary thickening and protective cortical sclerenchyma (Allen and Allen, 1958).

The effective and ineffective nodules are morphologically distinguished by the size, shape and colour. Though there is no difference in the early course of development in both these types of nodules, the meristem in the effective nodule shows prolonged activity without early disintegration of the bacteroid tissue. On the other hand, the ineffective nodule is characterized by short-lived meristem and meagre bacteroid tissue or none coupled with early disintegration (Thornton, 1954). According to Chen and Thornton (1940), the greater the mass and duration of the active bacterial tissue, the more effective the nodule would be. In the light of anatomical studies, this view is plausible. Instances of ineffective nodules with early degeneration and less or no bacteroid tissue are seen in *Trigonella* (Gothwal, 1962).

Atypical swellings, which are met with at the bases of secondary roots and in the root axiles (Allen and Allen, 1954), are composed of proliferated and hypertrophied cortical parenchyma with heavy deposition of starch. According to Allen and Allen (1940) such tubercles occur in plants of nitrogen deficiency. Atypical nodules with a pericyclic vascular supply are reported in *Cicer arietinum* (Arora, 1956 *c*). In *Canavalia gladiata* (Gothwal, 1962) these nodules are composed of hypertrophied parenchyma of the outer cortex with no sign of infection or any deposition of starch grains. No functional significance has yet been ascribed to them.

Non-Leguminous Root Nodules

The root nodule of the non-legume develops as a lateral swelling on the root. Later, it becomes pear-shaped, enlarges and may become variously branched. The branching of the nodule is somewhat characteristic of the species: progressive dichotomous in *Alnus*, *Elaeagnus*, *Shepherdia*, *Hippophae* and *Coriaria*; irregularly branching posterior to the apex in *Ceanothus*; and trichotomously branching in *Casuarina* and *Myrica* (Allen and Allen, 1958). A rootlet which is devoid of root hairs and which does not bear nodules emerges from each lobe. The nodules are endogenous because of pericyclic origin (Fletcher, 1955).

Although Fletcher (1955) concludes that the infection of the young root in non-legumes is through the deformed and branched root-hairs on the basis of the presence of micro-organisms in them similar to the one present in the inner lying host cells, the mode of infection has not yet been demonstrated by the use of pure cultures. Penetration by the infection strands is intracellular, and the strands show great affinity to the host nucleus, growing towards and adhering to it. The nucleus in turn becomes hypertrophied and amoeboidal.

The nodule consists of nodule meristem, central vascular cylinder, and nodule cortex. It emerges as a spherical structure with great disturbance in the cortical cells of the parent root, unlike the lateral root which has a digestive cap and so causes little disturbance.

The nodule meristem brings about growth of the nodule by adding cells to various regions. The presence of an endophyte in the meristem is uncertain due to its rich tannin content.

The central vascular cylinder is connected with the root stele and has xylem, poorly developed phloem, three to four layers of irregularly-shaped pericyclic cells and endodermis with its cells densely filled with tannin forming a "tannin barrier" beyond which the endophyte does not penetrate (Fletcher, 1955). In *Alnus glutinosa*, *Hippophae rhamnoides* and *Myrica cerifera* the tracheids are irregular and reduced in size (Allen and Allen, 1958). Secondary vascular growth as annual rings is seen at the base of the nodules (Spratt. 1912).

The cortex of the non-legume nodule shows three zones in the development of the endophyte: (1) the zone of infection immediately behind the nodule meristem; (2) the middle zone of infection; and (3) the basal zone of degeneration. The extent of infection in the nodule is variable in different species. In *Alnus* the infection is throughout the length of the nodule while in *Hippophae* and *Elaeagnus* it continues to the area immediately next to the apical meristem. In *Myrica* only three to four layers of cortical cells are invaded. The infection does not extend into the vascular cylinder or into the innermost cortical layers. The uninfected cells contain starch or tannin, and are small while infected cells are two to three times larger with the endophyte (Allen and Allen, 1958).

The degeneration of the nodule is marked by the disappearance of the endophytic cytoplasm, dissolution of the host cell nucleus, and aggregation of host and endophyte cell residues. It is not known whether the endophyte succumbs to phagic lysis or to enzyme action. It appears that the contents of the invaded cells are absorbed and assimilated by the neighbouring non-invaded tissues (Allen and Allen, 1958). However, Hawker and Fraymouth (1951) consider that the parasite absorbs the contents of the host cell and sporulates at the expense of its own cytoplasm and the spores escape to the soil through cracks in the nodule cortex.

Conclusions

The anatomical studies of the nodule not only have unravelled the important events regarding nodulation, but have evoked some points, physiological and otherwise, which need further investigation.

Infection by rhizobium is a prerequisite for nodulation. The modes of its entry are different in different hosts. There are also variations with regard to the number of infection threads that enter the root hair and their branched or unbranched nature in the root hair. It is not known whether all or a few root hairs are susceptible to infection by rhizobium and whether the plural infection threads and their branching in the root hair can be attributed to the presence of any susceptible factor or to the number of disomatic cells available in the cortex imme-

diately below the root; or again whether the mode of entry has anything to do with the chemical constitution of the wall of the root hair, the epidermis or the ruptured cortical cells and the enzyme system present in the rhizobium aiding in infection. The further penetration of the infection thread into the cortex has been shown to depend on the distribution of disomatic cells. However, no correlation has yet been attempted between the depth of rhizobial penetration and the extent of distribution of tetraploid cells in the cortex of roots and their branches of different orders. Occurrence of disomatic cells in the cortex of non-legumes which do not nodulate is known. In this context, the presence of disomatic cells in the root cortex of non-nodulating legumes is also worthy of investigation to evaluate the role of ploidy in nodulation in nodulating legumes. The infection thread shows special attraction to the host nucleus. It moves close to the nucleus, may branch and enclose the latter without causing any damage.

The nodule is initiated by the divisions of the infected and the uninfected cells situated in the vicinity of the infected cell. The nodule later differentiates into the central bacteroid zone surrounded by the nodule cortex, the network of vascular system and the nodule meristem. The nodule meristem may be diffused or may be distinct distally or may be diffused in the beginning and become distinct later at the apex of the nodule as seen in *Dolichos biflorus*. A detailed morphogenetic study of the nodule would reveal not only the factors that bring about differentiation in the nodule but also the maintenance of the distal meristem for a long period in woody members. The normal function and longevity of the nodule are attributed to the well developed vascular system. Detailed anatomical studies have led to the clear understanding of not only the structure of effective and ineffective nodules, but also the morphology of the nodule. Since the time of Malpighi, this structure has been variously interpreted. Anatomical studies support the view that the leguminous nodule is not a lateral root because of the cortical origin, the type of emergence, the absence of central vascular cylinder and the multiple vascular supply from the root stele. It is also not a tumour because it is not autonomous, but only a gall due to hyperplasia caused by the parasitic agent which later partakes in a symbiotic relationship. On the other hand, the non-leguminous nodule is distinctly a modified rootlet by virtue of pericyclic origin, prominent central vascular cylinder with secondary growth, and development of a rootlet from each nodule lobe with a root-cap. Otherwise, it appears to be a gall like the leguminous nodule.

The recent work on physiology of nodulation (Nutman, 1958; Sadasivan and Subramanian, 1960) is obviously the outcome of the anatomical studies done on nodules earlier. It must be admitted that the functional significance of several of the anatomical observations briefly reviewed here is far from clear. Further work will undoubtedly clarify many of these and a clearer and correlated picture of nodule structure and genesis on the one hand and nodule function on the other will emerge.

I am deeply indebted to Professor C. V. Subramanian, Head of the Department of Botany, University of Rajasthan, for initiating me into this line of work and to Professors J. Venkateswarlu and T. S. Sadasivan for providing me an opportunity to present this paper in the Symposium.

REFERENCES

ALLEN, E. K. AND ALLEN, O. N. 1958. Biological aspects of symbiotic nitrogen fixation. *Encyclopedia of Plant Physiology* **8**: 48–118. (Ruhland, W., Ed., Springer-Verlag, Berlin *1958).*

———. 1959. The scope of nodulation among members of the family Leguminosae. *Proc. 11th Int. bot. Cong.* (Montreal) **2**: 3-4.

——, GREGORY, K. F. AND ALLEN O. N. 1955. Morphological development of nodules on *Caragana arborescens* Lam. *Canad. J. Bot.* **33**: 139–148.

ALLEN, O. N. AND ALLEN, E. K. 1940. Response of the peanut plant to inoculation with rhizobia, with special reference to morphological development of the nodules. *Bot. Gaz.* **102**: 121–42.

———. 1954. Morphogenesis of the leguminous root nodule. In *Abnormal and Pathological Plant Growth, Brookhaven Symp. in Biol.* **6**: 209–34.

ARORA, N. 1954. Morphological development of the root and stem nodules of *Aeschynomene indica* L. *Phytomorphology* **4**: 211–16.

———. 1956*a*. Morphological development of root nodules on *Crotalaria juncea*. *Proc.* **43***rd Indian Sci. Congr.* (Agra), pp. 244.

———. 1956*b*. Morphological study of the root nodules on *Cajanus indicus*. *Ibid.* 244–45.

———. 1956*c*. Histology of the root nodules on *Cicer arietinum* L. *Phytomorphology* **6**: 367–78.

BIEBERDORF, F. W. 1938. The cytology and histology of the root nodules of some Leguminosae. *J. Amer. Soc. Agron.* **30**: 375–89.

BOND, G. 1958. Symbiotic nitrogen fixation by non-legumes. In *Nutrition of the Legumes*: 216–31. (Hallsworth, E. G., Ed., Butterworths, London, 1958).

BOND, L. 1948. Origin and developmental morphology of root nodules of *Pisum sativum*. *Bot. Gaz.* **109**: 411–34.

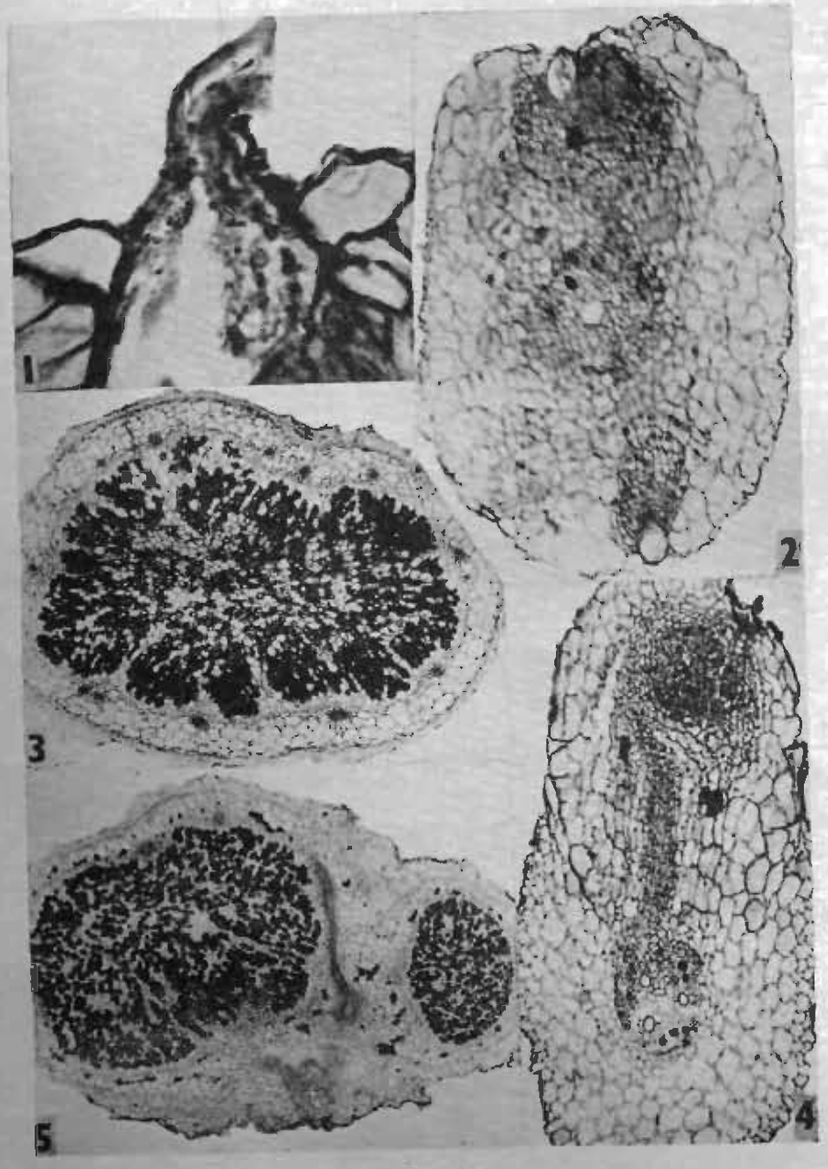
CHEN, H. K. AND THORNTON, H. G. 1940. The structure of "ineffective" nodules and its influence on nitrogen fixation. *Proc. roy. Soc. Lond.* **129B**: 208–29.

FLETCHER, W. W. 1955. The development and structure of the root nodules of *Myrica gale* L. with special reference to the nature of the endophyte. *Ann. Bot., Lond.* **29**: 501–13.

GOTHWAL, B. D. 1962. Morphology and development of root nodules in some legumes. M.Sc. Thesis. University of Rajasthan, Jaipur, India.

HARRIS, J. O., ALLEN, E. K. AND ALLEN, O. N. 1949. Morphological development of nodules on *Sesbania grandiflora* Poir. with reference to the origin of nodule rootlets. *Amer. J. Bot.* **36**: 651–61.

HAWKER, L. E. AND FRAYMOUTH, J. 1951. A reinvestigation of the root nodules of species of *Elaeagnus, Hippophae, Alnus* and *Myrica* with special reference to the morphology and life-histories of the causative organisms. *J. gen. Microbiol.* **5**: 369–85.



FIGS. 1–5

H. S. Narayana

JIMBO, T. 1927. Physiological anatomy of the root nodule of *Wistaria sinensis*. *Proc. imp. Acad. Japan* **3**: 164–66.

MCCOY, E. F. 1929. A cytological and histological study of the root nodules of the bean, *Phaseolus vulgaris* L. *Z. Bakt. Abt.* **79**: 394–412.

———. 1932. Infection by *Bact. radicicola* in relation to the microchemistry of the host's cell walls. *Proc. roy. Soc. Lond.* **110 B**: 514–33.

NARAYANA, H. S. 1963. A contribution to the structure of the root nodule in *Cyamopsis tetragonolobus* Taub. *J. Indian bot. Soc.* (in press).

NUTMAN, P. S. 1958. The physiology of nodule formation. *Nutrition of the Legumes*: 87–107. (Hallsworth, E. G., Ed., Butterworths, London, 1958).

SADASIVAN, T. S. AND SUBRAMANIAN, C. V. 1960. *Interaction of pathogen, soil, other micro-organisms in the soil and host. Plant Pathology* **2**: 273–313. (Horsfall, J. G. and Dimond, A. E., Ed., Academic Press, New York and London, 1960).

SCHAEDE, R. 1940. Die Knollchen der adventiven Wasserwurzeln von *Neptunia oleracea* und ihre Bakteriensymbiose. *Planta* **31**: 1–21.

SPRATT, E. 1912. The morphology of the root tubercles of *Alnus* and *Elaeagnus*, and the polymorphism of the organism causing their formation. *Ann. Bot., Lond.* **26**: 119–28.

THORNTON, H. G. 1930. The early development of the root nodule of lucerne (*Medicago sativa* L.) *Ibid.* **44**: 385–92.

———. 1954. The nodule bacteria and their host legumes: some problems that they still present. *Sci. Progr.* **42**: 185–204.

WIPF, L. AND COOPER, D. C. 1938. Chromosome numbers in nodules and roots of red clover, common vetch and garden pea. *Proc. nat. Acad. Sci., Wash.* **24**: 87–91.

———. 1940. Somatic doubling of chromosomes and nodular infection in certain Leguminosae. *Amer. J. Bot.* **27**: 821–24.

EXPLANATION of PLATE VIII

Vigna catjang (Figs. 1–3) and *Dolichos biflorus* (Figs. 4–5)

FIG. 1. Root hair with five infection threads, ×685.

FIG. 2. T.s. root with three regions of infection, *see* the differentiation of procambial strands below two of the infected regions, × 116.

FIG. 3. T.s. nodule, × 21.

FIG. 4. Early stage of the nodule with the infected root hair, × 142.

FIG. 5. T.l.s. mature nodule with two distinct bacteroid zones, ×12.

5-63. Printed at The Bangalore Press, Bangalore City, by T. K. Balakrishnan, Superintendent.