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PROCEEDINGS OF THE SUMMER SCHOOL OF BOTANY

held June 2-15, 1960

AT DARJEELING

Edited by

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MINISTRY OF SCIENTIFIC RESEARCH & CULTURAL AFFAIRS
GOVERNMENT OF INDIA
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FOREWORD

Summer Schools have become almost a normal feature of academic life in many western countries. Held during the period when universities have their long vacations, they provide an opportunity to workers from different institutions to meet together and discuss common problems in their special fields of study. They also offer the advanced students a chance to participate in courses dealing with research problems in which specialists from different universities and research organisations bring to their notice the latest methods and technique.

I am glad that a Summer School in Botany was held at Darjeeling from 2nd to 15th June, 1960, and inaugurated by Dr. B. C. Roy, Chief Minister of West Bengal. This School was the second in the series of Summer Schools organised by this Ministry and the first in Botany held in India.

The School was directed by Dr. P. Maheshwari, Professor of Botany, University of Delhi, who is one of our outstanding Botanists and a recognised authority on Plant Embryology. During the session of the School, fifty-three papers were read and discussed by thirty-six botanists from various Universities and other organisations including the Botanical Survey of India. Apart from this, the participants also made important recommendations for the promotion of teaching and research in Botany. These recommendations are engaging the active attention of the Government.

I hope it will be possible to organize such Schools at least once every two years if not more frequently, so that different disciplines in Botany may be accommodated. The life sciences, of which Botany is one, have gained considerable importance in recent years and we may expect some of the most significant advances in science in these fields. They have a direct bearing on the welfare of the human race with immediate applications in forestry, agriculture and health. I hope these Summer Schools will not only add to our knowledge but also help in bringing the results of science nearer to every day life.

15 October 1961
New Delhi

HUMAYUN KABIR

PREFACE

As the Indian Science Congress is now a very large organization, a need has been felt for a long time by scientists in India to have an opportunity to meet in smaller groups for an effective discussion of their problems. On the suggestion and initiative of Professor Humayun Kabir, Minister of Scientific Research & Cultural Affairs, Government of India, a Summer School of Theoretical Physics was held at Mussoorie from May 22 to June 18, 1959, in which teachers and research workers from all over India presented papers and held informal discussions. In 1960, Professor Kabir initiated a similar Summer School for Botany and this was held at Darjeeling from June 2 to 15, 1960. It was attended by 36 delegates representing the various Indian universities and the Botanical Survey of India (*see* Appendix I).

The School was inaugurated by Dr. B. C. Roy, Chief Minister, West Bengal. Dr. M. M. Das, Deputy Minister, Mr. A. K. Ghosh, Joint Secretary, and Mr. M. Kusari, Deputy Secretary, of the Ministry of Scientific Research & Cultural Affairs, Government of India, were also present at the time of inauguration.

In all 53 papers were read and discussed at the School of which 48 are being published in this volume. Apart from the papers read at the School (*see* Appendix II) and the lively discussions which followed them, the assembled botanists, all of whom stayed together in the same hotel, had plenty of opportunity for long informal and group discussions which were very helpful. The School was thus of great help in furthering mutual contacts between botanists who seldom have a chance to meet each other except for very brief intervals.

Several excursions were arranged to the Lloyd Botanic Garden, Happy Valley Tea Estate, Senchal Lake and other places in and around Darjeeling which show a very rich flora typical of Eastern Himalayas.

The better part of the last two days was devoted to a discussion on "The Promotion of Teaching and Research in Botany". Almost all the delegates took part in the discussion on the basis of which a short report was prepared and submitted to the Ministry of Scientific Research & Cultural Affairs, Government of India, for necessary action (*see* Appendix III).

The delegates attending the School unanimously agreed on its great value and the opportunity it provided for further thought. It was felt by all that similar Summer Schools in Botany be held at least once every two years.

Our grateful thanks are due to the Honourable Minister, Professor Humayun Kabir, for initiating the idea of a Summer School, and to Mr. A. K. Ghosh, Mr. M. M. Kusari and Mr. M. M. Malhotra (Under Secretary) for their constant co-operation.

P. Maheshwari
B. M. Johri
I. K. Vasil

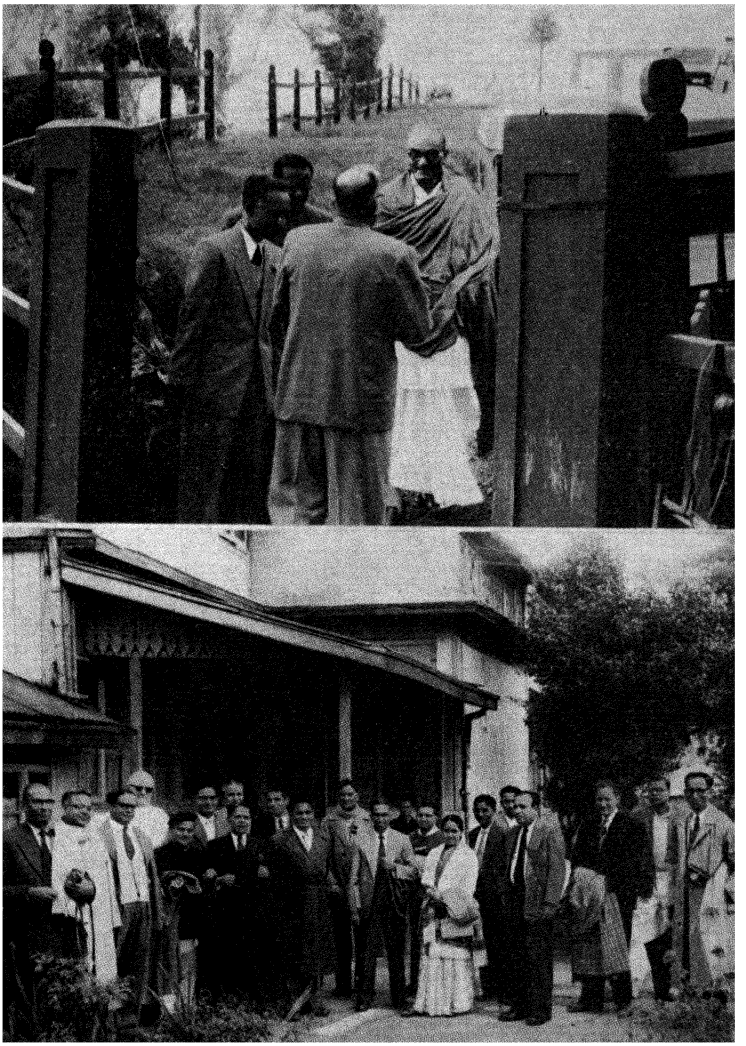
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Courtesy : Dr. T. K. Vasu

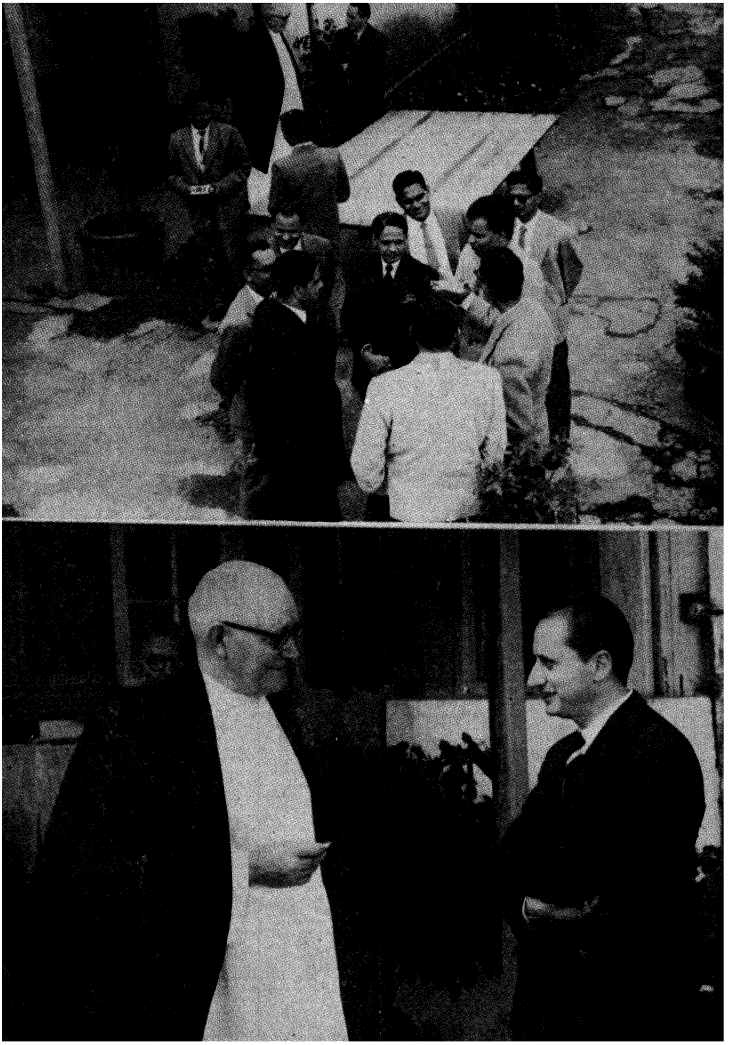
Top—Professor P. Maheshwari, Director of the School, receiving Dr. B. C. Roy. Also in the picture are Mr. A. K. Ghosh and Mr. M. M. Das of the Ministry of Scientific Research & Cultural Affairs, Government of India.

Bottom—A group of some delegates who attended the School.



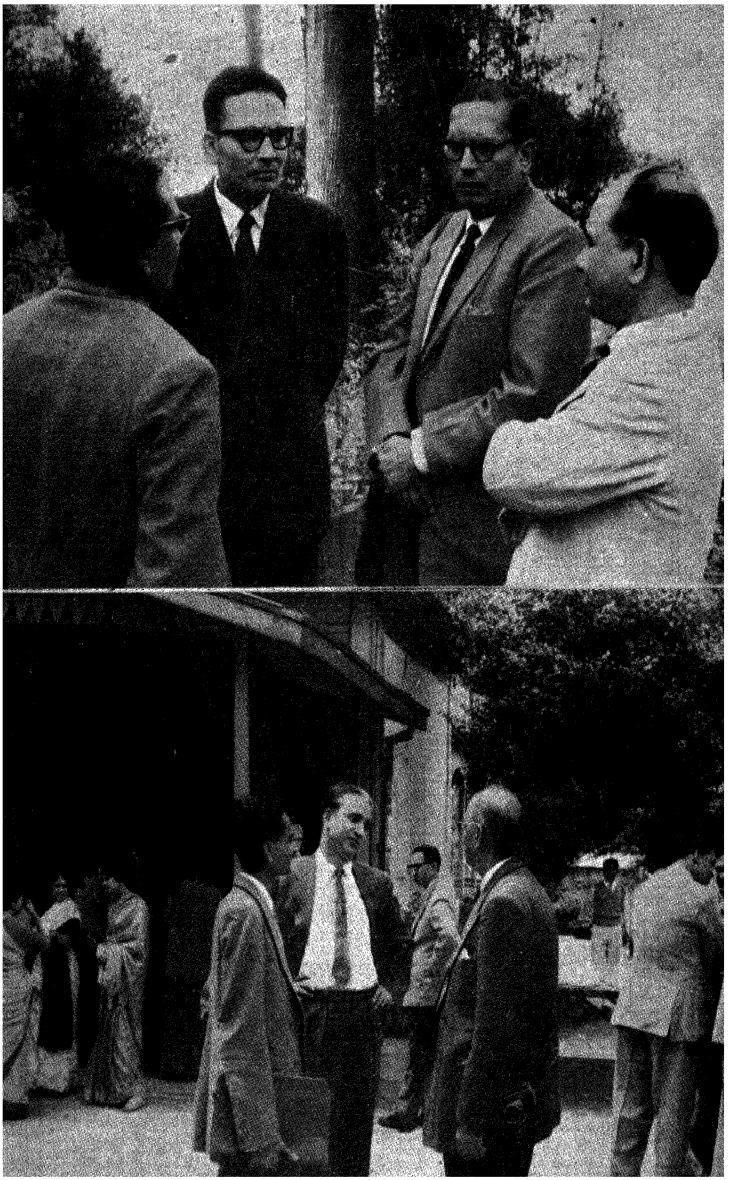
Courtesy : Dr. I. K. Vasil

Top—At the Llyod Botanic Garden, Darjeeling.
Bottom—I. K. Vasil, T. V. Desikachary, D. D. Pant, S. C. Agarwala.



Courtesy : Dr. I. K. Vasil

Top—An informal discussion amongst a group of delegates.
Bottom—H. Santapau, V. Garcia.



Courtesy : Dr. I. K. Vasil

Top—M. M. Das, K. S. Bhargava, D. Chatterjee, N. Das.
Bottom—A. K. Sharma, T. N. Khoshoo, B. M. Johri (foreground).

THE ROLE OF MICRONUTRIENTS IN PLANTS

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The term micronutrient element, for brevity referred to as micronutrient, was introduced by Arnon (1948, 1950a) for elements which were required by plants in very small quantities and fulfilled the following criteria of essentiality: (*i*) the plant must be unable to grow normally or complete its life cycle in the absence of the element, (*ii*) the element is specific and can not be replaced by another, and (*iii*) the element plays a direct role in metabolism.

Hewitt (1952) has retained this term for elements required in quantities often less than 1 p.p.m. in solution and which fulfil Arnon's criteria of essentiality. The essential micronutrients for plants comprise *manganese, copper, zinc, boron* and *molybdenum*. Due to its behaviour as a micronutrient element *iron*, which for historical reasons was treated with macronutrients has also been included in this category (Arnon, 1950 b). With advances in the techniques for establishing the essentiality of the nutrient elements additions are being made to the list of essential micronutrients. *Chlorine* has recently been shown to be essential for green plants (Arnon, 1959), *vanadium* for *Scenedesmus* (Arnon & Wessel, 1953) and *cobalt* for blue-green algae (Holm-Hansen *et al.*, 1954). Though claims have been made, yet the essentiality of boron for fungi and gallium for plants in general has not been conclusively shown (Steinberg, 1938, 1941, 1945, 1946). The essentiality of molybdenum with reduced forms of nitrogen supply was also considered doubtful but recent work (Agarwala, 1952a ; Hewitt & McCready, 1954) has shown that it is essential for the growth of higher plants irrespective of the nitrogen source.

EFFECT OF MICRONUTRIENTS ON GROWTH

In order to satisfy the criteria of essentiality laid down by Arnon, plants have to be raised in refined sand or water culture, where the concentration of the element under study is reduced to an extremely low value. The total omission of an element from the culture medium is not possible due to the difficulty in removing the last traces of an element from sand, water and nutrients and above all in getting seeds devoid of an element.

Visual Effects—Controlled culture experiments have shown that when a micronutrient is in short supply pathological conditions develop. These pathological conditions are so characteristic of the deficiency in a

particular species that particular names have been given to them. Thus, symptoms of manganese deficiency are known as 'Grey-speck' in oats, 'Speckled yellows' in sugar-beets, 'Frenching' in tung trees and 'Marsh-spot' in peas ; those of copper deficiency as 'Exanthema' or 'Die-back' in citrus, pears and other fruit trees, 'Reclamation disease' in farm crops of Netherlands ; those of zinc as 'Rosettes' in apples, pecans, vines and stone fruits, and 'Mottle leaf' in citrus ; those of boron as 'Heart-rot' in sugar beets and marigolds, 'Canker' in table beets, 'Brown-heart' or 'Raan' in swedes and turnips, 'Browing' or 'Hollow stem' in cauliflower, 'Cracked stem' in celery, 'Yellows' in alfalfa, 'Drought spot' or 'Corky pit' in apples, 'Hard-fruit' in citrus and 'Top sickness' in tobacco ; and of molybdenum deficiency as 'Whip-tail' in cauliflower (Wallace, 1951 ; Stiles, 1958). The characteristic symptom of iron deficiency is chlorosis of the younger growths but this condition is quite frequently met even when iron is abundant. In such cases the iron deficiency is considered to be a result of its unavailability to plants owing to one or the other cause, e.g. high pH, high bicarbonate ion concentration, macro- or micronutrient imbalance (Brown, 1956). To distinguish from iron deficiency, the chlorosis of younger growths resulting from unavailability of iron is generally termed 'induced iron deficiency'. A large number of induced iron deficiency effects have been observed by the author and his co-workers (Agarwala & Mehrotra, 1960 ; Agarwala *et al.*, unpublished).

Often, characteristic visual effects are also produced when the different micronutrient elements are present in excess quantities. One symptom common to excess of essential or even potential micronutrients, e.g. cobalt, nickel, chromium or selenium is chlorosis resembling that due to iron deficiency in addition to the specific metal effects (Hewitt, 1953 ; Agarwala & Kumar, 1960). Amelioration of the chlorosis resulting from excess concentration of heavy metals by the application of a suitable iron chelate shows the ability of the excess supply of different micronutrients and other heavy metals in inducing deficiency of iron (Vergnano & Hunter, 1952 ; Agarwala & Kumar, 1960).

The information of the pathological conditions produced by the deficiency or excess of a micronutrient element, singly or in combination, is of great value not only to the agriculturist and horticulturist but also to the physiologist as Stiles (1958) has put, 'in providing information regarding the functions of these elements'. The physiologist has to examine the precise manner in which metabolism is affected by the concentration of an element in the external nutrient medium and in the tissue and how the derangement in metabolism brought about by the deficiency or excess of a metal results in the development of the pathological effects—the visual symptoms.

Relation of External Supply to Uptake and Growth—From the

results of controlled culture experiments with graded levels of micronutrient supply it has been shown that within a certain range, which varies from species to species and at different stages of growth, there is an increase in the yield with an increase in the micronutrient supply. With yield expressed arithmetically and the nutrient supply logarithmically the relationship is generally linear. Above an optimum nutrient supply the different micronutrient elements generally exert toxic effects and the growth curve shows a negative slope. At times, however, instead of linear curve one gets a sigmoid curve (Hewitt & Hallas, 1951 ; Sharma, 1960). The sigmoid nature of the curve may perhaps be due to the difficulty in reducing the particular micronutrient element supply, by way of contamination, to zero.

The relationship between the internal nutrient concentration and the yield is important and is of great diagnostic value. In the case of the different micronutrient elements probably with the exception of iron, there is a curvilinear relationship between the nutrient concentration expressed logarithmically and yield expressed arithmetically. In the case of iron nutrition, the internal iron concentration may not be related to the plant yield (Sharma, 1960 ; Agarwala & Sharma, unpublished).

The tissue concentration of a particular nutrient element depends not only on its external concentration but also on cell division, cell expansion, differentiation and the total growth capacity of the plant (Hewitt, 1957). In case of manganese (Hewitt, 1957), copper (Piper, 1942) and molybdenum (Agarwala & Hewitt, 1954) the relationship between the logarithms of the external nutrient supply and the internal concentration has been shown to be curvilinear. No such relationship was found in the case of iron nutrition of barley where the tissue concentration of iron at lower levels of iron supply was often higher than at normal or sufficiency levels (Agarwala & Sharma, unpublished).

Relation of Micronutrients to Enzymes

The micronutrient elements play important but varied roles in the different metabolic processes of plants, e.g. in respiratory oxidation-reductions, photosynthesis, carbohydrate metabolism, nitrogen metabolism etc. Perhaps the most important role on which some precise information has been obtained is the role of the micronutrients in enzyme action. In many enzyme systems micronutrients are essential cofactors ; in some enzymes the micronutrient is a specific prosthetic group, in others the micronutrient can be replaced by one or more metals, with varying degrees of efficiency. The replacing metal may be an essential macro- or micronutrient, or a non-essential element. A list of the more important enzyme systems in which micronutrients function as cofactors is given in Tables 1-5, which have been mainly drawn up on the basis of information compiled by McElroy & Nason (1954) and Hewitt (1958).

TABLE 1

ENZYMES REQUIRING MANGANESE FOR ACTIVITY

Enzyme	Reaction	*Metal which can replace manganese*	*Reference**
Prolidase	Glycylproline → proline	Nil	Fruton (1946)
Nitrite reductase	nitrite → hydroxylamine	Nil	Nason *et al.* (1954)
Glycyl-glycine and alanyl-glycine peptidases	glycyl glycine or alanyl glycine → constituent amino acid	Nil	Berger & Johnson (1939)
(i) *Phytomonas tumefaciens*		Co	
(ii) *Bacillus megatherium*		Mg	
Glucokinase	Glucose + ATP → Glucose-6-phosphate	Mg	Cori & Slein (1947)
Hexokinase (yeast and higher plants)	(Fructose, Glucose or Mannose) + ATP → Hexose-6-phosphate + ADP	Mg	Berger *et al.* (1946) ; Bailey & Webb (1948) ; Saltmann (1953)
Phosphoglucokinase	Glucose-1-phosphate + ATP → Glucose-1,6-diphosphate + ADP	Mg	Paladini *et al.* (1949)
Phosphoglucomutase	Glucose-1-P ⇄ Glucose-6-P	Mg, Co and Cr	Cori *et al.* (1937) ; Najjar (1948) ; Stickland (1949)
Phosphoglyceric acid kinase	3-phosphoglyceric acid + ATP ⇄ 1,3-diphosphoglyceric acid	Mg	Bucher (1947)
transenolase	2-phosphoglyceric acid ⇄ 1,3-enolphosphopyruvic acid + H_2O	Mg, Zn	Warburg & Christian (1942)
Adenosine kinase	Adenosine + ATP → ADP + AMP	Mg	Caputto *et al.* (1950)
Arginine kinase	Arginine + ATP ⇄ Arginine phosphate + ADP	Ca and Mg	Szorenyii *et al.* (1949)
DPN kinase	DPN or $DPNH^+$ + ATP → TPN or $TPNH^+$ ADP	Mg	Kornberg (1950)
DPN kinase (liver)	DPN^+ only + ATP → TPN^+ + ADP	Mg	Wang & Kaplan (1954)
Yeast apyrase	ATP + H_2O → AMP + 2 PO_4	Nil	Meyerhof (1945)
Glutamyl transferase	Glutamine + NH_2OH + ATP → NH_3 + Glutamo hydroxamic acid	Nil	Stumf & Loomis (1950)
Glutamine synthesizing enzyme	Glutamic acid + NH_3 + ATP → Glutamine + ADP	Mg	Elliott (1951)
Dephospho CoA kinase	Dephospho CoA + ATP → CoA + ADP	Mg	Wang & Kaplan (1953)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

TABLE 1—(*Contd.*)

Enzyme	Reaction	*Metal which can replace manganese*	*Reference**
Pyruvic carboxylase • yeast	Pyruvic acid → Acetaldehyde + CO_2	Mg	Auhagen (1932) ; Green *et al.* (1941)
soya bean	,, → ,, ,,	Mg	Mee (1949)
a-ketoglutarate oxidase (plant)	a-ketoglutaric acid → Succinic acid + CO_2	Mg	Schales *et al.* (1950)
Condensing enzyme	Oxaloacetic acid + Acetyl CoA ⇄ Citric acid	Mg	Stern *et al.* (1950)
Yeast phosphatase	Glycerophosphate → Glycerol + PO_4	Mg, Co, Fe and Ni	Massart & Vandendriessche (1940)
Arginase	Arginine + H_2O → Ornithine + Urea	Co, Ni and Fe	Stock *et al.* (1938)
Lecithinase	Lecithin → Phosphorylcholine + diglyceride	Ca, Mg, Co and Zn	Zamecnik *et al.* (1947)
Cysteine desulfhydrase	Cysteine → $H_2S + NH_3 + CH_3CO$ COOH	Zn and Mg	Binkley (1943)
Desoxyribonuclease	Depolymerisation	Mg, Co and Fe	Miyaji & Greenstein (1951)
Isocitric dehydrogenase	1. Isocitrate + TPN+ ⇄ Oxalosuccinate + TPNH + H+(DPNH)	Mg	Euler *et al.* (1939)
Widespread higher plants & yeasts	2. Oxalosuccinate ⇄ α-ketoglutarate + CO_2	Mg	Ochoa (1945, 1948)
Oxalosuccinic decarboxylase (parsley root)	Oxalosuccinate ⇄ α-ketoglutarate + CO_2	Mg or Co Nil	Berger & Avery (1944) Ochoa Weisz-tabori (1945)
Malic dehydrogenase	1. Malate + TPN^+ ⇄ Oxaloacetate + TPNH + H^+	Nil	Vennesland *et al.* (1949)
	2. oxaloacetate ⇄ pyruvate + CO_2	Nil	Vennesland *et al.* (1949)
Oxaloacetic decarboxylase, widely distributed in plants, yeasts and bacteria	Oxaloacetate ⇄ Pyruvate + CO_2	Mg, Co, Ni and Ca Nil	Herbert (1950) Kalnitsky & Werkman (1943b) Plaut & Lardy (1949)
Microorganisms		Co and Zn	Vennesland & Felsher (1946)
Higher plants		Nil	Vennesland (1949)
Pyruvic oxidase complex	Pyruvate + PO_4 + DPN ⇄ Acetyl-PO_4 + DPNH + H^+ + CO_2	Mg	Korkes *et al.* (1950)
Galactokinase (Yeast)	ATP + galactose → galactose-1-phosphate + ADP	Mg	Trucco *et al.* (1948)
Flavokinase (Yeast)	Riboflavin + ATP or ADP → Riboflavin phosphate (FMN) + ADP or AMP	Co, Zn and Mg	Kearney & Englard (1951)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

TABLE 1—(*Contd.*)

Enzyme	Reaction	*Metal which can replace manganese*	*Reference**
Adenosine kinase (Yeast)	Adenosine + ATP → Adenylic acid + ADP	Mg	Caputto (1951)
Enol-phosphopyruvic acid phosphotransferase (Pyruvickinase) muscle	Enol-phosphopyruvic acid + ADP ⇄ Pyruvic acid + ATP	K and Mg	Lardy & Zeigler (1945), Kachmar & Boyer (1952)
Higher plants			Miller & Evans (1957)
Aspartylkinase (transferase) yeast	Aspartic acid + ATP ⇄ β-Aspartyl-phosphate + ADP	Mg and Fe	Black & Wright (1955)
FMN pyrophosphotransferase yeast	FMN + ATP ⇄ FAD + inorganic phosphate	Mg	Schreeker & Kornberg (1950)
Glucose-6-phosphate dehydrogenase (*E. coli*)	Glucose-6-phosphate + TPN^+ → 6-phosphogluconate + TPNH + H^+	Ca, Mg and Ba	McNair-Scott & Cohen (1953)
6-phosphogluconic dehydrogenase (*E. coli*)	6-phosphogluconate + TPN^+ → Ribulose-5 phosphate + TPNH + H+ + CO_2	Ca, Mg, Ba, Co	McNair-Scott & Cohen (1953)
Phosphoglucomutase (green gram)	Glucose-1-phosphate ⇄ Glucose-6-phosphate	Mg, Co	Ramasarma *et al.* (1954)
Inorganic pyrophosphatase (Yeast)	$P_2O_7^{-4} + H_2O$ 2 $PO_4^{-3} + 2H^+$	Mg, Co	Kunitz (1952)
Acid phosphatase (foliage)	Phosphate ester ⇄ inorganic phosphate	Ca, Co, Mg and Fe	Boroughs (1954)
(Yeast)		Co, Mg Fe, Ni and Zn	Massart & Vandendriesche (1940)
Alkaline phosphatase (*Penicillium* and intestine)	Phosphate ester → inorganic phosphate	Mg Mg, Fe and Zn	Sadasivan (1950, 1952) Thoai *et al.* (1947)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

TABLE 2

ENZYMES REQUIRING COPPER FOR ACTION

Enzyme	Reaction	*Metal which can replace copper*	*Reference**
Tyrosinase or polyphenol oxidase	Oxidation of tyrosine and various phenols.	Nil	Nelson & Dawson (1944)
Laccase	Oxidation of ortho and paradihydric phenols. p-phenylenediamine and ascorbic acid	Nil	Keilin & Mann (1939, 1940) Tissieres (1948, 1949)
Ascorbic oxidase	ascorbic acid → dehydroascorbic acid	Nil	Dawson (1950)
Butyryl CoA dehydrogenase**	Butyryl CoA—2e → Crotonyl—CoA	Nil	Mahler (1953)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

** Copper requirement now considered doubtful.

TABLE 3

ENZYMES REQUIRING ZINC FOR ACTION

Enzyme	Reaction	*Metal which can replace zinc*	*References**
Glycylglycine dipeptidase	Glycylglycine → glycine	Nil	Linderstrome-Lang (1934)
Carbonic anhydrase	$CO_2 + H_2O \rightarrow H_2CO_3$	Nil	Keilin & Mann (1940)
,, plant	,, → ,,	Nil	Sibley & Wood (1951)
Dehydropeptidase	Glycyldehydrophenylalanine → glycine + ammonia + phenylpyruvic A.	Nil	Yudkin & Fruton (1947)
Triose phosphate dehydrogenase	Glyceraldehyde-3-phosphate + DPN+ + inorganic phosphate → 1,3 Diphosphoglyceric acid + $DPNH^+ + H^+$	Nil	Valee *et al.* (1956)
Acid phosphatase yeast	Phosphate ester → inorganic phosphate	Co, Mg, Fe, Ni, Mn	Massart & Vandendriessche (1940)
Alkaline phosphatase	,, → ,,	Mg	Sadasivan (1950, 1952)
Aldolase yeast	Fructose 1,6 diphosphate → glyceraldehyde-3-phosphate + dihydroxyacetone phosphate	Co, Fe	Warburg & Christian (1942b, 1943)
Alcohol dehydrogenase (yeast)	Ethyl alcohol + $DPN^+ \rightleftharpoons CH_3CHO + DPNH + H^+$	Nil	Valee & Hoch (1955)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

TABLE 4

ENZYMES REQUIRING MOLYBDENUM FOR ACTIVITY

Enzyme	Reaction	*Metal which can replace molybdenum*	*References**
Nitrate reductase from	$\text{Coenzyme} \xrightarrow{2e} \begin{matrix}\text{FAD}\\\text{FMN}\end{matrix} \rightarrow \text{Enzyme} \rightarrow \text{Mo} \xrightarrow{e} \text{No}_3 \rightarrow \text{No}_2$	Nil	Nicholas *et al.* (1953, 1954); Nicholas & Nason (1954, 1955a, b)
(*Neurospora, Aspergillus,* Soya bean, *Escherichia coli*)	*Neurospora* requiring TPN, soya bean TPN or DPN, *E. coli* requiring in addition to coenzyme Cytochrome "b"		Taniguchi *et al.* (1956)
Molydoflavoprotein		Nil	Shug *et al.* (1954); Whitley & Ordal (1956) (*See* Hewitt, 1958)
Xanthine oxidase	$\text{Xanthine} + O_2 \rightarrow H_2O + \text{Uric acid}$	Nil	De Renzo *et al.* (1953); Green & Beinert (1953); Richert & Westerfeld (1953); Totter *et al.* (1953)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

TABLE 5

ENZYMES REQUIRING IRON FOR ACTIVITY

Enzyme	Reaction	*Metal which can replace iron*	*Reference**
Fumaric hydrogenase	Fumaric acid + $2H^+$ → Succinic acid	Nil	Harrison (1953)
Cytochromes	electron transport	Nil	Lamberg & Legge (1947)
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$	Nil	Granick & Gilder (1947)
Peroxidase	Oxidation of phenols and aromatic amines in presence of H_2O_2	Nil	Lemberg & Legge (1947)
DPNH—cytochrome-C reductase	DPNH + Cytochrome-C (Fe^{+++}) → DPN + Cytochrome C(Fe^{++})	Nil	Mahler & Elowe (1953)
Succinic dehydrogenase	Succinate → Fumarate + 2H	Nil	Green, Basford & Mackler (1956)
Cytochrome peroxidase	2 Cytochrome – $Fe^{++} + H_2O_2 + 2H^+ \rightleftharpoons$ 2 cytochrome $Fe^{+++} + 2H_2O$	Nil	Altachul *et al.* (1929)
Leucyl glycine peptidase (yeast)	Leucyl-glycine → leucine + glycine	dihydroxyphenylalanine	Schales & Roux (1950)
Arginase (jack bean)	Arginine + H_2O → ornithine + urea	Co, Mn, Ni	Stock *et al.* (1938)
Aspartyl kinase	Aspartic acid + ATP → β-aspartyl phosphate + ADP	Mg, Mn	Black & Wright (1955)
Aldolase (yeast)	Fructose 1,6 diphosphate → glyceraldehyde – 3 phosphate + dihydroxyacetone phosphate	Zn and Co	Warburg & Christian (1942)
Aconitase	Citrate $\rightleftharpoons$ *cis* aconitate $\rightleftharpoons$ isocitrate	Cysteine	Morrison (1954a, b)

* For references *see* McElory & Nason (1954) and Hewitt (1958).

The several ways in which these elements act in the enzyme systems have been recently discussed by McElroy & Nason (1954), Klotz (1954) and Hewitt (1958). Depending upon the manner in which the micronutrients affect the enzyme activity the following broad categories have been made:

(I). *Micronutrients that as such have very low activity but in combination with a protein moiety acquire very high catalytic efficiency :* The micronutrients involved in some oxidation-reductions come under this category, e.g. the oxidation of ascorbic acid by the inorganic form of copper and the decomposition of hydrogen peroxide by that of iron is very slow but combined with proteins, copper in ascorbic acid oxidase and iron in catalase and peroxidase carry out these reactions with manifold efficiency.

(II). *Micronutrients that change the properties of the proteins :* (*a*) *The elements that change the net charge of the proteins, thus affecting enzyme-substrate combinations.* The alteration in the pH optimum of certain enzymes, e.g. alkaline and acid phosphatase (Sadasivan, 1952) and fumarase (Massey, 1953) come under this category. This type of role of metals may also explain the effects of the various non-essential elements on the metabolism of plants. (*b*) *The elements that change the properties of the enzymes by the removal of inhibitory substances.* Survival of the activity of mitochondria of brain by the application of cobalt (Christie *et al.*, 1953) and the removal of cysteine inhibition of yeast aldolase by zinc, cobalt or iron (Warburg & Christian, 1942 a, b, 1943) are typical examples of this category.

(III). *The micronutrients that acquire high enzymatic activity in co-operation with proteins :* (*a*) This may occur by the metal acting as a bridge between substrate and protein, with or without the formation of chelate structure. Whether the metal and the protein will form an inhibitory complex or an active intermediate compound depends on the reactive groups of the proteins and the manner in which they react with the metal and the substrate group, e.g. decarboxylation and hydrolytic enzymes. (*b*) The micronutrient in combination with protein serves to catalyse the formation of an intermediate which is an essential transitional stage for the reaction, e.g. the peptidase activity in which manganese is a prosthetic group.

(IV). *Micronutrients that take part as electron couplers from one protein system to another :* Examples of these are several flavoprotein enzymes in which iron, copper or molybdenum are involved, e.g. butyryl CoA dehydrogenase ($Cu^{++} \rightleftharpoons Cu$), Nitrate reductase ($Mo^{VI} \rightleftharpoons Mo^{V}$), xanthine-oxidase ($Mo^{VI} \rightleftharpoons Mo^{V}$), Fumaric hydrogenase ($Fe^{+++} \rightleftharpoons Fe^{++}$) and DPNH-Cytochrome C reductase ($Fe^{+++} \rightleftharpoons Fe^{++}$).

It appears that copper, iron and molybdenum are involved in the electron transferring systems. These metals without combination with the

protein can to a limited extent mediate in electron transfer. On combination with protein this property is enhanced manifold. Iron and copper enzymes can function as terminal oxidases in respiration but efforts have generally failed to substantiate the claims that any copper enzyme could function as an effective terminal oxidase in any plant (Hackett, 1959) even when copper enzymes were abundantly present. Failures of the claims of copper enzymes functioning as a terminal oxidase may be explained by the observation of McElroy (1953) that "under physiological conditions some systems may be workng at a maximum rate whereas others may be operating at less than 1 per cent of the maximum efficiency".

Manganese is involved in the decarboxylation and hydrolytic actions. Zinc and magnesium also function in these processes to some extent. It is not clear whether these metals form a bridge between protein and substrate or whether the metal combines with the protein to increase the concentration of intermediates in the reaction. Nason *et al.* (1954) have shown that manganese is a specific co-factor for the flavoprotein enzyme nitrite reductase and manganese deficient plants accumulate large quantities of nitrites. An appreciable accumulation of free amino acids has also been reported to occur in manganese deficient plants (Possingham, 1956, 1957).

When the micronutrients involved in the enzyme activities are in short supply to plants the different enzymes activated by them become affected. Thus, the activities of catalase, peroxidase, cytochrome reductase and oxidase were depressed at low levels of iron supply (Nicholas & Goodmann, 1958 ; Agarwala *et al.*, unpublished). It is not necessary that all the enzymes activated by the metal present in short supply be affected in the same manner. In case of barley the iron requirement of peroxidase was met earlier than that of catalase, activity of which was severely depressed at low levels of iron supply (Agarwala *et al.*, unpublished). Waring & Werkman (1944) have reported that in iron deficiency of *Aerobacter* the iron requirement of cytochrome oxidase was met before that of catalase, peroxidase or formic dehydrogenase.

In some instances the micronutrient elements which are the specific activators of enzymes may themselves be necessary for the synthesis of the protein moiety, e.g. molybdenum is needed for the synthesis of protein moiety of nitrate reductase (Nicholas *et al.*, 1954, Candela *et al.*, 1957) and zinc for that of carbonic anhydrase (Wood & Sibley, 1952).

It is interesting to note that when a micronutrient is in short supply even enzymes not directly catalysed by the metal are affected. Thus, zinc deficiency decreases the activity of glutamic dehydrogenase (Nicholas & Goodmann, 1958) and increases that of DPNase in *Neurospora crassa* and polyphenol oxidase in tomato (Nason *et al.*, 1952). Deficiencies of iron, manganese, molybdenum and boron also increased polyphenol oxi-

dase activity (Nason *et al.*, 1952). Molybdenum deficiency depressed the activities of catalase and peroxidase (Nicholas, 1957) and dehydrogenases (Hewitt & Agarwala, 1952 ; Mulder, 1954 ; Nicholas *et al.*, 1954). Nason (1952) reported abnormal increase in the activities of isocitric dehydrogenase and oxlalosuccinic decarboxylase in tomato plants deficient in copper, iron or manganese. TPNH and DPNH diaphorases were depleted in zinc and iron deficient *Neurospora* and enzymes were reconstituted within 24 hours of returning the deficient metal to the deficient mycelial felts (Nicholas & Goodmann, 1958).

The excess supply of micronutrients and other heavy metals bring about changes in the enzyme pattern. In the case of cobalt toxicity in *Neurospora crassa* cytochrome oxidase, catalase, peroxidase and succinic dehydrogenase were markedly depressed (Healy *et al.*, 1955). The effect of excess cobalt on enzyme activity could be reversed by supplying excess iron. Excess of cobalt and manganese increased the isocitric dehydrogenase in snap beans (Anderson & Evans, 1956). An appreciable depression in the activity of catalase has been observed under conditions of copper, cobalt and nickel toxicities (Agarwala & Kumar, 1960). Experiments with seedlings of *Cucumis melo* and *Phaseolus radiatus* have shown that catalase and peroxidase were not necessarily depressed by the supply of heavy metals, and at times catalase and peroxidase followed reverse trends. Cobalt, nickel, molybdenum and chromium depressed peroxidase and stimulated catalase activity in barley seedlings at concentrations of the heavy metals at which germination was depressed. In mature plants the supply of the aforesaid elements at mM./L depressed catalase and stimulated peroxidase activity (Agarwala & Kumar, unpublished). The form in which iron was supplied and the concentration of iron made some quantitative changes in the effects produced. The different metals interacted differently with the iron source. Considerable amelioration of the toxic effects was produced by the discontinuance of the excess metal supply. Some ameliorative effect of Fe-EDTA was also observed. The results obtained by the author and his associates (unpublished) show that heavy metals derange the iron metabolism apart from having a specific effect.

Effect of Micronutrients on General Metabolism of Plants

There is hardly any known sphere of plant metabolism which is not affected by micronutrients but very little is understood about the precise manner in which these elements affect the various metabolic processes. Some of the more important effects which cannot be directly attributed to the known role of micronutrients as co-factors in certain enzyme systems are briefly discussed below. The effect of the different micronutrients

on the uptake and availability of the other nutrient elements would form a separate subject by itself and has not been incorporated here.

Iron : A deficiency or unavailability of iron results in a marked depression in chlorophyll synthesis. The precise manner in which iron is involved in chlorophyll formation is not known but it appears likely that it has some role in the synthesis of chlorophyll precursor, protoporphyrin —9: Recent work on the nutrition of barley has shown that when iron is in short supply there is a simultaneous and a parallel depression in the synthesis of chlorophyll and the iron porphyrin enzyme, catalase. In a large variety of plants heavy metal toxicities have been shown to produce chlorosis. There does not, however, seem to be sufficient justification for holding that in each case this effect is due to the change in iron metabolism brought about by the excess of heavy metal. Chlorosis is a phenomenon common to many macro- or micronutrient deficiencies.

Iron is also possibly involved in nitrogen metabolism of plants. Plants deficient in iron have been shown to accumulate large quantities of free amino acids and amides, particularly asparagine and glutamine (Possingham, 1956, 1957). Apart from its role in the synthesis of chlorophyll, iron, as a component of cytochrome-f and possibly another cytochrome, plays an important part in photophosphorylation (Arnon, 1959).

Manganese : There is considerable evidence to show that this element plays a role in the primary photochemical reaction in photosynthesis (Pirson, 1955 ; Arnon, 1954). Gerretsen (1949) believes that this action is independent of the effect of the element on chlorophyll synthesis. Lindberg & Ernster (1954) have held that manganese is a co-factor in oxidative phosphorylation.

Copper : Copper is essential for the formation of black pigment in the spores of *Aspergillus niger*. It also seems to be involved in nitrogen metabolism. Copper deficient plants accumulate large quantities of free amino acids (Possingham, 1956, 1957). In certain copper deficient plants like tung trees, even proteins tend to increase (Gilbert, 1951).

Whether copper is involved in photosynthesis is not clearly understood, but a large quantity of copper is localised in chloroplasts (Whatley *et al.*, 1951).

Zinc : Deficiency of zinc results in a depression in chlorophyll formation (Brown & Wilson, 1952). When in short supply, zinc brings about an increase in the amides and the free amino acids, particularly asparagine and glutamine (Possingham, 1956, 1957) and a decrease in the protein synthesis (Sebrell & Harris, 1954). It has been shown that zinc is necessary for the synthesis of tryptophane (Tsui, 1948) and thus indirectly for the synthesis of auxin. When present in excess concentrations zinc promotes nicotine production by tobacco plants (Steinberg & Jeffrey, 1956) and antibiotic production by *Fusarium* (Kalyansundaram & Saraswathi-Devi, 1955). These findings are of great interest and suggest

the possibility of economic exploitation of metabolic disturbances resulting from deficiency or excess of micronutrients.

Molybdenum : Molybdenum supply has an appreciable effect on the ascorbic acid content of plants. When present in short supply molybdenum depresses ascorbic acid synthesis (Hewitt *et al.*, 1950). Carbohydrate metabolism is also affected by this element. Molybdenum deficient plants are low in sugars (Agarwala & Hewitt, 1955). That molybdenum deficient cauliflower has poorly formed middle lamella suggests its role in pectin formation (Agarwala, 1952 b). Molybdenum is also involved in nitrogen metabolism of microorganisms and higher plants. Molybdenum deficiency results in a marked depression in the soluble nitrogenous compounds (Hewitt *et al.*, 1957 ; Possingham, 1956, 1957). Molybdenum deficiency depresses protein nitrogen in plants raised with nitrate but not with reduced forms of nitrogen. Plants grown with ammonia or urea had high levels of arginine and molybdenum deficiency increased this amino acid (Hewitt *et al.*, 1957).

Boron : Boron has not been shown to be directly involved as a cofactor in any enzyme action but seems to affect many physiological processes, e.g. water relations of plants (Schmucker, 1933, 1935 ; Gisiger, 1950 ; Baker *et al.*, 1956), pectin and fat metabolism (Schmucker, 1933, 1935) and absorption and utilisation of calcium (Hendersen & Veal, 1948 ; *see also* Stiles, 1958).

In the light of the findings of Sisler *et al.* (1956) that boron may be directly involved in the translocation of sucrose, it seems necessary to examine whether the other workers, most recent being McIlrath & Palser (1956), were justified in attributing the disturbance in the carbohydrate metabolism in boron deficient plants to the necrosis of phloem.

Recent studies have shown that when sugar is present, boron brings about very marked acceleration of the germination of pollen grains in many species (O'Kelley, 1957 ; Johri & Vasil, 1960 ; Vasil 1960). Vasil (1960) has concluded that the role of boron in pollen germination may be ascribed to the formation of sugar-borate-complexes which are more readily absorbed than sugar, to the promotion of the deposition of pectic materials, and increased oxygen uptake.

Vanadium : Deficiency of vanadium depresses chlorophyll synthesis in *Scenedesmus*. In vanadium deficient cells strong light inhibits photosynthetic oxygen evolution. On the supply of vanadium to deficient cells the oxygen evolution is restored.

Chlorine : Arnon (1959) has shown that chlorine is an essential cofactor in photophosphorylation in green plants and is thus involved in photosynthesis.

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FLORISTIC PATTERNS OF INDIAN VEGETATION

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The richness of the vegetational covering of India is well known. The great British botanist, Sir J. D. Hooker (1904) said about the Indian Flora that "The flora is more varied than of any other country of equal area in the Eastern Hemisphere, if not in the globe". In another place, Hooker (1905) described the vegetation of the Khasia hills of Assam as "the richest in India and probably in all Asia. We collected upwards of 2000 flowering plants within ten miles of the station of Churra, besides 150 ferns and a profusion of mosses, lichens and fungi". Similar views were also expressed by other botanists and plant geographers who visited India from time to time.

This great diversity of the vegetational types is possible due to the varied nature of rain fall, temperature, altitude and the substratum. If we follow the average annual rain fall of India and proceed in two directions from the southernmost point of Peninsular India, we find that as we move towards north-east, the annual precipitation gradually increases and we meet first tropical moist deciduous forests and later tropical evergreen forests. In Assam, where the rain fall is very heavy (150" to 450"), the forests contain lofty trees of such species like *Dipterocarpus macrocarpus, Shorea assamica, Vatica lancaefolia* and *Messua ferrea*. The trees are usually covered with a number of woody climbers (lianas) and many epiphytic ferns and orchids. The undergrowth contains evergreen shrubs, bamboos, palms, aroids and scitaminaceous plants. If on the other hand, we proceed directly towards the north, we meet the deciduous forests of Madhya Pradesh and then the desert, thorn and scrub forests of Raja[illegible] and East Punjab. Some of the common species of the deciduous fo[illegible] are *Terminalia tomentosa, T. belerica, Lagerstroemia parviflora, Sterculia villosa, Salmalia malabarica, Tectona grandis* and *Lannea grandis*. In the desert-thorn and desert-scrub forests of Rajasthan and East Punjab, the important plants are *Acacia arabica, A. leucophlaea, Prosopis spicigera, Dalbergia sissoo, Tamarix articulata, Phoenix sylvestris, Euphorbia* sp., *Capparis, Zizyphus, Grewia, Calotropis, Pluchea lanceolata* etc. (*see* Fig. 1).

In spite of the richness and great diversity of plants in India, botanists are somewhat divided in their opinion as to whether India has a characteristic flora of her own. According to Hooker, the flora of India is

*We regret that Dr Chatterjee passed away soon after the Summer School was held.

merely a mixture of floras of the surrounding countries like Malaya, Orient, Africa, Tibet, China and Japan. Champion & Trevor (1938) were also apparently influenced by Hooker's view, as they stated: "It is evident that there is no Indian flora as a separate entity, but our vegetation is compounded of several elements which are present in very different proportions in the different areas". The term "Indian Flora" would normally mean a collection of plants which are typical for India and which are normally not found outside the country. In other words, the proportion of plants which are called "endemic" would determine whether the country has a typical or characteristic flora or not.

Many plants and animals are called endemic when they are restricted in their distribution within a small area or region. Sometimes higher taxonomic groups like genera or even families are found restricted in a given area and would, therefore, be called endemic. For example, genera like

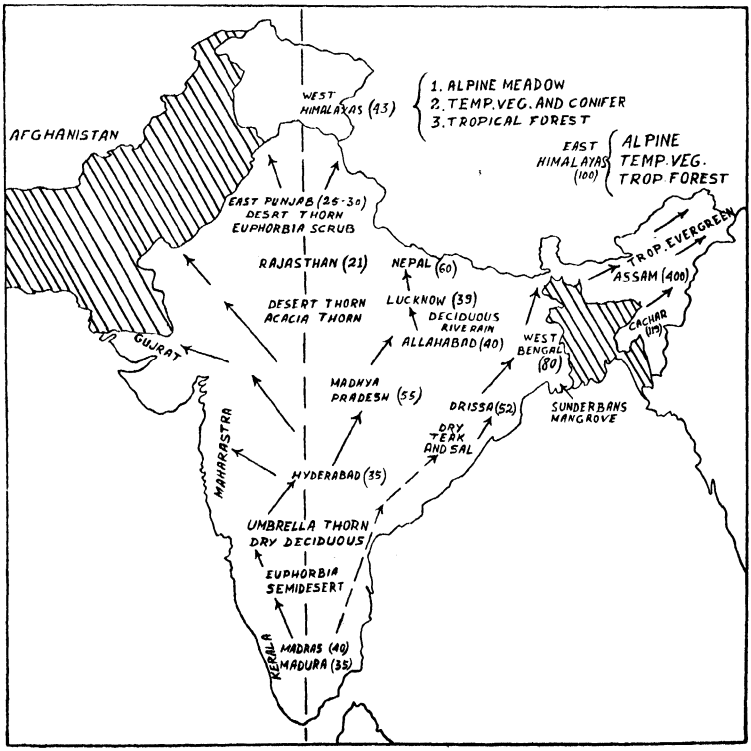


Fig. 1. Map of India showing the typical growth forms in various parts and their correlation with rainfall.

Chloroxylon (Rutaceae), *Beddomea* (Meliaceae), *Adenoon* (Compositae), *Jerdonia* (Gesneriaceae) and *Helicanthes* (Loranthaceae) are all confined to South India and are, therefore, endemic. In Madagascar, the families Chlaenaceae, Rhopalocarpaceae and Didieriaceae are localised within the island and should, therefore, be regarded as endemic.

The smaller range of endemic species, genera or families is probably due to two causes: (*i*) survival under favourable conditions of representatives of some ancient but widely distributed taxa (relict type), and (*ii*) formation of new species or groups, which due to their comparatively recent origin, have not had the opportunity of extending their distribution far and wide (neo-endemics). Such plants as *Ginkgo biloba, Metasequoia* and tree ferns may be cited as examples of the first group, while species of Himalayan *Gentiana, Impatiens* and *Primula* may be mentioned as examples of the second category. In oceanic islands, the surrounding sea acts as a barrier against long distance distribution and as such, the chances of increase in endemic species are greater in these islands. This will be evident from the fact that in the Hawaii islands the percentage of endemic species is as high as 82 per cent. The figure for New Zealand is 72 and for the Fiji islands 50. The indigenous flora of Madagascar and its associated islands abounds in endemic species, and the endemic value for the flora would also be high. Out of a total of 7,370 species of seed-bearing plants, as many as 6,765 species are considered endemic. It would be a matter of interesting investigation as to how the ancestral species of plants now found in these islands reached their destination. There are limitations of long distance dispersal beyond which effective dispersal of seeds or fruits would not be possible. Arrival of plants or ancestral plants in these islands might have been possible by one or more of the following causes:

(*i*) Arrival from continental areas through land connections which may have existed in the past.
(*ii*) Water currents.
(*iii*) Cyclones, storms and winds.
(*iv*) Migratory birds.

The continental drift, postulated by Wegener, might also be responsible for the presence of many ancestral plants, especially those showing affinities with plants in the continental areas.

In contrast to the restricted distribution of species in oceanic islands, the chances of distribution of species from one country to another, are much greater in continental areas. Both natural and assisted distribution of seeds and other propagating material through various agencies such as men, grazing animals, trans-continental trains and aeroplanes are much greater these days. As a result, we find that a large number of species found in one country of a continent are also found in the adjoining country. This, in turn, reduces the endemic percentage in relation

to the total number of species found in that country. For example, the percentage of endemic species in countries of Europe like Spain, France, England, Germany, Holland and Poland would hardly exceed 10 to 15 per cent. The same is probably true for countries in other continents which are, in many cases, floristically similar.

The position, however, is very different with respect to India, which is admittedly a country within the continent of Asia. A careful analysis of all species of dicotyledonous plants found in India was made (Chatterjee, 1940) and contrary to expectation, it was found that India has a high percentage of endemic species. In fact the proportion of endemic species is so high that it can compare favourably with some of the oceanic islands.

The causes which are responsible for the high endemic percentage of Indian species are worth investigation. It will be seen that the lofty Himalayan ranges act as a barrier against the migration of plants from the Tibetan area to the Indo-Gangetic plains. The Indian Ocean also

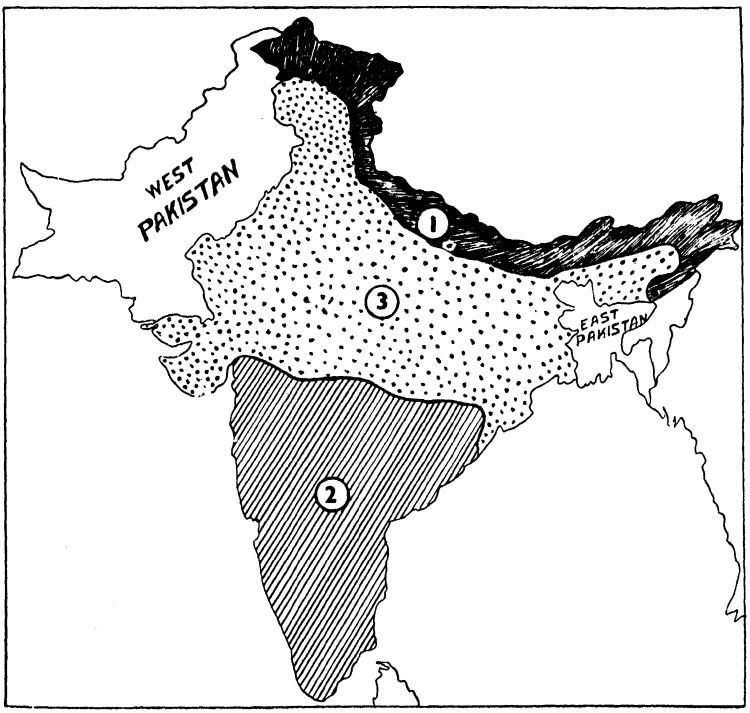


Fig. 2. Map of India showing areas of endemic plants in (1) Himalayas, (2) Peninsular India and (3) Indo-Gangetic plain.

acts as a barrier in the south against migration of plants from other countries south of India. Even the Indo-Gangetic plain with its warm alluvial soil acts as a barrier for migration of many Himalayan plants to the Nilgiri and other hills of South India. These natural barriers have helped in the development of a large number of species which are either localised in their distribution in the Himalayas or in the Peninsular India. The Himalayas undoubtedly contain the largest number of endemic species (3,169 dicots and about 1,000 monocots). South India comes next, with 2,045 species of dicots and about 500 monocots. The intervening area of plains which lies between the Himalayas and South India is, however, an area of high biotic concentration and equable climate. It is for this reason that this area is rather poor in endemic species and rich in pantropical weeds and other species which are widely distributed and which are commonly designated as "wides". In spite of this relative poverty of endemic species in the Indo-Gangetic plain, the all-India percentage of endemic species would still be very high and would be approximately 50 per cent (Chatterjee, 1940). Such high percentage of endemic species indicates that India has a typical flora of her own (Fig. 2).

An examination of a map of South-East Asia (Fig. 3) apparently shows that India occupies more or less a central position and might have faced invasion by a large number of species from the surrounding countries. African and East Mediterranean elements are often found in

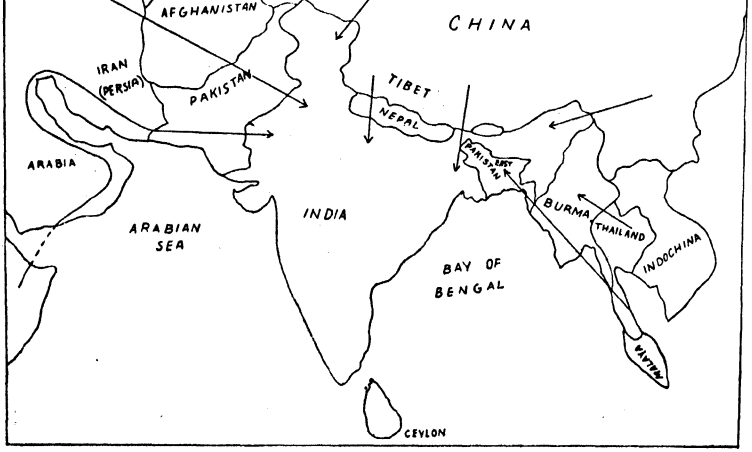


Fig. 3. Map of South-East Asia showing the possible lines of migration of flora from the neighbouring countries towards India.

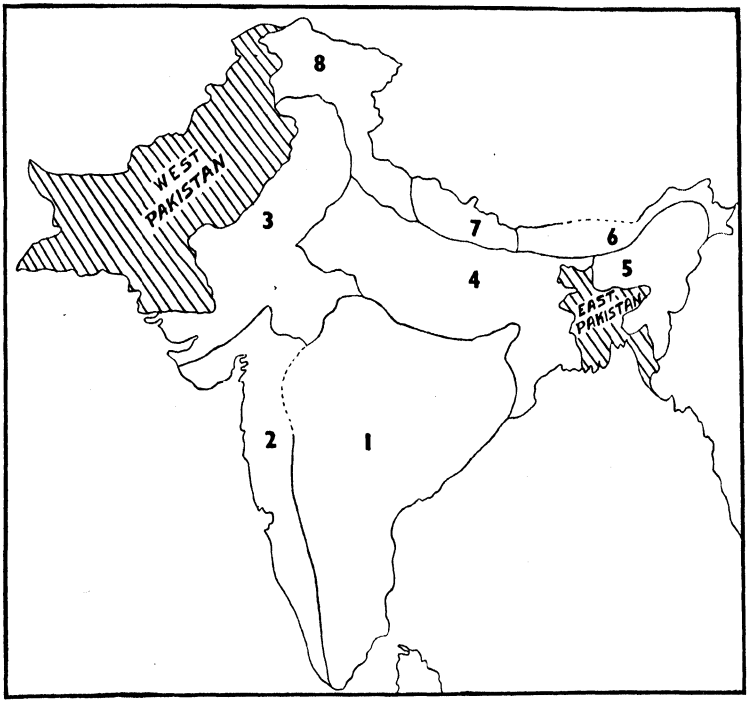


Fig. 4. Map of India showing the different phyto-geographical areas.

only in India and not distributed outside the country was considered a probable clue for a satisfactory solution of the problem. Examination made on these lines revealed that contrary to expectation, India has not only a rich endemic flora but also a large number of endemic species. It was found that more than 50 per cent of the dicotyledonous species of India were totally confined to India. It was, therefore, concluded that India *has* a flora of her own. Her unique geographical features and natural and physical barriers of lofty mountain chains in the north and ocean in the south have helped in the development of such a large number of endemic species. The endemic species and genera are largely concentrated in two regions, viz. (*i*) the Himalayas and (*ii*) South India. The Indo-Gangetic plain is relatively poor in endemic species. An attempt to divide the vegetation of India into phyto-geographical regions was also made and eight such regions are proposed as follows: (1) Deccan, (2) Malabar, (3) Indus Plain, (4) Gangetic Plain, (5) Assam, (6) Eastern Himalayas, (7) Central Himalayas and (8) Western Himalayas. These

regions are somewhat different from similar regions proposed by Hooker, Clarke and other earlier botanists.

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SOME ASPECTS OF THE CYTOTAXONOMY OF *AEGILOPS*

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The two genera, *Aegilops* and *Triticum* of Triticeae are closely related. Bowden (1959), however, has recently included the genus *Aegilops* with its constituent species in the genus *Triticum*. This lumping together of the two genera is neither in conformity with the rules of nomenclature nor is in agreement with the cytological and genetical studies.

Since the inception of the genus *Aegilops* by Linnaeus in 1737 only two important works have appeared on the systematic revision of these grasses. Of these the system by Zhukovsky (1928) includes 20 species in 9 sections. The monograph of Eig (1929a) has 22 species grouped into 6 sections and 2 subgenera. These two systems differ in some respects and there is also much confusion in the grouping of species into sections. The cytological approach has greatly helped the taxonomy of *Aegilops*. The first and most outstanding work in this direction is that of Senyaninova-Korchagina (1930, 1932). In this work which is an improvement over the previous systems, the species are grouped into 7 sections and a karyosystematic relationship is drawn for the species. Kihara's (1954) system which is mostly based on the genome-analytical work has 20 species in 6 sections.

The species of *Triticum* which have the chromosome series $2n = 14$, 28 and 42 fall into corresponding morphological groupings, namely Einkorn, Emmer and Dinkel. The genus *Aegilops* includes diploid, tetraploid and hexaploid species. The same chromosome series found in the wheats is also among the species of *Aegilops*, but the species in the latter can never be grouped into corresponding morphological series. This is because there is polymorphism within each chromosome series of *Aegilops*. This fact has confused the classical taxonomist and consequently his treatment is uneven. Thus, sometimes on account of certain minor differences, say in the nature of the awn or the hairiness etc., the taxa which are closely related have been made into separate species. For example, *Ae. aucheri* Boiss. differs from *Ae. speltoides* Tausch. in having only the apical spikelets with awns. Sometimes, the taxa which differ considerably with respect to the presence or absence of awns, the number of spikelets etc., are often lumped together as one species e.g., *Ae. triuncialis* L. In such cases, cytology and genetics can be of some use in forming reliable categories.

KARYOTYPES

Critical cytological studies have been made on all the known species of *Aegilops*. The chromosomes in *Aegilops* are more individual than those of the wheats (Sorokina, 1928). The diploid species represent in all seven basic karyotypes:

(1) The karyotype in *Ae. mutica* Boiss. and *Ae. speltoides* Tausch. consists of two pairs of chromosomes with fairly large satellites. The other five pairs in them have submedian centromeres. The difference between these species lies in the arm ratios.

(2) The type seen in *Ae. longissima* Schweinf. et Muschl. and *Ae. bicornis* (Forsk.) Jaub. et Spach. consists of two pairs of chromosomes with satellites of different sizes, the smaller one being slightly less than half the size of the larger one. All the other pairs have submedian centromeres. The close similarity of the karyotypes in these two species is also reported by Senyaninova-Korchagina (1930, 1932) and Riley *et al.* (1958).

(3) The third type is represented by *Ae. umbellulata* Zhuk. This has again two pairs of chromosomes with satellites of different sizes, the smaller one being approximately one-third of the larger satellite. Of the other five pairs of chromosomes, two have submedian centromeres, another two have submedian-subterminal centromeres and the fifth pair has a characteristic knob due to extreme subterminal centromere.

(4) *Ae. caudata* L. represents the fourth type. Its karyotype also consists of two pairs of chromosomes with satellites which differ only to a slight degree in size. Of the other five pairs, one has submedian centromere, another one has submedian-subterminal centromere and the rest three have subterminal centromeres.

(5) The fifth type is seen in *Ae. comosa* Sibth. et Sm. It has one pair with secondary constrictions with the arm ratio of approximately 1 : 1 : 1.5 and one pair with satellite on the short arm. The other five pairs of chromosomes, broadly speaking, have submedian centromeres.

(6) The karyotype in *Ae. uniaristata* Vis. which forms the sixth type has only one pair of chromosomes with satellites. Of the other six pairs, two have submedian centromeres, two have submedian-subterminal centromeres and the other two have subterminal centromeres.

(7) Lastly, *Ae. squarrosa* L. constitutes the seventh type, consisting of one pair with satellites and the other six pairs having submedian centromeres.

The karyotype analysis in the tetraploid and hexaploid species reveals that they consist of the above seven basic karyotypes, with or without

certain modifications. It may thus suggest that the tetraploid and hexaploid species of *Aegilops* are of interspecific hybrid origin, as understood till now.

APPLICATION OF KARYOTYPE TO SYSTEMATICS

Certain taxonomic deductions are possible from the karyotype study of this group and some important ones comprising the taxa of different ranks are presented here.

According to Zhukovsky (1928), Senyaninova-Korchagina (1930, 1932) and Kihara (1954), *Ae. mutica* Boiss. is placed in the section *Amblyopyrum* Eig (1929a) places it under the section *Anathera* and subgenus *Amblyopyrum* and considers that it closely approaches *Ae. longissima* and *Ae. bicornis*. Zhukovsky (1928) and Eig (1929a) have both expressed the view that the section with its species *Ae. mutica* is transitional to *Agropyron sensu latu*. In a later paper, however, Eig (1929b) separates it and places it under a new genus, namely *Amblyopyrum*. The chief morphological characters that distinguish it from the other species of *Aegilops* are its awnlessness and its glume characters. The karyotype of *Ae. mutica* seen here has close resemblance to that of *Ae. speltoides* and *Ae. aucheri*. This supports the view of Boissier (1844) that it is nearer to *Ae. aucheri*, rather than the view of Eig (1929a) that it is nearer to *Ae. longissima*. Whether it is related to *Ae. aucheri* or to *Ae. longissima*, *Ae. mutica* can not be included in the section *Sitopsis* to which the other two species belong, because it is completely awnless and keelless. It also stands in clear morphological contrast from the rest of the species of *Aegilops*. The creation of a new genus *Amblyopyrum* by Eig (1929b) seems justified.

The section *Sitopsis* with its constituent species, *Ae. speltoides*, *Ae. longissima* and *Ae. bicorne* stands in contrast from the other sections of *Aegilops* and is closely related to *Triticum*, especially to *T. aegilopoides*, by the presence of a rudimentary keel on the empty glume. It serves as a link between *Triticum* and *Aegilops*. The chromosomes of the various species of this section have submedian centromeres like those of the species of *Triticum*; and they have no chromosome with subterminal centromere characteristic of some species of *Aegilops*. On these grounds the section *Sitopsis* with its constituent species could be transferred to the genus *Triticum* or a new genus may have to be created for it.

In the section *Sitopsis*, *Ae. aucheri* Boiss. differs from *Ae. speltoides* Tausch. in having only the apical spikelets with awns. Both are diploids having similar karyotypes, except for slight differences in the arm ratios of the satellited pairs of chromosomes. According to Kihara & Lilienfeld (1932), the F_1 hybrid between *Ae. speltoides* and *Ae. aucheri* shows 7

bivalents during meiosis. Sears (1941) finds a complete homology of their chromosomes. He prefers to treat them as varieties. According to Kihara & Yamashita (1956), *Ae. aucheri* differs from *Ae. speltoides* in a single gene, the one for awning. According to Kihara (1951) there is a different degree of incompatibility between the genotype of the two taxa on the *longissima* plasma. The range of distribution is the same for both, namely Palestine, Syria, Mesopotamia and Asia Minor. Their reproductive isolation is incomplete. All these considerations in addition to their karyotypic similarity do not favour the treatment of the two taxa as separate species. They are treated as subspecies in the following references:

Aegilops speltoides Tausch. Flora **20** : 108-109. 1837. Syn.: *Triticum speltoides* (Tausch.) Gren., Fl. Massil. Adv. in Mem. Soc. Emul. Doubs. **2** : 434. 1857. *T. speltoides* (Tausch.) Gren. ex Richter, Plant. Europ. **1** : 129. 1890.

ssp. *speltoides*

ssp. *aucheri* (Boiss.) Chennaveeraiah, comb. nov., based on *Ae. aucheri* Boiss., Diagn. Plant. Orient. Nov., ser. 1. fasc. **5** : 74. 1844. Syn.: *Ae. speltoides* Tausch. var. *aucheri* (Boiss.) Fiori, Nuov. Fl. anal. d'Ital. **1** : 159. 1923-25. *Triticum speltoides* (Tausch.) Gren. ex Richter f. *speltoides* Bowden, Canad. J. Bot. **37** : 665. 1959.

Similarly, *Ae. longissima* Schweinf. et Muschl. and *Ae. sharonensis* Eig form another pair. The outer glumes of *Ae. sharonensis* are awned and those of *Ae. longissima* are not. Their karyotypes are very similar except for the difference to a slight degree in the arm ratios of the non-satellited pairs. Tanaka (1955) has obtained hybrids between them. The hybrids are awnless. The pairing in meiosis is regular. The pollen and seed fertility is practically normal. He has concluded that *Ae. sharonensis* may be considered as a variety of *Ae. longissima*. The distribution of the two is overlapping and both are found in Palestine and Egypt. The reproductive isolation is incomplete. These two taxa are also treated here as subspecies.

Aegilops longissima Schweinf. et Muschl., A Manual Flora of Egypt **1** : 156. 1912. Syn.: *Triticum longissimum* (Schweinf. et Muschl.) Bowden, Canad. J. Bot. **37** : 666. 1959.

ssp. *longissima*

ssp. *sharonensis* (Eig) Chennaveeraiah, comb. nov., based on *Ae. sharonensis* Eig, Notizbl. Bot. Gart. Berlin **10** : 489. 1928.

A similar situation is found in *Ae. comosa* Sibth. et Sm. and *Ae. heldreichii* Holzm. of the section *Macrathera*. The karyotypes of these are similar in most respects. Kihara (1937) and Kihara & Yamashita (1956) point out that they differ cytologically by a reciprocal translocation. Eig (1929a) treats *Ae. heldreichii* as a subspecies of *Ae. comosa*.

Lilienfeld (1951) considers it as a variety of *Ae. comosa*. Both these taxa are found in Greece, Syria and Asia Minor. Sears (1941) suggests that the two may be treated as subspecies. The same opinion is expressed in the following references:

Ae. comosa Sibth. et Sm., Fl. Gr. **1** : 75 & tab. 94. 1806. Syn.: *Ae. comosa* Steud., Synop. Plant. Gram. 356. 1855. *Triticum comosum* (Sibth. et Sm.) Richter, Plant. Europ. **1** : 128. 1890 ; Thell., Fl. adv. Montp.: 148. 1912.

ssp. *comosa*

ssp. *heldreichii* (Holzm.) Eig, Fedde Rep. Spec. Nov. Beih. **55** : 109. 1929. Syn.: *Ae. heldreichii* Holzm., Herb. norm. Plant. exs. flor. Hell.: 898 & 986. 1881 ; Halacsy, Zeit. d. bot. Gesel.: 763. 1886 ; Halacsy, Consp. Fl. Graec. **3** : 433. 1904. *Ae. caudata* var. *heldreichii* (Holzm.) Boiss., Fl. Or. **5** : 675. 1884. *Triticum heldreichii* (Holzm.) Richter, Plant. Europ. **1** : 128. 1890 ; Thell., Fl. adv. Montp.: 148. 1912. *Ae. turica* Azn., Fl. de Constant., Bull. Soc. Franc. **44** : 177. 1897.

Among the tetraploids, there seems to be mistaken identity between *Ae. kotschyi* Boiss. and *Ae. variabilis* Eig. The chromosome homology between them as shown by Kihara (1937) and Lindschau & Oehler (1936) and the inclusion of *Ae. kotschyi* with *Ae. variabilis* (Lilienfeld, 1951 ; Kihara, 1954, 1957a,b) is probably dependent on this mistaken identity. Probably they were dealing with different populations of *Ae. kotschyi*. The karyotypes in these two taxa studied by me differ significantly. *Ae. variabilis* has three pairs of chromosomes with satellites whereas *Ae. kotschyi* has only two such pairs. Also, there are differences in the arm ratios of the other chromosomes. The two taxa in my collection are morphologically distinct. If we consider that the chromosome differences are conclusive enough, the differences of the karyotypes in them should indicate that the two are distinct and separate species. They are not conspecific, contrary to the current opinion.

Ae. triuncialis seems to be a highly complex species among the tetraploids. There is a great diversity of morphological range in the taxa included in this species. The two subspecies studied, *Ae. triuncialis* ssp. *persica* and *Ae. triuncialis* ssp. *brachyathera* have different karyotypes. The former has three pairs of chromosomes with satellites, while the latter has only two satellited pairs. There are differences in the other chromosomes also. A decision now is not possible since all the taxa of this species complex have not been available for study. However, a revision of this species complex is highly desirable.

Ae. triaristata forms a tetraploid-hexaploid complex. In the tetraploid, i.e., *Ae. triaristata* ssp. *triaristata*, there is one pair of chromosomes with satellite on short arm, one pair with extreme subterminal centromere, four pairs with submedian-subterminal centromeres and eight pairs with

submedian centromeres. All these chromosomes are recognized in the hexaploid, i.e. *Ae. triaristata* ssp. *recta*. The additional seven pairs in the hexaploid never in any way form a duplicated set of either of the other two sets in the tetraploid. Kihara (1937) has observed 14 bivalents and seven univalents in the hybrid of 6x and 4x forms. This also shows that the third set of chromosomes is different from the other two sets. There are also certain morphological differences between them. In the tetraploid the number of spikelets is two or three, whereas it is four to six in the hexaploid. The awns in the tetraploid are spreading. In the hexaploid they are slanting. In the tetraploid the glumes are glabrous, but they are hairy in the hexaploid. The chromosome differences in them are thus supplemented by the morphological differences. The hybrid between the two is sterile. Therefore, there should be no hesitation to treat the two as separate species. They are as follows:

Ae. triaristata Willd. s. str. Sp. Pl. **4 :** 943. 1805. Syn.: *Ae. ovata* L. var. *triaristata* (Willd.) Griseb., Grisebach Spicil. Florae rumelicae et bithynicae **2 :** 425. 1844. *Triticum triaristatum* (Willd.) Godr. et Gren., Fl. Franc. **3 :** 602. 1856. *Ae. ovata* L. var. *triaristata* (Willd.) Cosson et Durieu, Fl. d'Alg.: 211. 1867 ; Boiss., Fl. Or. **5 :** 674. 1884 ; Post, Fl. of Syr., Pal. and Sinai: 899. 1896. *Triticum ovatum* (L.) B. *triaristatum* (Willd.) Asch. et Graeb., Asch. et Graeb. Synop. **2 :** 705. 1899-1902. *Ae. ovata* L. var. *triaristata* (Willd.) Halacsy, Consp. Fl. Graec. **3 :** 431. 1904.

Ae. recta (Zhuk.) Chennaveeraiah, comb. nov., based on *Ae. triaristata* Willd. ssp. *recta* Zhukovsky, Bull. Appl. Bot. Genet. & Pl. Breed. **18 :** 478. 1928.

Similarly, *Ae. crassa* forms another tetraploid-hexaploid complex. In the tetraploid *Ae. crassa* ssp. *crassa* the karyotype consists of two pairs of chromosomes with satellites, one pair with median centromere and eleven pairs with submedian centromeres. All these chromosomes are recognized in the hexaploid *Ae. crassa* ssp. *vavilovi*. The additional seven pairs in the hexaploid, consisting of one pair with satellites, one pair with median centromere and five pairs with submedian centromeres, are closely related to one of the two sets in the tetraploid, but never the same as the set in the tetraploid. *Ae. crassa* complex occurs in Palestine, Syria, Iraq, Afghanistan and Turkistan. According to Kihara *et al.* (1959), the tetraploid and hexaploid forms grow fairly mixed together. The hexaploid form must have arisen by chromosome doubling of the hybrid between the tetraploid form and an unknown diploid. There are certain morphological differences between them. In the tetraploid the node is covered by a sheath ; in the hexaploid it is naked. In the tetraploid the sheath is nonciliate at the margin ; in the hexaploid it is ciliate. The cytological and morphological differences between them are sufficient

to warrant the distinction of the tetraploid and hexaploid taxa as separate species. They are as follows:

Ae. crassa Boiss. s. str., Diagn. Pl. Or. Nov., ser. 1, fasc. **7 :** 129-130. 1846. Syn.: *Triticum crassum* (Boiss.) Aitch. et Hemsl., Trans. Linn. Soc. **3 :** 127. 1888.

Ae. vavilovi (Zhuk.) Chennaveeraiah, comb. nov., based on *Ae. crassa* Boiss. ssp. *vavilovi* Zhukovsky, Bull. Appl. Bot. Genet. & Pl. Breed. **18 :** 552-554. 1928.

Summary and Conclusions

Thus with the application of karyomorphology, the diploid species *Ae. aucheri* Boiss., *Ae. sharonensis* Eig and *Ae. heldreichii* Holzm. are treated as subspecific to *Ae. speltoides* Tausch., *Ae. longissima* Schwenif. et Muschl. and *Ae. comosa* Sibth. et Sm. respectively.

Among the tetraploid species, *Ae. kotschyi* Boiss. and *Ae. variabilis* Eig are not conspecific, but constitute two quite distinct species.

In the tetraploid-hexaploid complexes of *Ae. triaristata* and *Ae. crassa*, the tetraploid forms one species and the hexaploid another species in both the complexes.

The section *Sitopsis* with its constituent species needs to be transferred from *Aegilops* to *Triticum*, or a new genus may have to be created for it.

The formation of the new genus *Amblyopyrum* Eig for *Ae. mutica* Boiss. is justified.

As Darlington (1950) says, "changes in the chromosomes determine the isolation and divergent evolution of races, ecotypes, species and families . . . they determine not merely these present properties but all their future expectations". Further, a critical chromosome study is considered to be quite useful in modern taxonomy.

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GENOME ANALYSIS IN WHEAT

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The cereal scientists have evinced a great interest in the problem of the origin of the bread wheat. The bread wheat, *Triticum aestivum* (Syn. *T. vulgare*), is a hexaploid. All the hexaploid wheats are in the Dinkel group. Similarly the tetraploid and diploid wheats are in the Emmer and Einkorn groups respectively. Thus the wheats fall into three morphological groupings corresponding with the chromosome series and they have the genome formulae AA, AABB, and AABBDD. The species of *Aegilops* are considered to be closely related to wheats, and it was suspected that one or possibly two genomes have been contributed by *Aegilops* to the polyploid wheats. Many of the hybridization experiments of wheat and *Aegilops* point towards this direction.

The earliest intergeneric cross of *Aegilops* and wheat is that by Godron (1854). This and his later experiments (1856, 1870, 1877) were not in connection with the origin of bread wheat, but they concerned Fabre's (1852) and Jordan's (1855) conflicting views on the so-called *Aegilops* species, *Ae. triticoides*, proving that this species arose as a hybrid between *Ae. ovata* and wheat. According to Vilmorin (1911) another such successful intergeneric cross was made by his grandfather in 1856 and that was again between *Ae. ovata* and wheat.

METHODS

The earlier method of studying the relationships is the morphological comparison of the species involved. More recently, Anderson (1949) advanced an extrapolation method which also uses the morphological characters but more exactly. Applying this method Sarkar & Stebbins (1956) predicted that *Ae. speltoides* might have contributed the B genome to the tetraploid wheats. With the increased knowledge of cytology, chromosome homology became an important factor in genome analysis. The pairing in meiosis is generally taken as an indication of homology. This method has been extensively used by Kihara and his co-workers. There are certain inadequacies of this method and Gaul (1958) has discussed in detail why homology, as indicated by pairing, is not entirely reliable. Frequently, the karyotype of the involved species has also been taken into consideration in determining the relationship. By this method, Pathak (1940) suggested that *Ae. squarrosa* might be the donor of the D genome of the hexaploid wheats. Finally, the method is to produce

amphiploids of the suspected donors artificially and to compare them with the natural species, both morphologically and genetically.

The A Genome

The A genome is from the Einkorn wheats (*T. monococcum* and *T. aegilopoides*). By the formation of usually seven bivalents between the members of the Einkorns and Emmers, it is believed that the A genome of the Emmers is similar to the A genome of the Einkorns. Similarly the AB genomes of the Emmers and Dinkels are known to be alike. Thus the A genome of the Einkorns is found in the Emmers and Dinkels as well.

The D Genome

The suggestion of Percival (1921, 1923) that *Ae. ovata* and *Ae. cylindrica,* one or both, appear to have been involved in the ancestry of the Dinkel group of wheats gave a real impetus to cereal scientists to plan breeding experiments in the hope of synthesizing the bread wheat. He believed the bread wheats, or 21-chromosome wheats to be of hybrid origin and to have in their parentage the Emmer wheats and two *Aegilops* species, *cylindrica* and *ovata*. He arrived at these conclusions from the fact that the characters found in *Triticum aestivum,* not explainable by the Emmer group, can be accounted for by *Ae. cylindrica* and *Ae. ovata*. The results of breeding experiments of Percival (1926, 1928, 1930, 1932), Sax & Sax (1924) and Sax (1928), based mostly on morphological characters, support this view. This hypothesis was disproven when both the supposedly involved *Aegilops* species were definitely shown to be tetraploids (Kihara, 1929, 1930, 1931), demonstrating that neither *Ae. ovata* nor *Ae. cylindrica* could be the donors of the D genome in their entirety. However, Gaines & Aase (1926), Kagawa (1929), Aase (1930), Kihara (1931), Thompson (1931) and Sears (1944) have shown that *Ae. cylindrica* has the third genome D of the Dinkel wheats. The D genome is common to *Ae. cylindrica* and the Dinkel wheats.

Meister (1928) suggested that *T. aestivum* originated from a cross between *T. dicoccum* and *Secale cereale*. He claimed to have obtained a constant intermediate fertile hybrid in F_2. But Kihara (1924) found mostly univalents in wheat-rye hybrids. Longley & Sando (1930) also found no pairing in the cross *T. aestivum* × *S. cereale,* but in the cross *T. aestivum* × *S. montanum* one to three bivalents were seen rarely. This may be due to autosyndesis of wheat chromosomes among themselves. The results of Thompson (1926) and Bleier (1928, 1933) also show a lack of homology between the chromosomes of wheat and rye. Therefore, *Secale* could not have donated the D genome to the bread wheat.

Similarly, the suggestion by Gates (1931) that the hexaploid wheat arose as an amphiploid between the diploid and tetraploid wheats is discarded, as it has not been experimentally proved. The characters of the Dinkel wheats not found in the Emmers cannot be accounted for in the Einkorns.

Peto (1930, 1936) and Vakar (1934, 1935, 1936) believed that the bread wheat arose as a cross between *Triticum* and *Agropyron*. Excepting *A. elongatum*, most species of this genus have been known to contain chromosomes with subterminal centromeres, whereas their absence is significant in the wheats. Peto (1936) observed that approximately one set of chromosomes remained unpaired in the cross *T. dicoccum* var. *vernol* × *A. elongatum*, and approximately two sets remained unpaired in the cross between three varieties of *T. aestivum* × *A. elongatum*. It shows that the chromosomes of *A. elongatum* have affinities with one of the genomes in the tetraploid wheats, but not with the third genome of the hexaploid wheats.

On the basis of chromosome morphology, Pathak (1940) suggested that *Ae. squarrosa* could be the donor of the D genome of the hexaploid wheats. Sax & Sax (1924) noticed that seven *Aegilops* (*Ae. cylindrica*) chromosomes pair with seven wheat chromosomes. Kagawa (1928) also found seven bivalents in the F_1 of *Ae. cylindrica* × *T. aestivum*. Gaines & Aase (1926), Kagawa (1929), Aase (1930), Kihara (1931), Thompson (1931) and Sears (1944) all came to the conclusion that *Ae. cylindrica* has the third genome D of the Dinkel wheats, and not A or B of the Emmer wheats. Kihara & Matsumura (1941) speculated that the D genome was probably from *Ae. squarrosa*. Kihara (1944) and McFadden & Sears (1944) independently produced amphiploids between the Emmer wheat and *Ae. squarrosa*. The synthesized amphiploid resembled *T. spelta*, one of the hexaploid wheats. The experiments have been extended to other hexaploid wheats and though the evidences are only indirect, it is commonly believed that all the hexaploid wheats have the D genome in common derived from *Ae. squarrosa*. This view has received confirmation by field observations (Kihara & Yamashita, 1956).

The B Genome

The question regarding the B genome has been under a cross fire for a long time and the real truth remains to be unearthed. The suggestion of McFadden & Sears (1946) that the Emmer wheat might have arisen as an amphiploid between *T. monococcum* and *A. triticeum* is easily dispensed with, because the karyomorphological studies of Avdulov (1931), Sarkar (1955) and Matsumura & Sakamoto (1955) have shown that *A. triticeum* does not possess the desired type of chromosomes, i.e. with median and submedian centromeres. It may be recalled here that Peto

(1936) says that both auto- and allosyndesis takes place in *A. elongatum* and *Triticum* hybrids and that one set of chromosomes paired in a cross of *T. dicoccum* var. *vernol* and *A. elongatum*. It shows that the chromosomes of *A. elongatum* have affinities with one of the genomes in the Emmers. Matsumura & Sakamoto (1955) are of the opinion that *A. elongatum* has the desired type of chromosomes in respect to the B genome. However, further evidence has not been put forward in support of this view.

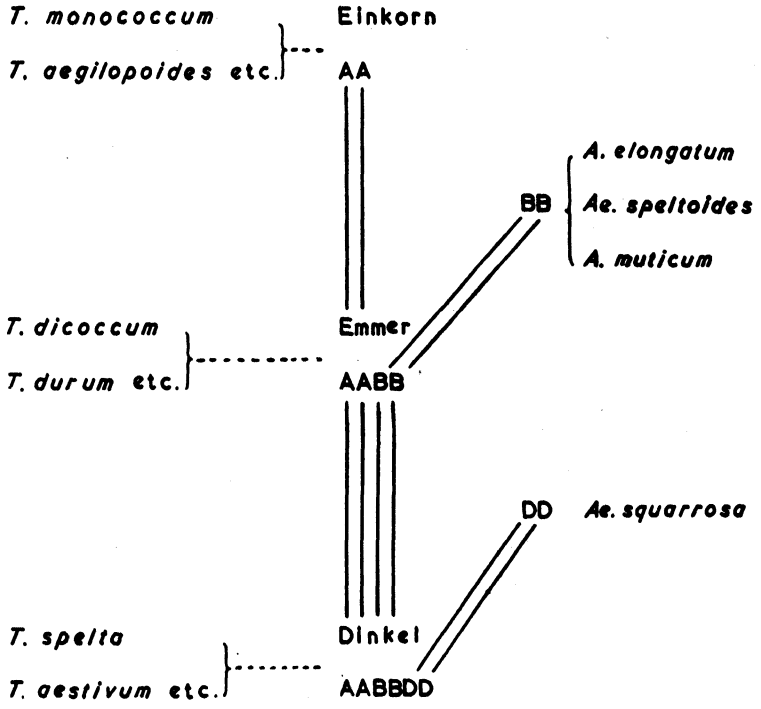
The morphological characters have been critically applied to this problem by Sarkar & Stebbins (1956) using Anderson's (1949) "Method of Extrapolated Correlates". They suggest that *Ae. speltoides* var. *ligustica* could be the donor of the B genome. This may be very near the truth.

In the karyotypes of the Emmer wheats there are two pairs of chromosomes with fairly large satellites. These are not seen in the Einkorns, but they are found again in the Dinkel wheats. Studies of Larson (1952) and Okamoto (1957a,b) have shown that both these pairs belong to the B genome. Therefore, the donor of the B genome should have these two satellited pairs, in addition to the other chromosome characters typical of the B genome. Studies by Riley *et al.* (1958) have shown that *Ae. speltoides* in the section *Sitopsis* has the desired type of chromosomes of the B genome. The SAT chromosomes of *Ae. speltoides* are similar to the SAT chromosomes in the tetraploid wheats. Thus they support the view of Sarkar & Stebbins (1956) that *Ae. speltoides* could be the donor of the B genome.

Much earlier, Jenkins (1929) observed the formation of seven bivalents in a cross between *Ae. speltoides* and *T. turgidum*. He came to the conclusion that *Ae. speltoides* chromosomes are homologous with A or B genome. Thompson (1931) also came to the conclusion that *Ae. speltoides* has A or B genome. Later Thompson *et al.* (1943) crossed *T. turgidum* with *Ae. speltoides* and the amphiploid of the hybrid was compared with the bread wheat, suggesting that *Ae. speltoides* might be carrying the D genome. This cancells Thompson's previous view.

The pairing behaviour of *Ae. speltoides* chromosomes is not entirely satisfactory in favour of the B genome. For example, Chizaki (1932) and Kihara & Lilienfeld (1932) have shown some degree of pairing in crosses of *Ae. speltoides* and *T. monococcum*, the latter consisting of the A genome but not the B genome. Sears (1956) has expressed the opinion that the S^s (*Ae. speltoides*) genome has more affinity to A than to B, because there is almost full pairing between A^m (*T. monococcum*) and S^s (*Ae. speltoides*). Therefore, the pairing data do not fully support the view that *Ae. speltoides* has the B genome.

Neither can the other members of the *Sitopsis* section be the donors of the B genome. Tanaka (1955, 1956) has proved that the S^1 (*Ae. longis-*



GENOMES IN WHEAT

FIG. 1

sima and *Ae. sharonensis*) genome is not homologous with the B genome. Secondly, the karyotypes of *Ae. bicornis, Ae. sharonensis* and *Ae. longissima* do not fit into the chromosome pattern of the B genome. This fact is made clear also by Riley *et al.* (1958).

In the light of the above circumstances, it is worthwhile making a further search for the donor of the B genome until something is definitely proved. I suggest that *Amblyopyrum muticum* (*Ae. mutica*) could be considered as a possible donor on the basis of its karyotype. According to Senyaninova-Korchagina (1930, 1932) the karyotype of *A. muticum* has four types of chromosomes, consisting of one pair with satellites and the rest of the pairs having submedian centromeres. With the better techniques now available, my studies in *A. muticum* show that its karyotype consists of two pairs of chromosomes with large satellites and the rest five pairs with submedian centromeres. This is exactly the type of chromosomes that the B genome consists of. Secondly, the karyotype of *A. muticum* corresponds well with that of *Ae. speltoides*. The size of the satellites also compares well with those of the Emmer wheats and of *Ae. speltoides*. Thus there is a good similarity of the karyotype of *A. muticum* with that of *Ae. speltoides* on the one hand and with that of the B genome on the other.

We may also look into the available pairing data. Kihara & Lilienfeld (1935, Figs. 21-23) have found good pairing in the F_1 of *T. durum* × *A. muticum* (*Ae. mutica*). The configurations are "$3_{III} + 5_{II} + 2_I$. $4_{III} + 3_{II} + 2_I$ (1 Univalentes fehlt), $5_{III} + 1_{II} + 3_I$ (1 Univalentes fehlt) and $1_V + 1_{III} + 4_{II} + 5_I$" (Kihara & Lilienfeld, 1935). Trivalents are frequently formed. The univalents vary from two to five. The formation of quadrivalents and trivalents not only explains autosyndesis of wheat chromosomes that may be taking place, but also the allosyndesis of *A. muticum* chromosomes with the wheat chromosomes. It is, therefore, suggested that *A. muticum* could as well be the donor of the B genome, but this needs confirmation by further experiments and other methods of study.

Conclusions

The genome analysis of the wheats as it stands to-day is presented diagrammatically in Figure 1. The A genome from the Einkorns occurs more or less in a linear fashion in the Emmers and the Dinkels. The D genome of the Dinkels is contributed by *Aegilops squarrosa*. There are three possible donors, viz. *Agropyron elongatum, Aegilops speltoides* and *Amblyopyrum muticum* for the B genome of the Emmers. Further research on the B genome will be required in order to determine whether all the above three have contributed to the different Emmer wheats, or any one of them is responsible universally, or whether there is some new source which we still do not know,

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STUDIES ON THE EFFECT OF AUXINS ON FLOWERING AND FRUITING OF PINEAPPLE

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Pineapple is one of the plants which can be forced to flower early by the application of auxins. The discovery that auxins are the effective chemical agents for inducing early flowering in pineapple was first reported from Hawaii in 1942 by Clark & Kerns. They observed that while low concentrations of the order of 10 ppm of NAA applied to stem apex accelerated flower formation, its relatively higher concentrations (1000 ppm) suppressed flowering even when the plants were exposed to short-day conditions (pineapple is a short-day plant). Cooper (1942) and Van Overbeek (1946) found that relatively low concentrations of sodium salt of NAA could readily induce early flowering in this plant. Cooper, however, observed that the degree of success in inducing early flowering depended largely upon the climatic conditions under which the experiments were conducted, for application of auxins in October yielded better results than applications in other months of the year under Florida conditions. Van Overbeek (1946) subsequently claimed to have forced pineapple to flower throughout the year.

Cooper's (1942) observation of a wide variation in the responses of pineapple towards the application of auxins under different environmental conditions prompted me to undertake work on this aspect under Indian conditions. The other reason for which this plant was selected as an experimental material is that stem apex and leaf-bases of pineapple contain a large amount of indoleacetaldehyde, the immediate precursor of the plant's endogenous auxin, IAA (*see* Van Overbeek *et al.*, 1947 ; Gordon & Nieva, 1949). As flowering of a plant is largely dependent upon its endogenous auxin level, studies on the induction of flowering in pineapple with the aid of auxins may throw new light on the problem of auxin-mechanism involved in the flowering of plants.

MATERIAL AND METHODS

An unirrigated hill slope was selected for the cultivation of pineapple. The layout of the experimental field was done strictly on statistical methods. Two replicates, each containing 27 plots, were made and each plot consisted of 20 plants. Pineapple suckers more or less of the same age of the Giant Kew variety were planted in April, 1958.

Aqueous solutions of three auxins (50 cc each)—NAA, 2,4-D and

IAA—at three levels (0, 5 and 10 ppm) were applied on the terminal vegetative bud and leaf bases with the help of a measuring cylinder. Application was made in mid-August, mid-September and mid-October, 1958. This was done in order to explore the probable variation in the responses of pineapple to auxin-treatment during different months of the year.

Until the appearance of flowers in the field, the measurement of the height of plant and counts of number of leaves (leaves longer than one foot were alone considered) of auxin-treated plants as well as of controls were taken at regular intervals of 30 days. When flowers appeared in March, 1959, flowers were counted in order to determine the difference in the percentage of flower formation in auxin-treated plants and controls. In this way the counting of flowers was continued till there was no more flower formation after September, 1959.

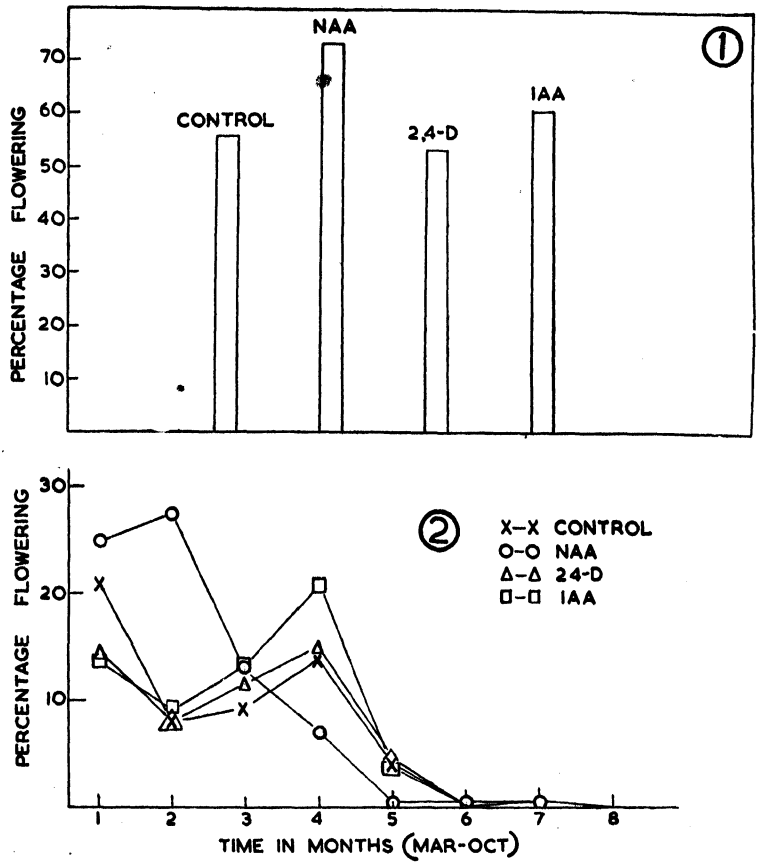
Experimental Results

Effect of Auxins on Flowering—The total effect of three auxins combined together shows a statistically significant difference in the percentage of flowers produced by auxin treatment. Plants treated with auxins showed about 62.48 per cent flower formation as against 56.00 per cent in the controls.

When the effect of individual auxins was examined it was found that both NAA and IAA promoted vegetative growth of plants with corresponding increase in leaf number. The effect of 2,4-D, however, was slightly inhibitory. Some formative effects, namely yellowing and curvature of leaves appeared after the auxin treatment.

NAA caused flowering in 73.63 per cent plants in contrast to 56.00 per cent in the controls ; IAA stands second in the list producing 60.33 per cent flowering. . 2,4-D, however, caused only 53.50 per cent flowering which is lower than that of the control (Fig. 1). The effect of 2,4-D, therefore, is slightly inhibitory.

When the percentage of flower formation in each month of the year was analysed, it revealed that there were two peak periods of flowering, the first in March and April and the second in June. The first flowering peak is much higher than the second, the former being the normal period of flowering, while the latter is of rare occurrence. No such two-peaked flowering period was observed with NAA-treated plants (Fig. 2) where the first peak was much higher, and the second peak had completely disappeared. This suppression of the second peak clearly indicates the induction of early flowering by NAA. Another significant feature of the action of NAA is that the flower induction by it is rapid and simultaneous and reaches the maximum level in the first two months, i.e. March and April, being 25.00 per cent and 27.50 per cent respectively as against 21.00 per cent and 8.00 per cent of controls. These results



Figs. 1-2.—Fig. 1. Histogram showing total percentage of flower induction throughout the year (1959) by a single treatment with three auxins—NAA, 2,4-D and IAA. The effect of each auxin indicates the sum total of the effects of auxin at 5 and 10 ppm combined together. Fig. 2. Percentage of flowering in each month of the year with three auxins—NAA, 2,4-D and IAA. The magnitude of effect of each auxin indicates the combined effect of two concentrations (5 and 10 ppm).

suggest that the penetration of NAA is possibly rapid. Plants treated with 2,4-D and IAA showed lower percentage of flower formation. In the month of May there was slight depression in the total flower formation in controls as well as in the auxin-treated plants which again rose in the succeeding month. This fluctuation in the number of flowers pro-

duced during one season is attributable to the changing environmental conditions.

IAA and 2,4-D treatment caused gradual rise in the number of flowers in May and June ; in June the maximum rise in flowering indicated the second flowering peak. The month of July first witnessed the sudden fall in the percentage of flower formation culminating in almost no flower formation in the subsequent months.

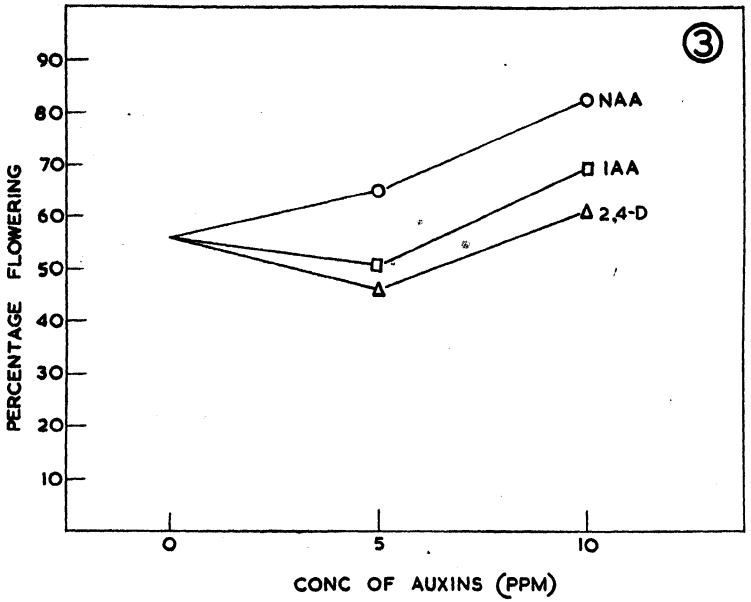
Relative Effectiveness of Various Concentrations of Different Auxins—An analysis of the extent of flower formation during the year by the three auxins at various concentrations was also investigated. It was found that NAA at 10 ppm evoked the highest percentage of flower formation which is of the order of 82.30 per cent, while at 5 ppm it is 64.96 per cent (Fig. 3). IAA stands second on the basis of number of flowers produced, where the value is 69.83 per cent at 10 ppm and 50.83 per cent at 5 ppm (this is lower than the controls). 2,4-D induced 61.00 per cent flowering at 10 ppm and 46.00 per cent at 5 ppm (this is also lower than the controls). Flower formation of the untreated conrols is about 56.00 per cent. It is thus seen that for all the three auxins, the concentration of 10 ppm is the optimum. Investigations of the effect of still higher concentrations of auxins are in progress.

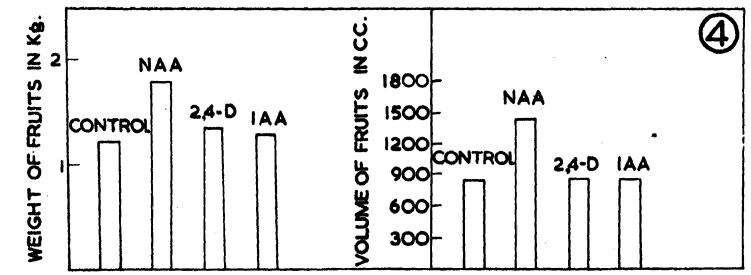
Month to month effect of the three auxins at the same concentration (10 ppm) suggests that the effect of NAA far exceeds the effect of 2,4-D and IAA. NAA evoked 35.00 per cent and 37.00 per cent flowering in March and April as against 15.00 per cent and 9.00 per cent with IAA, and 19.00 per cent and 10.00 per cent with 2,4-D.

Similar noticeable variation in the effectiveness of auxins at 5 ppm is also evident.

Effect of Three Auxins (at 5 and 10 ppm) on Fruit Size, Fruit Weight and Number of Fruitlets Along the Longest Spiral of a Fruit—The initial observations concerned the size and number of leaves of plants treated with auxins and in the controls. It was observed that on the basis of fruit size and fruit weight, there is a positive correlation between the size and the number of leaves a plant bears and the size and the weight of its fruits. It was observed that robust plants bear larger fruits. This correlation is still more pronounced in auxin-treated plants.

Harvesting of fruits was started from June, 1959, onwards. Immediately after harvesting, fruits with the crowns were transferred to laboratory for weighing. Fruits raised from plants treated with auxin and those from controls were kept in separate lots. Weighing was immediately followed by counting number of fruitlets along the longest spiral of each fruit. The average number was determined on the basis of 12 fruits. Number of fruitlets was taken into account in view of the fact that auxin application may very likely cause alteration in number of florets produced in a day-length sensitive plant like pineapple. Number





Figs. 3-4.—Fig. 3. Graph showing percentage of total flower formation throughout the year as function of concentration of three auxins—NAA, 2,4-D and IAA, applied by a single treatment. Fig. 4. Average increase in weight and volume of fruits per 12 fruits raised from each treatment of three auxins—NAA, 2,4-D and IAA with both the concentrations (5 and 10 ppm) combined together.

of florets in a pineapple plant corresponds well with number of fruitlets.

Volume of fruits was determined by employing Archimedes' principle (by the displacement of equivalent volume of water). The total effect of individual auxin with two concentrations taken together was first considered. It was revealed that NAA-treated plants produced fruits whose average weight was 1.79 Kgm and volume 1435 cc and the number of

fruitlets 13. In IAA and 2,4-D treated plants also the fruit weight and size were found to be slightly higher than those of controls (Fig. 4).

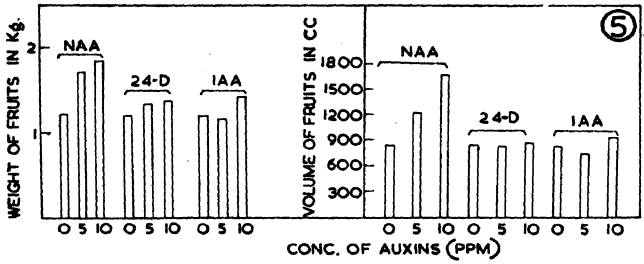
On analysing the individual effect of two concentrations of each auxin it was found that 10 ppm of NAA is the most effective concentration. At this particular concentration the fruit weight increased to 1.87 Kgm and the volume correspondingly increased to 1684 cc. The number of fruitlets along the longest spiral of the fruit increased to 14. A concentration of 5 ppm of NAA also brings about an increase in fruit weight to 1.72 Kgm and the volume to 1222 cc. With the other two auxins there was no significant difference in the fruit weight between auxin-treated and control plants (Fig. 5).

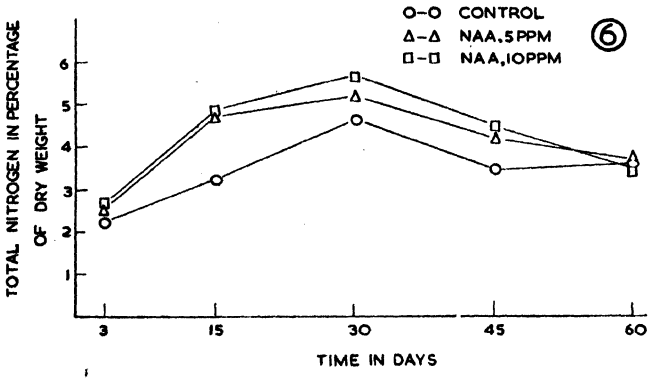
Investigations on Induced Metabolic Changes in Pineapple Fruits due to Application of Auxins—Some reports have appeared in recent years regarding certain metabolic changes in cereals and other crop plants as a result of auxin application. Åberg *et al.* (1948) reported an increase in protein content of auxin-treated wheat plants. Sugarcane sprayed with 2,4-D showed one per cent increase in sugar content (Beauchamp, 1950). The fact that auxin (IAA) stimulates the synthesis of nucleotides and phosphorylation reactions has been confirmed with compounds labelled with P^{32} (Sen, 1959).

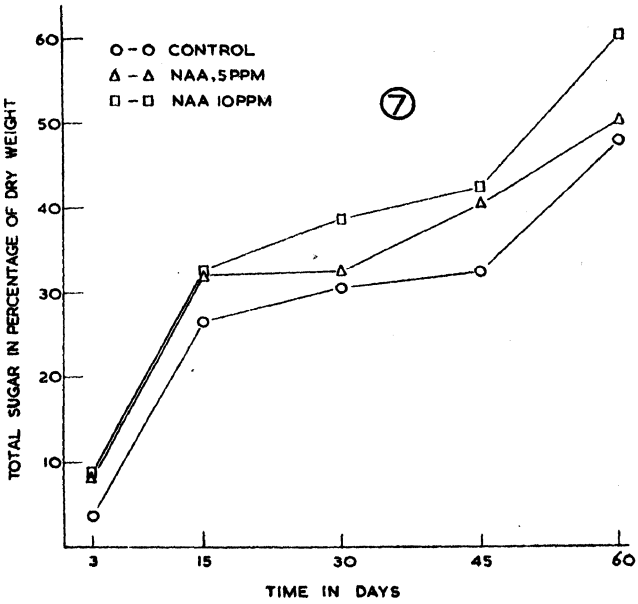
Organic Acid—The abundance of organic acids in succulent leaves of pineapple has been known from very early days. Auxin-induced changes in organic acid metabolism have not yet been elucidated clearly. Experiments were conducted with a view to isolate, identify and estimate various organic acids. Up till now three acids—tartaric, citric and malic—have been chemically isolated and identified. Tests for oxalic acid gave negative results. The method of extraction used was that of Pucher *et al.* (1941). Paper chromatographic technique aiming at further separation and identification of organic acids is in progress.

Nitrogen Metabolism—The effect of NAA (0, 5 and 10 ppm) on total nitrogen of fruits at different stages of maturity was determined. Fruit samples were cut into small pieces and dried on petri-dishes at 60°C in a thermostatically controlled oven. Estimation of total nitrogen was performed according to the method of Ashton (1936) with slight modifications.

Fortnightly analyses carried out for the estimation of total nitrogen of fruits collected from NAA-treated and control plants at different stages of maturity revealed that at the initial stage the level of total nitrogen in all these cases was more or less of the same order. Fifteen days after flowering there appeared a significant change in both auxin-treated and untreated plants. It was 4.73 and 4.88 per cent at 5 and 10 ppm respectively as against 3.26 per cent of controls. The upward rise reached a maximum level after 30 days, being 5.20 and 5.69 per cent at 5 and


⑤
WEIGHT OF FRUITS IN Kg.
NAA
24-D
IAA
VOLUME OF FRUITS IN CC
0 5 10 0 5 10 0 5 10
CONC. OF AUXINS (PPM)


⑥
O–O CONTROL
Δ–Δ NAA,5PPM
□–□ NAA,10PPM
TOTAL NITROGEN IN PERCENTAGE OF DRY WEIGHT
TIME IN DAYS


⑦
O–O CONTROL
Δ–Δ NAA,5PPM
□–□ NAA 10PPM
TOTAL SUGAR IN PERCENTAGE OF DRY WEIGHT
TIME IN DAYS

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AN OUTLINE OF ARGENTINE PHYTOGEOGRAPHY

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The great extension of Argentina (the second largest in South America), its varied topography, as well as its length, ranging from 22° to almost 60° of southern latitude, makes possible the existence of many types of climates. As vegetation follows climate very closely, all kinds of formations are found, such as forests, grasslands, tundras and so on.

Many systems have been developed in an attempt to divide the country in phytogeographical regions. The earlier divisions were made on a vegetational basis with no particular emphasis on the floristic composition. In other words, they followed an ecological or physiological system of classification.

References to Argentina are found in many standard books such as those by Engler (1936), Good (1947) and others, but in general they are so inaccurate that most of the Argentine plant geographers are not willing to make use of them. According to Cabrera (1953), maps concerning Argentina given in several texts including that of Good (1947) are unacceptable. In botanical works published in the Old World, Argentina has been considered superficially, without keeping in view an account of the literature on the country.

The first allusion to Argentina was made by Grisebach as early as 1872 in his book entitled "Die Vegetation der Erde", in which he considered two domains, the Pampean and the Antarctic. This was followed by a series of almost a dozen systems based on the vegetation. The increased knowledge of flora gradually replaced these systems by others in which the floristic composition was the important factor. An example of this type of classification is the scheme of Cabrera (1953). His system takes into consideration the floristic composition of the different areas as well as the communities that are supposed to be at the climax and importance is given to the endemism of families and genera.

According to Cabrera (1953), Argentina may be sub-divided into a series of floristic units such as regions, domains, provinces and so on. However, there is no coincidence between the term province as used by Cabrera and others. In this sense Cabrera's province would correspond to what Gaussen (1933) and Braun-Blanquet (1932) call sector. An approximate definition of the province would be: "a sub-division of a domain based on endemism of important species and the predominance of certain genera poorly represented in other provinces".

Cabrera (1953) divided Argentina into thirteen provinces included in

the South African and Antarctic Kingdoms of Good's (1947) classification. However, for simplicity only the most important nine provinces are included in the present paper.

The Western and Eastern sub-tropical provinces are included in the domain of sub-tropical America. In Argentina this domain occupies a reduced area in the north-west and north-east. The climate of this domain is hot and humid. It has rich flora with the following characteristic families: Araceae, Aquifoliaceae, Betulaceae, Cyatheaceae, Eriocaulaceae, Hymenophyllaceae, Juglandaceae, Lauraceae, Moraceae, Parkeriaceae and Xyridaceae. Among the important families are Bignoniaceae, Bromeliaceae, Euphorbiaceae, Graminae, Orchidaceae and Palmae.

1. WESTERN SUB-TROPICAL PROVINCE—This forms a narrow band extending through the low mountains up to 28° latitude. In altitude it extends up to 2,000 m. above sea level depending on the latitude and topography. The climate is humid and hot with summer rains and winter frost. The soil is rich in humus with half disintegrated rocks covered with a very thick layer of plant residue. The predominant vegetation is a forest but woods and prairies are also found. Some parts of this province are quite unexplored. Several genera have so far been reported exclusively from this province, such as *Tipuana, Cascaronia, Calycophylum, Amburana* etc.

Among the important trees in the forest climax could be listed *Piptadenia excelsa* (Gris.) Lillo (horco-cebil), *Tipuana tipu* (Benth.) OK. (tipa), *Ocotea puberula* Nees (laurel), *Tabebuia avellanedae* Lor. et Gris. (lapacho) and *Juglans australis* Gris. (nogal). There are also bamboos belonging to the genus *Chusquea*, shrubs, lianas and epiphytes like *Epidendrum, Oncidium, Aechmea, Rhipsalis, Tillandsia* etc. The slopes facing the east between 1,500 and 2,000 m. have a cooler climate and the predominant types of vegetation are woods and prairies. Amongst the commonest trees mention may be made of *Alnus jorullensis* var. *spacchii* Regel (aliso), *Podocarpus parlatorei* Pilg. (pino) and *Ilex argentina* Lillo. The flora of the prairies is very rich with grasses and dicotyledons having showy flowers such as *Cosmos peucedanifolius* Wedd., *Zinnia peruviana* (L.) L., *Calceolaria teucrioides* Griseb. etc. Also woods of a drier type are found along the river sides formed mostly by *Acacia aroma* Gill. (tusca).

2. EASTERN SUB-TROPICAL PROVINCE—This covers the north-eastern part of the country and stretches along the two big rivers Paraná and Uruguay up to near Buenos Aires. The climate is hot and humid with rain throughout the year and frost in winter. The soil is red. Forests and savannas are the major formations. In the forest climax the most characteristic trees are *Balfourodendron riedelianum* (Engl.) Engl., *Patagonula americana* L., *Holocalyx balansae* Micheli, *Ocotea suaveolens*

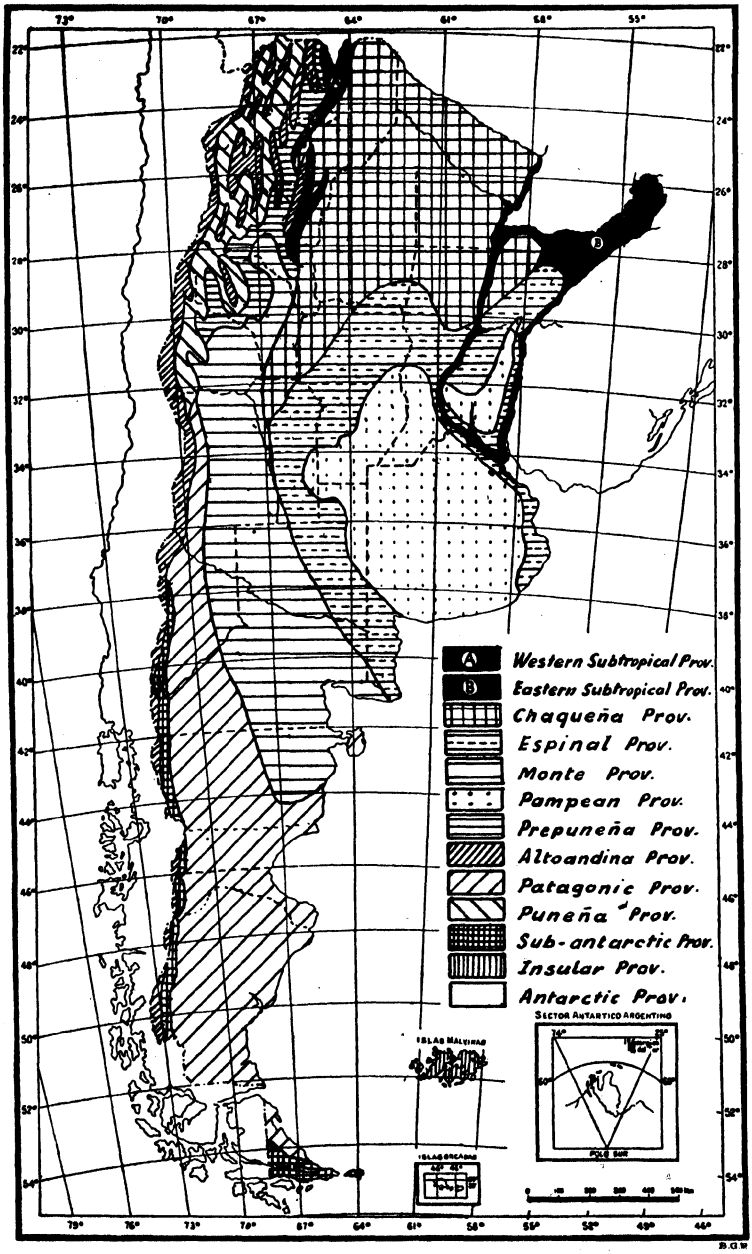


FIG. 1. Phytogeographical provinces of Argentine Republic (*After* Cabrera, 1953).

(Meissn.) Hassl. and *Luehea divaricata* Mart. (sota-caballo). Many shrubs as different species of *Eugenia,* tree ferns as *Alsophyla procera,* bamboos like *Chusquea* and *Guadua* are also found. Even lianas and epiphytes are very common.

In the eastern part and up to 800 m. above sea level there is an arborescent stratum in which the predominant species is *Araucaria angustifolia* (Bert) OK. (pino) accompanied by some other trees. Only two species of *Araucaria* grow in Argentina, i.e. *A. araucana* (Mol.) Koch. in the south, and the already mentioned *A. angustifolia* in two reduced areas, very far away from each other. The flora of the river-side is a little different and becomes poorer and poorer to the south. It reaches up to 34° latitude with only a dozen arborescent species. It is still possible to find *Guadua* here, this being the southern limit of the bamboos on the Atlantic coast.

3. CHAQUEÑA PROVINCE—It occupies a broad band in the northern part that becomes narrower in the centre of the country. The topography is mainly formed by plains and low mountains (up to 2,000 m.). The climate is hot and dry with rains accompanied by frost confined to winter. The predominant type of vegetation is a deciduous forest but savannas, steppes and pure associations of palms are also found. Among the important trees, mention may be made of *Schinopsis balansae* Engl. (quebracho colorado)—one of the most valuable timbers, *Aspidosperma quebracho-blanco* Schlecht, *Prosopis nigra* (Gris.) Hieron. (algarrobo negro) and *Tabebuia nodosa* (Griseb.) (palo cruz). There are also shrubs and Cactaceae like *Opuntia* and *Cereus.* The palms are found in salty and low levels and are represented by only one species, *Copernicia alba* Mor. et Britt. The savannas are formed by grasses and a number of herbaceous mono- and dicotyledons. There are also woods along the river side with *Salix humboldtiana* W. and several species of *Tessaria.*

4. ESPINAL PROVINCE—This extends like an arc in the centre of the country. It is a plain with sandy or loessic soil. The climate is hot and humid. The predominant type of vegetation is a xerophytic wood but there are also savannas and steppes. This province has a close relationship with the Chaqueña Province and could be considered as a continuation of the same with a poorer flora and without *Schinopsis.* The predominant trees belong to the Mimosoideae and are represented by *Prosopis* and *Acacia* accompanied by *Celtis, Jodina* and *Schinus.*

5. PREPUNEÑA PROVINCE—It covers the slopes of the north-western part of the country. The altitude of this province ranges from 800 m. in the south up to 3,400 m. in the north. The soil is rocky and sandy, loose and highly permeable. The climate is dry and warm with small amount of rain confined to the summer months. The predominant type of vegetation is a steppe with isolated shrubs and giant Cactaceae like different species of *Trichocereus* and *Cereus.*

6. MONTE PROVINCE—It extends in the form of a plain with some slopes from the centre of the country to 44° of southern latitude. The soil is sandy and highly permeable and the climate ranges from dry and hot in the boreal part to a cooler in the austral part. The predominant type of vegetation is a steppe with shrubs. Among these the Zygophyllaceae (e.g. *Larrea*) and shrubby *Prosopis* predominate. Cactaceae are also found in this area but belong to the short or creeping types like *Opuntia* and *Echinocactus*.

7. PAMPEAN PROVINCE—But for some isolated mountains the landscape appears like a horizontal plain. The soil is of chernozion type with a loessic subsoil, in the western part it may be sandy. The climate is temperate and humid with rains throughout the year, but chiefly in spring and autumn. The vegetation type is a grassland climax called 'pampa', a kind of steppe characterized by the predominance of grasses, specially of Festuceae and Agrostideae accompanied by some spring dicotyledons. This region has endemisms of no importance, most of its components coming from the neighbouring areas, mainly from the Chaqueña Province. It has also some components from the Andes. A remarkable feature is the absence of spontaneous trees in this enormous plain.

8. PATAGONIC PROVINCE—It stretches across the southern part of the country between the Atlantic Ocean and the Andes, with a topography represented by low mountains and terraces with a rocky or sandy soil. The climate is dry and cold with snow and frost in winter. The vegetation type is a herbaceous or shrubby steppe.

The sub-antarctic domain extends along the Andes from 37° to almost 60° of southern latitude. In Argentina it occupies a very narrow band along the western side. It also includes the Malvinas Islands and some others. The climate of this domain is cold and humid with snow during winter and frost throughout the year. The flora of this domain bears a close relationship with those of Australia and New Zealand. The most salient feature is the dominance of the genus *Nothofagus*. There is also an abundance of conifers, some like *Dacrydium* are represented by only one species. Some of the conifers found in this domain are also represented in the northern hemisphere, e.g. *Libocedrus*, which has recently been changed into a new genus *Austrocedrus*. Some conifers are endemic to this area, e.g. *Fitzroya*, *Pilgerodendron* and *Saxegothaea*. Among other characteristic families of this domain mention may be made of some Liliaceae like *Philesia* and *Lapageria*, Proteaceae like *Embothrium* and *Lomatia*, Monimiaceae like *Laurelia* etc. There is evidence that this domain occupied a larger area in the past, since some of its genera like *Gunnera* extend as far as Brazil and others like *Lagenophora* have been recently reported from Panama and Venezuela.

9. SUB-ANTARCTIC PROVINCE—It is the most important province of

this domain and occupies a narrow band from 37° to the south. The topography is mountainous with rocky and loose soil. The climate is cold and humid with snow in winter and frost throughout the year. The type of vegetation is a deciduous forest but prairies and tundras are also found. This province can be sub-divided into four districts:

(*a*) VALDIVIAN DISTRICT with an evergreen forest represented by *Nothofagus dombeyi* (Mirb.) Blume, *N. antarctica* (Foerst.) Oerst., *Eucryphia cordifolia* Cav. (urmo.), *Flotovia diacanthoides* Less. (palo santo), *Saxegothaea conspicua* Lindl., *Podocarpus nubigena* Lindl. (maniu) etc. This district is characterized by the presence of bamboos (*Chusquea*), parasites like *Phrygilanthus tetrandrus* (R et P.) Eichl., *Myzodendron* spp., a great number of lianas like *Mitraria, Dioscorea,* and epiphytes like ferns, lichens and mosses.

(*b*) MAGELLANIC DISTRICT with a poorer flora characterized by the predominance in the evergreen forest of *Nothofagus betuloides* (Mirb.) Blume (guindo) accompanied by *Drymis winteri* Forst. (canelo), *Maytenus magellanica* (Lam.) Hook. etc. There are no bamboos and lianas and the epiphytes are of rare occurrence. Not uncommon are peat-bogs with *Sphagnum,* some sedges and other angiosperms.

(*c*) PEHUEN DISTRICT is named after *Araucaria araucana* (Mol.) Koch. which is commonly known as pehuen. This species is found in the northern part of the province in almost pure associations but sometimes accompanied by species of *Nothofagus, Pernettia mucronata* (L. f.) Gaud., *Escallonia virgata* (R et P.) Pers., *Quinchamalium chilense* Mol. etc.

(*d*) DECIDUOUS FOREST DISTRICT is characterized by the presence of different species of *Nothofagus* like *N. nervosa* (Phil.) Dim et Mil (rauli), *N. obliqua* (Mirb.) Blume (roble), *Lomatia hirsuta* (Lam.) Diels., *Aristotelia maqui* L'Herit etc.

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CONTROLLED GROWTH OF OVARY AND OVULE

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The tissue culture techniques have been successfully employed in the artificial cultivation of ovary and ovule. Under controlled conditions it is possible to follow the morphogenetic effects of chemicals, influence of calyx and corolla on ovary growth, impact of the development of ovules on the growth of ovary and *vice versa,* and of endosperm on embryo. The influence of physical factors like temperature, humidity, photoperiod and monochromatic light on plant organs is equally important and the *in vitro* procedure offers a suitable field of investigation in this direction also.

LaRue (1942), who first cultured the flowers of several angiosperms, reported extensive growth of ovaries and rooting from excised flower parts. In some preliminary trials Nitsch (1949a, b) also successfully cultured the excised ovaries of tomato. While working with the ovaries of *Lycopersicon pimpinellifolium* Jansen & Bonner (1949) confirmed the results of Nitsch. Pollinated ovaries were grown on a synthetic medium containing mineral salts, trace elements, sucrose and some organic supplements. Maximum rate and extent of development occurred in the media containing casein hydrolysate, indoleacetic acid and a mixture of 11 B-vitamins. The ovaries having an initial diameter of about 1 mm at the time of inoculation reached maturity including lycopene formation in one or two months and the diameter increased to 4-8 mm. The cultured tissue revealed normal development of pericarp and gelatinous placental tissue but the ovules failed to mature. The fruits *in vivo* show rapid formation of pigments after 5-6 weeks and at maturity vary in diameter from 4.5-10 mm depending on the number of seeds which mature.

The credit for refining and firmly establishing the technique of ovary culture goes to Nitsch (1951) who experimented with the ovaries of bean (*Phaseolus vulgaris*), gherkin (*Cucumis anguria*), strawberry (*Fragaria chiloensis, F. virginiana*), tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*). Unpollinated ovaries failed to develop in the nutrient medium unless growth substances were added. Ovaries, excised several days after pollination, showed satisfactory growth on a simple medium composed of sucrose and mineral salts only. As a rule the size of the artificially cultured fruits was smaller than the field-grown fruits and viable seeds developed only in gherkin. Callussing and rooting of pedicel were very common.

Following Nitsch several other investigators have cultured the ovaries

of different plants: Leopold & Scott (1952) of a self-sterile strain of tomato, Rédei & Rédei (1955) of wheat, de Capite (1955) of *Fragaria* and *Pisum*, and Anantaswamy Rau (1956) of *Phlox*.

As compared to ovary culture the work on ovule culture is very meagre. White (1932) and LaRue (1942) obtained callus from the ovules of *Antirrhinum*, while those of *Erythronium americanum* (LaRue, 1942) increased to four times their size. Withner (1943) reared orchid ovules which showed satisfactory growth. Muzik (1956) failed to grow immature ovules of *Hevea braziliensis*. Mature seeds inoculated after the removal of integuments germinated a day or two sooner on agar than in soil. In cultures the perisperm remained intact even after three to six months although in seeds planted in soil it is absorbed in three to four weeks. In cultured seeds, if the perisperm got separated from the cotyledons it turned green but further development did not occur. Pieces of perisperm cultured separately increased in volume but did not regenerate roots and buds although they remained healthy for six to eight months.

There is a recent report of the normal *in vitro* development of ovules of *Calanthe, Cypripedium, Dendrobium* and *Phalaenopsis* (Poddubnaya-Arnoldi, 1959).

Materials and Methods

During the last five years, at the University of Delhi, several research workers have been engaged in the artificial culture of ovaries and ovules of the following plants:

Ovaries: *Aerva tomentosa, Allium cepa, Althaea rosea, Anethum sowa, Carum copticum, Foeniculum vulgare, Iberis amara, Linaria maroccana, Lycopersicum pimpinellifolium, Ranunculus sceleratus, Syzygium cumini, Triticum aestivum, Tropaeolum majus* and *Zephyranthes*.

Ovules: *Abelmoschus esculentus, Argemone mexicana, Citrus lemona, C. microcarpa, C. reticulata, C. sinensis, Dianthus plumeria, Eschscholzia californica, Gossypium hirsutum, Hyoscyamus niger, Momordica charantia, Nicotiana rustica, N. tabacum, Opuntia dillenii, Papaver rhoeas, P. somniferum* and *Zephyranthes*.

Not all of this work has so far been published and reference will be made here only to our studies on the ovaries of *Iberia amara* (N. Maheshwari & Lal, 1958), *Althaea rosea* (Chopra, 1958), *Linaria maroccana* (Sachar & Baldev, 1958), *Zephyranthes* (Sachar & Kapoor, 1959), *Aerva tomentosa* (Murgai, 1959) ; and ovules of *Papaver somniferum* (N. Maheshwari, 1958), *Argemone mexicana* (N. Maheshwari *et al.*, 1959), *Zephyranthes* (Sachar & Kapoor, 1959 ; Kapoor, 1959) and *Opuntia dillenii* (Sachar & Iyer, 1959). There are three other articles published from this laboratory on "Test-tube fruits and seeds" (P. Maheshwari,

1959), "Artificial culture of fruits" (Sachar & Chopra, 1959) and "Chemical stimulation of excised ovules" (N. Maheshwari *et al.*, 1959).

The nutrient medium includes major and trace inorganic elements, ferric citrate, and a mixture of vitamins and glycine. This medium will henceforward be referred to as NBV. Sucrose (2-5 per cent) is added for the carbon source and 0.8 per cent bacto-agar for jelling the medium. With a view to stimulate growth the medium is fortified with a number of supplements, singly or in combination: casein hydrolysate (casamino acids), yeast extract, indoleacetic acid (IAA), indolebutyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, gibberellin or gibberellic acid and coconut milk. The pH is adjusted to 5.5-6.0. The material is sterilized with chlorine water and inoculated in 6″ × 1″ "Pyrex" test tubes containing 10 or 20 ml of sterile medium. The cultures are stored in diffuse light at 25°C and the relative humidity in the culture room varies from 45 to 55 per cent.

Ovary Culture

In nature the ovaries of *Iberis amara* (N. Maheshwari & Lal, 1958) enlarge to twice their size in a week after pollination and by the end of the second week the fruits reach the maximum size. Differentiation of the pericarp and maturation of seeds takes another week. Flowers excised one day after pollination (Fig. 1) (at this time the ovules contain the zygote or a 2-celled proembryo and some endosperm nuclei) show normal growth on NBV and the fruits are comparable to those produced in nature. The calyx and corolla persist for well over a week, later the corolla turns yellowish and withers but the calyx persists. The growth pattern and the maturation of fruit is similar to that in nature. Addition of IAA (5 mg/l) to NBV results in fruits which are even larger than natural ones (Fig. 2). The incorporation of kinetin (0.1-0.6 mg/l) alone or in combination with IAA (5 mg/l) in NBV does not appreciably improve the growth of the ovary. When 2,4-D (2 mg/l) and kinetin (0.5 mg/l) are added to NBV the growth of both the embryo and endosperm is arrested. This is evidently due to 2,4-D or a combined effect of 2,4-D and kinetin.

If calyx and corolla are removed at the time of inoculation the size of the ovary remains stunted.

Besides seeded fruits, *Althaea rosea* (Chopra, 1958) also produces parthenocarpic fruits in nature. Unpollinated ovaries grown on NBV supplemented with IBA (20 mg/l) give rise to parthenocarpic fruits comparable in size to that of field controls (Fig. 3). The seeds also match in size but they are empty. Addition of kinetin (0.5 mg/l) + IAA (5 mg/l) to NBV produces similar results (Fig. 4) but in the presence of only one of these chemicals the fruits do not reach the normal size. Irrespective of

the supplements, occasionally the ovules enlarge and protrude beyond the ovary wall or they abort and the ovary wall proliferates.

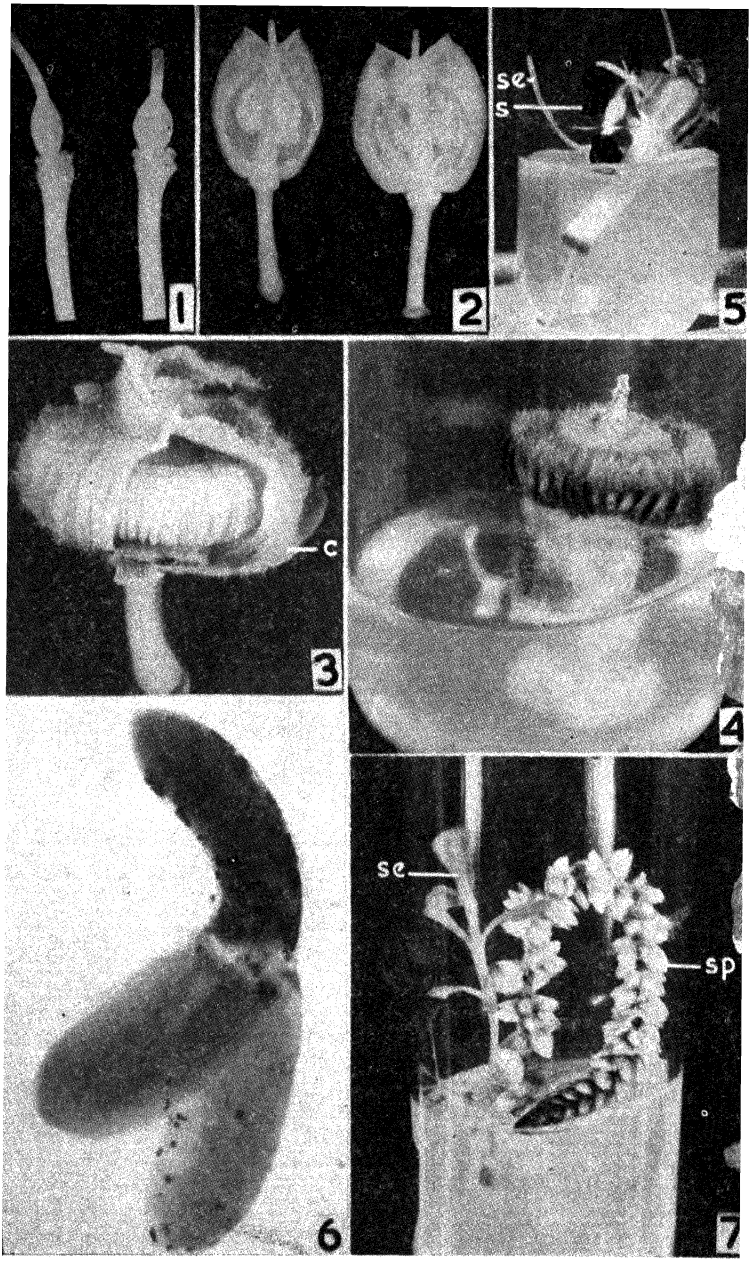
Ovaries excised three days after pollination produce normal-sized, seeded fruits on NBV + IBA. The retention of calyx has a beneficial effect on the growth of ovaries. Swelling of the pedicel occurs in the presence of kinetin and IAA and this is much more marked in unpollinated ovaries.

The ovaries of *Linaria maroccana* (Sachar & Baldev, 1958) were grown on NBV. The unpollinated ovaries do not show any response even with the addition of growth substances to the medium. Ovaries excised two days after pollination, when the ovules contain zygote and two to six endosperm nuclei, develop satisfactorily although the fruit is of a smaller size than the control in the field. Supplementing the medium with kinetin (0.1-1.0 mg/l) in combination with IAA (5 mg/l), IBA (5 mg/l), 2,4-D, (2 mg/l) and adenine (5, 10 mg/l) results in slightly larger fruits but they are invariably smaller than the field controls. In yeast extract (0.25, 0.5, 1.0 per cent) the fruit growth is better than in all the other media. In nature maximum fruit size is attained 10-12 days after pollination and the fruits mature in 21-23 days. Under *in vitro* conditions also the maximum fruit size is reached in 10-12 days but maturity is attained in 15-17 days.

The development of endosperm and embryo in the cultured fruits is similar to those growing in nature except that there is greater accumulation of starch in endosperm. The *in vitro* seeds take a slightly longer time (66 hours) to germinate than the natural seeds (48 hours). The cultured fruits show a greater proportion of abortive seeds and sometimes only the testa develops but the seed is empty inside. The chief cause of the reduced fruit size appears to be low seed setting.

Kinetin in conjunction with IAA, IBA, 2,4-D or adenine induces callus formation from the cut end of the pedicel, and subsequently all along the pedicel, at the base of the ovary, and on the inner and outer surfaces of the calyx lobes. In cultures where the medium contains kinetin, IAA, IBA and 2,4-D, after about 18-20 days root primordia differentiate within the callus and one or two of them grow and branch profusely. Root hairs appear over the entire length of the root. The development of roots does not affect the growth of the fruit since the latter reaches maturity before the root system is fully developed.

The ovaries of *Zephyranthes* (Sachar & Kapoor, 1959) excised two days after pollination contain ovules at the zygote stage with undivided primary endosperm nucleus. On NBV, in two weeks, the ovaries mature into normal but somewhat smaller fruits. The seeds have a fully differentiated embryo and germinate while still enclosed within the ovary (Fig. 5). The seeds from the cultured ovaries are much larger than the size attained when the ovules alone are cultured but are slightly smaller than in nature.


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CYTOGENETICAL EVOLUTION IN THE GYMNOSPERMS —KARYOTYPE

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In recent years it has become increasingly clear that the Gymnospermae constitute a heterogeneous assemblage of taxa and that the term has "outlived its usefulness" (Arnold, 1948 ; Florin, 1955 ; Turrill, 1959). However, the group being with naked seeds represents an intermediate step in the evolution between vascular cryptogams on one hand, and angiosperms on the other. It may, however, be emphasized that the recognition of such an intermediate step does not necessarily mean any linear relationship between the three groups.

The first concrete evidence of the presence of gymnosperms is found in the upper Devonian or lower Carboniferous. At that time the two major phyletic lines, Cycadophyta and Coniferophyta, were well represented and possessed an elaborate structural organization, indicating that these lines had their inception some time in Silurian or Ordovician, if not earlier. During all this period the group had seen many vicissitudes. Its most dynamic phase lasted up to Jurassic period, when it was a dominant element of the earth's vegetation. The decline started in Cretaceous and since then it has remained rather static and stable. The present day genera are mostly end points in their respective evolutionary series. In a word they constitute "remnants" and no living genus can be regarded as a direct ancestor or a direct descendant of any other living genus. Our present-day understanding of the evolution within the group is based on the valuable information from the palaeontological, morphological and distributional studies. Contribution of the cytogenetical approach to the understanding of the evolution within gymnosperms is rather sketchy. The reason is evident ; such studies can be made only on the living forms, which as pointed out earlier, offer but a mere apology for the glorious past of the group as a whole. We can never know the cytogenetical features of such important groups as Cycadofilicales, Bennettitales, Cordaitales, Voltziaceae etc. All these taxa hold key positions in any phyletic treatment of the gymnosperms. Therefore, cytogenetical approach to the understanding of gymnosperms, by itself, may have some limitations. This in fact is true of all such antique groups of plants. However, since the chromosomes are the bearers of genes, we may reasonably expect that the changes in the chromosomes would bear a more direct relationship to the "genetic-evolutionary processes than do any other types of changes". Furthermore, since the publication of the works of Professor

Karl Sax and his collaborators on gymnosperm chromosomes (Sax, 1932 ; Sax & Sax, 1933 ; Sax & Beal, 1934 ; Flory, 1936), enough data has accumulated (Mehra, 1946 ; Stebbins, 1948 ; Mehra & Khoshoo, 1956a, b ; Hunziker, 1955 ; Hair & Beuzenberg, 1958 ; Khoshoo, 1959), to make it worthwhile to analyse and to draw possible conclusions.

If we consider plant evolution from a cytogenetical view point, we find that mutation, recombination, selection and reproductive isolation are the factors that count. The word mutation has been used here in an evolutionary sense, i.e. "a mutation is a discontinuous chromosomal change with a genetic effect". Following this viewpoint mutations may occur at three levels: firstly, in the genes at submicroscopic level affecting the molecular structure ; secondly, these may occur in chromosomes involving changes in number and phenotypic characters of the chromosomes, i.e. in karyotype ; thirdly, mutations may involve duplication or addition of whole chromosome sets or genomes, i.e. auto- or alloploidy (*see* Stebbins, 1959).

Out of these, the role of polyploidy in the evolution of gymnosperms has been already evaluated in the first paper of this series (Khoshoo, 1959). In the present communication, I propose to deal with some general features of the karyotype. This leaves gene mutations, recombination, apomixis, selection and reproductive isolation, which will be taken up elsewhere, and with that a comprehensive analysis of the genetic systems in the group will be brought out. This along with other data would pave way for discussing the interrelationships within and among the gymnosperm families. Such a consideration will be taken up in due course.

So far 264 species (42 per cent) and 55 genera (82 per cent) of the gymnosperms have been worked out cytologically. All these data have been consolidated from diverse sources by Khoshoo (1961) ; unless otherwise stated, all chromosome numbers have been taken from this work. A study of these data reveals that various workers have used different tissues, namely root and stem tips, leaf primordia, pollen mitoses and endosperm for investigating mitotic chromosomes. Furthermore, pretreatments and staining procedures used also vary considerably and so do the magnifications of the drawings of mitotic chromosomes. In view of this, critical phyletic comparisons based on such data may not be altogether safe. I am, however, trying to standardize a system for a statistical comparison of the karyotypes in gymnosperms from the available data. Pending such a uniform treatment, a comparison of the mitotic chromosomes reveals that karyotypic changes may be apparent or may be cryptic. In the visible changes are included phenotypic characters of the chromosomes, like alterations in basic number, size of chromosomes, location of centromere, number and position of secondary constrictions and satellites and lastly the amount and location of heterochromatin. Out of

these, studies on the last aspect are almost lacking. The location of centromere has been denoted by V, J and I types. The V type (or median-submedian) has an arm ratio from 1:1 to less than 1:3, the J type (or subterminal) has 1:3 or more, and the I type (or terminal) has 0:1 or nearly so.

The cryptic changes involve repatterning of chromosomes due to segmental interchanges, paracentric inversions and cryptic structural hybridity. These changes are not externally visible in the karyotypes, because they leave the basikaryotype phenotypically almost unaltered. These are, however, apparent during meiosis as inferred from the structural hybridity for long and short regions in species and species hybrids.

Alteration in Number

If we regard 12 as the basic number of the Coniferales, then at the family level ideal examples of change in basic number are Araucariaceae (x = 13), Taxodiaceae and Cupressaceae (both x = 11) and Sciadopityaceae (x = 10). At generic level are the genera within Cycadaceae (x = 8, 9, 11, 13), Podocarpaceae (x = 9-13, 15, 17-19), *Torreya* and *Amentotaxus* (both x = 11) in Taxaceae (x = 12) and *Pseudotsuga* (x = 13) and *Pseudolarix* (x = 22) in Pinaceae (x = 12). Lastly, at the specific level are some isolated examples within the genera *Cycas*, *Encephalartos* and *Zamia* (*see* Khoshoo, 1961), but the best examples are found in the genera *Dacrydium* and *Podocarpus* (Hair & Beuzenberg, 1958). In the genus *Dacrydium* the haploid number varies from 9 to 12 and 15, while in *Podocarpus* it varies from 10 to 13 and 17 to 19. All the foregoing examples of change in basic numbers can be relegated to reduction or addition in chromosome number due to unequal translocations and fragmentation-fusion process.

Reduction and Addition—The mechanism involved is the same as suggested by Darlington (1937, 1958) and further elaborated by Stebbins (1950). It involves a "system of unequal translocations" by which a karyotype could be numerically altered. The direction of the alteration depends on the nature of chromatin present in the proximal portion of the chromosomes. The number would decrease if this material is largely inert, but would increase if it is genetically important. Former process has been experimentally verified in *Crepis* by Togby (1943) and Sherman (1946).

The cases of reduction in chromosome number are more than those of addition. At the family level reduction in number seems to have taken place in Taxodiaceae and Cupressaceae (both x = 11) from the ancestral condition of x = 12, still preserved in Ginkgoales, Taxales and Pinaceae. The Sciadopityaceae (x = 10) is an allied family of Taxodiaceae

(x = 11) and the two have probably arisen from a common ancestor with x = 11. The Sciadopityaceae is extremely specialized in some morphological features, and so is it in its chromosome number. At the generic level reduction has taken place in *Torreya* and *Amentotaxus* (both x = 11) from x = 12 which is ancestral and is still preserved in *Taxus*, which on morphological grounds is regarded as more generalized than the former two genera (*see* Florin, 1948).

In brief, therefore, in three cases reduction appears to have taken place from 12 to 11, while in one case from 11 to 10. An outright reduction of a chromosome pair is not possible at the diploid level, because it would be deleterious. The reduction of a chromosome pair may be preceded by translocation of all the genetically active material on other chromosomes of the karyotype. This would leave the chromosomes too small, probably in the from of a small genetically inert centric-fragment, that could be lost without any ill-effects.

Cases of addition of a pair of chromosomes in gymnosperms are very few. This is also true of angiosperms. Furthermore, to date we have no clear and precise experimental proof of how such an increase takes place. In gymnosperms, Araucariaceae (x = 13) is the only probable example at the family level showing an increase in number provided, however, we believe that it arose from ancestors with x = 12. This means no commitment with regard to the family being advanced or derived in comparison to its rival, Pinaceae (x = 12). At the generic level addition of a pair of chromosomes has taken place in *Pseudotsuga* (x = 13) from the prevailing condition of x = 12 in the family Pinaceae to which it belongs. A comparison of the karyotype in *Pseudotsuga* (6V + 6J + 1I) with other Pinaceae indicates that the additional pair of chromosomes is the one with terminal centromere, otherwise its karyotype resembles *Larix* (6V + 6J).

Fragmentation-Fusion—With the passage of years, enough data have accumulated which reveal convincingly that fragmentation of a chromosome with localized centromere into two subtelo- or telocentrics is possible, as is also the fusion of latter to give rise to former. Darlington (1956) has discussed the mechanism of these phenomena. He believes that "successive interchanges of segments of chromosome near the centromere with losses of small fragments would account for the apparent fusion. The fissions, on the other hand, might occur more directly by misdivision at the centromere itself giving rise to telocentrics and hence to isochromosomes which may lose most of one arm ; thus a V could become replaced by two I's". It may be possible to decide about the direction of the change (whether fragmentation or fusion) after a careful karyotypic comparison with the allied taxa ; however, it is often worthwhile to take into consideration the data on the morphological evolution of the group in question, since the series can be also read with equal ease in the reverse

order. In this category are included *Pseudolarix* and the various genera of Cycadaceae and Podocarpaceae.

The case of *Pseudolarix amabilis* (n = 22 = 2V + 20I) is relatively simple. It belongs to Pinaceae (x = 12) and was at one time regarded as a hypo-autotetraploid (4x – 4) (Sax & Sax, 1933). However, after a comparison of the karyotype of this genus with its allied genera, like *Larix, Cedrus* and *Pinus,* evidence has been put forward (Khoshoo, 1959) in support of the fact that *Pseudolarix* has arisen from x = 12 by fragmentation of 10 (out of 12) metacentric chromosomes. This would result in 20I's and 2 metacentrics, which actually is the karyotype in the genus.

The situation in cycads is not easy to interpret. On one hand are genera like *Ceratozamia* and *Zamia* (both x = 8 = 6V + 2J) which are both low-numbered and with symmetrical or unspecialized karyotypes, and on the other *Cycas* (x = 11 = 2V + 4J + 5I) and *Microcycas* (x = 13 = 1V + 1J + 11I) which have high-numbered and asymmetrical or specialized karyotypes. The asymmetry is due to the preponderence of I's, i.e. subtelo- or telocentrics. This is particularly true of *Microcycas.* Apparently from the cytogenetic view-point alone, one is rightly tempted to rate *Cycas,* and in particular *Microcycas,* as specialized or advanced, and *Ceratozamia* and *Zamia* as unspecialized or primitive.

On morphological grounds the general impression is that *Cycas* because of the lack of an ovulate cone and possession of large leaf-like megasporophylls is the most primitive genus in the family. *Microcycas* possesses multiple male cells which is recognized as a primitive feature. *Ceratozamia* and *Zamia* lack all such features and are regarded as advanced in the scale of evolution. Superimposing the cytological data on the above morphological views, we find that *Cycas* and *Microcycas* are specialized karyotypically but primitive morphologically. On the other hand, the reverse is true of *Ceratozamia* and *Zamia.* The two trends, morphological and karyotypical, run diametrically opposite to each other. Without going into further morphological details, it may be pointed out that if the vegetative and anatomical characters are also considered, we find that no single genus shows an all round advancement or primitiveness (*see* Arnold, 1953 ; Florin, 1955). *Cycas* (x = 11 = 2V + 4J + 5I) is antique in reproductive features, *Stangeria* (x = 8 = 6V + 1J + 1I) in vegetative characters and *Zamia* (x = 8 = 6V + 2J) occupies a primitive position in vegetative characters and anatomy. A situation like this, where it is impossible to arrange genera in a linear series taking all characters into account, is quite understandable when we consider that Cycadaceae, though a compact alliance, had its inception sometime in Paleozoic. The existing genera are only remnants and to quote Arnold (1953): "each genus represents the terminal branchlet of a phylogenetic tree and all connecting portions have disappeared". There is, however, no doubt that the present day karyotypic heterogeneity at the generic

level shows that in this family much karyotypic evolution has taken place in the past. This is particularly clear from the karyotypes of *Cycas* and *Microcycas*. Their karyotypes are without doubt derived and not original. Judging from the evidence gathered in plants so far (Stebbins, 1950), such karyotypes appear to have evolved from more or less symmetrical ones. Therefore, one is tempted to speculate that in the ancestors of this family the karyotype was low-numbered and symmetrical. It is of course not possible to speculate about the chromosome number of such ancestors, because there is no strict numerical correlation between the V, J and I chromosome types found in the various genera. Possibly the intermediate stages are extinct. In any event there evolved from such an ancestor genera like *Ceratozamia* and *Zamia*, chiefly by gene mutations and repatterning without much change in the karyotype, and the same purpose was achieved by radical karyotypic alterations in *Cycas* and *Microcycas*. While karyotype evolved in these directions, the morphology followed its own independent course of evolution.

Mehra & Khoshoo (1956b) remarked that the Podocarpaceae is a "taxonomically complex and a morphologically very variable" family and in agreement with this statement are the recent cytological observations of Hair & Beuzenberg (1958). The haploid numbers vary from 9-13, 15 and 17-19 and there is a "regular numerical relationship" between the number of metacentric or V-shaped and subtelocentric or I-shaped chromosomes. In this process one metacentric replaces or is replaced by two subtelocentric chromosomes. The evidence from intraspecific heterozygotes and an interspecific hybird reveals that the direction of the change is, in all probability, fusion of I's into V's. Furthermore, they believe that the process possibly began with 20I's and by continuous fusions the number was reduced. According to them, this would result in economy of centromeres and stability of chromosomes. In terms of karyotypic symmetry, it evidently means that the original or the ancestral condition was 20I or 1V + 19I, which has progressively given rise to 10V's. In other words the trend has been asymmetry to symmetry.

In view of our present understanding of karyotypes in plants in general (*see* Stebbins, 1950), there can be no two opinions about the karyotype 1V + 18I being extremely specialized and derived in nature. If, however, we believed it to be ancestral, we shall have to put up a supplementary hypothesis for its coming into being from some earlier, now extinct, unspecialized and original condition. This is an objection to an otherwise cytogenetically fine scheme proposed by Hair & Beuzenberg (1958). This objection could be apparently met with by considering a comparable situation found in animals. It has been noticed that in *Drosophila* (Patterson & Stone, 1952), the Orthoptera (White, 1954) and various groups of vertebrates (Matthey, 1951) the most primitive karyotype con-

tains almost exclusively telocentrics and reduction in chromosome number takes place by centric-fusions in the same manner as proposed for Podocarpaceae by Hair & Beuzenberg (1958), resulting in forms with more metacentrics in the karyotypes. However, even here, the latest opinion is that the most primitive animals, which were ancestors of the present day forms, possibly had karyotypes with metacentrics, which during the course of evolution gave rise to asymmetrical karyotypes found at present (Stebbins, 1958).

An alternate view could be as follows: A perusal of the data on Podocarpaceae (Hair & Beuzenberg, 1958) shows that karyotypes of all the normal species could be relegated to those containing 8, 9 and 10 metacentrics. All the karyotypes in the large and heterogeneous genus like *Podocarpus* and other genera like *Microcachrys, Acmopyle, Pherosphaera, Saxegothaea* and *Dacrydium* (mostly) could be simplified to only 10, of *Phyllocladus* to 9 and two species of *Dacrydium* to only 8 metacentrics. It may be pointed out that out of the 45 normal species that have been analysed karyotypically, the numbers 9 and 10 are actually found in 21 species (48 per cent). The remaining species possess the derived conditions with mixture of V's and I's. As in Cycads, it is possible that the ancestral condition (10V's and 9V's) in Podocarps has been retained in some, but altered in others by fragmentation. Furthermore, even the intermediate conditions may themselves have been the starting points of new lines. A point in favour of the revised scheme is that while the ancestral condition of 10V's and 9V's is still retained in 48 per cent species, the ancestral condition of 20I's under Hair & Beuzenberg's scheme has not been discovered so far and possibly may not exist. Furthermore, the next ancestral condition, i.e. 1V + 18I is found only in 4 species (9 per cent). In conclusion, it may be remarked that the situation is rather complex and only a closer study involving data from all aspects (including the nature of centromere in the Podocarpaceae) is likely to reveal the true situation. At present, therefore, it is not safe to support any one of the above schemes. However, in this laboratory we are exploring the possibility of producing an integrated picture of evolution within the family after taking into consideration the cytological data in conjunction with the data from general morphology, cone-organization, wood-microstructure and embryology.

Alteration in Size

The change in size of individual chromosomes due to fragmentation-fusion is not considered here, because it does not alter materially the absolute chromosome size. Other alterations in size in gymnosperms have been noticed at two levels. Firstly, there may be size difference between chromosomes of the same complement and secondly, there may be altera-

tions in the absolute chromosome size of the complements of various taxa.

More often the intrakaryotypic size-differences are associated with the change in form of the chromosomes due to unequal interchanges. These would be detailed in the next section. The differences in chromosomes when their form remains almost the same are seen in four species: *Pinus radiata, Callitris verrucosa* (Mehra & Khoshoo, 1956a), *Ephedra gerardiana* and possibly also *E. sinica* (Mehra, 1946). A comparison of the karyotypes of these species with the allied species reveals that one of the chromosomes is reduced in size. Evidently it was originally of the usual size found in other species.

The alterations in absolute size of chromosomes of various taxa in gymnosperms have not received the attention they deserve. The preparation of histograms revealing such differences is rendered very difficult because of the different techniques used by the various workers. Pending a unified treatment, it may be remarked that there is a general decrease in size with phylogenetic advancement in the Coniferales. For instance, even after taking into account the reduced chromosome number in Taxodiaceae and Cupressaceae ($x=11$), the absolute chromosome size in these families is smaller than in Pinaceae ($x=12$) from which the two families have probably arisen. Even in the family Pinaceae there is a general reduction of chromosome size and species of *Pinus* possess larger chromosomes than any of the remaining 8 genera. Similarly, the genera within Taxodiaceae and Cupressaceae show size differences.

Within genera, the species also exhibit significant differences in the size of chromosomes. Apart from the species of *Pinus, Podocarpus* (*P. falcatus* and *P. gracilior*) and *Ephedra* (Hunziker, 1959), this is particularly true of the genus *Callitris* in which the species resemble each other in the morphology of their karyotype in every detail (Mehra & Khoshoo, 1956a, b).

So far we do not know how exactly the phylogenetic alteration in size of chromosomes is brought about. However, it is increasingly held that size differences are controlled by factors within the chromosomes themselves. Furthermore, it is possible that deletion of non-genic material may be one such factor.

The correlation of chromosome size and the habitat in gymnosperms is not vivid, but may not prove rewarding, because most of them occupy relatively stable habitats in temperate zones. There is, however, a general correlation between the chromosome size and the habit. Trees in general are known to have small chromosomes. Due to the general constancy of the nuclear-cytoplasmic ratio, a check to the alterations in chromosome size is likely to be exercised by the smallest cells in the life of a plant. In the woody and long-lived plants these are the cambial cells particularly those that form wood fibers. The gymnosperms are an exception, since they have larger chromosomes than most of the dicoty-

ledonous trees. It is believed that the larger size of the chromosomes in gymnosperms (particularly in conifers) has been rendered possible by the lack of wood fibers (*see* Darlington, 1937 ; Stebbins, 1938 ; Khoshoo, 1959).

Alteration in Morphology

The alterations in morphology due to fragmentation-fusion processes have been dealt with earlier. Only those changes will be dealt here in which chromosome number remains the same, but morphology changes with the shifting of the centromere and/or change in number, nature and location of nucleolar organizers. The centromere is easily shifted from median to terminal position by unequal translocations and pericentric inversions. How alteration in nucleolar organizers is brought about is not clear so far!

The various genera within Pinaceae, Taxodiaceae and Cupressaceae possess the same chromosome number, but often differ in morphology of their karyotypes. This is particularly true of Pinaceae. *Pinus* possesses the most generalized karyotype of 12V's. It appears to have retained the ancestral condition. All karyotypes found in other genera of the family could be derived from such an ancestral condition. The various karyotypes differ in the number of the different types of chromosomes (*see* Table II of Mehra & Khoshoo, 1956a). However, it is not possible to correlate these karyotypic changes with morphological advancement.

Similar karyotypes are often found in groups of genera within various families: *Ceratozamia—Zamia* (Cycadaceae), *Araucaria—Agathis* (Araucariaceae), *Picea—Tsuga* (Pinaceae), *Cryptomeria—Cunninghamia, Taxodium—Sequoiadendron* (Taxodiaceae), *Actinostrobus—Callitris—Tetraclinis—Widdringtonia—Thuja* and *Cupressus—Chamaecyparis—Juniperus—Biota* (Cupressaceae). In several instances these groups of genera form compact taxonomic alliances within their respective families. Furthermore, among some of the alliances like *Ceratozamia—Zamia* (Chamberlain, 1926), *Picea—Tsuga* (Campo-Duplan & Gaussen, 1949) and *Cupressus—Chamaecyparis* (Osborn, 1941), intergeneric hybrids have also been recorded, which in some cases are reported to be fertile. Evidently, apart from the karyotypic similarities, these groups of genera are genetically very alike.

The various species within the gymnospermous genera, except *Podocarpus* and *Dacrydium,* possess the same basikaryotype. In *Podocarpus* and *Dacrydium,* particularly the former, species within some sections have the same karyotype. This is true of the sections Polypodiopsis and Dacrycarpus of the genus *Podocarpus.*

The various species within genera like *Pinus, Cupressus* (Mehra & Khoshoo, 1956a) and *Ephedra* (Mehra, 1946 ; Hunziker, 1955) differ in

number, nature and location of the nucleolar organizers. In *Ephedra* even two nucleolar organizers may be found on the same chromosome (Mehra, 1946 ; Hunziker, 1955). If the original condition was that of two nucleolar organizers in a diploid complement, then higher number of these organizers in some species of *Pinus* (up to 8) and in *Ephedra* (up to 12) may be considered to be a derived condition. How this has been brought about in gymnosperms is not as yet vivid!

Evidence of the karyotypic changes within a single species is found in *Ginkgo* in which though the basikaryotype is more or less constant, yet there are differences in the precise location of centromeres and in number and location of the satellites (Sax & Sax, 1933 ; Resende & Rijo, 1948 ; Tanaka *et al.*, 1952 ; Lee, 1954 ; Newcomer, 1954 ; Pollock, 1957). The differences centre round the cytological basis of sex differentiation. Possibly these karyotypic differences may also be connected with the varietal diversity found even in this "living fossil" (*see* Dallimore & Jackson, 1948). Recently, Hunziker (1958) has described the occurrence of two karyotypes in *Cupressus sempervirens* and four in *C. lusitanica*. The diploid number in all cases is 22. Again, both these species are polymorphic (Dallimore & Jackson, 1948) and as such it is not surprising to find a measure of chromosomal polymorphism in them.

Repatterning Without Change in Basikaryotype

As is clear from the foregoing discussion, the repatterning due to unequal translocation, pericentric inversions and fragmentation-fusion processes results in alteration in number, size and symmetry of chromosomes. Reciprocal translocation of equal segments or segmental interchange and paracentric inversions would not alter the basikaryotype appreciably. However, these changes can be inferred from meiotic analysis. That such alterations have been at work at and below specific levels is clear from the consideration of the following cases, which are all diploid in constitution.

Hirayoshi *et al.* (1943) have noted in the hybrids between *Pinus densiflora* and *P. thunbergii* that during meiosis the most frequent association is $0_{I} + 10_{II}$, while in the parents there are always formed 12_{II}. It is evident, therefore, that the two parental species differ from each other in one segmental interchange involving two non-homologous chromosomes. The hybrid being heterozygous for such an interchange forms a translocation-quadrivalent.

Below the specific level same phenomenon has been observed to varying extent in *Taxus cuspidata* (Matsuura & Suto, 1935) *Cephalotaxus drupacea* (Sugihara, 1940) and *Cupressus lusitanica* (Câmara & de Jesus, 1946). In *Taxus cuspidata* ($2n = 24$) a ring of 4 chromosomes is organized, while in *Cephalotaxus drupacea* out of the 34 individuals

studied, 9 had 12_{II} and the remaining 25 plants showed 4 different types of associations, namely 0_4+10_{II}, $0_4+0_4+8_{II}$, $0_6+0_4+7_{II}$ and $0_6+0_4+0_4+5_{II}$. A particular association was usually characteristic of a particular individual. Unfortunately no data on the further course of meiosis are available in this species. The extent of fertility and mode of perpetuation are also unknown.

Sometimes trivalent associations have also been found in *Cupressus lusitanica* (Câmara & de Jesus, 1946). The nature of these associations reveals that they are cases of segmental interchange between non-homologous chromosomes.

All the above three cases exhibit structural hybridity to varying degrees. These cases, particularly of *Cephalotaxus drupacea*, may represent transitional or "floating" stages from which different chromosomal biotypes may evolve in future by the institution of balanced lethals. Alternatively, these may be intraspecific hybrids between different chromosomal biotypes that have evolved in past due to chromosomal repatterning. Anyway, in no case they exhibit any marked difference in the basikaryotype or change in the genic content. However, the altered genic arrangement could be the starting point of a new line.

The meiotic studies and fertility data in the intra- and interspecific hybrids in *Pinus, Larix, Cupressus, Juniperus* etc. (Sax, 1932 ; Gopala Ayengar, 1942 ; Câmara & de Jesus, 1946 ; Ross & Duncun, 1949 ; Toyama, 1950 ; Guilline, 1952) show that intra- and interspecific differentiation may also be due to inversions as evidenced by the bridge-fragment configurations, and cryptic structural hybridity as indicated by perfect pairing but reduced fertility. Neither of these changes can be detected in the mitotic chromosomes.

In brief, some data have accumulated which reveal that in gymnosperms, morphologically undetectable karyotypic repatterning is possible by means of segmental interchanges, paracentric inversions and cryptic structural hybridity. What initiates these phenomena in gymnosperms is unknown at present!

Concluding Remarks

Morphologists regard gymnosperms as a heterogeneous assemblage of taxa. Some obvious reasons for it are, polyphyletic origin of the group, heavy extinction of taxa at various levels with the result that the originally continuous pattern becomes discontinuous, and parallel evolution in otherwise unrelated evolutionary lines. Furthermore, evolution has not been uniform in all directions, the various organs, including the nucleus, appear to have advanced at different rates. The direct result of all these factors is that it is not possible to form an evolutionary series taking all characters into consideration. Often what is the beginning of any one

sequence, constitutes the end of the other. In simpler words, no one living genus can be derived from any other. Obviously, the modern families and genera in gymnosperms are the end points of long and continued natural selection that has been acting on various evolutionary trends which began millions of years back. In view of this, we may reasonably expect to achieve a better understanding from the comparisons of the basal stocks, rather than the end points. Furthermore, the picture from the chromosome phylogeny in gymnosperms is likely to be of limited value by itself, "but as a part of the frame-work of evolutionary speculation and investigation", it may prove to be very helpful. To borrow a figure of speech from Darlington (1956), it "adds a new dimension to the argument". While reviewing the data on karyotype we have examined the alterations in basic number, size and morphology of the chromosomes. From this some conclusions of a general nature emerge which may now be considered.

In general the present cytological data follow the taxonomic groupings in gymnosperms. In the living gymnosperm families except Cycadaceae (x=8, 9, 11, 13) and Podocarpaceae (x=9-13, 15, 17-19), the various evidences generally point out that there is a single basic number: Ginkgoaceae, 12 ; Cephalotaxaceae, 12 ; Taxaceae, 12 ; Araucariaceae, 13 ; Pinaceae, 12 ; Sciadopityaceae, 10 ; Taxodiaceae, 11 ; Cupressaceae, 11 ; Ephedraceae, 7 ; Welwitschiaceae, 21 and Gnetaceae, 22. If it is proved that the karyotypic change in the Podocarpaceae has been unidirectional and that the family constitutes a monophyletic and morphologically compact alliance, then the basic number could be relegated to either 20 or 10 (and 9), depending on whether fusion or fragmentation respectively has been the physical basis of evolution of the karyotype. The familial distinctions in Coniferophyta are apparently based on gain or loss of a pair of chromosomes. The families are generally homoploid except Cycadaceae and Podocarpaceae and genera like *Torreya* and *Amentotaxus* (x=11) in Taxaceae (x=12), and *Pseudotsuga* (x=13) and *Pseudolarix* (x=22) in Pinaceae (x=12). The basikaryotype remains generally constant within a genus, except in *Podocarpus* and *Dacrydium*. In the latter genera it remains constant in sections. In some genera like *Juniperus* (partly), *Sequoia* and *Ephedra* (partly) the gametic number is the multiple of the basic number of the families, i.e. these are polyploid. The various genera within a family may or may not resemble one another in their basikaryotype. When they resemble, they often form compact taxonomic alliances and in some cases they are also genetically very similar as evidenced by the fertile intergeneric hybrids. Since such genera are morphologically distinct and well recognised, it means that in these cases genotypic differentiation has lagged behind the phenotypic differentiation. However, when distantly related or unrelated genera have almost similar karyotypes, it indicates that in these cases chromo-

somes show only an outward resemblance and are not alike in genic content and hereditary potentialities.

The species within a genus differ in matter of details, e.g. absolute size of chromosomes and/or number, nature and location of secondary constrictions and satellites. The intraspecific differences though nearly similar in nature are, nevertheless, less striking. Furthermore, apart from gene mutations, the specific differences may be due to segmental interchanges, inversions and cryptic structural hybridity.

In Coniferales the decrease in chromosome number and absolute size of chromosomes of a complement generally appears to be correlated with the phylogenetic advancement. On the other hand, the increase in asymmetry cannot always be correlated with morphological advancement. Here it is pertinent to mention that some of the taxa like *Cycas, Ginkgo, Pseudolarix,* some species of *Podocarpus* and *Welwitschia* have highly asymmetrical karyotypes. Some of these are no doubt highly specialized in morphology and/or in habitat.

It is clear from the foregoing account (*see also* Khoshoo, 1959) that in the gymnosperms there is a general cytogenetic stability in comparison to the angiosperms. Coupled with such a stability, the group in general shows stability in the habitat preferences, habit, morphology, pollination, reproductive systems etc. The probable causes and implications of such a stability or conservatism will be discussed in an integrated manner elsewhere in due course.

The processes responsible for karyotypic evolution are unequal translocations, segmental interchanges, peri- and paracentric inversion and cryptic structural hybridity. Although these processes may keep the hereditary potential almost constant, nevertheless, they reshuffle the genetic material often significantly. In this manner, such chromosomes are organized whose total genotypic influence is different from what it was before such reshuffling occurred. This is chiefly due to the reshuffling in the linkage groups and the consequent position effects. These altered gene sequences are an important evolutionary feature. The new gene sequences or combinations with an adaptive value would be favoured and moulded further by natural selection. Possibly the karyotypic changes exercise their evolutionary effects in this manner, although we are not as yet in a position to grasp their full evolutionary meaning, particularly in the gymnosperms.

Lastly, a question arises as to why some genera in gymnosperms show chromosomal polymorphism while others are stable. For instance, in *Podocarpus* the karyotype is extremely flexible whereas in *Pinus* it is nearly inflexible, although both resemble each other in other traits of their genetic system. To simplify the question, we may ask as to what promotes variability in karyotype! Apart from other aspects, possibly a comparative study of heterochromatin in the major phyletic lines of

gymnosperms may prove rewarding in this direction. It is of significance to point out that it is essential to have a reasonable amount of heterochromatin, because our present understanding shows that its presence is associated with centric fusions, loss of centromeres etc. For the proper understanding of the karyotypic evolution it is not only necessary to make a comparative study of karyotypes in various taxa but also special attention should be given to the karyotypic and cytogenetic analyses of the hybrids. Also DNA and protein comparisons may add new information. It must be emphasized that data from morphology, cytogenetics, cytochemistry and other disciplines need to be synthesized before a true picture of the evolution in the group can emerge. This has to be done in spite of the fact that at present we have reasons to believe that evolution in various directions has progressed rather independently.

SUMMARY

So far about 82 per cent genera covering nearly 42 per cent species of gymnosperms have been studied cytologically. This is a reasonably good sample for estimating the roles of various cytogenetic factors in the evolution of the group. The role of polyploidy has been already evaluated (Khoshoo, 1959). In the present communication data on the karyotype have been reviewed from all the pertinent aspects. The processes responsible for the karyotypic evolution and differentiation in the group (in particular in the conifers) at various levels are translocations, fragmentation-fusion processes, segmental interchanges, peri- and paracentric inversions and cryptic structural hybridity. These have resulted in alterations in the number and morphology and repatterning of chromosomes without significant changes in the karyotype.

It has been concluded that in the gymnosperms the various organs (including the nucleus) seem to have advanced at different rates. As such it is not possible to form a rigid evolutionary series taking all characters into consideration. However, in general, cytological data follow the taxonomic groupings and in the various families (except Cycadaceae and Podocarpaceae) a single basic number can be recognised. In these two families the basic numbers in the present day genera form a dysploid series resulting from fragmentation and/or fusion of centromeres. Judging from our present day understanding of the karyotypic evolution in plants in general, it is tempting to speculate that the ancestral karyotype in these families was low-numbered and symmetrical.

The various genera is a family may or may not resemble one another in their basikaryotype which remains generally constant within genera except in *Podocarpus* and *Dacrydium*. However, in the latter genera basikaryotype remains constant only in some sections. The inter- and intraspecific differentiation is due to changes in absolute size of the chromo-

some complement and/or number, nature and location of secondary constrictions and satellites. Furthermore, apart from gene mutations, such differentiation involves other changes like segmental interchanges, inversions and cryptic structural hybridity.

The decrease in chromosome number and absolute size of chromosomes of a complement seems to be generally correlated with the phylogenetic advancement in conifers. Similarly the increase in asymmetry can also be correlated in some cases with specialization in habitat, habit and morphology.

In comparison to angiosperms, the gymnosperms are cytogenetically extremely stable. Such stability is also found in their habitat preferences, habit, morphology, pollination and reproductive systems etc.

In the end avenues for further work have been pointed out.

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BUDS IN SOME INDIAN FERNS

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Vegetative buds are known to occur in ferns. A classical example is the buds in the axils of pinnae in *Cystopteris bulbifera* or the buds at the ends of flagellae in the so-called walking ferns. These buds occur in a number of species and help in vegetative propagation. They are known to be present in about 219 species belonging to different families of the Filicales. The position of such buds varies from base to the apex of a leaf in different species. A few are axillary as in *Botrychium* and some arise on stipules as in *Angiopteris evecta*. They are rarely found on the stipe, but not uncommonly at the base of the pinnules or the whole leaf, as in *Asplenium bulbiferum, Cystopteris bulbifera, Anisogonium esculentum, Hemionitis arifolia* etc. In a number of ferns they occur on the margin of leaflets as in *Gymnopteris subcrenata* or occupy a terminal position as in *G. contaminans*. In *Aneimia, Adiantum, Asplenium* and *Athyrium* they occur at the end of the rachis. In *Ceratopteris* they occur anywhere on the margin, base or the apex of the leaf. In many species of *Ophioglossum, Platycerium, Botrychium* and *Anisogonium* they occur on roots.

Since they arise in a variety of positions, the question naturally arises: are they different in point of origin, morphology and behaviour? If they are, then what are the causes responsible for their growth? It is quite obvious that they are so different in certain genera, e.g., the root buds of *Ophioglossum* and the leaf buds of *Hemionitis*. Wardlaw (1943, 1952) and his associates made intensive studies on the shoot apex in ferns and conclude that removal of or injury to the shoot apex induces development of lateral buds or their rudiments ; especially so if the shoot apex is excised and treated with IAA. It has been shown that shoot apex has inhibitory effect on the growth of buds.

These buds develop either endogenously from the middle cortex or superficially. Both the types of growth are known. In *Ophioglossum aitchisonii* and *Anisogonium esculentum*, it was observed that the root buds develop endogenously from cells situated a little behind the apical cell of the root. The same phenomenon was observed in the first formed root of *Ophioglossum* and in the embryonic root of *Equisetum*. Naturally, the question arises as to what is the relation of leaf-apex or root-apex to the formation of buds?

OBSERVATIONS

To study the mutual effects of these organs, *Goniopteris prolifera* Roxb.—a singular fern in this respect belonging to the Polypodiaceae, sub-family Aspidieae—was selected. This fern produces large flagellae which are sometimes 7 or 8 feet in length, producing one bud in the axil of each leaflet. All these buds, however, are not alike. Some bear sporophylls, and others develop into vegetative shoots ; some do not develop either way and fall off. To the best of my knowledge such dimorphism in buds has not been known so far.

At a very early stage during the investigations on this fern by Joshi (1959), it was observed that it produces vegetative and fertile shoots alternately. The fertile shoots are generally produced soon after the monsoon in August, September and October and they produce a few sporophylls and fall off. At other times of the year, only the vegetative shoots having sterile pinnae are formed. In order to study the factors which regulate the production of these two kinds of buds, they were isolated from the rachis of flagellum and were cultured in nutrient media separately. The buds excised from the parent plant germinated well in artificial media. Some of them even produced sporophylls in cultures with IAA. But it was observed that small isolated buds directly put in the soil were not capable of growing independently for a long time, possibly due to the lack of food material or a growth hormone. On the other hand, buds of nearly the same age growing on a parent plant with flagellum apex intact, grew normally. Buds on a flagellum of which the apex had been removed died slowly. Perhaps the rachis apex, like the shoot apex, develops some hormone at a certain period which is passed on to the buds, but is not so dominant as the one in the shoot apex. Some of the buds, therefore, were later cultured on IAA-agar medium. A concentration of 0.003 per cent IAA was found to be suitable for the growth of such buds in cultures.

Chromatographic analysis showed that the young buds contain cystine, phenyl-alanine, proline, threonine and leucine. In addition to these amino acids, they also contain valine, methionine and iso-leucine. Glucose and sucrose were also present. Application of IAA showed inhibitory effect on the growth of roots in buds. A similar effect inhibiting growth of roots of a young bud in the axil of a pinna was seen. Possibly, a bud-forming substance is formed here at the apex of the flagella, but is very small in quantity. It alone seems to be responsible for the production of sporophyllous buds or may be the photoperiod is coupled with it in its effect.

The qualitative analysis of vegetative and sporophyll-bearing buds showed very little difference in amino acids like leucine, phenyl-alanine,

methionine, threonine, cystine or sugars like glucose and sucrose, but the last sugar always gave a faint spot.

Another kind of morphogenetic change introduced in a plant as a result of chemical treatment or surgical operations is the change that takes place in its stelar structure. The type of stelar structure developing in the stem of *Dryopteris* and *Matteuccia* has been studied by Wardlaw (1943). A large number of small buds develop at the periphery of the stem in these ferns. No such buds at the periphery of excised rachis were seen in *Goniopteris prolifera* or in the roots of *Anisogonium esculentum*.

This clearly indicates that the causes for the growth and formation of buds are not the same in all the cases and that the nature of the buds, their origin, behaviour and composition in different species need to be investigated separately in each plant.

Possible Factors

The factors responsible for these differences can only be inferred at present. It may be the difference in nutrition, bud-forming substances, photoperiod or heredity. For example, leaflets having intermediate structure of veins and elongated tips were developed when *Asplenium ebenoides* was crossed with A. *rhizophyllum*. These elongated tips formed buds at their apex. Wagner & Boydston (1958), who tried this experiment, observed that the elongated tips of these leaves formed small buds, not very different than those seen in *Goniopteris prolifera* or *Adiantum caudatum*. According to them this is due to hybridity in their plants. Whether it is possible to attribute all such cases where species propagate vegetatively by such leaf buds to hybridity is a moot point and needs further studies.

Further work on the isolation of factors and their effect on the morphogenesis of buds is in progress.

Summary

The paper draws attention to different kinds of buds found in Indian species of ferns and gives a detailed account of two kinds of buds, vegetative and fertile, found on the proliferous rachis of *Goniopteris prolifera* and the probable factors responsible for their production. It is suggested that occurrence of these two kinds of buds is due to some substance produced at the tip of the rachis which seems to have a profound effect on the production of pinnae and buds in their axils.

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OBSERVATIONS ON THE PROTHALLI OF SOME INDIAN FERNS

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The Filicales comprise a group of plants having a continuous history from the Middle Devonian to modern period. They form an important element in the flora of the Carboniferous period, though the then conspicuous genera such as *Botryopteris, Zygopteris* or *Metaclepsydropsis* have disappeared. They gained greater predominance in subsequent epochs, and have remained as an important group till today, although in comparison to angiosperms they often do not seem to be so dominant everywhere. But in tropical forests, in sub-tropical jungles, in the foot hills of a tall mountain to a height of about 8000′, they occupy a prominent position. As a matter of fact, in tropical forests they are relatively as much rich in genera and species as are angiosperms. The largest variety of them, of course, occur in tropics but several genera occur elsewhere too, being capable of withstanding very hot or cold conditions, and can evade unfavourable conditions of life by their peculiar adaptations and modes of vegetative propagation. A few have taken to aquatic habit and some to epiphytic. This variety of habitats naturally provides several features of both adaptative and phylogenetic significance, especially when the adaptations concerned are associated with spore-producing parts, e.g. the nature and kind of protective indusium, hairs in the sori, ability of spores to withstand submergence as in *Azolla*, or the desiccation of leaves and sori in a number of Polypodiaceae growing epiphytically or under xeric conditions, drying up in summer and aestivating in winter.

It is generally believed that they consist of about 305 genera and 10,000 species, three-forths of which are found in tropical or semi-tropical regions.

Like the sporophyte of ferns, well known for its continuous growth and anatomical peculiarities, the gametophyte also has several ways of continuous growth and it also shows adaptations to different conditions of life. Most of the gametophytes are ephemeral but some are quite perennial, e.g. the prothalli of *Ophioglossum, Cheilanthes, Gymnogramme,* some species of *Pteris, Scolopendrium, Aneimia* etc. Prothallia growing under humid conditions, e.g. those of *Osmunda, Gymnopteris* and *Gleichenia,* also have a long lease of life. The general impression, therefore, that prothalli are short-lived is only broadly true. The gametophyte, however, being rather simple in construction offers only a few criteria either for phylogenetic considerations or for understanding the problem of their perennation. In this connection, the prothalli of

Antrophyum or *Vittaria* deserve special mention ; they propagate by gemmae formed irregularly on the mid-rib, like the antheridia-bearing lateral branches of *Targionia*. Most of the perennial prothalli, however, grow by apical meristem like liverworts, and become ribbon-shaped later.

The subterranean prothalli of *Ophioglossum aitchisonii* sometimes bear as many as eight to nine dichotomised branches, bearing not less than 200-300 archegonia. A prothallus on an average forms about 20-30 archegonia, which would mean that such prothalli would be of 10-15 years of age. In course of perennation, some prothalli become tuberous, their mid-ribs becoming swollen. This happens particularly in asymmetrical prothalli of *Pteris* species, *Scolopendrium*, *Gymnogramme* and sometimes in *Angiopteris evecta*. They get associated with endophytic fungi attributed to such genera as *Rhizoctonia*, *Fusarium*, *Completoria* etc. Such underground prothalli prefer to grow either in extreme humid conditions as do the prothalli of *Botrychium* and *Helminthostachys* or in calcarious soils as do the prothalli of *Ophiglossum aitchisonii* or *O. reticulatum*. The tuberous mycorrhizic prothalli of *Ophioglossum fibrosum*, *O. gramineum*, *Botrychium lunaria* and *B. lanuginosum* grow in moist humus or in marshy soil. The great majority of other prothalli, however, grow above the surface of the soil, turn green and assume a cordate form. Out of about 300 species of prothalli spread over 70 genera, more than 250 have a green two-lobed, symmetrical prothallus, but examples to the contrary are also not uncommon, e.g. in *Nephrolepis* species, *Ceratopteris thalictroides*, several species of *Gymnopteris*, *Pteris*, *Asplenium*, *Aspidium* etc. Here one always comes across prothalli having asymmetrical lobes, more than two lobes, or prothalli having no mid-rib. Some of the prothalli are very short and ameristematic. An extreme case of asymmetrical prothallus is seen in *Gymnopteris variabilis* var. *lanceolata* (Syn. *Leptochilus decurrens*) where no two prothalli have the same shape. They vary from spatulate to irregularly 2-lobed, multi-lobed or ameristic prothalli. On the other hand, lobes of the prothallus in the perennial prothalli of *Gymnogramme*, and in some species of *Gymnopteris* and *Cheilanthes* tend to be suppressed. The causes for such irregularities could be many, either nutritional or disturbances in the normal growth-promoting substances, injury, space, apogamy, etc. In this connection the prothalli of *Cheilanthes farinosa* deserve special mention. In this fern complex—a 2n diploid form from Ceylon (*grisea*) where $2n=60$ ($n=30$)—there is a mid-rib. But in tetraploid form from Ceylon (*anceps* with $2n=58$), there is no mid-rib. Similarly in triploid apogamous form from Kenya ($3n=87$; $n=29$), the prothallus is very small and lacks mid-rib (cf. Panigrahi, 1956).

It is well known that prothalli of filmy ferns growing under extremely moist conditions look like algal filaments. It is generally presumed that the prothalli of primitive ferns such as *Ophioglossum* are also considered

to be primitive in structure. The main reasons for this are their cylindrical shape, dichotomy of apex, radial distribution of reproductive organs, embedded antheridia and archegonia, and reduced green leafy outgrowths, at the top. But quite a number of these features can as well be considered as adaptive, especially the shape and distribution of reproductive organs due to subterranean habit and association with mycorrhiza. The shapes developed in them bear a close similarity to the shape of the embryos of orchids which also get infected by fungal association and then grow. It is not true, therefore, to say that the shape of the prothallus of *Ophioglossum* is more primitive than the shape of a simple cordate prothallus having a mid-rib. An analogy may be provided here by thallose and foliose liverworts like *Sewardiella* and *Fossombronia*. The former has a two-lobed thallus but the latter may be cauliflower-like or with a cylindrical axis having leafy lobes at its apex. The original prothallus probably may have been a multi-lobed structure with a prominent mid-rib, green in colour, and with no mycorrhiza. Such a prothallus is seen even today in *Gymnogramme calomelanos* (Syn. *Pityrogramme calomelanos*), *Ceratopteris thalictroides*, *Osmunda regalis*, *Cheilanthes farinosa* etc.

It has long been known that the prothalli of some ferns such as *Aneimia*, *Adiantum*, *Gymnopteris* and *Acrostichum* develop collenchymatous thickenings. The exact significance of this, however, has not been clear. The main fact is that the same processes which bring about thickening of cell walls into collenchyma in a sporophyte are also seen at work in the prothalli. Sometimes there are similar hairs on the sporophyte as well as on the gametophyte.

Another feature of considerable interest is the presence of tracheids in the prothalli of ferns such as *Nephrolepis*, *Aneimia*, *Adiantum* and *Lomaria* (Syn. *Plagiogyria*). These are by now known in about a dozen Indian genera. They appear to have been differentiated from a cell—fourth or fifth from the meristem of the prothallus—and are quite clear when stained with safranin. They occur isolated or continuous in older prothalli. Their occurrence in the midst of gametophytic tissues is quite intriguing. In this connection it is tacitly assumed, that the cell or cells getting so differentiated are diploid ; but even after a careful search this has not yet been proved. Naturally, the questions arise: why do they occur in old prothalli? What is their significance? We generally believe that formation of xylem elements like tracheids is a function of a sporophyte which is essentially a land plant. However, the finding of various chromosome numbers in plants growing in temperate and tropical regions having the same morphological appearance but different chromosome valency, or numbers, has belied this conviction. Is it not likely that the gametophyte also originally possessed tracheids but lost them later, as its very existence comes to a close by the superimposition of a leafy

sporophyte which quickly establishes itself in the soil and brings the life of the prothallus to an end? Viewed in this light, the gametophyte is only an utterly reduced phase of the sporophyte and, therefore, the presence of tracheids in prothalli is vestigial. The diploid and higher polyploid plants having become successful land plants tend to become perennial and, therefore, this character, viz. vascularity, has become more and more important in them. But the gametophyte of fern and fern allies which is often a short-lived hygrophyte is of no use and is ultimately lost. The presence of tracheids in prothalli should, therefore, be considered as an archaic feature.

I have already commented above on the fungal association of fern prothalli especially of the Eusporangiatae. Recent studies of Harley (1959) and others on the significance of mycorrhiza and plant nutrition are yet to be extended to the fungal association of prothalli of ferns ; but one thing is certain, that many of them develop either starch or oil as storage products which are utilized by fungi which help them to perennate. Curiously enough, in some isolated cells of irregularly shaped prothalli of *Gymnopteris variabilis* (Syn. *Leptochilus decurrens*), one also notices members of Cyanophyceae such as *Nostoc*. What is its real significance is yet to be found out. May be the latter helps the former in nitrogen fixation.

There are various nutritive media in which the fern spores readily germinate and form green filaments. The first division of the germ plant seems to be characteristic in many genera. Knop's solution ($\frac{1}{2}$), Beanecke's medium with or without agar, and pumice with peat are the favourite media for culturing fern prothalli of many Leptosporangiatae. The prothalli of *Ophioglossum, Botrychium* or *Helminthostachys*, however, do not yield results by this method. Their sopres remain dormant in cultures for quite a long time and germinate only when a few-celled prothallus gets firmly associated with mycorrhiza found in the roots of the same plant. The nature of this association is obligatory and there is some growth factor which is provided by the fungus to the germinating spores. This has not yet been determined.

The prothalli of water ferns such as *Marsilea* or *Azolla* are of extremely specialized type and grow in a frothy or mucilaginous mass. The characters of this mass provide taxonomic features for the classification of species of *Azolla*. The shape of mucilaginous cord liberating mega- and microspores, and their characteristic apices and appendages are used in the diagnosis of the members of the Marsileaeceae.

Problems concerning the life of prothalli of many leptosporangiate ferns, their hybridity and the ability to withstand dessication like mosses, are yet to be studied. The microclimate which largely governs the life of this tiny stage in the life-cycle of ferns is highly critical for many species. But it has not received much attention at the hands of pteridologists.

Recent morphogenetic studies on the prothalli of *Pteris longifolia* have conclusively proved the extraordinary capacity for regeneration and activation possessed by the prothallial cells. The prothalli of *Nephrolepis acuta* may be mentioned here as an example (Javalgekar & Mahabale, 1959). Any cell from the older prothallus of this species is capable of forming a gemma, propagating it vegetatively again and again. Is it not true then that the prothallus is merely a meriphyte, an adaptive phase of the sporophyte in its life cycle, and that the two are homologous—a view proposed by Bertrand (1947).

Summary

The paper gives a resume of the work done by the author and others on the fern prothalli in India and indicates their main features. It also points out the unsolved problems on prothallial cultures and emphasises the importance of the vascular prothalli in ferns. It is suggested that occurrence of tracheids in fern prothalli is an archaic feature, and that the primitive fern prothallus was probably a multi-lobed cauliflower-like body as is found in the prothalli of some ferns like *Pittyrogramme calomelanos*.

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CLEISTOGAMY IN ANGIOSPERMS

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Cleistogamy is a common phenomenon in flowering plants. By this is meant the behaviour of flowers which do not open, but which nevertheless produce fruits and seeds as a result of autogamy. Such flowers were known to the early botanists (Hoffmann, 1660 ; Dillenius, 1719, 1732 ; Ruppius, 1726 ; Linnaeus, 1737, 1753) who recognized this condition in *Viola, Ruellia* and *Campanula*. The subject has been treated in detail by Uphof (1938) and Rickett (*see* Camp & Gilly, 1943), who have listed the following families in which this phenomenon is known: Papaveraceae, Cruciferae, Papilionaceae, Oxalidaceae, Malpighiaceae, Polygalaceae, Balsaminaceae, Malvaceae, Cistaceae, Violaceae, Polemoniaceae, Labiatae, Scrophulariaceae, Acanthaceae, Rubiaceae, Campanulaceae, Polygonaceae, Illecebraceae, Caryophyllaceae, Portulacaceae, Annonaceae, Ranunculaceae, Droseraceae, Elatinaceae, Primulaceae, Gentianaceae, Nyctaginaceae, Nymphaeaceae, Rosaceae, Vitaceae, Tiliaceae, Hypericaceae, Lythraceae, Onagraceae, Ericaceae, Asclepiadaceae, Convolvulaceae, Boraginaceae, Solanaceae, Gesneriaceae, Compositae, Plantaginaceae, Podostemonaceae, Alismaceae, Liliaceae, Iridaceae, Gramineae, Mayacaceae, Commelinaceae, Pontederiaceae, Juncaceae, Amaryllidaceae and Orchidaceae. Some well known genera where cleistogamous flowers have been observed are: *Commelina, Juncus, Streptocarpus, Cardamine, Oxalis, Impatiens, Viola, Lamium, Ajuga, Salvia, Dipteracanthus, Specularia, Saxifraga, Scrophularia, Lycopus, Drosera, Subularia, Lathraea, Stellaria, Montia* and *Ononis* (Clute, 1898 ; Brandegee, 1900 ; Zederbauer, 1904 ; Goebel, 1905 ; Nieuwland, 1916 ; Gorczynski, 1929, 1930, 1932 ; Kellett, 1935 ; Uphof, 1938). Further, it is widespread among grasses and orchids, e.g. *Bromus, Poa, Panicum, Stipa, Danthonia, Leersia, Muehlenbergia, Triplasis, Cottea, Pappophorum, Triodia, Sorghum, Aciachne, Oryza, Cephalanthera, Cattleya, Dendrobium, Bulbophyllum, Thelasis, Microstylis, Oberonia, Liparis* etc. (Darwin, 1877 ; Reichenbach, 1877 ; Moore, 1877 ; Forbes, 1884, 1885 ; Ridley, 1888 ; Smith, 1905, 1928, 1929 ; Hackel, 1906 ; Ritzerow, 1908 ; Schlechter, 1914 ; Chase, 1918, 1924 ; Kircher, 1922 ; Weatherwax, 1928, 1929 ; Poulter, 1932 ; Piacco, 1935 ; Ayangar, 1936).

Cleistogamous flowers are usually associated with a reduction in the number and size of floral parts. In all cases, it is the result of an arrested development of the flower bud during its earlier stage or at a late one

when the flower is almost ready to unfold. In some members of the Gramineae (Uphof, 1938 ; Brown, 1949) and Malpighiaceae (Uphof, 1938), these flowers are so greatly modified that even the correct identity of the plant becomes difficult to establish. *Stipa leucotricha* (Brown, 1949) is an example where the cleistogamous spikelets borne in the axils of lower leaves are considerably reduced. Frequently, the corolla is poorly developed or entirely absent, as in *Cardamine chenopodifolia,* and it usually lacks the normal colour. A reduction in the number of stamens and anther sacs is a common accompaniment. In *Oxalis acetosella* the inner stamens, which are by no means strongly developed even in chasmogamous flowers, are still less conspicuous in the cleistogamous flowers. In the family Commelinaceae, cleistogamous and underground flowers are produced on the rhizomes in *Tradescantia erecta* (syn. *Tinantia fugax*), *Commelinantia pringlei, Commelina virginica, C. nudiflora, C. indehiscens, C. forskalaei* and *C. benghalensis* and have been studied by Wight (1853), Henslow (1879), Calvino (1922, 1923), Tharp (1927), Hagerup (1932), Trochain (1932), Uphof (1934), P. Maheshwari & Singh (1934), Parks (1935), Barnes (1949), P. Maheshwari & J. K. Maheshwari (1955), and S. C. Maheshwari & Baldev (1958).

Observations

In *Commelina forskalaei* (Figs. 1-4) the cleistogamous flowers are produced on long, etiolated branches, which are provided with scarious leaves usually reduced to their sheathing bases. Some of the branches grow downward in the loose, sandy soil and produce underground spathes developing in the axils of the leafy scales. There is usually a single bisexual flower, rarely two, in each spathe. This flower resembles the upper chasmogamous, bisexual flowers, except for its reduced size, absence of pigment and lack of any clear distinction between the sepals and petals. In the closed flower, elongation of the style results in a spiral coil and the stigma is brought in contact with the large, yellow, curved anther. While a large number of pollen grains germinate on the stigma in the aerial as well as the underground flowers, a few might do so while still within the anther sac. In comparison to the aerial flowers, which occasionally fail to ripen, the underground flowers are uniformly fertile. The fruits, protected by the characteristic spathe, contain a single indehiscent seed with a hard testa instead of one large and two small seeds as in the aerial fruits. The ovules in the other chambers remain undeveloped and ultimately degenerate. The single seed is of about the same size as that produced in the posticous locule of an aerial fruit. The underground seeds, which are self-sown in nature, germinate in the laboratory with the same rapidity as the aerial ones. Towards the end of November, when the night temperature goes down to 10°C, even the aerial



Figs. 1-4. *Commelina forskalaei* (*ch*, chasmogamous flower ; *cl*, cleistogamous flower). Fig. 1. Part of a prostrate branch showing underground cleistogamous flowers (*cl*) and aerial chasmogamous flowers (*ch*). × 1½. Fig. 2. Underground branch with cleistogamous flowers. × 3½. Fig. 3. L.s. cleistogamous flowers showing one large, curved anther, two smaller anthers and a spirally coiled style. × 11. Fig. 4. Seed produced from underground flower. × 7. (*After* Maheshwari & Maheshwari, 1955).

spathes remain closed and may occasionally set seeds cleistogamously. According to Hagerup (1932) the third flower of the aerial spathe is also cleistogamous in the warm climate of Timbuctu, whereas at Delhi, although variable in its sexual characters, it is invariably chasmogamous.

Some of the branches destined to produce underground flowers fail to grow down due to the presence of rocks or other impediments in the soil. They usually show a single chasmogamous flower (Fig. 1). Such transitional types, which occupy an intermediate position between the aerial and underground flowers, have also been reported in *Commelinantia pringlei* (Parks, 1935) and *Commelina benghalensis* (Barnes, 1949 ; Maheshwari & Maheshwari, 1955). The presence of these transitional flowers is significant indicating that cleistogamy is derived from chasmogamy, the former being a later development.

Commelina benghalensis is similar in many respects to *C. forskalaei*. A number of axillary shoots arise from the lower portions of the plant. Many of them penetrate the loose, damp, sandy soil and develop into long runners. There is a single, pear-shaped spathe in the axil of a leafy scale. It encloses a solitary, bisexual flower on the outer cyme branch, the inner cyme branch being absent. The small, bluish petals remain

within the sepals. At the time of pollination, the style elongates in the form of a spiral and the stigma becomes pollinated. The fruits usually contain three seeds (occasionally two or four) in contrast to the one-seeded condition in *C. forskalaei*. The seed in the posticous chamber is always the largest. This germinates as vigorously as the seeds from the aerial fruits ; the two smaller seeds show no sign of germination. The transitional flowers are borne on shoots arising near the base and which fail to enter the soil. The spathes borne on them resemble the underground ones except for their green colour. In addition, there occur short, green, slender branches, one at a node, bearing two or three, smaller, urn-shaped spathes. They contain a solitary, bisexual, self-pollinated flower on the outer cyme branch, the inner cyme branch being absent as in the underground spathes. Such flowers usually remain enclosed inside the narrow-mouthed spathe, although a partial opening may occur sometimes. In branches, which are only partially buried under the soil, the upper portion often curves up and produces capsules above the ground. The solitary fruits produced in these spathes are usually three-seeded as in the underground ones. The behaviour of the third flower in the upper aerial spathes has been described differently by various authors. Trochain (1932), who studied this species from Senegal, writes that the third flower is cleistogamous. However, our observations show that the third flower, if present, is always chasmogamous (Maheshwari & Maheshwari, 1955).

Ecological Cleistogamy

In some cases cleistogamy is inherent, but in others and in a great number of tropical and subtropical species, it is ecological, i.e. the presence or absence of cleistogams is controlled by environmental factors. Such cases have been described as "ecological cleistogamy", "pseudo-cleistogamy", "photo-cleistogamy" etc. (Hansgirg, 1891 ; Uphof, 1938). Some of these factors are: submersion in water (in *Ranunculus moseleyi, Mayaca fluviatilis, Elatine hexandra, E. triandra,* and *E. hydropiper*), drought (in *Impatiens parviflora* and *Dianthera sessilis*), high atmospheric moisture (in *Liparis caespitosa*), cold (in *Specularia perfoliata, Viola pinnata, V. biflora,* and *Thlaspi rotundifolium*), excessive heat (in *Aechmanthera wallichii* var. *gossypina, Eranthemum cinnabarinum,* and *E. crenulatum*), shade (in *Viola arvensis*), surrounding soil (in *Cardamine chenopodifolia*) and so on. By far, the greater number of cleistogamous plants may be called facultative. They are of the mixed type and bear both kinds of flowers, either as a regular feature or occasionally, when conditions determine it. Among the latter are the seasonal cleistogams, such as *Lamium amplexicaule,* whose cleistogamous flowers appear in early spring. In *Oxalis acetosella* and *Viola odorata,* growing in shady

places, the flowers produced in the spring are chasmogamous but later, when overgrown by other vegetation, they bear only cleistogamous flowers.

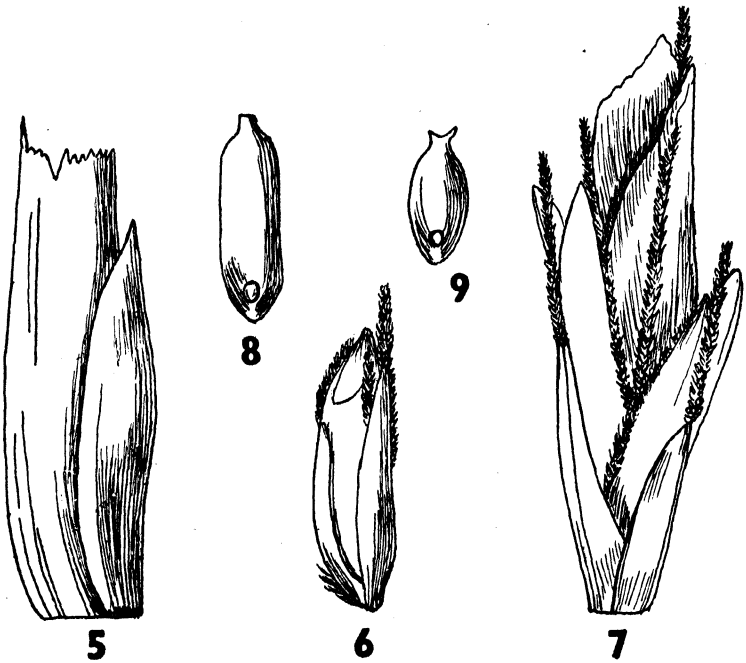
In *Commelina forskalaei* and *C. benghalensis* (Maheshwari & Maheshwari, 1955) a larger number of underground flowers are produced with the onset of unfavourable winter conditions. Similarly, in *Commelinantia pringlei* (Parks, 1935) and *Commelina forskalaei* (Maheshwari & Maheshwari, 1955) if the cleistogamous flowers or the branches bearing them are accidentally or otherwise exposed to light, chasmogamous flowers are produced. In *Bromus catharticus* (Brown, 1949) also the inflorescences, which are produced late in the spring under dry, hot conditions, contain a larger proportion of cleistogamous florets. These observations clearly demonstrate the important role of environment in the expression of cleistogamy. Moreover, several workers in this field have been able to induce experimental cleistogamy in a number of ways. Ritzerow (1908) secured cleistogamous blossoms in *Pavonia hastata* and *Specularia perforata* by subjecting them to poor soil conditions. In his extensive studies on *Lamium maculatum, Viola odorata, Linaria spuria, Stellaria media* and other plants, Vöchting (1893) found that these plants produce different stages of cleistogamous flowers depending upon the intensity of the light. Of interest in this connection are the experiments of Schiveley (1904) with *Amphicarpa monoica.* He buried the flower-buds in the soil and obtained subterranean pods from flowers which had all the morphological and anatomical characters usually associated with underground environment. Likewise, subterranean legumes were developed from chasmogamous aerial flowers artificially kept in the soil. However, cleistogamy is frequently caused by more than one stimulus.

Origin and Significance of Cleistogamy

Two hypotheses have been put forth regarding the origin and significance of cleistogamy. Ritzerow (1908), Goebel (1923), and Uphof (1934, 1938) are of the opinion that cleistogamous flowers represent retarded forms of chasmogamous flowers, but endowed with the power of precocious pollination and, therefore, capable in spite of their imperfect development, of forming seeds. According to Darwin (1877), this phenomenon is an acquired adaptation in the struggle for life and cleistogamous flowers show special modifications to ensure self pollination. As stated earlier, a number of cleistogamous variations are induced by environmental factors, such as temperature, food supply, light and water. There are also numerous examples of partial fluctuations where cleistogams as well as chasmogams occur in the same inflorescence or at least in the same plant, e.g. *Linaria canadensis* (Brandegee, 1900). In *Antirrhinum cooperi, A. watsoni,* and *A. subsessile,* the early flowers are frequently

cleistogamous, but at a later stage different types of transitions are found, up to the chasmogamous condition. Similar examples have been found in various species of *Viola* by Goebel (1904), Kerner & Hansen (1913), Bergdolt (1932) and others.

The theory that cleistogamous flowers are simply normal flowers inhibited in development, does not hold good for a number of species belonging to the family Gramineae, for in them the cleistogams are not a part of the normal inflorescence, but are isolated flowers, produced in the axils of the lower leaf-sheaths on flowering shoots. They are produced after the maturity of the panicled spikelets, and the seeds they form are longer and narrower than those of the chasmogamous flowers, e.g. in *Triplasis purpurea* (Figs. 5-9). It seems probable that they germinate *in situ* being close to the ground and that they are a regular means of propagation. In *Amphicarpa* (Schiveley, 1904), the chasmogamous fruits are lanceolate or falcate in shape, sparingly hairy and contain two to three



Figs. 5-9. *Triplasis purpurea*. Fig. 5. Base of internode showing cleistogamous spikelet enclosed by prophyll. × 14. Fig. 6. Cleistogamous spikelet. × 10. Fig. 7. Spikelet from terminal panicle. × 10. Fig. 8. Grain from cleistogamous spikelet. × 8. Fig. 9. Grain from terminal panicle. × 6. (*After* Chase, 1918).

seeds. The fruits of the subterranean cleistogams are pyriform and one-seeded, while those of the aerial cleistogams are densely hairy and one- to three-seeded. The anatomical structure of the two kinds of fruits is different. These findings, therefore, support the belief that cleistogamy involves changes going deeper than a simple arrest of development. Goebel's theory, therefore, might apply in such cases where cleistogamy appears at a certain stage in the life history of the plant and is not heritable.

In *Salvia cleistogamica, Poa chapmaniana, Leersia oryzoides, Liparis cleistogama, Thelasis capitata, Bulbophyllum scrobiculilabre, B. cleistogamum, Chloreae inconspicua, Appendicula cleistogama, Dendrobium cleistogamum, Subularia aquatica,* and *Polycarpon tetraphyllum* cleistogamy is a fixed specific character (Uphof, 1938). Burck (1906) considered this to be genetic and suggested that in many species such a condition might have originated by mutation. As examples may be cited *Ammannia latifolia* and *Salvia cleistogamica* (Uphof, 1934) where even under the most favourable conditions the plants failed to produce any chasmogamous flowers. For five years Ascherson (1871) cultivated successive progenies of *S. cleistogamica* and regularly obtained cleistogamous flowers, thus demonstrating that the condition was inheritable. It is noteworthy to mention that a number of well established cleistogamous varieties are known among some chasmogamous species, and the distinction appears to be genetic and heritable, e.g. *Calanthe veratrifolia* var. *cleistogamia* and *Plocoglottis glaucescens* var. *cleistogamia* among the orchids. Thus, local populations of a species may be wholly cleistogamous and others wholly chasmogamous.

Weatherwax (1929) regards the cleistogamous *Poa chapmaniana* as having originated from the chasmogamous *Poa annua* by mutation, or by some selective process. Cleistogamy, according to him, is an effective barrier which prevents the new species from hybridizing with its parent. In some South American species of *Plantago* cleistogamy shows a sex-linked inheritance. Some plants have only hermaphrodite, cleistogamous flowers, while others are wholly male and chasmogamous. The hermaphrodite flowers cleistogamously pollinated, give only cleistogamous offspring, but if pollinated from male flowers the offspring are all male (Schürhoff, 1924). Mather & Vines (*see* McLean & Cook, 1956) found two cleistogamous plants among the offspring of a cross between *Antirrhinum majus* and *A. glutinosum*. These plants had distinct foliage and set good seed, the cleistogamy being heritable. Thus, two species—one an obligatory cross-breeder and the other a facultative cross-breeder—possessed between them all the genetical material for producing an obligatory inbreeding race. In such cases it appears, that the cleistogamous line or lines ("cleistogameons" of Camp & Gilly, 1943), developing from a single, heterozygous individual, will be quite constant in their morphological characters. The flowers capable of cross pollination, sporadically

or seasonally produced on these same plants, will open the way for the production of new genetic combinations, some of which in turn—through cleistogamy—will become stabilized and produce local or sometimes widespread races distinguished by different sets of characters.

The fact that in the genus *Commelina* only a few species show a cleistogamous-chasmogamous type of floral dimorphism, is evidence for some form of genetic control. Moreover, Uphof (1934) has shown experimentally that cleistogamy is a constant feature in *Commelina virginica*. If uprooted plants are grown without soil in a damp jar and subjected to strong sunlight, the aerial stems normally produce chasmogams, but the subterranean rhizomes continue to produce cleistogamous flowers even in the air. It is, therefore, concluded that there is a hereditary factor for cleistogamy in those species which produce subterranean cleistogams in addition to the aerial chasmogams.

SUMMARY

Cleistogamy is a common phenomenon in flowering plants and the role it plays in the production of complex specific populations has been fully recognized. Such flowers or cleistogenes, as they are called, have been observed in several genera: *Commelina, Juncus, Streptocarpus, Cardamine, Oxalis, Impatiens, Viola, Lamium, Ajuga, Salvia, Dipteracanthus, Specularia, Saxifraga, Scrophularia, Lycopus, Drosera, Subularia, Lathraea, Stellaria, Montia, Ononis, Bromus, Poa, Stipa, Triplasis, Dendrobium* and *Liparis*. A cleisto-chasmogamous type of floral dimorphism is exhibited by some species of *Commelina*, e.g. *C. benghalensis* and *C. forskalaei*.

In some of the above-mentioned examples, cleistogamy is constitutional, but in others it is controlled by environmental factors (pseudo-cleistogamy or ecological cleistogamy).

Two hypotheses have been put forward regarding the origin and significance of cleistogamy. It is considered by some to be genetic and in many species such a condition might have originated by mutation. There are others who believe that cleistogamous flowers represent retarded forms of chasmogamous flowers. The latter theory is applicable only in those cases where cleistogamy appears at a certain stage in the life history of the plant. The observations presented here show that in *Commelina* there is a hereditary factor for cleistogamy in those species which produce subterranean cleistogams in addition to aerial chasmogams.

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STUDIES ON THE NATURALIZED FLORA OF INDIA

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Although India has a flora of its own, the introduction and subsequent naturalization of foreign plants on our soil has occurred, largely by coastal and land invasions from other countries. This was further aided by man and animals. History tells us that the early Aryan settlers from the countries lying north-west of India, brought with them a number of economic plants. It is quite possible that they also brought other plants or seeds, either by accident or on purpose. Their methods of life were primitive. The felling of trees on the area selected for development, burning the felled material and then planting a crop was practised. Man, thus, began to change the flora of this country by agriculture and by clearing the ground of its primary vegetation, right from the dawn of civilization. He also created ample opportunities for the introduction of aliens even prior to the settlement of Jesuit missionaries and early European explorers. During the nineteenth century, large numbers of plants were transported in the tropics between botanic gardens, while scientific institutions, wealthy patrons, seedsmen and nurseries sent collectors to the far corners of the earth to bring back novelties for their hothouses and for sale. In India, the Sibpur Botanic Garden played a prominent role in the distribution of a large percentage of plants and, therefore, our study of the migration and naturalization of foreign plants may be said to date as far back as 1787, the date of the foundation of the Botanic Garden. Thus, by the time Brühl (1908) wrote his 'Flora Advena of Bengal and Bihar', a large number of exotic species had already become naturalized in this part of the country. In the following pages I have attempted to summarise my preliminary observations on the naturalized plants of India. In the course of an exploration of the flora of Delhi (Table 1) and some parts of Madhya Pradesh, it was found that there has been a considerable increase in the number of genera and species due to the introduction and naturalization of many foreign elements (Maheshwari, 1957, 1960, 1961). An analysis of the naturalized plant aliens will, therefore, give some idea of the additions to our flora, many of which are changes for the worse. It will also reveal how the naturalized content of the flora is a reflection of historical, climatic, ecological and occupational factors. I have included here only such species which following their immigration or introduction during the pre-historic and recent years, have become more or less wild and may now be called as the permanent denizens of this country.

TABLE I

ORIGIN OF PLANTS OF DELHI STATE

	Plants of	Number	Percentage
1.	N. African-Indian Desert Belt ...	22	4.60
2.	Tropical Africa	37	7.74
3.	Tropical Africa and N. African-Indian Desert Belt	23	4.81
4.	Africa or West Asia	17	3.56
5.	Mediterranean Region	10	2.09
6.	Indian Region	81	16.95
7.	Indo-Malayan Region and East Asia	33	6.90
8.	Indo-Malaya and Africa ...	33	6.90
9.	Temperate Region	8	1.67
10.	New World	14	2.93
11.	Tropical or Cosmopolitan ...	200	41.84

METHODS OF ENTRY AND DISTRIBUTION

It is only rarely that we can definitely date the actual introduction of any species, and a good number have been repeatedly brought into different parts of the country. Many areas have been imperfectly studied, especially in the important early periods and, therefore, their time of arrival can in most cases be fixed only with a rough degree of approximation. India is a country with land connections on the north and west, and plants have migrated from North-East Africa or West Asia, and South-West China by way of Arabia, Afghanistan, Kashmir, and North Burma and North-East India respectively. The presence of these land connections together with iso-climatic conditions tends to distribute plants from one to the other and *vice versa* by the natural processes of plant distribution. Such a process viewed through thousands of years of geological time increases the range of distribution of many species and gradually decreases their endemic nature. The surrounding countries which have contributed to the Indian Flora are Ceylon, Burma, Malaysia, South-West China, Eastern China, Japan, West Asia and Africa. A large number of species have come to India from these surrounding countries (Fig. 1). Although the vegetation of most of these regions is very different from that of India, none the less, the great influence of these countries upon the flora of India is evident (Chatterjee, 1939 ; Mukerjee, 1940). Broadly speaking, it is regarded that families like Cruciferae, Caryo-

phyllaceae, Capparidaceae (partly), Fumariaceae (partly), Boraginaceae (partly), Rosaceae (partly), and Labiatae (partly) had their origin in the East Mediterranean Region and migrations have taken place both east and westwards, and the number of migrants becomes thinner and lesser as we go farther and farther from the main source (Fig. 2). Some of these common plants which are found extending from West Asia to India are as follows: *Hypecoum procumbens* Linn., *Dianthus caryophyllus* Linn., *D. fimbriatus* Biebr., *Silene conoidea* Linn., *S. arenosa* C. Koch, *Stellaria aquatica* Scop., *S. bulbosa* Wulf., *Cerastium trigynum* Vill., *C.*

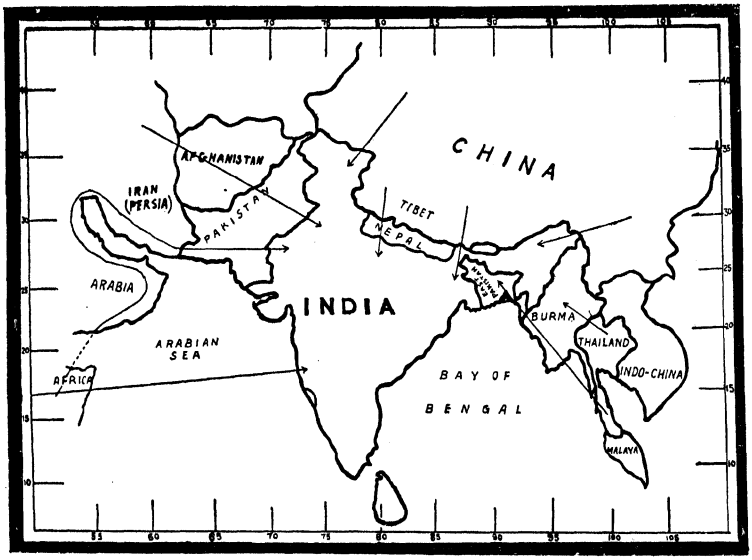


Fig. 1. India and the surrounding countries. Possible routes of the immigration of plants are shown by arrows. (*After* Chatterjee, 1939).

dahuricum Fisch., *C. vulgatum* Linn., *Arenaria serpyllifolia* Linn., *Drymaria cordata* Willd., *Polycarpaea spicata* W. et A., *Sisymbrium irio* Linn., *S. wallichii* Hook. f. et T., *S. sophia* Linn., *S. loeselii* Linn., *Matthiola odoratissima* Br., *Cardamine impatiens* Linn., *Erophila vulgaris* DC., *Brassica tournefortii* Gouan, *Capsella bursa-pastoris* Moench, *Lepidium latifolium* Linn., *Malcolmia africana* Br., *Erysimum repandum* Linn., *Farsetia jacquemontii* Hook. f. et T., and *F. aegyptiaca* Turr. (Chatterjee, 1947). Besides, we find that a large number of weeds have been imported as 'accidental' introductions with food grains, ballast, packing materials, seeds of economic plants or merely by adhering to the clothing of man and to the hairs of domestic animals. When they arrived at their new home, they were usually further distri-

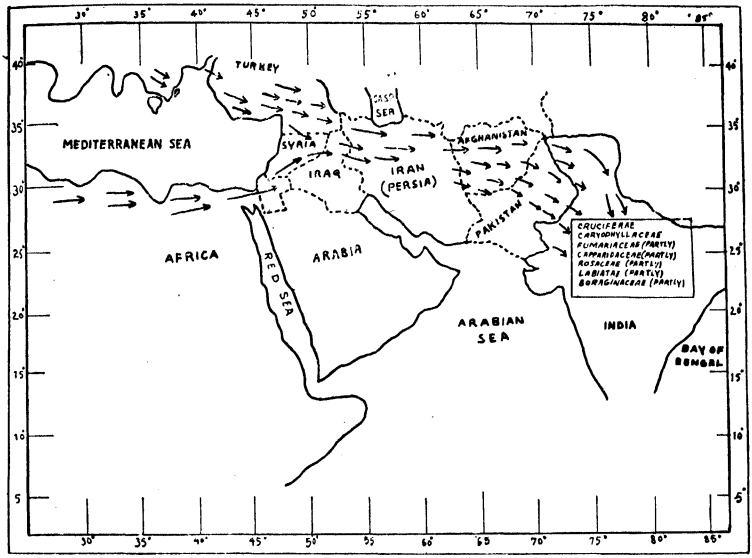


Fig. 2. Map showing the influence of East Mediterranean region flora on that of India. Possible route of the migration of plants from West Asia and North-East Africa is shown by arrows. (*After* Chatterjee, 1947).

buted by natural causes like wind and water, and aids provided by man and animals, e.g. deforestation, pasturage methods, shifting cultivation, methods of harvesting, construction of roads and railway lines, and continual sowing of impure seeds on cultivated and uncultivated lands.

The neotropical plants, i.e. those hailing from the tropics of the New World have been found to naturalize on Indian soil in a remarkably short period of time. Of such may be mentioned the euphorbiaceous South American *Croton bonplandianum* Baill. (*C. sparsiflorus* Morong.). About the year 1897 a ship arrived at Chittagong (East Pakistan) from La Plata with ballast of mud from S. America. To get rid of the mud, it was supplied to a local gardener for soil. In it were seeds of *Croton*, which germinated and grew. The plant travelled along the railway lines and by steamer to Calcutta and is now-a-days common in many parts of India, chiefly following the roads, railway lines and canal banks (Brühl, 1908). A similar history attaches to the introduction of *Eupatorium odoratum* Linn., from the West Indies to India, East Pakistan and Burma, by seeds confined to the ballast heaps of cargo boats calling at Singapore. Of late years, it was observed growing in the teak plantations of Kerala State. It is believed that the seed of this weed was brought down to Kerala from Assam by the labourers returning from the Assam Front. The seeds stuck to their beddings and clothes, and thus bridged the big gap from

Assam to Kerala (Biswas, 1934 ; Moni, 1959). In the year 1917, *Acanthospermum hispidum* DC. was first noticed round about Tirupattur Railway Station in the Salem district and at Nileshwar in South Kanara, suggesting that a few seeds of the plant must have been carried along with the packing material of some imported goods. Since then, it has been spreading very rapidly by roads and railways, and has now established in different parts of the country. Among other examples of recently introduced weeds and weedy plants may be cited, *Nicotiana plumbaginifolia* Viv., *Datura suaveolens* H. et B. ex Willd., *D. quercifolia* H.B. et K., *Alternanthera repens* Link, *A. paronychioides* St. Hil., *Gomphrena celosioides* Mart., *Chenopodium ambrosioides* Linn., *Evolvulus nummularius* Linn., *Heliotropium curassavicum* Linn., *Euphorbia prostrata* Ait., *E. geniculata* Orteg., *E. graminea* Jacq., *Jatropha gossypifolia* Linn., *Flaveria australasica* Hook., *Parthenium hysterophorus* Linn., *Eupatorium glandulosum* H.B. et K., *Erigeron mucronatus* DC., *E. bonariensis* Linn. (*E. linifolius* Willd. ; *Conyza ambigua* DC.), *E. annuus* Pers., *Carthamus oxyacantha* Bieb., *Tithonia diversifolia* A. Gray, *T. tagetiflora* Desf., *Lagascea mollis* Cav., *Synedrella nodiflora* Gaertn., *Gnaphalium purpureum* Linn., *Ximenesia encelioides* Cav. (*Verbesina encelioides* B. et Hk. f.), *Crassocephalum crepidioides* S. Moore (*Gynura crepidioides* Benth.), *Galinsoga parviflora* Cav., *Aeschynomene americana* Linn., *Oxalis latifolia* H.B. et K., *O. pes-caprae* Linn., *O. martiana* Zucc. (*O. corymbosa* DC.), *Scoparia dulcis* Linn., *Herpestis chamaedryoides* Linn., *Mecardonia dianthera* Pennell (*Lindernia dianthera* Sw.), *Calceolaria mexicana* Benth., *Hyptis suaveolens* Poit., *Talinum paniculatum* Gaertn. (*T. patens* Willd.), *Mikania cordata* B.L. Robins. (*M. scandens* Clarke, non Willd.), *Asclepias curassavica* Linn., *Peperomia pellucida* H.B. et K., *Sagina apetala* Linn. and *Apium leptophyllum* F. Muell. ex Benth. (*see* Prain, 1890 ; Collett, 1902 ; Brühl, 1908 ; Gamble & Fischer, 1915-1936 ; Calder, 1919 ; Kashyap, 1924 ; Jouget, 1928 ; Mayuranathan, 1929 ; Fyson, 1932 ; Biswas, 1934 ; Raizada, 1935, 1936, 1950 ; Santapau, 1945, 1946, 1953 a, b, 1957 ; Santapau & Randeria, 1955 ; Sandwith, 1946 ; Chatterji & Raizada, 1948 ; Mooney, 1950 ; Datta, 1954 ; Tadulingam & Venkatanarayana, 1955 ; Srivastava, 1955 ; Sundararaj, 1956 ; Pattnaik, 1956 ; Rao, 1956 ; Maheshwari, 1957, 1960, 1961 ; Matthew, 1959 ; Chaudhuri, 1959 ; Mirashi, 1960 ; Chatterjee, 1960).

Factors Influencing Naturalization and Distribution

One would have expected that an adventive plant was more suited ecologically to its country of origin than to its new home. On the other hand, it has been observed that an organism often multiplies rapidly when carried to a new environment. Water hyacinth (*Eichhornia*

crassipes Solms.) in South-East Asia, the Nile and the Congo (Pirie, 1960), *Lantana* in India, and prickly pears (*Opuntia*) in Australia and India (Burkill, 1911), are familiar examples. One of the major reasons is that when an alien plant has been introduced without many of the normal pests and diseases, it has more chances of flourishing in the new area. We can only contemplate what would happen to the Malayan rubber industry, if the South American Leaf Blight (*Dothidella ulei*) were accidentally introduced (Purseglove, 1959).

Various factors favour the spread and increase of alien plants in India and they may be grouped under the following heads:

DEFORESTATION—In considering the introduction and naturalization of alien plants in India, the factor of greatest importance would include man himself. Since the post-glacial period through pre-historic and historic times, the climax evergreen vegetation over most parts of India has suffered greatly from natural causes and biotic interference, resulting in grasslands, deserts and scrubs (Puri, 1959). Thus, plants growing under the shade of larger trees suffer when the shade is removed. As soon as the land is cleared, other plants both indigenous and introduced make their appearance and in a short time, a new growth consisting of the old and the new flourishing side by side is seen.

FAULTY PASTURAGE METHODS—Overstocking and overgrazing of pastures aids the spread of indigenous as well as introduced weeds. The animals wander to and fro, distributing the burred, prickly, hairy or adhesive seeds of various weeds and continually select and eat out the better pasture plants. The obnoxious, prickly, woody and poisonous plants are usually left untouched and in this way they are at a great advantage over fodder plants which are being continuously grazed down.

SHIFTING CULTIVATION—The common practice of cultivating a small portion of land for a few years and abandoning it when the crops give a smaller return, and in due course shifting to another portion of land, will eventually destroy the original flora of the area. This is the history of the present botanical condition in India (Ridley, 1930). As the country so treated becomes denuded of its primary vegetation and with the micro-climate and the community structure altered, plants adapted for the changed conditions invade the devastated area.

METHODS OF HARVESTING—Clean, thorough and timely harvesting is as important a factor in keeping the ground clean and free from weeds as is cultivation. It is among the standing straw that all kinds of pernicious weeds ripen and shed their seeds.

SALE AND INTRODUCTION OF IMPURE SEEDS—A regrettable feature in the spread of alien plants has been the lack of control in regard

to their introduction from abroad and the way in which these plants have been spread with impure agricultural seeds. This is of very common occurrence in South India. The European plants were undoubtedly introduced as impurities, at some time with the vegetable seeds or seeds of garden plants. Important among them are the following plants: *Stellaria media* Cyr., *Spergula arvensis* Linn., *Sagina apetala* Linn., *Convolvulus arvensis* Linn., *Anagallis arvensis* subsp. *arvensis*, *Sonchus arvensis* Linn., *Trigonella corniculata* Linn. and *Melilotus alba* Desr. It is, therefore, high time to prohibit the sale of material containing impure seed mixtures.

Phytogeographical Considerations

The statistical data on this subject are lacking. The Botanical Survey of India has undertaken intensive field surveys and the results are likely to yield precise and accurate figures. For the present, it is believed that of the total number of Indian flowering plants, *ca* 40 per cent are foreign and now naturalized in different parts of the country. These plants could be classified as:

(1) Pluri-regional species (paleotropical, neotropical, pantropical and cosmopolitan groups).
(2) Weeds of cultivation and other introduced weeds.
(3) Exotics and escapes from cultivation.
(4) Species of limited distribution in India and adjoining regions.

The first three groups may be considered to represent 10 per cent of the total figure. The plants belonging to the fourth group have immigrated into India from Malaysia, China and West Asia. It is assumed that 10 per cent of the Indian flora has come from West Asia and an almost equal proportion respectively from the Chinese and Malayan sides. This, altogether, makes up the total to 40 per cent for all the foreign plants.

As of botanical interest may be mentioned the following phytogeographical groups:

(1) Pluri-Regional Species or "Wides"—The plants belonging to this category have very wide distribution in Europe, Asia, Africa and America. Many of them are characterized by pantropical, paleotropical, neotropical or cosmopolitan type of distribution. In the Indian flora, a large number of pluriregional species or "Wides" exist in the following families of dicotyledons (the number of pluri-regional species is given in parentheses): Ranunculaceae (61), Annonaceae (52), Menispermaceae (30), Cruciferae (78), Capparidaceae (30), Polygalaceae (25), Caryophyllaceae (44), Guttiferae (20), Malvaceae (81), Sterculiaceae (47), Tiliaceae (42), Elaeocarpaceae (27),

Balsaminaceae (21), Rutaceae (48), Meliaceae (27), Aquifoliaceae (21), Celastraceae (24), Rhamnaceae (25), Ampelidaceae (31), Sapindaceae (34), Anacardiaceae (25), Papilionaceae (372), Caesalpiniaceae (78), Mimosaceae (53), Rosaceae (76), Saxifragaceae (27), Combretaceae (26), Myrtaceae (59), Melastomaceae (42), Lythraceae (26), Cucurbitaceae (58), Umbelliferae (49), Araliaceae (23), Rubiaceae (187), Compositae (330), Campanulaceae (24), Primulaceae (31), Myrsinaceae (29), Oleaceae (29), Apocynaceae (30), Asclepiadaceae (62), Loganiaceae (20), Gentianaceae (42), Boraginaceae (62), Convolvulaceae (90), Solanaceae (42), Scrophulariaceae (135), Acanthaceae (88), Verbenaceae (60), Labiatae (160), Amaranthaceae (31), Chenopodiaceae (37), Polygonaceae (25), Lauraceae (33), Loranthaceae (26), Euphorbiaceae (161), Moraceae (70), Urticaceae (42), and Cupuliferae (22). Among the monocotyledons, the families Liliaceae, Cyperaceae and Gramineae are very rich in the number of "Wides".

(2) WEEDS OF CULTIVATION AND OTHER INTRODUCED WEEDS—The chief centres of origin of tropical weeds are Mexico and some parts of South America, especially eastern Brazil in the New World and parts of India and Africa in the Old World, i.e. those areas within the tropics with a limited rainfall, where an open type of vegetation has developed rather than a dense tropical forest. Naturally areas in which the practice of agriculture is an ancient art form a part of the picture (Merrill, 1946). In the dicotyledonous families, the number of weeds is very large, for several hundred species are involved and nearly all of these are of more or less broad distribution in all tropical countries. The families Compositae, Amaranthaceae, Papilionaceae, Malvaceae, Tiliaceae, Sterculiaceae, Rubiaceae, Acanthaceae, Euphorbiaceae, Convolvulaceae, Labiatae, Scrophulariaceae, Cyperaceae and Gramineae are notably rich in weeds. Many of them are found in waste lands, pastures, garden beds, cultivated and fallow grounds, rivers, marshes and wet lands. Typical examples are as follows:

I. TROPICAL AMERICAN—*Nicotiana plumbaginifolia* Viv., *Martynia annua* Linn., *Iseilema laxum* Hack., *Chloris virgata* Sw., *Digitaria adscendens* Henr., *Tridax procumbens* Linn., *Eupatorium odoratum* Linn., *Synedrella nodiflora* Gaertn., *Euphorbia prostrata* Ait., *E. geniculata* Orteg., *Sida veronicifolia* Lam., *Corchorus aestuans* Linn., *Ruellia tuberosa* Linn., *Lantana camara* var. *aculeata* Mold., *Talinum paniculatum* Gaertn., and *Herpestis chamaedryoides* H.B. et K.

II. MEXICAN—*Erigeron mucronatus* DC., *Tithonia tagetiflora* Desf., *Oxalis latifolia* H.B. et K., *Argemone mexicana* Linn., *Datura suaveolens* H. et B. ex Willd., *Opuntia cochinelifera* Mill., and *Calceolaria mexicana* Benth.

III. SOUTH AMERICAN—*Passiflora foetida* Linn., *P. morifolia* Mast., *Acanthospermum hispidum* DC., *Galinsoga parviflora* Cav., *Gomphrena celosioides* Mart., *Alternanthera repens* Link, *A. paronychioides* St. Hil., *Croton bonplandianum* Baill. (*C. sparciflorus* Morong.), *Hyptis suaveolens* Poit., *Asclepias curassavica* Linn., *Opuntia monacantha* Haw., *O. elatior* Mill., and *O. dillenii* Haw.

IV. EURASIAN—*Melilotus alba* Desr., *Lathyrus aphaca* Linn., *Sonchus arvensis* Linn., *Anagallis arvensis* subsp. *arvensis* (*A. arvensis* Auct., non Linn.), and *Setaria glauca* Beauv.

V. AFRO-ASIAN—*Phaseolus trilobus* Ait., *Emilia sonchifolia* DC., *Laggera aurita* Sch.-Bip., *Merremia emarginata* Hal. f., *Ocimum americanum* Linn., *Jatropha glandulifera* Roxb., *Pupalia lappacea* Juss., *Digera alternifolia* Aschers. (*D. arvensis* Forsk.), *Eragrostis plumosa* Link, *Cymbopogon martinii* Wats., *Eremopogon foveolatus* Stapf, and *Cleome monophylla* Linn.

VI. AUSTRO-ASIAN—*Indigofera enneaphylla* Linn., *I. trita* Linn. f., *I. trifoliata* Linn., *I. glandulosa* Willd., *Desmodium parvifolium* DC., *Crotalaria medicaginea* Lam., *Wedelia calendulacea* Less., *Salvia plebeia* R. Br., *Ottelia alismoides* Pers., *Scleria tessellata* Willd., *Sporobolus diander* Beauv., and *Dimeria ornithopoda* Trin.

VII. EURASIAN AND AFRICAN—*Sisymbrium irio* Linn., *Medicago polymorpha* Linn. (*M. denticulata* Willd.), *Vicia sativa* Linn., *V. hirsuta* Gray, *Lathyrus sativus* Linn., *Sonchus oleraceus* Linn., and *Sorghum halepense* Pers.

VIII. TEMPERATE REGION—*Stellaria media* Cyr., *Veronica anagallis-aquatica* Linn., *Convolvulus arvensis* Linn., *Polygonum hydropiper* Linn., *Potamogeton crispus* Linn., *P. pectinatus* Linn., and *P. perfoliatus* Linn.

IX. PALEOTROPICAL—*Cleome viscosa* Linn., *Abelmoschus ficulneus* Wt. et Arn. (*Hibiscus ficulneus* Linn.), *A. moschatus* Medic. (*Hibiscus abelmoschus* Linn.), *Hibiscus panduraeformis* Burm. f., *H. vitifolius* Linn., *Corchorus trilocularis* Linn., *C. fascicularis* Linn., *Indigofera linifolia* Heyne, *I. cordifolia* Heyne, *I. hirsuta* Linn., *Ludwigia parviflora* Roxb., *Glinus oppositifolius* DC. (*Mollugo oppositifolia* Linn.), *Borreria stricta* Sch., *B. hispida* Sch.,

Canscora diffusa R. Br., *Heliotropium ovalifolium* Forsk., *Ipomoea eriocarpa* R. Br., *I. aquatica* Forsk., *I. pestigridis* Linn., *Physalis minima* Linn., *Datura innoxia* Mill., *Solanum xanthocarpum* Sch. et Wendl., *Achyranthes aspera* var. *porphyristachya* Hook. f., *Polygonum barbatum* Linn., *Acalypha ciliata* Forsk., *Sebastiania chamaelea* Muell.-Arg., *Commelina forskalaei* Vahl, *Cyperus pumilus* Linn., *C. alopecuroides* Rottb., *C. triceps* Endl., *C. difformis* Linn., *C. iria* Linn., *C. flavidus* Retz., *Saccharum spontaneum* Linn., *Eragrostis tremula* Hochst., and *Ischaemum laxum* R. Br.

X. PANTROPICAL—*Gynandropsis gynandra* Briq., *Polycarpaea corymbosa* Lam., *Sida cordifolia* Linn., *S. spinosa* Linn., *Corchorus olitorius* Linn., *C. tridens* Linn., *C. capsularis* Linn., *Triumfetta bartramia* Linn., *Tribulus terrestris* Linn., *Biophytum sensitivum* DC., *Cardiospermum halicacabum* Linn., *Sesbania bispinosa* F. et R., *Tephrosia purpurea* Pers., *Zornia diphylla* Pers., *Desmodium triflorum* DC., *Abrus precatorius* Linn., *Crotalaria verrucosa* Linn., *Teramnus labialis* Spr., *Mucuna prurita* Hook., *Rhynchosia minima* DC., *Cassia tora* Linn., *C. occidentalis* Linn., *C. pumila* Lam., *Oldenlandia corymbosa* Linn., *Vernonia cinerea* Less., *Ageratum conyzoides* Linn., *Eclipta prostrata* Linn., *Blainvillea latifolia* DC., *Elephantopus scaber* Linn., *Ipomoea pentaphylla* Jacq., *Cressa cretica* Linn., *Boerhaavia diffusa* Linn., *Celosia argentea* Linn., *Amaranthus spinosus* Linn., *A. gracilis* Desf. (*A. viridis* Hook. f., non Linn.), *Euphorbia hirta* Linn., *Cyperus pygmaeus* Rottb., *C. compressus* Linn., *Fimbristylis miliacea* Vahl, *Dactyloctenium aegyptium* Beauv., *Hackelochloa granularis* Kuntze, and *Rottboellia exaltata* Linn. f.

XI. COSMOPOLITAN—*Coronopus didymus* Sm. (*Senebiera pinnatifida* DC.), *Portulaca oleracea* Linn., *Oxalis corniculata* Linn., *Bacopa monnieri* Penn., *Chenopodium album* Linn., *C. murale* Linn., *Ceratophyllum demersum* Linn., *Vallisneria spiralis* Linn., *Cyperus rotundus* Linn., *Fimbristylis diphylla* Vahl, *Setaria verticillata* Beauv., *Digitaria setigera* Roth, and *Cynodon dactylon* Pers.

(3) EXOTICS AND ESCAPES FROM CULTIVATION—A large number of plants, originally under cultivation in gardens and fields, are found as escapes and are so thoroughly established by natural agencies like wind, water, and animals that they now form a part of the Indian flora, distinguishable from the indigenous flora

only by their known history. Typical examples are: *Lantana camara* var. *aculeata* Mold., *Ipomoea angulata* Lam. (*I. coccinea* Clarke), *Ageratum conyzoides* Linn., *Eupatorium glandulosum* H.B. et K., *Helianthus annuus* Linn., *Tithonia tagetiflora* Desf., *T. diversifolia* A. Gray, *Barleria cristata* Linn., *Adhatoda vasica* Nees, *Clitoria ternatea* Linn., *Euphorbia geniculata* Orteg., *Jatropha gossypifolia* Linn., *Pedilanthus tithymaloides* Poir., *Eichhornia crassipes* Solms., *Plumbago zeylanica* Linn., *Oenothera rosea* Soland., *Peperomia pellucida* H.B. et K., *Allamanda cathartica* Linn., *Cryptostegia grandiflora* R. Br., *Agave angustifolia* Haw., *Opuntia cochinelifera* Mill., *O. monacantha* Haw., *O. elatior* Mill., *O. dillenii* Haw., and *Dioscorea alata* Linn. (Burkill, 1911 ; Burkill & Prain, 1936 ; Raizada, 1935, 1936, 1950 ; Bor & Raizada, 1954).

Several exotic trees, shrubs and grasses have been introduced and naturalized in connection with the soil conservation and afforestation of dry and desert areas of India. The mesquite (*Prosopis juliflora* DC., and *P. glandulosa* Torr.) of the arid regions of Mexico and Central America, is a familiar example. The earliest record of its cultivation in India dates as far back as 1877 when the first consignment of seeds was received from Kew, England. It was successfully grown in Jodhpur State (Rajasthan) as early as 1913 and seeds were supplied by the State Forest Department to the adjoining states and to the Ajmer Forest Department. At present, five forms of this plant, viz., Argentine form, Arid form, Mexican form, Peruvian form and Australian form, are reported to have naturalized in Punjab, Bombay State, Rajasthan, Madhya Pradesh and Madras State (Raizada & Chatterji, 1954). Among the successful exotic species, mention may be made of *Acacia decurrens* Willd., *A. mollissima* Willd., *Pithecellobium dulce* Benth. (*Inga dulcis* Willd.), *Parkinsonia aculeata* Linn., *Agave americana* Linn., *Ailanthus altissima* Swingle (*A. glandulosa* Desf.), *Anacardium occidentale* Linn., *Casuarina equisetifolia* Linn., *Eucalyptus citriodora* Hook., *E. globulus* Labill., *E. camaldulensis* Dehnh., *Pennisetum purpureum* Schum., *Spinifex hirsutus* Labill., *Brachiaria mutica* Stapf, and *Chloris gayana* Kunth (Parker, 1925 ; Blatter & McCann, 1935 ; Badhwar *et al.*, 1948 ; Mathauda, 1959).

(4) Species of Limited Distribution in India and Adjoining Regions:

I. Indo-Malayan—The invasion of the sea-shore or maritime plants of the Indian coast was by a different route than the terrestrial ones. A large number of these plants (e.g., from the Lauraceae, Guttiferae) were evolved in the coral islands of Polynesia and Malaysia, and their seeds drifted along the south coast of Asia settling on the eastern shores

of India, rarely travelling up to the western coasts. Typical examples are: *Mammea, Samadera, Xylocarpus granatum* Koen., *Colubrina asiatica* Brongn., *Desmodium umbellatum* DC., *Derris uliginosa* Benth., *Afzelia bijuga* A. Gray, *Rhizophora conjugata* Linn., *Bruguiera eriopetala* W. et A., *Pemphis, Scyphiphora, Guettarda, Wedelia biflora* DC., *Ochrosia, Tournefortia argentea* Linn. f., *Avicennia officinalis* Linn., *Cassytha, Hernandia, Flagellaria, Remirea maritima* Aubl., *Spinifex squarrosus* Linn., etc. (Ridley, 1942).

II. NORTH-EAST AFRICAN OR WEST ASIAN—After the closing of the east end of the Mediterranean probably considerably later than the Miocene Age and the desert formation of Arabia and Baluchistan, much of the west coast of India became xerophytic and sub-desert. A desert flora hailing from Africa via Arabia, and the western parts of South Asia, invaded Rajasthan, Sindh, Bombay and as far south as Kerala. The characteristic ones are: *Salvadora, Azima, Dodonaea, Acacia, Heliotropium, Indigofera, Farsetia, Malcolmia,* many Crassulaceae, Zygophyllaceae, Capparidaceae, Cruciferae and grasses (Ridley, 1942).

III. INDO-CHINESE—Lately, mainly through the efforts of Janaki Ammal (1954), it has been established that North-East India and South-West China are another source of plants for India. The extreme Eastern parts of the Mishmi Hills in Assam and the adjacent portions of China are centres of distribution for many of our Asian plants. Of Asian Buddleias, the largest number of species with the greatest of diversity is found in the Chinese provinces of Yunnan and Szechuan (Janaki Ammal, 1954). It is regarded that the family Rosaceae as represented in India has two centres of origin, West China and West Asia. Plants have migrated from both these centres into India by the eastern and western routes respectively. Some of these plants common to India and China are as follows: *Stranvaesia glaucescens* Lindl., *Pourthioea arguta* Dcne., *Potentilla fragarioides* Linn., *Spiraea callosa* Thunb., *Verbascum chinense* Sant. (*Celsia coromandeliana* Vahl), *Torenia vagans* Roxb., *Vandellia mollis* Benth., *Ilysanthes hyssopioides* Benth., *Bonnaya tenuifolia* Spreng., *Craniotome versicolor* Reichb., *Anisochilus pallidus* Wall., *Pogostemon glaber* Benth., *Mosla ocymoides* Buch.-Ham. ex Benth. etc.

Summary

This paper is a contribution to our knowledge of naturalized flora of India. It deals with such exotic species which following their immigration or introduction during the historic or recent years, have by now become more or less permanent denizens of this country. Their possible routes of immigration, approximate dates of arrival and means of distribution have been discussed. The main factors influencing naturalization and distribution of plant aliens in India are: (1) deforestation, (2) pasturage methods, (3) shifting cultivation, (4) method of harvesting, (5) sale and introduction of impure seeds, (6) construction of roads and railway lines, and (7) continual sowing of impure seeds on cultivated and uncultivated lands.

The statistical analysis reveals that *ca* 40 per cent of the Indian flowering plants are foreign and now naturalized in different parts of the country. These plants could be sorted as: (1) pluri-regional species or "wides", (2) weeds of cultivation and other introduced weeds, (3) exotics and escapes from cultivation, and (4) species of limited distribution in India and adjoining regions. It is felt that adequate check or control over the introduction and spread of noxious weeds and pests is urgently needed.

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CONTACTS BETWEEN EMBRYOLOGY, PHYSIOLOGY AND GENETICS

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Ten years ago, when speaking to the Botany Section of the Indian Science Congress at Poona, I had chosen the above as the title of my Presidential Address (Maheshwari, 1950a). During this decade more work has been done on this topic than ever before and a fresh review seems to be appropriate. It is perhaps particularly suited to this occasion when we have a distinguished audience of botanists representing all the three fields.

Amici (1824, 1830) made a discovery of great importance when he found pollen grains bursting on the stigma and the pollen tubes passing down into the ovary, one tube for each ovule. Schleiden (1837) confirmed this in many other plants but imagined that the tip of the tube itself became converted into the embryo. Hofmeister (1849) demonstrated the existence of a germinal vesicle in the embryo sac and believed that a fluid discharged by the pollen tube activated it to grow into an embryo. Strasburger (1884) studied the nuclei in the pollen tube and found that it is the fusion of the egg and the sperm which is responsible for the origin of the new embryo. Nawaschin (1898) completed our knowledge of fertilization by showing that while one of the male nuclei fuses with the egg, the other joins with the two polar nuclei to form a 3n structure, the endosperm, which is a nurse tissue for the embryo. The endosperm does not normally develop without triple fusion and the maturation of the embryo is arrested if there is a disfunction of the endosperm.

Two years after the publication of Nawaschin's paper there came the rediscovery of Mendel's laws and an unprecedented activity in the hybridization of different varieties, species and genera with a view to produce newer and more useful types. Earlier, breeders could merely put the pollen upon the stigma and pray for results in the ovary, but now the stage was set for a careful study of the causes leading to the failure of certain crosses and the ways of remedying the difficulties as far as possible.

DIFFICULTIES OF THE BREEDER

The chief difficulties which the breeder has to contend with are: (*a*) loss of viability by pollen during storage, (*b*) failure of pollen grains to germinate, (*c*) slow growth of the pollen tubes so that they do not reach the ovules in time, (*d*) a disfunction of the endosperm, (*e*) hyperplasia of the endothelium, and (*f*) degeneration of the embryo.

Lewis (1949) writes that in *Brassica oleracea, Raphanus sativus* and *Secale cereale*[1] incompatible pollen tubes hardly penetrate even the stigma. In *Nicotiana sanderae, Petunia violacea, Nemesia strumosa* and *Beta vulgaris* the tubes grow to varying lengths but are usually unable to reach the ovary.

In certain cases of interspecific hybridization like those between *Brassica oleracea* and *B. rapa* it is the endosperm which breaks down due to mitotic irregularities (Håkansson, 1956). Its growth is arrested and it is unable to penetrate into and draw upon the underlying tissue which remains more or less intact. This slows down and interrupts embryo development. In incompatible crosses in *Lilium* and *Hyacinthus,* Brock (1954b, 1955) has called attention to the formation of chromosome bridges and other abnormalities during the mitoses in the endosperm resulting in its total degeneration.

In the cross *Hordeum jubatum* × *Secale cereale* (Brink *et al.,* 1944 ; Cooper & Brink, 1944) not only does the endosperm develop abnormally but the antipodal cells also do not behave in the normal manner. In grasses the antipodal cells multiply considerably, their nuclei enlarge and they probably aid the nutrition of the endosperm and embryo in early stages. Their disfunction results in a disturbance in the nutritive relations of the embryo sac and embryo.

In some solanaceous crosses like those of *Datura* and *Lycopersicon* the trouble begins with the failure of the endosperm but is followed by a hyperplasia of the endothelial cells resulting in a filling of the embryo sac cavity (*see* Avery *et al.,* 1959). On the other hand, in certain interspecific crosses in *Gossypium* the hybrid embryo itself produces some substances which cause cessation of growth of the endosperm. Or, possibly both embryo and endosperm compete for some essential substance which promotes growth (Weaver, 1957).

Germination and Growth of Pollen Tube

With the modern advances in our knowledge of photoperiodism, vernalization, and auxin effects, we are now well on our way to control the flowering behaviour of a plant. It is also possible to store the pollen for long periods by using low temperatures and a relative humity ranging from 30 to 50 per cent (*see* Johri & Vasil, 1961).

Several workers have observed that at least in artificial media a dense sowing of the pollen grains results in better germination than if only a few grains are present (Holubinsky, 1945 ; Visser, 1955). It is not unlikely that what happens in vitro also occurs in vivo. Experiments with

[1] It is to be noted that while diploid rye is self-incompatible, the tetraploid is fully compatible. The genetics of this has been explained by several authors (*see* Lundquist, 1956).

apple and pear have shown that even mixtures, containing 98 or 99 per cent dead pollen and only 1 or 2 per cent living pollen, give a fairly good fruit set provided a reasonable quantity is used for pollination (*see* Visser, 1955). The Arabs in ancient times did not throw away old date pollen but mixed it with new stock and then sprinkled it liberally (*see* Stout, 1924).

In mixtures of pollen grains of different species there may either be a mutually promoting or inhibiting effect, or none at all. Russian botanists have specially reported on the beneficial effects of mixed pollinations. This must obviously be due to the diffusion of some chemicals which have to reach a certain concentration before germination can take place. Trace elements like boron are also known to have a significant effect on the growth of pollen tubes. Ever since Schmucker's (1935) discovery of its role in the germination of the pollen of *Nymphaea,* this element (in the form of boric acid or borax) has been widely used as an aid in the artificial germination of pollen, and Batjer & Thompson (1949) employed boric acid sprays to augment the fruit set in some trees. More recently O'Kelley (1957) has shown that pollen is almost always deficient in boron so that the feeding of boron to the plant itself or to the pollen improves germination and tube length.

In artificial cultures Smith (1942) and Addicott (1943) obtained stimulation of both pollen germination and tube growth by the addition of hormones and vitamins to the media. Resnik (1956) has found that the germination of citrus pollen is promoted by the addition of 2,4-D, thiamine, boric acid, and indole-butyric acid ; and Raghavan & Baruah (1956) state that in *Areca catechu* the length of the pollen tubes is twice or thrice that of the controls when the culture medium is supplemented with IBA.

Recently gibberellic acid (Kato, 1955 ; Chandler, 1957) and kinetin (Konar, 1958) have been found to have a marked effect on the growth of the pollen tube in artificial media. Minute doses of antibiotics like penicillin and streptomycin may also improve germination to some extent (Pulvertaft, 1946 ; Vasil, 1960).

On the stigma the conditions are different because this is a living tissue and has its own secretions which may promote or inhibit germination and tube growth (Linskens, 1955). Many plants are self-sterile, i.e. pollen grains do not germinate on own stigma although they germinate readily on the stigma of a related variety.

Pearson (1929) was able to overcome such self-sterility in cabbage by opening the buds and pollinating from 1 to 5 days before the flowers would normally open. Yasuda (1930) and Eyster (1941) did the same in *Petunia.* When pollination is carried out in early bud stage, the pollen tube has more time to grow down the style and reach the ovules before the flowers fall off. It is also possible that at this stage the inhibiting

substance is either not produced or produced only in insufficient concentration. On the other hand, in some plants it is late pollination which serves the same purpose although such a condition is less frequent.

However, normally the difficulty lies not so much with the germination of the pollen grains but with the rate of growth of the pollen tube which is too slow to enable fertilization to take place in time. To circumvent this various methods may be employed, both physical and chemical. These will be considered under the following heads: (*a*) temperature effect, (*b*) splicing and grafting of style, (*c*) intraovarial pollination and (*d*) role of chemicals in stimulating the growth of the pollen tube.

Temperature Effect

The germination of pollen grains and the growth of the pollen tube barely occur at 5°C but take place freely above 10°C. After this and up to about 30°C the rate of tube growth increases in accordance with Vant Hoff's law. However, this is not true of incompatible pollinations in which the tube actually grows slower than before. This suggests that incompatibility is probably due to a chemical reaction similar to an antigen-antibody reaction in animals and that its speed, like that of all chemical reactions, is increased by a rise in temperature[1] (Lewis, 1955). In such cases the relation between the pollen tube and the style is like that of an intercellular parasite and the host.

Linskens (1954) further found that during the course of the inhibition process the oxygen consumption of self-pollinated styles is about 10 per cent more than that of the cross-pollinated styles. Haurowitz (1953) suggests that immunity reactions are endothermic processes so that the respiration of pollinated styles is not merely a total of that of the style and the pollen tube but greater than that.

Splicing and Grafting of Style

When two species are crossed, one having flowers with short styles and the other with long styles, the cross—short styled ♀ × long styled ♂—may take place more easily than that in the reverse direction. Kostoff & Prokofieva (1935) write: "The longer a style a species has, the greater the growth potency its pollen tubes have." Thus, the pollen tubes of *Nicotiana rustica* having a 10 mm long style are unable to bring about fertilization of the pistils of *N. paniculata* which have a 23 mm long style, although the reciprocal cross is quite easy. Similar observations

[1] El Murabaa (1957) reports, however, that in self-incompatible clones of radish the fruit set and total seed yield were higher at 26°C than at 17°C. Here high temperature seems to weaken the incompatibility reaction between the pollen and the style.

have been made in *Datura* (Avery *et al.*, 1959) and some other plants. To give an example, when *D. stramonium* is pollinated by D. *meteloides* fertilization occurs normally. However, when *D. stramonium* is used as the male parent, germination is very poor and the pollen tubes grow so slowly in the style of *D. meteloides* that the cross is unsuccessful.

The simplest method of getting over such a difficulty is to amputate the style and reduce it to a suitable length. In a cross between *Zea* and *Tripsacum*, Manglesdorf & Reeves (1931) shortened the style of the former to a length suitable for the pollen tubes of *Tripsacum* and thereby obtained intergeneric hybrids. However, since the cut end of the style is not always as suitable for pollen germination as the stigma, sometimes it is desirable to use a different method in which the middle portion of the style is removed and the upper and lower portions are joined together and held in place by means of a closely fitting grass straw (Buchholz *et al.*, 1932). Although there is no union of the tissues, the pollen tubes pass down the joint and are able to reach the ovules. In some crosses, between species having long and short styles, it has been possible to obtain fertilization by cutting away a large portion of the long style and replacing on the stump the tip portion containing the pollen tubes from the short-styled male parent. In Blakeslee's laboratory crosses were made by this method between *Datura discolor* and *D. quercifolia*, and *D. innoxia* and *D. ferox* (*see* Avery *et al.*, 1959).

Yasuda (1931) went one step further and attempted an actual graft of the style upon the ovary in *Petunia violacea*. He cut off the style of one flower and glued it with gelatin to the ovary of another flower whose style had been amputated. In order to prevent the falling apart of the grafted style, this was supported against an iron wire, the support and the style being tied together with a spider's thread. When the operation was successful, the grafted style grew normally and attained its usual size. On the other hand, if the tissues failed to unite, the style turned brown and withered away.

A grafting of the style of the species used as the paternal parent on to the ovary of the maternal parent certainly seems to be a promising method of combating incompatibility factors between two species or varieties. However, it requires a great deal of manipulative skill and has apparently never been successfully attempted. It seems to be impracticable with plants having thin styles, but may be tried when the styles are reasonably stout.

Swaminathan (1955) used a different method to achieve the cross between *Solanum pinnatisectum* and *S. bulbocastanum*, and between *S. pinnatisectum* and *S. lanciforme*. He removed the stigma along with a small portion of the style from the flowers of *S. pinnatisectum* and then applied a drop of an agar-sucrose-gelatin medium to the decapitated stump. Pollen was sown on this medium and the artificial stigma covered with

a piece of moist cotton wool. The same technique was later employed by Swaminathan & Radhakrishna Murty (1957) for two interspecific crosses in tobacco—*N. tabacum* × *N. rustica* and *N. tabacum* × *N. debneyi*. Under ordinary conditions these crosses fail because of the longer style of *tabacum* in comparison with the styles of *rustica* and *debneyi*. When much of the style of the *tabacum* parent was cut away and pollen was applied to an agar-sucrose medium smeared on the stump, fertilization was successful. Davies (1957) obtained several hybrids from the cross *Lathyrus odoratus* × *L. hirsutus* by cutting away the entire style and pollinating the cut stump. He also successfully grafted the styles of *L. hirsutus* on the ovaries of *L. odoratus*.

In his extensive studies on self-incompatibility in *Oenothera organensis* Emerson (1940) cut the styles about 50 mm below the stigma. After this he reunited the portion having the stigma to the lower portion of the style of the same or a different flower. The graft union was contained inside a capillary tube wedged into the hypanthium. Pollen tubes growing through a compatible scion frequently continued their growth through the graft union and into a stock portion of the style provided this was also compatible. However, when the stock was incompatible, the pollen tubes were arrested. Emerson concluded that here the incompatibility reaction could take place at any level in the stigma or style. Hecht (1960) used a different technique. In his experiments styles with attached stigmas were removed by cutting through the base of the hypanthium. Some of these were then pollinated with compatible and others with incompatible pollen, after which all were placed in large petri dishes on moistened filter paper. After a 15-hour stay at 27°C the styles were removed, squashed between glass slides and stained in iodine. It was found that while compatible pollen grew 100 mm down the styles incompatible pollen either did not germinate or failed to penetrate even the lobes of the stigmas. In another set of experiments Hecht took stylar scions and united them with stocks of different compatibility types. The graft union was held together in a drop of 10 per cent gelatin containing 6 per cent lactose ; at the same time a square of moistened lens paper was used as a splint to maintain the two halves of the graft in contact. It was interesting to find that in such grafts the pollen tubes grew for an appreciable length through the graft unions into the incompatible styles suggesting three possible mechanisms: (1) the incompatibility reaction is much stronger in the stigma than in the style ; (2) in the growth of the pollen tubes through compatible scions some stimulus is provided which at least partially counteracts the inhibitory effect of the incompatible stock ; (3) the inhibitory substance of the stock diffuses out into the gelatin-lactose mixture. Hecht's technique is so simple that it deserves a thorough trial in physiological studies on incompatibility.

Intraovarial Pollination

Another possible device for overcoming incompatibilities is to inject the pollen grains directly into the ovary. The possibilities of this technique were first indicated by the experiments of Dahlgren (1926) on *Codonopsis ovata,* Capelletti (1937) on *Digitalis purpurea,* and Bosio (1940) on some species of *Paeonia* and *Helleborus.* In *Paeonia* some viable seeds were also obtained. More recently Kanta (1960) has described the technique and the results obtained by her with *Papaver rhoeas.* She prepared a pollen suspension in 2 cc of sterile, double distilled water with 0.01 per cent boric acid, each drop containing nearly 100 pollen grains. The flowers were castrated and the surface of the ovary wiped with cotton soaked in rectified spirit. A puncture was made on one side of the ovary to allow the air to escape and another on the opposite side through which the pollen suspension was injected till the ovarian cavity was filled with it. After the operation the punctures were sealed with petroleum jelly. The injected ovaries grew normally and finally produced fruits with viable seeds containing fully developed embryos. Similar results have been obtained by Maheshwari & Kanta (1961) with *Papaver* and *Eschscholzia.*

This technique may prove useful in overcoming such incompatibilities where the zone of inhibition lies in the style.

Roles of Chemicals in Stimulating the Growth of the Pollen Tube

Since physical methods are not always successful, we may now consider what role chemical treatments may have on the rate of growth of the pollen tube. A reference has already been made to trace elements, specially boron. More important still are certain growth hormones and a few examples of their action are given below.

Several varieties of *Petunia* are self-incompatible but by spraying a solution of 10 ppm naphthaleneacetamide on the flowers immediately before or shortly after self-pollination Eyster (1941) was able to obtain fertilization. He reported similar success with some self-sterile races of *Tagetes erecta, Brassica oleracea* and *Trifolium pratense.* In *Lilium* also many varieties fail to set seed when own pollen is used although they do so readily when pollen of another variety is applied. Emsweller & Stuart (1948) applied a 1 per cent lanolin paste of naphthaleneacetamide to the wound at the base of the ovary formed by breaking a petal at the point of its attachment to the flower. All the hormone-treated flowers set fruit but none of the others. In the U.S.S.R. Britikov & Petropavlovskaia (1954), who did some self-pollinations in winter rye and beet, found that the treatment of the flowers with small amounts of vitamins, enzymes

and auxins considerably increased the probability of fertilization as well as the weight of the seed.

Wester & Marth (1949) obtained an increased yield of seeds by auxin treatments in some crosses of *Cucumis melo* made to evolve disease-resistant strains. Similarly Evans & Denward (1955) were also able to carry out some successful interspecific hybridizations in *Trifolium* with the use of NAA. Frimmel (1956) has reported that in potato the treatment of the stigmas with hormone extracts prepared from the pollen of *Lathyrus* sp. or with an aqueous solution obtained by crushing the anthers of potato was effective in remedying the low seed set observed in certain intravarietal crosses. This is attributed to the deficiency of hormone in the pollen of the male parent.

Crane & Marks (1952) and Brock (1954a) produced hybrids between pear and apple through the use of hormones. Immediately after pollination the pear ovary was painted with a 40 ppm aqueous solution of β-naphthoxyacetic acid, care being taken that the hormone solution did not come in contact with the stigma or the pollen. It is to be noted that in the compatible pear-pear crosses fertilization occurred in 5-7 days after pollination. In the hormone induced pear-apple crosses the pear pollen tubes took 10-12 days to reach the embryo sacs of apple. When no hormone was applied the pear pollen tubes did not reach the embryo sacs of apple even 14 days after pollination.

In the blueberry (*Vaccinium*) heteroploid crosses involving parents with different chromosome numbers are usually sterile. With the use of naphthaleneacetamide, Darrow *et al.* (1954) and Darrow (1956) succeeded in obtaining seeds in crosses between hexaploid and diploid species.

Using a 0.01 per cent lanolin paste of parachlorophenoxyacetic acid Rick (1951) crossed the tomato with a wild species of *Solanum* native to Southern Peru. Similarly, Dionne (1958) has recorded that when a cross was made between a diploid hybrid (*Solanum chacoense* × S. *rybinii*) and *S. stoloniferum*, the untreated ovaries gave only a few seedless berries of a very small size while from ovaries treated 24 hours after pollination with a drop of 3-6 ppm of 2,4-D several hundred fruits were obtained. These produced thousands of viable seeds.

In India and Pakistan, for several years, botanists have attempted to cross the two cultivated species of jute, *Corchorus capsularis* and *C. olitorius*. Ganesan *et al.* (1957) showed that although fertilization occurs, viable seeds are not formed due to an early degeneration of the embryo and endosperm. Recently, Islam & Rashid (1960) were able to remedy this by wrapping the pedicels of the flowers of the female parent (*C. olitorius*) with cotton wool soaked in 300 ppm of IAA. By this method they obtained a few seeds of which three grew to mature plants. The authors state: "The factor which led to the degeneration of embryo and endosperm at an early stage may have been counteracted by 3-indole

acetic acid. The inhibitory effect being removed, the embryo and endosperm developed fully."

Mention may finally be made of a rather unusual method of growing the embryo of one parent upon the endosperm of another and using the seedlings obtained in this way for the cross. Hall (1954, 1956) increased the crossability of a variety of spring wheat with a spring rye by raising the wheat plants from embryos which had been excised and cultured on the endosperms of either rye or a Chinese spring wheat which normally has a high crossability with rye.[1] On the other hand, Szteyn (1959) reports that repeated reciprocal grafting of *Lycopersicum esculentum* on *L. glandulosum* and *vice versa* did not increase the number of successful matings in any way. Nor was he able to confirm some Russian claims of changing the genetic characters of the scion under the influence of the stock.

Although it is not claimed that chemical agents will overcome all incompatibilities, their use may sometimes be of distinct value. This is perhaps due to four factors: (1) auxin stimulates ovary growth and thus provides the supporting case or receptacle in which the ovules can continue their growth ; (2) auxin delays the abscission of the style or the flower and thus gives more time for the pollen tubes to continue their growth ; (3) possibly it has a direct stimulating effect on the rate of growth of the pollen tube ; and (4) it may counteract the effect of inhibitory substances in the style and ovary which arrest the growth of the pollen tubes.

Embryo Culture

In some cases of self-sterility the pollen tube enters the embryo sac, but the male and female nuclei may nevertheless fail to fuse. Thus, in a variety of the black currant (*Ribes nigrum* L.) the pollen tubes reach and enter the ovules but the male and female nuclei do not fuse and hence no fruits are formed (Ledeboer & Rietsema, 1940). *Theobroma cacao* is another good example of the same kind (Cope, 1958). Since no remedy is known for this situation, it need not be discussed further. More frequently, however, syngamy occurs but the fusion nucleus is in such a state of imbalance that the young embryo often aborts without undergoing its full development. Brock (1954b) finds this to be true of some self-sterile species of *Lilium*. Its occurrence is more common in cases of interspecific and intergeneric hybridization and the abortion of the hybrid embryo is attributed to the harmful influence of the maternal tissues. While Hannig (1904) and Dietrich (1924) successfully excised the embryos of certain plants and reared them in artificial media, it was Laibach (1925, 1929) who foresaw the utility of this method for plant

[1] In this connection see also Pissarev & Vinogradova (1944).

breeders and now it has become a common practice to employ the embryo culture technique before declaring any cross to be inviable.

Microscopic examination has shown that embryo abortion is correlated with a disturbed relationship between the embryo, the endosperm and the maternal tissues lying outside the endosperm. This usually takes the form of a hyperplasia of the nucellus or the endothelium (when the nucellus has already disorganized) and an atrophy of the endosperm—the so-called somatoplastic sterility of Cooper & Brink (1940).

While Laibach used very simple media, it soon became clear that not only do younger embryos need very careful handling so as to avoid damage and infection but more complex media are required also for their growth and maturation. There were soon reports of the beneficial effects of malt and yeast extracts and of coconut milk all of which were believed to contain an 'embryo factor'. Kent & Brink (1947) reported the presence of the 'embryo factor' in casein hydrolysate and pointed out its usefulness in checking precocious germination of immature embryos under artificial conditions. In Steward's laboratory at Cornell University the liquid contents of the immature fruits of *Aesculus woerlitzensis* have been used with advantage for in vitro cultures of plant issues. Among other substances that have been tried from time to time are pear extract, tomato juice, and decoctions of date, banana, almond and the endosperms of maize and barley. It is to be noted that these sources of growth-promoting substances are not always mutually interchangeable and embryos of different species are often lacking in different growth factors.

It is of interest that sometimes embryos grow better on foreign endosperms rather than their own. This was recorded many years ago by Stingl (1907) who showed that embryos of *Hordeum* grew better when transplanted to the endosperm of *Triticum*. Gamara (1943) noted a strong influence of the endosperm upon the embryos so that the plants raised from them showed a different texture and external morphology and even early maturity. Some Russian investigators (Glutschenko, 1950) have even claimed heritable changes of a far-reaching character but such reports remain unconfirmed (Szteyn, 1959). Mention must also be made of many attempts to grow the endosperm itself. In *Zamia floridana* it was cultured for 10-12 months and finally gave rise to adventitious buds which produced both roots and shoots, but so far this has not been possible with the endosperm of any angiosperm (LaRue, 1954).

Working on the embryos of *Datura*, Van Overbeek *et al.* (1942) prepared an extract from coconut milk which showed an activity of as much as 170 times of the original sap. Mauney *et al.* (1952) used coconut meat instead of milk and isolated from it an organic compound which was 4,350 times more active than the original material. Cutter & Wilson (1954) found that only the milk from young fruits was stimulatory for the

dormant embryos of coconuts, whereas the milk and soild endosperm from older fruits had an inhibitory effect on embryo growth.

According to Steward & Schantz (1959) the favourable response of coconut milk results from the interaction of several substances. These fall into three principal categories: (*a*) free amino acids (these can be replaced by casein hydrolysate, or a synthetic mixture, or even urea) ; (*b*) a "neutral" fraction which is not absorbed by ionic resins or by charcoal and is unable to induce any growth by itself but has a highly synergistic effect with the active fraction ; (*c*) an active fraction composed of growth-promoting substances which are effective in very low concentrations and show strong ultraviolet absorption.

With the recent isolation of kinetin by Miller, Skoog and others (1956) and its property of promoting cell division it was suspected that this might be the long sought for 'embryo factor'. However, in his work on cotton embryos Mauney (1958) found kinetin to be detrimental to normal embryonic growth.

It is important to know what role growth-regulators play in the development of the embryo in a normal compatible cross. Sanders (1950) was unable to detect any marked effect of IAA ; but Rappaport *et al.* (1950) found a slight improvement in the growth of embryos of *Datura stramonium* by the addition of 0.05-0.1 ppm NAA. In *Capsella* Rijven (1952) found that 0.001 ppm IAA stimulated growth while concentrations above 1.0 ppm inhibited it.

Dure & Jensen (1957) studied the effect of IAA and gibberellic acid on embryos of cotton with average weights of 37 mg and 64 mg which had been partly inserted into an agar medium containing mineral salts and sucrose. The embryos of the first series showed no growth, but gibberellic acid as well as gibberellic acid and IAA used together had a favourable effect on those of the second series ; IAA alone inhibited growth.

In general the effect of auxin is to inhibit the growth of young embryos although the endosperm no doubt contains auxins which have no such damaging effect in vivo. Presumably both auxins and anti-auxins are present and their combination brings about a balanced growth of the embryo to the stage of maturity.

While engaged in the culture of young embryos, Van Overbeek *et al.* (1942) had hoped that the experience gained thereby would eventually enable them to follow the development of the egg in vitro. However, up till now it has not proved feasible to isolate very young embryos and perhaps the nearest one has gone is the rearing of embryos of *Capsella* with 16-32 cells (Castle ; quoted in Naylor, 1952). A somewhat simpler approach to the problem is the culture of whole ovules. This has been done quite successfully in *Papaver somniferum* by N. Maheshwari

(1958) and in certain orchids, namely *Dendrobium nobile, Calanthe veitchii, Phalaenopsis schillereiana* and *Cypripedium insigne,* by Poddubnaja-Arnoldi (1959).

Inspite of the imperfections in our understanding of the nutritive requirements of young embryos this technique has already proved useful in rearing many hybrids from crosses which had hitherto proved unsuccessful. Skirm (1942) successfully cultured 414 embryos from 116 different interspecific crosses of *Prunus,* and 300 embryos from 29 crosses of *Lilium.* Among other examples may be mentioned several interspecific crosses in *Datura* (Avery *et al.,* 1959), *Lycopersicum* (Smith, 1944 ; Chowdhury, 1959), *Gossypium* (Weaver, 1957), *Chrysanthemum* (Kaneko, 1957), *Trifolium* (Keim, 1953) and some intergeneric crosses such as *Hordeum* × *Secale* (Brink *et al.,* 1944), *Datura* × *Brugmansia* (Blakeslee & Satina, 1944), *Triticum* × *Elymus* (Iwanowskaya, 1946) and *Tripsacum* × *Zea* (Farquharson, 1957). The culture of hybrid embryos has indeed become a standard practice in all plant breeding laboratories.

Embryo culture is also useful to the breeder in shortening the life cycle of seeds which have a prolonged period of dormancy. This has been done with success with certain rosaceous fruit trees to obtain seedlings in eight months which normally take twenty months or more to reach this stage (Nickell, 1951). Using this method Randolph & Randolph (1955) shortened the seed-to-flower interval in *Iris* from several years to less than one year. Further, seed physiologists now use the embryo culture technique as a quick means for testing the viability of dormant seeds.

Artificial Induction of Parthenogenesis

Since Loeb's initial success in inducing the parthenogenetic development of the ova of certain animals, numerous methods—physical, chemical and biological—have been tried for plants without any conspicuous success except in some algae (*see* Hiroe & Inoh, 1954).

In plants the first 'natural' haploid was reported in 1922 by Blakeslee *et al.* Since then numerous examples of a similar nature have been brought to light from time to time and it appears that parthenogenesis is much more common in nature in plants than in animals. Although of little value in themselves haploids are of significance in that it is possible to produce from them homozygous diploids in a single step by colchicine treatment of seeds, seedlings, or shoot apices((*see* Morgan & Rappleye, 1950). No satisfactory method has, however, been found of obtaining haploids at will. Among the varied techniques used are the use of foreign or dead pollen or of irradiated pollen. Exposure of the inflorescences to high or low temperatures and delayed pollinations have also been tried, but none of these treatments can be considered to be of general value.

Either the treatment is successful for only a particular species or the percentage of success is too low to be of any practical value.

More promising perhaps would be a method of chemical treatment of the ovary or ovules but the only successful report of this kind is that of Yasuda (1940) who obtained a division of the egg and an enlargement of the antipodal cells in ovaries of *Petunia* injected with a solution of 'Belvitan' (probably IAA) manufactured by "Bayer". Others, who tried injections of ovules with various chemical preparations, have not so far reported any positive results, and even Yasuda obtained only a young proembryo whose capacity for further development remains uncertain.

However, Tschermak-Seysenegg (1949) claimed a large-scale induction of parthenogenetic embryos in a number of plants belonging to 25 different families by the application of such common substances as dead pollen, talcum powder and even dust! This was apparently supported by Raniger (1951). However, other investigators (Jungfer, 1952 ; Wallensick *et al.*, 1952 ; Bandlow, 1953) have cast serious doubts on the validity of the observations of Tschermak-Seysenegg who later admitted that his experiments had been done without bagging ("ohne Schutzung") the flowers.

Chase (1947) pollinated diploid maize plants with pollen containing a dominant genetic marker and looked for plants of the maternal type from the F_1 progeny. A relatively high proportion of these were found to be haploids. About 10 per cent of them later gave rise to homozygous diploid lines by spontaneous doubling of the chromosome complement (Chase, 1952).

A major difficulty in the production of haploids by chemical treatment is that even if the egg can be made to divide without the stimulus of fertilization, the embryo is likely to abort at a young stage unless there is a supporting endosperm to provide the necessary nutrition. In other words, it is not enough to stimulate divisions of the egg ; it is equally necessary to cause the secondary nucleus to divide and form an endosperm.

Since little or no endosperm is formed in orchids, Heslop-Harrison (1957) tried to provoke the development of the egg in unfertilized ovules of species of *Dactylorchis* by the application of NAA. While so far there has been no success, more experiments under different conditions and with other chemicals are necessary before a decisive conclusion can be arrived at.

It must be noted that haploids do not always arise from unfertilized embryo sacs. Even in such embryo sacs, in which normal double fertilization has taken place, an unfertilized synergid sometimes develops side by side with the zygote so as to give rise to haploid-diploid twins. Or, only the secondary nucleus is fertilized and not the egg, i.e. there is single

fertilization instead of the normal double fertilization. The numerous haploids of *Zea mays* obtained by Chase (1952) seem to have arisen in this fashion.

Androgenesis

The origin of a haploid is ordinarily attributed to the parthenogenetic development of the egg and perhaps this is true in the majority of plants. However, a few examples are known of haploids which reproduce the characters of the male parent (*see* Maheshwari, 1950b). Campos & Morgan (1958) have recently recorded an androgenic haploid in *Capsicum frutescens*. This was the result of an attempt to cross two varieties differing in their foliar and leaf characters. Since the F_1 haploid showed the characters of the male parent, it is concluded that only a sperm cell functioned in its development. Davies (1958) has similarly obtained androgenic plants by pollinating autotetraploid *Hordeum bulbosum* with *H. vulgare*.

A method of producing androgenic haploids at will is very much to be desired but none has been found. Possibly the exposure of the female plants to X-rays so as to kill the egg nucleus but not its cytoplasm, followed by pollination with untreated pollen, might be of use but no large scale attempts have yet been made.

Adventive Embryos

While normally the embryo arises from the zygote, there are also formed sometimes the so-called 'adventive' embryos from the adjacent cells of the nucellus or integument. Since no gametic fusion is involved and such embryos originate from purely maternal tissues, they must give rise to seedlings which recapitulate the characters of the maternal parent. Horticulturists have naturally utilized such seedlings on a large scale in the propagation of desirable varieties of *Citrus*. It would be a boon if adventive embryony could be induced in other plants where it does not occur naturally. On the other hand, there are occasions when it is necessary to eliminate the adventive embryos. For example, if a breeder is crossing two varieties, his work would be made difficult if he has to deal with a mixed offspring consisting partly of zygotic and partly of nucellar origin and it would indeed be rendered infructuous if the zygotic embryo is suppressed by nucellar embryos.

Many years ago Haberlandt (1921, 1922) made the observation that in plants showing adventive embryony the proliferation of the embryo-initiating cells is invariably preceded by a degeneration of some of the adjoining nucellar cells. He was led by this to propound the so-called 'necrohormone theory' according to which the stimulus for cell division

and proliferation is supplied by certain substances liberated from the adjacent degenerating cells. Proceeding on this assumption he tried pricking the ovules with a fine needle (Anstichmethode) or gently squeezing the ovary (Quetschmethode) so as to damage the cells slightly but not excessively. In an ovule of *Oenothera* he claimed to have obtained two embryos, one of which he believed to be of nucellar origin. Several workers, namely Němec (1935), Doak (1937), Ivanov (1938) and Beth (1938), attempted to duplicate Haberlandt's results but without any success. Beth, who made the most careful study, concludes that the additional embryo in Haberlandt's experiments must have originated from the accidental fertilization of a twin embryo sac.[1]

Van Overbeek *et al.* (1941) injected a number of substances into the ovaries of *Datura stramonium*. In those treated with 0.1 per cent NAA and IBA tumour-like structures or "pseudo-embryos" were found to arise from the endothelium. Experiments carried out at Delhi with *D. fastuosa* yielded similar results. However, no one has yet succeeded in making the tumours differentiate into embryos.

A very interesting work in this connection is that of Ranga Swamy (1958) who excised the nucelli of *Citrus microcarpa* from pollinated ovules and cultured them on a modified White's medium supplemented with casein hydrolysate. The nucelli first gave rise to proliferating masses of pseudobulbils which on sub-culturing produced normal seedlings. Since this work was done with a species which is known to be naturally polyembryonic, it remains to be seen whether a similar technique can be used to induce adventive embryony in plants in which normally only the zygote develops into an embryo. It is also to be noted that while the Malaysean and Philippine varieties of mango are polyembryonic, most of the Indian varieties produce seeds with a single embryo. Is it possible to induce adventive embryony in these varieties by chemical treatments or by making cultures of the nucelli? Our experiments have so far been only negative but the problem needs to be investigated more thoroughly.

Earlier, Curtis & Nichol (1948) cultured the embryos of *Vanda tricolor* and a *Cymbidium* hybrid and obtained from them a callus-like tissue which was capable of potentially unlimited growth if subcultured. True germination in the sense of the production of a shoot and root was not common but sometimes normal plants were produced with stems, roots and leaves.

Using embryos of *Cuscuta reflexa* Maheshwari & Baldev (1961) have obtained similar callus-like masses and proliferations which are capable of giving rise to new plants. Apart from its academic interest such work is of practical value because of the possibility of producing clones in unlimited numbers. Mention must also be made of Reinert's (1959) work on a strain

[1] It may be noted that twin embryo sacs are common in *Oenothera* (*see* Maheshwari, 1950b).

of callus from *Daucus carota*. For many years this was grown on media containing coconut milk and formed only roots but on transferring it to a suitable synthetic medium it was induced to form bipolar adventive embryos which developed into complete plantlets. Steward *et al.* (1958) have gone still further in raising embryo-like structures and finally mature plants from freely suspended cells of the phloem of carrot roots. The essential stages in this process are: (*a*) the formation within the cultured mass of cells of a spherical sheath of cambium-like cells enclosing a nest of lignified elements ; (*b*) differentiation of the spherical nodule into root and shoot initials, the shoot initial arising at a point diametrically opposite to the root apex so as to produce a simple embryo-like structure ; and (*c*) the growth of the embryo into a plantlet. It must be noted, however, that the freely suspended cells do not give rise to the embryo-like structures except when they are enclosed within the wall of cambium-like cells. Freely exposed cells show only random growth.

Mention must also be made of the work of Haccius (1955) on *Eranthis hiemalis*. In this plant the seeds are shed at a stage when the embryo is still undifferentiated and pear-shaped. In nature approximately 0.3 per cent of the seeds show twin embryos, but when the seeds were treated with 0.1 per cent 2,4-D the frequency of twin embryos rose to 3-8 per cent. The artificially induced twins were invariably fused at the radicular end and are, therefore, considered to be the result of cleavage. If they can be separated and reared to maturity, they should produce seedlings which are indentical with regard to their genetical make-up.

Conclusion

There is a great scope for further research in experimental embryology. The artificial culture of embryos, ovules and ovaries involves techniques similar to those used for culturing other materials like root tips and shoot apices. Here the relationship between the morphologist, physiologist, and geneticist becomes particularly close. For the inactivation of incompatibilities between the pollen tube and the pistil we need a better knowledge of the bio-chemistry of both. A proper understanding of the physiology of reproduction requires much team work. It will be a major landmark in botany when the fertilized egg is removed from the embryo sac and made to grow in an orderly manner to form a normal embryo. The next step will be to grow the unfertilized egg whether in the ovule or outside it to form a parthenogenetic embryo.

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HABITATS, PLANTS AND THEIR INDICATOR VALUE

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The term habitat is very widely used by biologists. McDougall (1927) defined it thus: "by the habitat of a plant or plant association is meant the kind of situation in which the organisms live. It is practically equivalent to what would be meant by the immediate environment. It is the place of abode of the organism together with all the environmental factors that are operative within the abode." But Clements & Shelford (1939) recommended that the concept be restricted to the place of habitation with its physical (abiotic) elements only. According to Udwardy (1959) habitat should refer to the abode of an individual species or other smaller, natural population unit. He also suggested that habitat of a bigger unit of population or biotic community should be known as biotope, a word used by German ecologists as equivalent to habitat. He believes that biotope should never be used to express habitat of individual species or a small population but always for bigger units. Odum (1953) differentiated between the meaning of habitat used for a single species or a population and a bigger unit, the biotic community. In case of the former usage it should include both biotic as well as abiotic environment but in case of its use for latter, only the physical or abiotic complexes of the environment should be taken into consideration. In spite of these differences in details, its general usage to convey the meaning suggested by McDougall remains unchanged despite several pleas. Small differences in location in a particular habitat are known as microhabitat.

Every habitat is a complex in which the habitat factors such as water, gases used directly by plants, nutritive salts, light, temperature etc. are almost inextricably interwoven. Each factor of a habitat influences every other factor, and is in turn affected by it. Nevertheless, some factors are of paramount importance and plant growth can be related to them directly.

There can be three major types of habitats, viz. hydric, mesic, and xeric, with regard to availability of water. Hydric habitat can be marine or freshwater which in turn can be divided into numerous minor divisions based on different properties, both physical and chemical, of water. Likewise, mesic and xeric habitats can be classified into numerous subdivisions. The object at present is not to deal with many of them but to confine to some interesting ones, like man made road side ditches and pools, which remain full of water during rainy season or a little later and subsequently dry up, passing through various stages of water content. Ultimately,

during May and June they are very dry, much below wilting point. These areas thus pass through all the three major stages, hydric, mesic and xeric.

A comparable habitat can be found on the bank of a lake, a bigger and perennial water reservoir. At the banks of the Sagar Lake (Madhya Pradesh, India) the bed is exposed relatively much later, i.e. in March and April and is sufficient to cause a great difference in vegetation. None of the plants found in the former habitat appears here although apparently identical conditions seem to be prevalent. This points to the complex nature of a habitat. A slightly different temperature obtained at the time of drying of water seems to be responsible for this change.

The plants which appear in both the types of habitats can be said to begin their life as hydrophytes, mature as mesophyte and persist for some time after the onset of drought as xerophyte. This particular type of habitat attracted my attention and an ecological investigation of this was, therefore, undertaken. The main features of the habitat and the community developing thereon have been analysed. The soil is mostly alluvial, silty clay varying in its depth. The temperature of the bed during the appearance of the plant community has been noted to range between 10° to 27°C. In all such habitats, where the characteristic community develops, full sunlight is available throughout the daytime. Microflora of the soil is relatively poorer than those of the surrounding areas. The base status of the soil is appreciable. Carbonate content and pore space in soil is quite high.

The plant community has a special character. Its constituent members are mostly prostrate, in many cases appressed to the ground. Near about Sagar 30 species of plants are found growing in such habitats. Of these, 15 are such which are unrepresented in the neighbouring upland areas. They are *Heliotropium supinum, Polygonum plebejum, Chrozophora prostrata, Crypsis aculeata, Gnaphalium pulvinatum, Sphaeranthus indicus, Trigonella occulta, Cochlearia flava, Polanisia chelidoni, Scirpus michelianus, Nasturtium indicum, Glinus lotoides, Gnaphalium luteoalbum, Althaea ludwigii* and *Polypogon monospeliensis*. Judging from their fidelity they can be considered as exclusive species. The next six species are such which occur abundantly here, but are also occasionally found in the neighbouring uplying areas. They are termed as 'selectives' as they are found especially in one community but occasionally met with in others. They are, *Sutera glandulosa, Argemone mexicana, Caesulia axillaris, Potentilla supina, Eclipta erecta* and *Asteracantha* sp. Species of both the categories can be included among characteristic ones. Some other species are such which can be classed as indifferents, i.e. without a pronounced affinity for any community. They are, *Chrozophora rottleri, Volutarella divaricata, Merremia emarginata, Xanthium strumarium, Cynodon dactylon, Euphorbia hypercifolia* and *Phyllanthus* sp. Some-

times *Cassia tora* appears as ephemeral adventies, i.e. only a stranger appearing accidentally and being not able to complete its life cycle.

The communities of depressions are mostly similar but some differences are also discernible which can give a clue to the successional trend. Relatively younger depressions or even older ones, whose top soil is removed for various purposes, show mostly *Argemone mexicana, Polygonum plebejum* and *Heliotropium supinum* while the older ones show preponderance of *Crypsis aculeata* and *Trigonella occulta*. It could not definitely be said whether this is due to differences in microhabitat or due to aggressive nature or some special adaptability of the last two species. One special feature of *Crypsis aculeata* is that its seedlings appear even when there is some free water on the surface of soil. This is quite a special character and is certainly enough to give an early start in life—a feature quite effective in enabling the species to gain supremacy over other associates, which are able to start their life a little later.

A brief reference to an apparently similar habitat, already alluded to, may not be out of place. The Sagar Lake, which is silting at a very rapid rate starts exposing part of its bed towards its margin from the end of March or the beginning of April. The detailed phytosociological study of the community appearing there is yet to be undertaken but a preliminary survey has revealed that the constituent species are entirely different and none seems to be common with those occurring in other depressions.

Habitat and Adaptations in Plants—Every plant is a product of the conditions under which it grows. Under the influence of the habitat quite often there develop features, both morphological and physiological, which are of distinct survival value in that habitat. These features of an organism or its parts, which are of definite value in allowing that organism to exist under the conditions of its habitat, may be called adaptations. These may ensure a degree of success either by allowing the plant to make full use of the amounts of nutrients, water, heat or light available to it or by bestowing a significant amount of protection against some adverse factor, such as extremes of temperature, drought or parasites. By accumulating adaptations, organisms utilise the earth's resources ever more efficiently. It is as a result of this that problems presented by a particular kind of habitat have been resolved by the same type of adaptations in a variety of taxonomically unrelated species. Thus, under the influence of habitat, succulent habit of the stem has arisen independently in the Cactaceae, Euphorbiaceae and Asclepiadaceae. When we devote ourselves to think how these adaptations come about, a serious contrast appears between the older view points and the new ones. The former seem to be dominated by anthropomorphism and teleology and the latter by the principle of general variation and natural selection. Anthropomorphism is the habit of attributing human characteristics to non-human objects.

This is reflected fully in such expressions as "Sal avoids basalt" or a particular species prefers calcium. Teleology is the doctrine that considers adaptation to be purposive as is implied in the statement that the spines are for protection against browsing animals. These views are now considered as almost entirely philosophical and not based upon a careful analysis of certain important facts. One of such facts is the finding of certain structures and functions that appear to be definitely related to one type of environment in an entirely different environment. Examples of such cases can be very well found in xeromorphic *Pinus rigida* var. *serotina* inhabiting hydrophytic ponds and occurrence of succulent *Sedum ternatum* in mesophytic winter deciduous forest. All these point to the fact that the organism and its habitat do not present a closed system of cause and effect.

According to the newer viewpoint, most adaptations are the result of variation and natural selections. Variation may be what is known as environmentally induced (somatic) or genetically fixed. Environmentally induced variation can be met with among plants which are genetically identical but grow in diverse habitats. Plants of *Marsilia* growing in water or in drier substratum show great difference in morphological characters. Similarly *Polygonum amphibium* exhibits very different forms when grown in water and on land. These changes are produced readily and remain in equilibrium with their corresponding habitat indefinitely but no permanent changes are induced and the process of modification remains reversible. These distinct forms, or ecophenes as they are some times called, change their distinctiveness when transplanted into the same habitat. Frequently, these variations are beneficial and may be classed as adaptations but there are instances where these environmentally induced variations are not useful.

Variations may be genetically fixed and, therefore, ordinarily irreversible. It is a decided fact that origin of genetic variations is subject to the laws of chance. If the new character is detrimental in a habitat the organism is less successful than others of its kind and hence the change is not likely to be preserved. On the other hand, if the innovation confers an advantage upon the recipient, it has better-than-average chances of survival. In this way, natural selection operating upon genetic variations tends to produce new forms ever more closely adapted to the habitat which ultimately result in maximum harmony between organism and its habitat. This provides the only satisfactory explanation for the countless instances of ecological similarity among taxonomically diverse species.

Plant Indicators—Every plant is the product of the conditions under which it grows and is thereby a measure of these conditions. Also, practically every species has an optimum habitat in which it exhibits typical indicator value. As expressed earlier, some of the habitat factors are of paramount importance and decide the nature of community which would

develop there. Various component species of a community restricted to a particular habitat have different degrees of fidelity, i.e. the degree with which a species is restricted to a particular kind of community. The exclusive, selective and preferent species are considered to be characteristic species and can have indicator value of a community and habitat. It is believed that a community is a better indicator of some ecological factor, of process or of a future possibility.

Indicators of Ecological Factors—Water Indicators: Water requirements of different plants vary and they indicate water relation of the habitat. In Sagar area *Terminalia glabra* is planted along roads but when it grows in nature it is always found along water courses. From a distance one can make out the course of a water channel by marking these trees. *Eugenia heyneana* is another species indicating the same fact.

Indicators of Solute: The ordinary nutrient salts of the soil rarely leave a distinctive impress upon plants, owing to lack of concentration. When the concentration reaches a point where absorption is interfered with, there appears a definite physiological and structural response to the saline or alkaline conditions. Saline indicators are typical of sea shores the world over, but their most striking development is found in the arid basins of the interior of continents. Halophytes are very well known to indicate saline conditions.

Indicators of Aeration: Some species grow in well aerated soil. *Cassia obtusifolia* grows well only on such soils which are disturbed and there is adequate aeration. So is the case with *Geranium* and *Impatiens*. 'Sal' also grows in well aerated soil.

Indicators of Fires: Certain vegetation forms appear to owe their character or at least their dominance to fire. Certain genera and species are almost universal indicators of its action. The best known examples are *Populus tremuloides, Pteris aquilina, Pinus contorta, Agrostis hiemalis, Epilobium spicatum, Pyronema confluens* and *Bryum argenteum*.

Indicators of Cultivation: In fallow and abandoned fields appear some characteristic plants in different climatic conditions. In the lower terai regions of Himalayas *Leucas aspera* has been found commonly in fallows. *Helianthus annuus* and *H. petiolaris* indicate this condition in southern Texas. *Amsinckia intermedia, Sisymbrium altissimum, Eschscholtzia californica* are such indicators in California.

Indicators of Grazing: In Sagar the heavily grazed lands are dominated by *Andropogon pumilus, Heteropogon contortus* and *Alysicarpus* spp. Throughout the grassland climax of U.S.A. there occur three indicators of over-grazing, which outrank all others in importance. These are *Gutierrezia sarothrae, Aristida purpurea* and *Artemisia frigida*. The cacti which serve to indicate over-grazing belong almost wholly to the genus *Opuntia*.

Practice Indicators: Practice indicators are those plants or communi-

ties which point out the possibility or desirability of a particular practice. The basic practices concerned in a system of indicators are agriculture, grazing and forestry. Since successful agriculture brings the largest returns per unit area, the first question is whether the land is agricultural. If not, the next question deals with its value for forestry or grazing, or for a combination of the two. In addition to distinguishing areas as primarily agricultural, grazing, or forest land, practice indicators serve also to indicate particular types of agriculture, grazing, or forestry, as well as to suggest the crop of the greatest promise. Thus, in the case of agriculture, indicators may be used to denote the greater feasibility of humid, dry, or irrigation farming, or the importance of combining grazing with dry-farming. Where grazing is concerned, the type of vegetation not only determines whether cattle, sheep or goats are preferable, or a combination of two or three is possible, but it also indicates whether the introduction of other dominants is possible or desirable. In similar fashion, indicators may be employed to determine the possibility of afforestation or reforestation, as well as the most promising dominants for any particular region. Finally, practice indicators have value for reclamation projects.

Edaphic Indicators of Types of Farming: They are primarily related to soil and water content. The river valleys in America are occupied by a community of which *Andropogon, Agropyron, Calamovilfa, Elymus* or *Spartina* are the dominants and indicate the possibility of a subhumid farming. Communities of *Kochia vestita* or *Atriplex confertifolia* generally indicate the necessity of irrigation. According to Hilgard (1906) *Sporobolus airoides, Spirostachys, Salicornia, Suaeda, Sarcobatus, Frankenia, Cressa* and *Distichlis* are indicators of the necessity of underdrainage as a prerequisite to a successful irrigation farming. The maximum production of corn is within the main area of the subclimax and true prairies. Winter wheat has its centre in the true prairies of Kansas and Nebraska in which *Andropogon* plays an important role, spring and durum wheat reach their maximum production in mixed prairies or the northern portions of true prairies where *Stipa spartea* and *Andropogon glaucum* are especially important.

Pasturing Indicators: *Stipa* and *Bouteloua* in North Dakota and Montana, *Bulbilis, Agropyrum* and *Bouteloua* in the region of the Black Hills indicate its value only as grazing field.

Indicators of Afforestation: 'Sal' forests of our country are inseparably connected with grasslands. *Saccharum narenga* is a typical grass which indicates a soil where 'sal' will flourish. *Anthistiria gigantea* is another grass of the above type. Bessey expressed in 1895 that there are evidences that the sand hills of Nebraska could be forested. This evidence consisted wholly of valley and canyon relicts of wood land, chiefly yellow pine. Bates & Pierce in 1913 pointed out that certain shrubs of sandhill

regions indicate possibility of afforestation. *Yucca glauca, Salix humilis, Ceanothus ovatus* and *Symphoricarpos occidentalis* are typical examples of such shrubs. Similarly, *Arctostaphylos uva-ursi,* which grows only in limited localities on moist north slopes, is thought to be indicative of conditions favourable for western yellow pine, since it is an almost invariable associate of that tree in the Rocky Mountains.

Summary

Concept of habitat, characteristics of temporary pools during dry phase and their vegetation, and the relationship between the habitat and adaptations in plants have been discussed. Plants and plant communities are the products of conditions under which they grow and as such they have indicator value. They indicate certain ecological factors like water content, solutes and aeration of soil, incidence of fire, grazing etc. which could enable agriculture, grazing and forestation.

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PHOTOPERIODIC BEHAVIOUR IN SOME VARIETIES OF RICE GROWN IN INDIA

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Although the rudiments of the effect of day length on plants were known to the farmer from the time he took to agriculture, the credit goes to Garner & Allard (1920) for the scientific disclosure and demonstration of this outstanding phenomenon of the plant world. Scores of papers have since been published on this subject. From the theoretical point of view, they have stimulated basic research of varied types in the developmental physiology of plants and have been largely responsible for giving a new insight into many a physiological process. These studies have been immensely profitable in increasing the economy of crop production.

Rice being the staple food crop of India has attracted the attention of Indian scientists. Alam & Saran (1938) for the first time in India noted the importance of relative day length to induce earlier flowering while crossing two varieties of rice which normally differ in their flowering time. Alam (1940-41) by applying short days to several varieties of rice grown in Bihar came to the conclusion that all varieties require a minimum period of 30 days for vegetative growth and a succeeding period of about 30 days of short-day treatment for coming into flower. Sircar (1942) using a winter rice ('Bhasmanik') grown in Bengal, observed an earliness of 20 days in ear emergence by application of short days of 8 hours. Sircar (1944, 1946) subjected the winter rice 'Bhasmanik' to short days of 6, 8 and 10 hours for varying periods in the seed bed and also to prolonged treatment after transplantation. All treatments resulted in acceleration of flowering. Kar & Adhikari (1944-45) obtained a significant earliness in eight varieties of winter rice of Bengal by application of 8-hour short days while a delay in ear emergence was noted in a 17-hour long day. Sen Gupta & Sen (1943-44, 1945-46) obtained a retardation in ear emergence in a late-sown winter rice by application of short photoperiods. Saran (1945) investigated the photoperiodic behaviour of five varieties of rice grown in Bihar. He came to the conclusion that by application of short days, any variety of rice, irrespective of the class or maturity period to which it belonged, could be induced to flower within 60-63 days of germination. Sircar & Parija (1945, 1949) tested the photoperiodic response of three varieties of winter rice and two varieties of summer rice. Short-day treatments resulted in the acceleration of flowering of all the varieties except 'Jhanji', a summer variety. Kar (1946) observed an

earliness in ear emergence in two summer varieties of rice of Bengal by short as well as long days. Sircar & Ghosh (1947) observed in two summer varieties of rice grown in Bengal that short days delay flowering by 7-12 days and annul the effect of vernalisation by high temperature. Sen (1948) observed an earliness of 6-7 days in two varieties of late-sown winter rice of Bengal by 16 hour long-day exposure, applied for one month. Saran (1950) reported that short days for 15-30 days bring about conspicuous earliness in a winter rice of Bihar.

Material and Methods

Altogether eighteen varieties of rice (*Oryza sativa* sub-species *indica*), twelve from Uttar Pradesh and six from Orissa, have been used in the present investigation. The grains, after preliminary selection for uniformity of size and colour, were sterilised in a 0.2 per cent formalin solution for 3 hours, after which they were thoroughly washed in tap water. All the experiments were conducted by sowing the sterilised seeds in earthen pots containing a fresh mixture of well pulverised garden loamy soil and cow dung manure in the proportion of 8:1 by volume.

Short-day treatment consisted of a daily 10-hour exposure to natural day light from 8.00 A.M. to 6.00 P.M. For the rest of the 24-hour cycle the pots were removed to a well-ventilated dark room. Long-day treatment consisted of 24 hours continuous illumination. This was obtained by supplementing the natural day length with artificial illumination from a 1000 watt gas-filled 'Osram' bulb giving a light intensity of 30-40 foot candles. The control set in each case received the normal day length and the natural environmental conditions in the growing season at Allahabad (Lat. 25° 27'N Long. 81° 44' E). All other conditions except photoperiod were kept identical as far as practicable in all the treatments.

Experimental Results

The exsertion of inflorescence through the last leaf (flag leaf) sheath of the main shoot was taken as the criterion of ear emergence. Observations were made each day at about 3.00 P.M.

Experiment 1—Short days were given to four early varieties, T. 136, T.N. 22, T.N. 27 and Ch. 10, at the age of 18 days for one month in one set and prolonged till ear emergence in another. Long days were given to 10-day old seedlings for 20 days in one set and for 30 days in another. Data for ear emergence are presented in Table I.

TABLE I

Time from sowing to ear emergence of the main shoot in days. Sowing date May 7, 1949.

	(No. of days from sowing to ear emergence)				
	T. 136	T.N. 22	T.N. 27	Ch. 10	Mean
Control	90.68	88.30	87.18	90.43	89.14
S. Day for 30 days	97.13	96.60	94.96	95.61	96.07
S. Day till ear emergence	98.95	102.87	100.50	102.00	101.08
L. Day for 20 days	91.08	91.26	—	—	91.17
L. Day for 30 days	93.11	93.31	—	—	93.21

A study of Table I shows that there is a significant delay in ear emergence in both sets receiving short-day treatments, the average delay being 6.98 days in plants treated for one month and 11.94 days in those treated till ear emergence. The difference between the two short-day sets is also significant, the prolonged short-day treatment producing a longer delay than the other. The treatment effects do not seem to vary with the varieties because the variety × treatment interaction was non-significant.

In addition to the delaying effect of the short days on the emergence of the ears, a peculiar behaviour was noted in the case of the experimental set where short-day treatment was given till ear emergence. In this case the emergence of the ears on the main shoot was totally suppressed in a number of plants. This behaviour was noticed in all the four varieties. The suppression was as high as 50 per cent in the two varieties T. 136 and T.N. 22, dropping down to 41.6 per cent in variety T.N. 27 and still to a smaller value of 21 per cent in variety Ch. 10. On dissection of the culms in these plants it was observed that although the ears were formed, their growth was arrested inside the boot. Thus, the effect of the prolonged short-day treatment may be resolved into two groups: (*i*) delay in the ear emergence of the main shoot, and (*ii*) suppression of the ear emergence due to failure of stem elongation.

Long-day treatment for 30 days produced a significant delay in ear emergence.

Experiment 2—Short days were given to two early varieties, T.A. 64 and T.N. 32, at the ages of 10, 20, 30, 40 and 50 days for a period of one month and also till ear emergence in separate sets. Long days were given to 10, 20 and 30 days old seedlings for one month. The time taken from sowing to ear emergence of the main shoot under different treatments is presented in Table II.

TABLE II

Time taken for ear emergence in days. Sowing date April 20, 1950. Each figure is average of 20 plants.

	No. of days from sowing to ear emergence					
	Treatment for one month			Treatment till ear emergence		
	T.N.32	T.A.64	Mean	T.N.32	T.A.64	Mean
S. Day from 10th day	99.24	99.54	99.39	103.32	104.64	103.98
S. Day from 20th day	97.84	97.08	97.46	99.34	102.52	100.93
S. Day from 30th day	95.98	96.90	96.44	98.98	98.40	98.69
S. Day from 40th day	91.42	92.70	92.06	91.94	94.40	93.17
S. Day from 50th day	91.02	92.22	91.62	91.22	93.22	92.22
L. Day from 10th day	100.54	—	—	—	—	—
L. Day from 20th day	100.88	—	—	—	—	—
L. Day from 30th day	102.54	—	—	—	—	—
Control	90.36	91.32	90.84	90.36	91.32	90.84

A study of Table II reveals that short photoperiods bring about a delaying effect in ear emergence in both the varieties. The delay is greatest when the treatment is applied to 10 days old seedlings. As the treatment is commenced with older seedlings of 20, 30, 40 and 50 days, there is a gradual lessening of the delaying effect. At every increase of 10 days in the age of the seedlings at the time of the commencement of treatment, there is a decrease in the mean time of heading by 2.56 days within the treatment range of 10-50 days old plants. Furthermore, the time taken for ear emergence in the series where the short photoperiod is given till heading is always longer than the other series where the photoperiod is limited to a month, irrespective of the age of treated seedlings but the difference seems to be much less when the age of the treated seedlings gets more and more advanced. The two varieties do not give significantly different response.

The 24-hour continuous illumination for one month given to 10, 20 and 30 days old seedlings brought about a significant delaying effect in ear emergence of the main shoot, irrespective of the age of the seedlings at the time of the commencement of the treatment.

Experiment 3—Short days were given to three medium-early rice varieties T. 3, T. 12, and T. 21, to four early winter rice varieties T. 1145, T. 23, T. 36 and T. 17 and to four late-winter rice varieties C. No. 3, S.R. 26B, F.R. 13A and F.R. 43B at the age of 7 days for 3, 4, 5 and 6 weeks

in the seed bed in separate sets and also prolonged in one set till ear emergence. Long days were similarly given to one week old seedlings for 3 to 6 weeks. The time from sowing to ear emergence is presented in Table III.

TABLE III

Time from sowing to ear emergence of the main shoot in days. Sowing date June 18, 1949.

	Days from sowing to ear emergence					
	Control	S. Day for 3 weeks in seed bed	S. Day for 4 weeks in seed bed	S. Day for 5 weeks in seed bed	S. Day for 6 weeks in seed bed	S. Day prolonged till ear emergence
Medium-Early						
T.3	106.25	106.30	107.70	109.75	112.45	128.00
T.12	99.30	107.55	108.90	110.25	111.85	121.25
T.21	108.70	111.15	112.05	113.75	115.85	121.55
Mean	104.75	108.33	109.55	111.25	113.38	123.60
Early-Winter						
T.1145	119.55	120.95	123.30	127.80	128.00	101.80
T.23	119.10	124.10	126.60	128.30	131.75	108.50
T.36	119.50	123.90	127.00	129.60	131.90 in 65% 69.20 in 35%	80.00 in 50% 65.30 in 50%
T.17	115.90	119.40	122.50	123.00	124.90 in 75% 68.00 in 25%	99.20 in 85% 74.00 in 15%
Mean	118.51	122.08	124.85	127.17		
Late-Winter						
C.No.3	142.20	145.20	148.70	149.20	149.80	110.40
S.R.26B	136.30	138.50	142.70	144.15	146.40	125.45
F.R.13A	137.45	137.80	138.35	140.10	141.50	116.25
F.R.43B	137.90	141.80	142.40	142.70	143.60	102.80 in 85% 69.60 in 15%
Mean	138.46	140.82	143.03	144.03	145.32	

Table III shows that the effect of the short days on the three varieties of medium-early rice is of a similar nature as their effect on early varieties. There is a gradual dalay in the time of ear emergence as the duration of the short-day treatment is increased in the seed bed and thereafter. With three weeks' short days in the seed bed the average delay

is 0.5, 8.25 and 2.45 days respectively in the three varieties T. 3, T. 12 and T.21. With 6 weeks' short days in the seed bed the average delay is 6.20, 12.55 and 7.15 days respectively for the same three varieties. When the short days were prolonged after transplantation till ear emergence the delay is much more, being 21.75, 21.95 and 12.85 days respectively for the three varieties T.3, T.12 and T. 21. Although short days greatly delayed the ear emergence in all the three medium-early varieties, in the prolonged short-day treated set they did not produce any inhibitory effect by way of complete suppression of some of the main shoot ears as they did in some early varieties.

All the four early-winter varieties do not behave uniformly towards the short days. In the two varieties T. 1145 and T. 23, the short-day treatment in the seed bed retards the time of ear emergence. The retardation is more with longer duration of the treatment in the seed bed, but in the set where the short-day treatment is given for 6 weeks in the seed bed and after transplantation is prolonged till heading, the ear emergence was greatly accelerated. In the varieties T. 36 and T. 17, short-day treatment for 3, 4 and 5 weeks delayed ear emergence. The seed-bed treat ment for 6 weeks produced a delaying effect in 65 per cent of the plants in T. 36 and in 75 per cent of the plants in T. 17, while in the rest of the plants acceleration is noted. In the prolonged treatment set earliness in ear emergence is observed in all the plants, but here also two periods of heading are noted ; much greater earliness is noted in certain percentages of the total number of plants and a small amount of earliness is noted in others.

Out of the four late-winter varieties, only C. No. 3, S. R. 26B and F. R. 13A react to short photoperiod likewise and the other variety F. R. 43B behaves differently. Short days for 3 weeks during the seed bed stage bring about a delaying effect in ear emergence in the above three varieties. As the duration of the treatment increases to 4, 5 and 6 weeks there is more and more delay in the ear emergence. The interesting point is that when the short days were given to the seedlings for 6 weeks and then continued after transplantation till ear emergence, a reverse effect, namely, a marked earliness in ear emergence is noted. Variety F. R. 43B behaved a little differently. In the prolonged short-day treated set, 85 per cent of the plants showed an average earliness of 35 days in ear emergence while the rest 15 per cent showed an earliness of 68 days in ear emergence.

Although in the three varieties, T. 36, T. 17 and F. R. 43B, the short-day treatment in the seed bed for 6 weeks and that prolonged till ear emergence induced a much greater earliness in the ear emergence of the main shoots in certain percentages of the plants, the remaining tillers in the very same plants flowered much later than the main shoots (Table IV).

TABLE IV

Time of ear emergence of the main shoots and tillers

	Days from sowing to ear emergence					
	T.36		T.17		F.R.43B	
	Main shoot	Remaining tillers	Main shoot	Remaining tillers	Main shoot	Remaining tillers
Control	119.50	122.00	115.90	118.00	137.90	140.00
S. Day for 6 weeks in seed bed	69.20	132.14	68.00	126.60	143.60	146.00
S. Day prolonged till ear emergence	65.30	103.44	74.00	98.66	69.60	102.00

Flowering in the tillers of the control plants was completed within 4 days. Thus, it appears that short-day exposure of these varieties results in uneven flowering of the main shoot and tillers. The tillers of the plants which received the short-day treatment for 6 weeks in the seed bed in these varieties invariably flowered a few days later than the corresponding tillers of the control plants. It is interesting to note that tillers of the plants which received short days till ear emergence flowered much earlier than the tillers of the plants which received the treatment for 6 weeks only in the seed bed.

The results of the long-day series are presented in Table V.

TABLE V

Days from sowing to ear emergence of the main shoot
Sowing date June 18, 1949

	No. of days from sowing to ear emergence		
	Control	L.Day for 3 weeks in seed bed	L.Day for 6 weeks in seed bed
Medium-early			
T.3	106.25	104.80	112.50
T.12	99.30	98.00	110.15
T.21	108.70	104.35	114.75
Mean	104.75	102.38	112.46
Early-winter			
T.1145	119.55	118.90	120.15
T.23	119.10	120.25	123.90
T.36	119.50	119.50	120.65
T.17	115.90	115.80	116.10
Mean	118.51	118.61	120.20
Late-winter			
C.No.3	142.25	144.50	145.25
S.R.26B	136.30	135.15	137.10
F.R.13A	137.45	136.55	137.65
F.R.43B	137.90	139.15	138.30
Mean	138.47	138.83	139.57

Table V shows that long-day treatment for 3 weeks in the seed bed does not bring about any significant change in the time of flowering in almost all the varieties but the treatment for 6 weeks brings about a significant delaying effect in the three medium-early varieties and also in one variety T. 23 of the early-winter group. In the rest it has got no effect.

Experiment 4—Short days were applied to two early-winter varieties T. 36 and T. 23, after the seedlings were transplanted at the ages of 30, 40, 50, 60, 70 and 80 days and were continued till ear emergence in the individual sets. The results are presented in Table VI.

TABLE VI

Time from sowing to ear emergence of the main shoot in days. Sowing date June 26, 1949.

	Days from sowing to ear emergence .		
	T.36	T.23	Mean
S.Day to 30-day old seedlings	67.6	82.8	75.2
S.Day to 40-day old seedlings	77.0	83.2	80.1
S.Day to 50-day old seedlings	86.9	92.7	89.8
S.Day to 60-day old seedlings	95.9	102.3	99.1
S.Day to 70-day old seedlings	107.0	112.2	109.6
S.Day to 80-day old seedlings	109.4	113.4	111.4
Control	113.7	112.7	113.2

Table VI shows that short days in general reduce the period from sowing to ear emergence. The reduction as a matter of fact is more when the treatment is started earlier. The reduction in the duration for ear emergence is, however, more in variety T. 36. The relation between the age of the seedlings at the time of the commencement of the treatment and the reduction in the flowering duration from the control is practically linear. For every 10 days delay in the commencement of the treatment, flowering duration is increased by 9.77 days within the treatment range of 30 to 70 days old plants. In variety T. 23, 30- and 40-day old seedlings gave practically the same response and with 70- or 80-day old seedlings there was no earlier ear emergence than the control. With 40 to 70 days old seedlings, for every 10 days delay in the commencement of the treatment, flowering duration is increased by 9.46 days agreeing well with the value for T. 36.

Another interesting observation that emerges from a study of Table VI is that in variety T. 36, an exposure to short days for a period of 37 days

is necessary to bring about ear emergence in all sets excepting the 80-day old set. In other words, whether the short days are begun with 30-, 40-, 50-, 60- or 70-day old seedlings, another period of 37 days of short-day exposure is the minimum requirement in each case for the exsertion of the ear through the flag leaf. Only in 80-day old seedlings, exposure to 30 short days suffices to bring about ear emergence. Variety T. 23 presents a slightly different picture. Here, for the 30-day old seedlings 53 days exposure of short days is the minimum requirement ; for the 40-, 50-, 60- and 70-day old seedlings, 42-43 days exposure and for the 80-day old seedlings, 33 days exposure only is sufficient to bring about the emergence of ears.

Experiment 5—8-hour and 10-hour short days were applied to one variety of spring rice, D. I. 4, at the age of 7 and 14 days and were continued till ear emergence. 24-hour long days were also applied to one week old seedlings for 20 and 30 days in separate sets. The data on ear emergence are presented in Table VII.

TABLE VII

Time from sowing to ear emergence of the main shoot in days.
Sowing date February 19, 1949.

	No. of days from sowing to ear emergence
8 hr. S.Day from 1st week till heading	87.51
8 hr. S.Day from 2nd week till heading	86.63
10 hr. S.Day from 1st week till heading	88.13
10 hr. S.Day from 2nd week till heading	83.68
24 hr. L.Day from 1st week for 20 days	215.85
24 hr L.Day from 2nd week for 30 days	213.03
Control	216.86

Table VII shows that short days have brought about a considerable earliness in ear emergence. There is no significant difference between the effects of the two periods of day lengths, 8 hour and 10 hour, used in the present investigation. Short-day treatment is slightly more effective when applied to two week old seedlings than to one week old seedlings. Treatment with long days for 20 and 30 days has no marked effect different from the controls. Because the sowing date in the present investigation was greatly delayed from the normal sowing season of December-January, the control plants took an unusually long time of nearly 7 months to come to flower.

Discussion

It is clear from the effects of short days on the six early varieties that short-day treatment not only produced a significant delaying effect in the time of ear emergence of the ears of the main shoot but in some cases inhibited their emergence. In these cases of inhibition the vegetative apices had all changed to reproductive spikelets but they remained arrested inside the flag leaf because of the failure of the axis to elongate. Arrest of ears of a similar nature inside the flag leaf was observed by Purvis (1934) in rye. The stem has apparently specific photoperiodic requirements for stem elongation (Wanser, 1922) in the absence of which such failure in elongation probably takes place.

The degree of delay was more with seedlings still younger at the time of treatment initiation and also with prolonged duration of treatment. Results similar in trend to that of these six varieties of early rice were obtained by Sircar & Ghosh (1947, 1954) in two early varieties of rice of Bengal, by Roy & Subramanyam (1955) in some early varieties of Bihar and by Misra & Samantarai (1955) in an early variety of Orissa. Sen & Mitra (1957) obtained both delaying effect and earliness in two varieties of rice of Bengal depending upon the stage of growth of the plant at which treatment is given. Sircar & Parija (1949) and Ghose & Shastry (1954) could not get any delaying effect in some early varieties by short-day treatment. The results of the present investigation with six early varieties of rice where short days prolonged the time of ear emergence call for a modification of Kar's (1946) generalisation that in different varieties of paddy high temperature associated with short day lengths was inducive to earliness.

The photoperiodic behaviour of the three medium-early varieties was somewhat akin to that of the early varieties in that there was a gradual delay in the onset of ear emergence with a longer duration of the short photoperiod. An important result obtained from the present investigation is that so far as their photoperiodic response is concerned the early and medium-early varieties may be classified as "intermediates" since these flower the soonest under the natural day length of 11-13 hours and produce flowers less readily when the days are either shortened or lengthened (Allard, 1938 ; Allard & Garner, 1940). Eguchi (1937) and Sen & Mitra (1957), however, consider some early varieties of rice as "day neutral".

From a standpoint of photoperiodic sensitivity the eight late varieties of rice of the present investigation can be grouped into two main categories, one comprising the varieties T. 1145, T. 23, C. No. 3, S. R. 26B and F. R. 13A and the other comprising the varieties T. 36, T. 17 and F. R. 43B. The first group is photoperiodically a much less sensitive group than the second one. None of the eight varieties, however, is as

sensitive as the two varieties 'Rupsail' and 'Patnai' studied by Sircar & Parija (1949).

The differential behaviour between short-day treatments in the seed bed up to 6 weeks and that continued after transplantation beyond the sixth week stage observed in these late varieties of rice is possibly due to the fact that short days are only effective in bringing about earliness when applied at or beyond a particular stage of the plant. Exactly a similar phenomenon though of opposite nature was recorded by Purvis & Gregory (1937) in rye. That even in the same variety the photoperiodic response is dependent on the age of the seedlings at which the treatment is given is also borne out by investigations of Misro *et al.* (1953) and Dutta & Misra (1958 b). The temperature prevailing up to 6 weeks from the day of treatment initiation and also after seed bed stage might have marked influence on the photoperiodic response in rice as is suggested from the work of Sircar & Sen (1951).

The anomalous situation as to why in certain percentages of the short-day treated plants in varieties T. 36, T. 17 and F. R. 43B earlier ear emergence is seen and not in the whole lot of the experimental set may be due to the fact that the photoperiodic stimulus might not be uniformly perceived by all the plants under a particular treatment. It might be possible that all the plants under a particular treatment may not be at the same stage of development to receive the photoperiodic stimulus conducive to earliness. Sircar & Sen (1950, 1953) noted such a case in their study on the photoperiodic induction and the development of the growing apex in a winter variety of rice of Bengal.

It was also observed in these three late varieties of rice that whereas the main shoots showed much earlier ear emergence, the remaining tillers flowered near about the same time or even later than the controls. Such uneven flowering of the tillers and the main shoot has also been observed by Beachell (1943) and Sircar & Parija (1949) in rice. This differential response in the flowering behaviour between the main shoot and the remaining tillers is presumably due to the fact that the photoperiodic stimulus was perceived by the main shoot at the time of exposure to short photoperiods, while the tillers whose cell initials were perhaps formed after the period of exposure did not receive the stimulus conducive to flowering.

Whatever be the behaviour of the tillers, it can definitely be concluded from the present investigation that considerable earliness is observed in the main shoot ear emergence of all the eight late varieties as a result of short-day treatment prolonged until heading. Similar observations have been made by subsequently extending the investigation into the photoperiodic response of several other late varieties of rice (Dutta & Misra, 1957, 1958a, 1959). Gangulee (1954, 1955) in his studies on the flowering behaviour of rice has brought out certain distinctiveness of the

late variety of rice, a finding which corroborates the results of the present investigation.

SUMMARY

The response of eighteen varieties of rice to short and long photoperiods has been studied. As short day, a 10-hour day length and as long day, a 24-hour day length was used. There is great difference between the response of different varieties to the same photoperiod. Early and medium-early varieties show a delay in flowering in short days whereas early-winter and late-winter varieties show the opposite effect of earliness under the same photoperiods. With regard to the effect of the dose of photoperiod it was observed that a greater dose of the photoperiod is more efficacious than a smaller dose of the photoperiod. Another interesting observation was that short days for 3, 4, 5 and 6 weeks in the seed bed brought about a delay in flowering in the early-winter and late-winter varieties of rice but a prolonged photoperiod produced the reversed effect of earliness. The stage of the plant at which the photoperiod is administered is, therefore, a very important factor in studies of photoperiodism since the same photoperiod given at different stages of the plant produces different results. Judging from their behaviour towards short and long photoperiods in general, it may be concluded that the early and medium-early varieties are "intermediates", whereas the winter varieties are predominantly "short-day" ones.

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HORMONES IN RELATION TO FLOWERING BEHAVIOUR IN RICE

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The mechanism of flowering in plants is still an unsolved problem. The fact that photoperiodic stimuli are perceived by the leaves and then translocated to the buds where the response takes place has been adduced as an evidence for the existence of a flowering hormone. This led Cajlachjan (1936) to suggest that a hormone was responsible for photoperiodic induction. The nature of the flowering hormone has been the subject matter of serious studies by biochemists and plant physiologists alike. The suggestion that the flowering hormone might be in some way related to an auxin has led to a series of investigations on the flowering behaviour of different plants by the influence of different auxins.

Bonner & Bandurski (1952), van Overbeek (1952), Lang (1952), Liverman (1955) and Leopold (1958) have reviewed recent works on this topic. It was first Cholodny (1936) who obtained increased earliness by treatment of seeds with auxins. Thimann & Lane (1938) reported that soaking the seeds with IAA produced a better vegetative growth and hastened flowering in oats and wheat. Clark & Kerns (1942) and Cooper (1942) reported the remarkable case of the induction of flowering with auxin in pineapple. Van Overbeek (1946) reported a similar effect of earliness in pineapple with 2, 4-D. Galston (1947) obtained an increase in flowering of soybeans by the application of 2,3,5-triiodobenzoic acid which is a growth regulator but not included among the auxins. Shigeura (1948) obtained copious flowering in litchi by auxin sprays. Leopold & Thimann (1949) obtained promotion of flowering in Wintex barley with low concentrations of auxins and inhibition with higher concentrations. Leopold & Guernsey (1953, 1954) found that treatment of seeds with auxin followed by temperature of 4°C for 4-15 days produced earliness in soybean, oats, corn, barley and peas. Hussey & Gregory (1954) obtained promotion in flowering in Wintex barely by NAA, but no effect was seen in Petkus rye. Howell & Wittwer (1954) brought about flower initiation in sweet potato by 2,4-D. Chakravarti (1954) found inhibitory effect of several auxins upon vernalisation of flax. Asana *et al.* (1955) reported that wheat responded to IAA and NAA treatment of the seed with increased earliness. Chakravarti & Pillai (1955) obtained earliness in mustard when auxin was applied after chilling. Salisbury (1955) put forward evidence to show the dual role of auxin ; flowering of *Xanthium* was inhibited by auxins only if applied before the translocation of the flowering stimulus

from the leaf was complete. Auxin applied after this time caused an increased rate of floral bud development. Sircar & Kundu (1955) obtained earlier heading in a winter rice by the application of auxins. Liverman & Lang (1956) induced flower initiation with auxin in long-day plants, *Hyoscyamus* and *Silene*.

Material and Methods

Rice (*Oryza sativa* sub-species *indica*) was chosen as the material for the present investigation because the species comprises different varieties which vary in their normal length of vegetative periods ; the autumn varieties have a short vegetative period and winter varieties a long one. Furthermore, the autumn, i.e. early and medium-early varieties, are generally intermediate in their photoperiodic response while winter ones are predominantly short-day plants (Misra, 1961). The present investigation has been designed to study the effect of three plant growth regulators on the growth and reproduction of four different varieties of rice, one each from early, early-winter, mid-winter and late-winter varieties.

Seeds of pure strains of four varieties of rice—N.136 (early), T.1145 (early-winter), T.141 (mid-winter) and J.192 (late-winter)—were procured from the Central Rice Research Institute, Cuttack. After a preliminary selection for uniformity the seeds were soaked separately in five different concentrations—500, 250, 100, 10 and 1 ppm—of each of the three plant growth regulators, indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA) and 2,3,5-triiodobenzoic acid (TIBA) for 72 hours. The seeds thus treated were washed thoroughly in water and sown in seed-bed pots containing a mixture of soil and cow-dung manure in the proportion of 8 : 1. At the age of 21 days the seedlings were transplanted to 10″ earthen pots containing the same mixture of soil and manure. The plants were next subjected to weekly foliar sprays with auxin solutions of the same strength in which the seeds had been soaked, commencing with four week-old seedlings and were continued till panicle emergence.

Experimental Results

The number of days taken from sowing to ear emergence is presented in Table I. A study of the table indicates that IAA has brought about earliness in ear emergence in the early variety at the lower concentrations of 1, 10 and 100 ppm and in the late-winter variety at all the concentrations. It is without any effect in the early-winter and mid-winter varieties. NAA brought about a significant delay in ear emergence in the early and early-winter varieties at the two higher concentrations of 500 and 250 ppm but produced earliness in the late-winter variety at the

lower concentrations of 10 and 1 ppm. It does not show any effect on the mid-winter variety. TIBA was without any effect in the early, early-winter and mid-winter varieties, but produced an earliness in the late-winter variety at a concentration of 100 ppm. The results of the present investigation agree fairly well with those of the previous years (Misra & Sahu, 1957, 1959).

TABLE I

Time from sowing to ear emergence in the main shoot in days

(Average of 24 plants ; Sowing date July 3, 1957)

Treatments		Days from sowing to ear emergence			
		Early variety N. 136	Early-winter variety T. 1145	Mid-winter variety T. 141	Late-winter variety J. 192
IAA	500 ppm	67.31	102.06	109.81	116.06
	250 ppm	67.00	101.38	108.63	115.81
	100 ppm	66.63	102.06	108.75	115.69
	10 ppm	66.13	101.19	109.25	115.69
	1 ppm	65.63	101.44	109.50	115.63
NAA	500 ppm	70.63	102.88	110.06	116.38
	250 ppm	69.94	102.69	109.38	116.56
	100 ppm	68.50	101.69	109.88	116.50
	10 ppm	68.00	101.88	109.75	116.25
	1 ppm	67.13	101.63	109.31	116.06
TIBA	500 ppm	69.13	100.75	109.63	116.63
	250 ppm	68.06	101.13	109.69	116.63
	100 ppm	67.38	100.19	109.44	116.13
	10 ppm	68.31	100.38	109.81	116.63
	1 ppm	67.88	101.75	110.31	116.69
Controls		68.13	100.63	109.56	117.13
S. E. Mean		0.36	0.45	0.29	0.23
C. D. at 1% level		1.38	1.71	1.08	0.88

DISCUSSION

Although the present study on the effect of hormones on rice is by no means complete, nevertheless, it throws some interesting light on the developmental physiology of the rice plant.

The differential response of different varieties of rice, *Oryza sativa* subspecies ***indica,*** to the same auxin is most outstanding. The early variety showed slight earliness in flowering when treated with IAA, the early-winter and mid-winter varieties were not affected by IAA, whereas the late-winter variety came to earlier heading by treatment with IAA. With NAA it was similarly found that the early and early-winter varieties are delayed, the mid-winter variety remained unaffected and the late-winter variety came to earlier heading. The varietal response of rice to auxin

is, therefore, established beyond doubt. It is, therefore, not correct to generalize that auxins cause earliness in rice (Sircar & Kundu, 1955). Furthermore, so far as the quantitative aspect of earliness is concerned, none of the varieties included in the present investigation is as sensitive to auxin response as that of Rupsail, a winter rice of Bengal where Sircar & Kundu (1955) obtained a very high value of 18 to 27 days earliness in ear emergence.

The present investigation also brings out the differential effects of different auxins on the same variety of rice. For example, IAA brought about an earliness in the early variety, whereas NAA brought about delay in the same variety. Taking the early-winter variety, we see that IAA did not produce any marked change but NAA brought about delay in flowering.

Various concentrations of the same auxin bring about different results in the same variety of rice. For example, IAA brought about earliness in the early variety only at the three lower concentrations of 100, 10 and 1 ppm, but was without any significant effect at higher concentrations. NAA brought about delay in the early and early-winter varieties only at the two higher concentrations of 500 and 250 ppm. In the late-winter variety NAA brought about earliness at the two lower concentrations of 10 and 1 ppm and no significant change at the higher concentrations.

It is seen that late-winter variety of rice which is predominantly a short-day one, responds better to auxin treatment. This points out to some interesting relationship of auxin response with photoperiodic response and requires further elaboration.

The late-winter variety of rice which is short day in its response to photoperiod can be safely added to the small list of short-day plants like Cocklebur and Biloxy soybean in which auxins bring about promotion in flowering (de Zeeuw & Leopold,[1] 1956).

The present investigation again emphasizes that auxin response of the rice plant is more of a theoretical interest than of any agricultural utility.

SUMMARY

From the standpoint of individual hormones averaged over all concentrations, the general conclusion is that IAA induced earliness in ear emergence in the early and late-winter varieties but was without any significant effect in the early-winter and mid-winter varieties. NAA and TIBA did not produce any significant effect on ear emergence in any of the four categories of rice. When, however, the results were analysed laying emphasis on the concentration of the hormones, a different picture was obtained. The significant earliness that was obtained by the applications of IAA in the early variety was mainly due to lower concentrations but in the late-winter variety there was a general earliness in all con-

centrations with the maximum effect in the lowest one. On segregation of the concentration effect, NAA was found to bring about a significant delay in ear emergence in the early and early-winter varieties at higher concentrations of 500 and 250 ppm but brought about the opposite effect of earliness in the late-winter variety in the two lower concentrations of 10 and 1 ppm. TIBA brought about a significant earliness in the late-winter variety only at the concentration of 100 ppm. Thus, in studies of hormonal effect on flowering it is not only that the hormones play different roles but their relative concentrations are also of great significance.

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SEED STRUCTURE IN THE AIZOACEAE

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The study of seed structure forms an interesting aspect of general morphology apart from the importance of surface markings of seeds in taxonomic diagnosis. Martin (1946) has classified the seeds on the basis of relative size and position of embryo in the seed and believes that it is indicative of certain phylogenetic trends. The works of Corner (1951) on the leguminous seed and Singh (1953) on the seed structure of the Cucurbitaceae have shown the systematic value of seed characters up to the generic level. Such a study is felt useful for the Aizoaceae in view of the fragmentary work on *Mesembryanthemum crystallinum* (Woodcock, 1931) and *Mollugo verticillata* (Payne, 1935) which does not throw much light on the relationship of the Aizoaceae with the Cactaceae. The contributions of Bhargava (1934, 1935) on *Mollugo nudicaulis* and *Trianthema monogyna*, Joshi & Rao (1936) on *Gisekia pharnaceoides* and Raghavan & Srinivasan (1940) on *Sesuvinum portulacastrum* and *Trianthema decandra*, are too meagre to warrant any conclusions regarding affinities of the Aizoaceae.

MATERIALS AND METHODS

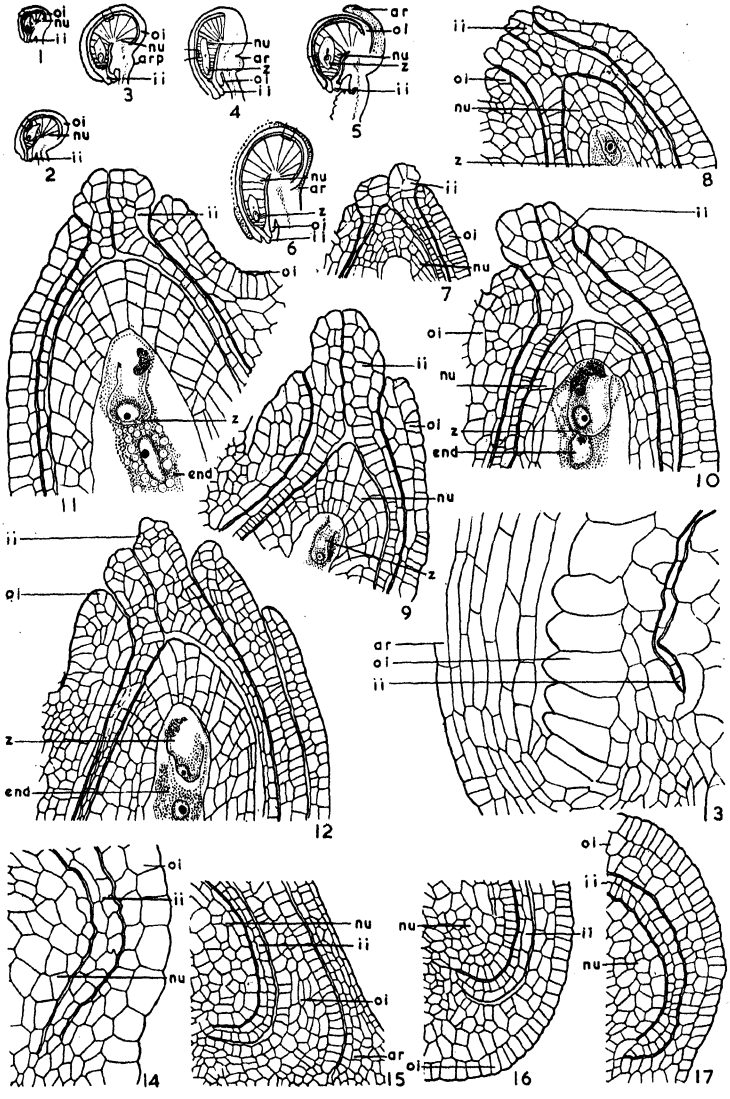
Mature flowers and fruits of different ages of *Mollugo cerviana*, *M. nudicaulis*, *Gisekia pharnaceoides*, *Orygia decumbens*, *Glinus lotoides* and *Trianthema pentandra* were collected round about Jodhpur and fixed in F.A.A. Some old seeds were kept separately either in glacial acetic acid or picric acid for 10-12 days before infiltration. Embedding was done by the customary methods, sections were cut 10-15 microns thick and stained with safranin-fast green. Hand sections of the mature seeds were also made wherever necessary.

OBSERVATIONS

OVULE—The ovules are bitegmic and campylotropous (Figs. 1-6). They are arranged in two rows in each loculus on an axile placenta. In *Trianthema*, however, only two are found in each locule borne at the base.

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(Fig. 10), *Glinus lotoides* (Fig. 11) and *Trianthema pentandra* (Fig. 12) in l.s. × 269. Figs. 13-17. Integumentary bases from ovules of *Glinus* (Fig. 13), *Mollugo nudicaulis* (Fig. 14), *Trianthema* (Fig. 15), *Orygia* (Fig. 16) and *Gisekia* (Fig. 17). × 269.



Figs. 1-17. (*ar*, aril ; *arp*, arillate primordium ; *end*, endosperm ; *ii*, inner integument ; *nu*, nucellus ; *oi*, outer integument ; *z*, zygote)—Figs. 1-6. L.s. ovules of *Mollugo cerviana* (Fig. 1), *M. nudicaulis* (Fig. 2), *Gisekia pharnaceoides* (Fig. 3), *Orygia decumbens* (Fig. 4), *Glinus lotoides* (Fig. 5) and *Trianthema pentandra* (Fig. 6). × 28. Figs. 7-12. Magnified micropylar portions of *Mollugo cerviana* (Fig. 7), *M. nudicaulis* (Fig. 8), *Orygia decumbens* (Fig. 9), *Gisekia pharnaceoides*

The ovules of *Mollugo cerviana* are very small in contrast to those of *Trianthema pentandra* which are large, while those of the others intergrade between these two limits. The funiculus is generally short with the exception of *Trianthema* and *Glinus*.

The ovules are bent away from the placenta, i.e. towards the septum and are horizontally oriented to the longitudinal axis in *Mollugo, Orygia* and *Glinus* and parallel to the long axis of the gynoecium in *Gisekia* and *Trianthema*.

An obturator is met with in *Orygia, Mollugo* and *Glinus*. It is placental in the first two and funicular in the last-mentioned genus.

The nucellus is massive forming a sort of cap by the divisions of the epidermal and subepidermal cells and is bent towards the funiculus.

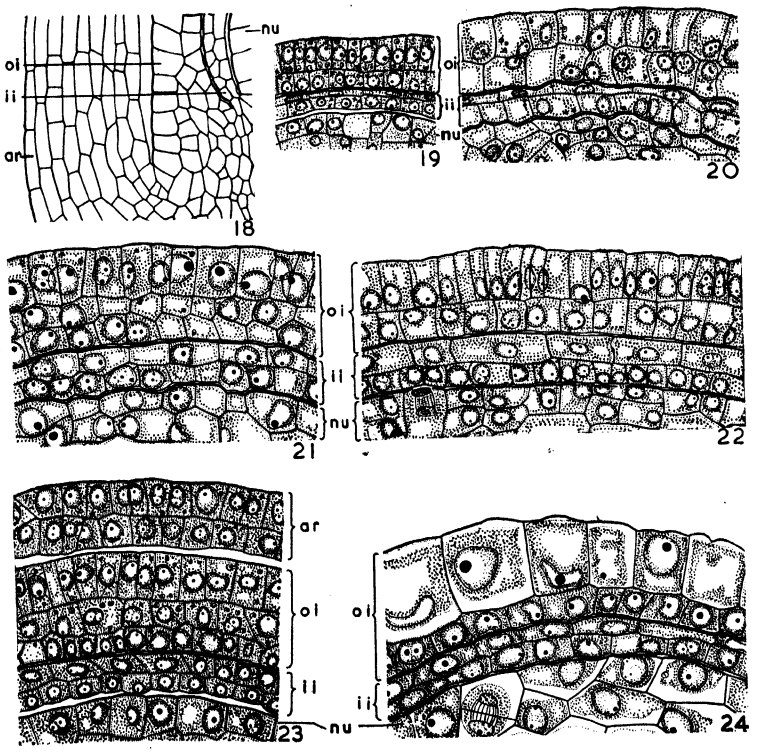
The inner integument forms the micropyle (Figs. 1-6) and is two-layered in the lower two-thirds of the ovule but three- to four-layered in the region of the micropyle (Figs. 7-12). This increase in thickness is generally brought about by the periclinal and subsequent irregular divisions of the cells in the inner layer. Rarely, the cells of the outer layer also divide once or twice in *Trianthema pentandra* (Fig. 12). Of the two layers, the outer consists of cells tangentially elongated twice or thrice the size of those of the inner layer (Figs. 19-24). This distinction is lost near the micropyle in *Gisekia* and *Trianthema* (Figs. 10, 12) while it is maintained in *Mollugo nudicaulis, Orygia* and *Glinus* (Figs. 8, 9, 11) coupled with radial enlargement of the components of the inner layer.

The composition of the outer integument is variable. It is uniformly two-layered in *Mollugo cerviana* (Figs. 7, 19) and *M. nudicaulis* except at the base of the integument in the latter where it is three to four cells thick (Figs. 8, 14), a feature not reported by Bhargava (1934) ; it is two-layered in the middle but three to four cells thick at both ends in *Orygia* (Figs. 9, 16, 22). In *Glinus* (Figs. 11, 24) and *Gisekia* (Figs. 10, 21) also it is two-layered towards the micropylar half but three- to five-layered elsewhere in the last-mentioned species (Figs. 10, 17, 21). This is contrary to the report of Joshi & Rao (1936). In *Trianthema* the outer integument is generally three-layered and somewhat massive at the extremities (Figs. 12, 15). According to Bhargava (1935) and Raghavan & Srinivasan (1940), it is 3-layered in both *T. monogyna* and *T. decandra*. This variability in thickness is the result of divisions of the subepidermal cells at the base in *Trianthema*.

The cells of the outer layer of the outer integument are radially distended when compared to those of the square or rectangular cells of the inner layer. They may be twice as broad and thrice as large as the cells of the inner layer in *Glinus* (Fig. 24). Starch grains may be present in both the layers.

The aril which was first reported by Bhargava (1935) in *Trianthema monogyna* is well developed, completely enclosing the ovule as the latter

matures in *T. pentandra* (Figs. 6, 12). It is quite distinct on the funicular side (Fig. 36) besides the counterparts of both the integuments and develops belatedly, though Bhargava (1935) has erroneously indicated the outer integument as the aril. In *Glinus* it differentiates at the stage of megaspore formation and encloses the lower one-third of the ovule at the time of fertilization (Fig. 5). In *Orygia* this occurs before fertilization. In *Mollugo nudicaulis* the primordial swelling has been observed at the base of outer integument. This distends into a knob-like structure at the stage of filamentous proembryo and persists even in the late globular stage (Figs. 25, 26). In *Gisekia* also, the primordium-like swelling is discernible in the mature ovule (Fig. 3), but it remains arrested during subsequent development of the seed. However, Bhargava (1934) and



Figs. 18-24. (*ar*, aril ; *ii*, inner integument ; *nu*, nucellus ; *oi*, outer integument)—Fig. 18. Integumentary base from the ovule of *Orygia*. × 380. Figs. 19-24. Magnified diagrams of marked portions from Figs. 1-4, 6 and 5 respectively. × 532.

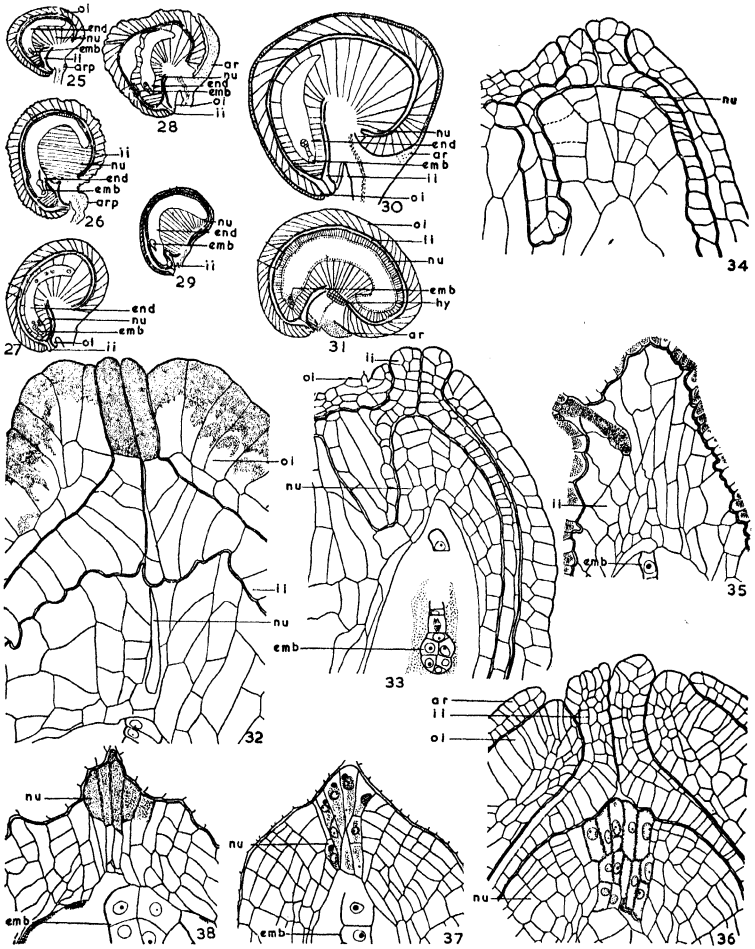
Joshi & Rao (1936) have never ascribed any significance to this swelling.

The thickness of the aril is also variable. It is two-layered in the upper half and three- to five-layered in the lower half in *Trianthema* (Figs. 12, 15), five- to seven-layered in *Glinus* (Fig. 13) and seven- to nine-layered in *Orygia* (Fig. 18). The cells are tangentially elongated and thin-walled, filled with dense contents in the latter two genera. However, in *Trianthema* the cells of the upper layer are slightly elongated or more or less square, similar to those of the inner layer (Fig. 23) and are filled with starch grains.

DEVELOPMENT OF THE SEED—As the seed develops, changes take place in the inner integument resulting in the gradual crushing and obliteration of the outer layer and the accumulation of darkly-stained contents in the cells of inner one. In *Orygia* (Figs. 18, 31, 41), *Gisekia* (Figs. 27, 42) and *Trianthema* (Figs. 30, 44) the outer layer of the inner integument although compressed, persists till about the formation of the proembryo. In *Mollugo nudicaulis*, as the seed matures and the embryo becomes globular, this layer is further crushed out (Fig. 40). In still more mature seeds as in *M. cerviana* this layer totally disappears (Figs. 29, 39). It is obliterated quite early on the convex side in *Glinus* (Figs. 11, 13, 43). The distal half of the inner integument on the funicular side is uniform in thickness in all the members except *Mollugo nudicaulis* (Fig. 26) and *M. cerviana* (Fig. 29) where it is enlarged and is composed of radially distended cells of the outer layer which is later crushed leaving behind a compressed loop of the inner layer (Figs. 33, 34).

The outer integument also exhibits some changes similar to those of the inner one as the seed matures. The cells of the outer layer are distended radially developing a deep, yellowish-brown colour. Vacuolation of the cytoplasm also takes place. Reserve food substances appear prominently (Figs. 39-44). In a slightly older seed of *Mollugo nudicaulis* the thickening commences on the outer tangential and radial walls. The latter may show reticulate thickenings (Fig. 40). The cells of inner layer enlarge and remain thin-walled containing abundant reserve food.

The meristematic activity of the cells of the nucellar epidermis together with that of the subepidermis results in profuse growth of the nucellus on the convex side forming a J-shaped structure. The chalaza becomes laterally shifted. The enlarging embryo sac is initially surrounded throughout its length by the thick nucellar tissue. Later, the reduction of nucellus in thickness is considerable on the convex side and negligible elsewhere (Figs. 25-31). In *M. nudicaulis* the cells of the nucellar epidermis above the micropylar end of the embryo sac divide periclinally. These together with the adjacent cells form the nucellar hood which is prominently seen up to the late globular stage of the embryo (Figs. 8, 25, 26). Similar is the case in *M. cerviana* (Figs. 7, 33, 34). In *Glinus*



Figs. 25-38. (*ar,* aril ; *arp,* arillate primordium ; *emb,* embryo ; *end,* endosperm ; *hy,* hypostase ; *ii,* inner integument ; *mi,* micropyle ; *nu,* nucellus ; *oi,* outer integument)—Figs. 25, 26. L.s. seed of *Mollugo nudicaulis.* × 21. Figs. 27-31. L.s. seeds of *Gisekia* (Fig. 27), *Glinus* (Fig. 28), *Mollugo cerviana* (Fig. 29), *Trianthema* (Fig. 30) and *Orygia* (Fig. 31). × 21. Fig. 32. Micropylar region of the seed of *Glinus lotoides.* × 241. Figs. 33, 34. Micropylar half of the seeds of *Mollugo cerviana* at early and late globular stages of the embryo. × 241. Fig. 35. Nucellar hood of *Orygia.* × 241. Fig. 36. Micropylar region of the ovule of *Trianthema* at the globular stage of embryo. × 241. Figs. 37, 38. Same, nucellus from the micropylar region ; see the radially elongated cells above the embryo sac. × 241.

lotoides also the cells of the nucellar epidermis divide transversely at the micropylar end of the embryo sac (Figs. 11, 32). However, according to Raghavan & Srinivasan (1940) there is only radial distension of the epidermal cells at this end in *Mollugo oppositifolia* (Syn. *Glinus oppositifolia*). In *Orygia* the nucellar epidermal and subepidermal cells are radially elongated in this region (Fig. 35). On the other hand, in *Trianthema* the epidermis as well as the underlying cells of the nucellus show radial elongation at the upper end. They may also divide periclinally. The cells of the outer layer become thickened later and persist for a considerable period forming an epistase-like structure (Figs. 36-38). In *Orygia* a group of cells differentiates as hypostase at the chalazal end when a linear proembryo is organised (Fig. 31).

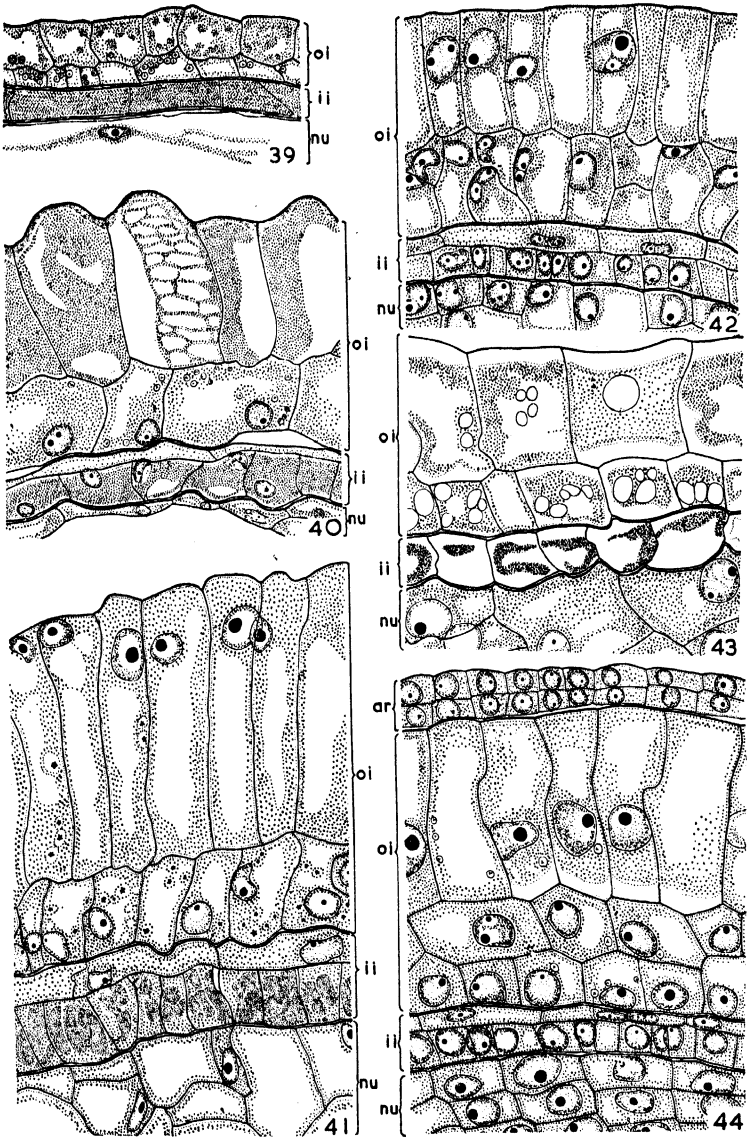
MATURE SEED—There is great uniformity in the general feature of the seeds, both in shape and texture, as well as in the broad outline of internal structures suggesting the family character.

The seeds are distinctly campylotropous and ring-marked. The embryo is well developed with two cotyledons and encloses the massive nucellus which persists as a perisperm on the inner side while the same is crushed elsewhere. The endosperm is scanty and occurs as a single layer of cells in the micropylar half (Figs. 45-48).

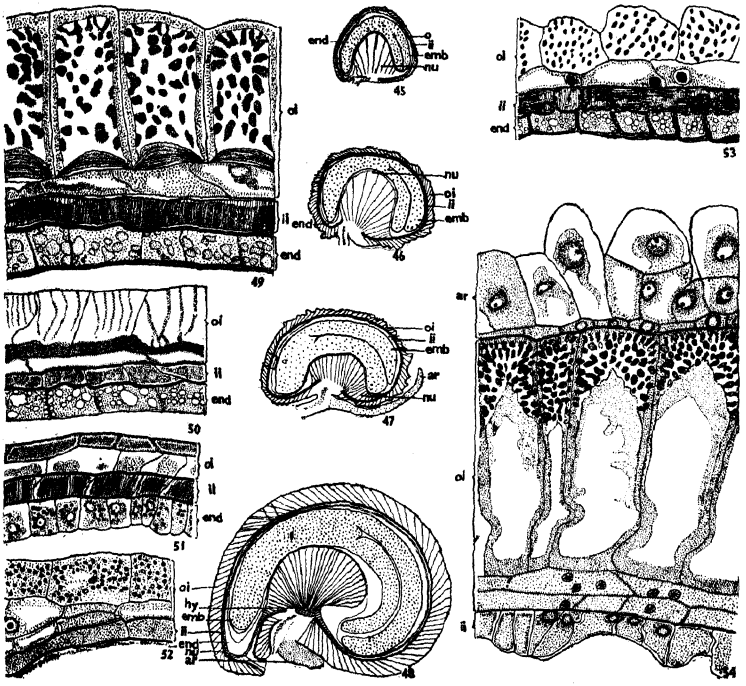
The seed coat is composed of the entire outer integument and the inner layer of the inner integument. The cells of inner integument show fibrous thickenings either connecting the tangential walls as in *Mollugo cerviana* (Figs. 45, 51) and *Orygia decumbens* (Figs. 48, 49) or radial walls as in *Mollugo nudicaulis* (Figs. 46, 53) besides the darkly-stained contents. In *Gisekia* (Fig. 52), *Trianthema* (Fig. 54) and *Glinus* (Figs. 47, 50) the thickening is along the walls.

The cells of the outermost layer of the outer integument are radially distended in *Orygia* (Fig. 49) and *Trianthema* (Fig. 54) and to a lesser extent in *Glinus* (Fig. 50). In *Mollugo cerviana, M. nudicaulis* and *Gisekia* the cells are tangentially enlarged. The thickening is enormous with the thin circular areas aligned in alternating rows as in *Mollugo nudicaulis, Glinus* and *Gisekia* or irregularly as in *Orygia*. In the latter the inner tangential wall is also greatly reinforced and lamellate. In *Trianthema* and *Mollugo cerviana* the thickening is along the walls but in the former it is considerable in the upper half. The cells of the underlying layers remain thin-walled with scanty contents. In *Trianthema* these possess druses.

The aril extends only to a short distance in *Orygia* (Fig. 48). It encloses one-third of the seed in *Glinus* (Fig. 47) while it completely envelops the seed in *Trianthema*. Of the two layers of aril, the cells of the outer layer in *Trianthema* are radially enlarged whereas those of the inner layer remain tangentially compressed (Fig. 54). A few cells of the outer layer may divide once or twice.



Figs. 39-44. (*ar*, aril ; *ii*, inner integument ; *nu*, nucellus ; *oi*, outer integument)—Figs. 39-44. Enlarged diagrams of the seed coat marked in Figs. 29, 26, 31, 27, 28 and 30 respectively. × 532.



Figs. 45-54. (*ar*, aril ; *emb*, embryo ; *end*, endosperm ; *hy*, hypostase ; *ii*, inner integument ; *nu*, nucellus ; *oi*, outer integument)—Figs. 45-48. V.s. mature seeds of *Mollugo cerviana* (Fig. 45), *M. nudicaulis* (Fig. 46), *Glinus* (Fig. 47) and *Orygia* (Fig. 48). × 20. Figs. 49-54. Magnified diagrams of seed coat of *Orygia* (Fig. 49), *Glinus* (Fig. 50), *Mollugo cerviana* (Fig. 51), *Gisekia* (Fig. 52), *Mollugo nudicaulis* (Fig. 53) and *Trianthema* (Fig. 54). × 364.

Conclusions

Raghavan & Srinivasan (1940) consider that the Molluginoideae are characterised by the occurrence of two-layered outer integument, absence of aril and the periclinal divisions of the cells of nucellar epidermis above the embryo sac. The Ficoideae are distinguished by the three-layered outer integument, presence of aril and absence of the periclinal divisions of the cells of nucellar epidermis at the apex. According to them *Mollugo oppositifolia*, a member of the Molluginoideae, forms a bridge between these two subfamilies owing to the presence of an aril and lack of divisions in the nucellar epidermis at the micropylar end.

The present investigation, however, has revealed that there is no such rigid set of characters delimiting the two subfamilies. The three-layered

outer integument characteristic of the Ficoideae is also observed in *Gisekia* and *Orygia*. Aril of various dimensions ranging from primordium-like swelling of *Mollugo nudicaulis* and *Gisekia*, and short and stumpy structure of *Orygia* to a thin investment over the lower one-third of the seed of *Glinus* is met with in the Molluginoideae. As regards the epidermal cells of the nucellar tip there may also be periclinal divisions besides radial distension as observed in *Trianthema pentandra* (Ficoideae). The radial distension of cells of the nucellar epidermis, characteristic of the Ficoideae, is also prevalent in epidermal and subepidermal cells of the nucellus of *Orygia* (Molluginoideae). Nevertheless, this feature may alone characterize the Ficoideae without the bridge of *Glinus* as envisaged by Raghavan & Srinivasan (1940) in view of the periclinal divisions observed in *Glinus lotoides*.

Features like the presence of bitegmic and campylotropous ovule with an air space between the outer and inner integuments at the base, the micropyle consisting of only the endostome, presence of curved embryo enclosing massive perisperm on the inner side, thin layer of endosperm at the micropylar region capping the radicle of embryo, seed coat comprising the outer integument but only the inner layer of the inner integument as in the Aizoaceae, are also met with in the Chenopodiaceae (Bhargava, 1936 ; Mahabale & Solanky, 1954), Amarantaceae (Joshi & Rao, 1934 ; Joshi & Kajale, 1937), Phytolaccaceae (Joshi, 1936), Portulacaceae (Kajale, 1942) and Caryophyllaceae (Bakshi, 1952). Similarly, the presence of air space at the base of integuments, the formation of micropyle by the endostome, the presence of a curved embryo enclosing the perisperm on the inner side, hard seed coat formed by the outer layer of the outer integument and inner layer of the inner integument together with the presence of tannin-like substances in both these layers (Maheshwari, 1950 ; Maheshwari & Chopra, 1955), indicate a close affinity of the Aizoaceae with the Cactaceae.

Summary

In the Aizoaceae the ovules are bitegmic, campylotropous and crassinucellate. The micropyle is organised by the endostome. The inner integument is two-layered but massive in the micropylar region. As the seed matures the outer layer gets crushed and obliterated leaving behind the inner layer which becomes strengthened either by fibrous or uniform thickening. The outer integument is two- to three-layered in the middle but massive at the extremities. The cells of the outer layer distend either radially or tangentially and develop thickenings with circular or irregularly-shaped thin areas whose alignment imparts a definite pattern. The presence and size of aril are variable. The nucellus is curved. The epidermal and subepidermal cells at the micropylar end divide periclinally

to form a nucellar cap which gets absorbed gradually in the mature seed. In *Trianthema pentandra* cells of the upper layer at the nucellar apex extend radially, develop thickenings and persist for a long time forming an epistase-like tissue. In *Orygia* a hypostase is formed at the base of nucellus. In general the structure of the seed in the Aizoaceae is in conformity with the other families of Centrospermae and also shows great affinities with the Cactaceae.

My sincere thanks are due to Professor B. Tiagi for facilities and encouragement during this investigation.

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MORPHOLOGICAL VARIATIONS IN GROWTH AND DIFFERENTIATION OF EMBRYOS *IN VITRO*

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Our knowledge on the origin of the embryo, its architecture and the ultimate destinies of the various embryonal parts is quite extensive and almost complete ; but that of the growth behaviour of young embryos excised from their confines in the ovule or seed and cultured artificially under *in vitro* conditions, is rather inadequate and fragmentary. This is because investigations on embryo culture have mostly been physiological with no adequate attention to the histo-morphological changes accompanying embryo differentiation. It has been found that in artificial cultures, embryos seldom follow the normal path and develop typically or atypically in different circumstances. Although there is no direct reference in literature to growth patterns as such, various authors have called attention to one phase or the other of abnormal organogenesis *in vitro* but there has been no consolidated account on the subject. Abnormalities of embryos could be caused by anomalous environmental conditions especially with chemicals as Mizushima *et al.* (1956) have shown in their investigations on the rice plant.

Similar studies of growth in culture of excised portions of lupine embryos from mature seeds have been carried out under different cultural conditions involving the use of NAA and adenine sulphate (Lee, 1955 a) and the regenerative capacity of the fragments recorded. Potentially unlimited growth of lupine callus was obtained from a fragment A (about 0.5 mm) and has been maintained for over three years (Lee, 1955 b). Tissue fragments of vernalised seeds of mustard T.102, cultivated *in vitro*, have regenerated complete plants especially from apical meristems or seedling hypocotyls (Sen & Verma, 1957). Furuya (1956) and Furuya & Soma (1957) have investigated the direct effects of auxins on the development of bean embryos cultivated *in vitro* and the behaviour of decotylated bean embryos or its excised portions under several experimental conditions.

The nutritional requirements of an embryo are complex. The younger the embryo is on excision, the more abnormal is its growth behaviour. The purpose of this paper is to present an account of abnormal organo-

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genesis of embryos in media containing known growth chemicals as compared to that *in vivo*.

Attempts to induce the unfertilised egg to develop into a haploid organism have not been successful. Heslop-Harrison (1957) tried to induce parthenogenetic development of the egg in unfertilised ovules of *Dactylorchis* by the application of NAA but without success. The single-celled zygote, or embryos at the quadrant or octant stages and even the multicellular proembryo fail to develop to maturity *in vitro*. Culture of proembryos of orchids gave undifferentiated tissue masses capable of apparently indefinite growth (Curtis & Nichol, 1948). Normal morphogenesis was considerably affected with the use of certain barbiturates in the media (Curtis, 1947).

Literature on embryo culture pertains mostly to experiments conducted to obtain successful growth of young immature embryos into seedlings. Nearly mature embryos grow normally in a medium containing only minerals, water and sugar solidified by agar ; but if young enough (less than 0.5 mm) when planted, these either fail to grow or show abnormal organogenesis, unless supplemented by trace elements, vitamins, hormones and embryo factors. The tendency of young embryos to develop abnormally in culture and to germinate precociously into a miniature weak seedling instead of passing through the normal embryonic growth has been observed by many workers.

Coconut water (liquid endosperm) has largely been used as a growth adjuvant in embryo culture. However, the results obtained have been at variance depending upon whether coconut milk was autoclaved or Seitz-filtered ; whether milk from tender or mature coconut was used and its concentration. Norstog (1956) cultured embryos of barley (smallest size, 0.15 mm-0.2 mm) using 90 per cent Seitz-filtered milk. The embryos grew as small balls of undifferentiated tissue, akin to callus, later producing roots and leaves. Investigations to obtain undifferentiated tissue of embryos for subsequent demonstration of host-parasite relationships of *Claviceps purpurea* and rye have been carried out by Carew & Schwarting (1958). Embryos of rye, 10 to 20 days old, were grown on a modified Heller's medium containing 2 per cent sucrose, 0.5 per cent yeast extract and 1 mg/l, 2,4-D. Callus tissue of unlimited growth, capable of being subcultured, was obtained on this medium.

Seitz-filtered liquid endosperm from young green coconuts promoted normal growth of embryos of *Cocos nucifera* but mature endosperm proved inhibitory (Cutter & Wilson, 1954). Extensive morphological variations have been observed in cultures of coconut embryos in a variety of treatments involving the use of coconut milk but these have not been thoroughly investigated.

Datura embryos (2 mm size, 14 days after pollination) never developed cotyledons on agar media containing mineral salts and sugar only, but

with the addition of Seitz-filtered coconut milk, viable seedlings were produced. However, non-autoclaved coconut milk precluded root formation, but caused enlargement and thickening of cotyledons and hypocotyl accompanied by lobe formation and branching (van Overbeek *et al.*, 1942) of heart-shaped embryos and embryos less than 0.25 mm. in size. With autoclaved coconut milk, the young embryos grew as unorganised masses. Tetraploid embryos of *Datura* of the same size and shape induced a callus type of growth showing no differentiation in a medium containing autoclaved coconut milk. Halved ovules containing embryos did not grow but caused considerable callus formation at the cut surfaces. Ziebur & Brink (1951), however, report that addition of coconut milk to the culture medium did not have any favourable effect on the growth of immature barley embryos.

In investigations on the morphogenetic response of immature grass embryos of some of the cultivated Indian millets like *Pennisetum* (Narayanaswami, 1959 a, b), *Echinochloa* etc., certain features were observed which are worthy of report. In both liquid and agar media using White's solution (White, 1943) to which adenine in concentrations up to 60 ppm was added, overgrowths of scutellum of *Pennisetum* embryos were observed, compared to that of the control in which the growth was normal. Incorporation of even as low as 1 ppm of adenine, caused the scutellar tumor. The growth of the scutellum was accentuated by the addition of exogenous IAA (2 ppm). In such embryos the embryonal axis was inhibited in growth. Another substance of the purine group, viz. guanine, also gave identical results. Both the substances serve to furnish a factor for the growth of scutellum *in vitro*. In embryos showing overgrowths of scutellum, when transferred to a medium containing coconut milk (20-40 per cent), adenine (40 ppm) and IAA (2 ppm), enhanced growth of the scutellar tissue was observed, followed by the inhibition of the root-shoot axis. This would indicate the possibility of obtaining growth of the scutellum for unlimited periods.

Culture of the embryonal axis alone without the scutellum showed some growth in size but the plumule never emerged out of the coleoptilar sheath even after 2-3 weeks of treatment nor were lateral roots ever observed to develop. LaRue (1952) explored the possibility of prolonging the embryonic growth of scutellum alone *in vitro* by excising the plumule-radicle axis of maize embryos. Significant increase in wet and dry weights of scutelli over the control was observed though not over that from ripe seeds. LaRue's experiments also indicated the formation of outgrowths of the nature of partial roots or partial stems.

Culture of the whole caryopsis of *Pennisetum* and wheat containing young differentiating embryos was made in WM containing kinetin (0.5 ppm), IAA (2 ppm) and adenine (40 ppm). The scutellum grew into a

tumor tissue showing bizarre shapes followed by degeneration of the embryonal axis.

Supernumerary buds, each enclosed within separate coleoptiles originating from the mesocotyl region, and development of twin buds in the region of the shoot apex were other abnormalities of the embryos of *Echinochloa* in media containing adenine (40 ppm).

Another phenomenon observed was the rooting of scutellum in artificial cultures. Root-like processes were formed from the tissue in liquid media to which 2,4-D (5 ppm) and IAA (2 ppm) were added but not in treatments of either 2,4-D or IAA alone. Rooting was also initiated in old cultures of tumoroid scutelli of *Pennisetum*. Stimulus to endogenous root formation from the perivascular sheath of the scutellum was indicated in cultures containing tomato juice or coconut milk (15-20 per cent). Profuse rooting of maize embryos was observed in WM containing 2,4-D (0.5 ppm). Endogenous root primordia were initiated in abundance from the scutellum of immature embryos of *Echinochloa* in cultures of 'entire' caryopsis, akin to the initiation of roots from petioles of leaves or of stem cuttings kept in damp chambers.

Submerged cultures of *Avena* embryos in liquid medium (WM) showed strikingly elongated coleorhizeae but not so on those that were floated or inoculated on agar surface. Coleorhizal elongations of excised oat embryos (1.0-1.5 mm) cultured in WM have been reported by Norstog (1955). Elongation was found to be inversely proportional to the amount of air present in the immediate environment, greatest length being attained in liquid media. Lower concentrations of IAA (0.1-1.0 ppm) in liquid cultures stimulated coleorhizal elongation while higher concentrations (5.0-10.0 ppm) proved inhibitory. Coleorhiza detached and cultured on White's agar medium showed no elongation but hairs were produced in abundance from the exposed portions of the epidermis. Greatly lengthened coleorhizeae in subsurface cultures of excised oat embryos were reported by Dietrich (1924). Considerable swelling of the coleorhiza to form a spongy structure by hypertrophy of the cells was observed in my studies with *Pennisetum* and *Triticum* embryos in WM to which kinetin (0.2 ppm) and IAA (2 ppm) were added.

Experimental studies on growth of excised grass embryos in a medium containing maleic hydrazide (MH) gave interesting results. All concentrations of MH proved fatal to root meristems in treated embryos of all ages and of several differentiating phases. The group of apical initials in the plerome region was necrosed. The calyptrogen layer, if formed, was in a state of collapse. The scutellum had swollen up largely due to radial elongation of its cells (Narayanaswami, 1959b).

Culture of embryos undergoing differentiation in media with low concentrations of MH (10^{-6}M and 10^{-7}M) resulted in abnormal growth. The root-shoot axis developed protuberant masses of parenchyma over

its entire length indicating distinct centres of growth activity. Fasciated embryos were thus formed. Others developed swellings from the tips of the coleoptile. The present evidence indicates that the younger the embryo is the more abnormal is its growth behaviour (*see also* Tukey, 1938).

Surgical treatments of embryos indicated certain features worthy of record. Excised scutelli of *Pennisetum* grew in culture by cell proliferation around the procambial strand in WM containing coconut water (20 per cent) and IAA (1 ppm). If excision of the root-shoot axis were complete, a new root was initiated mostly from the cut end as has also been reported by Blociszweski (1875) in his experiments with maize, rye and oats. Abundant callusing from the wounded end of the scutellum on nutrient agar followed by initiation of fresh roots was observed. Papillate surface outgrowths from the scutellum of maize were noted by LaRue (1952) on a medium containing tomato juice. However, no regeneration of adventitious buds ever occurred.

Excision of primary root resulted in the development of seminal roots in barley embryos, which grew out as elongated, wiry structures bearing lateral roots. van Tieghem (1873) reported extensive development of adventitious roots in maize embryos but no adventitious buds were ever formed. This would indicate that the isolated scutellum is incapable of independent development. Excision of part or all of the cotyledons of an embryo of the weeping crab apple had no effect on subsequent germination and growth of the seedlings provided the stem and root-growing points were not injured (Nickell, 1951).

INFLUENCE OF AUXIN—Loo & Wang (1943) observed cell divisions to a limited extent in young embryos of *Pinus yunnanensis* and *Keteleeria davidiana* caused by the addition of IAA (10 ppm) or thiamin in the culture medium. Embryos of *Capsella bursa-pastoris* were inhibited by IAA at 1 to 10 ppm, but at 10^{-3} ppm, slight stimulation was observed (Rijven, 1952).

Tukey's (1938) investigations on embryos of peach, apricot etc., did not give a consistent response at the concentrations used. van Overbeek *et al.* (1942) suggested that IAA is responsible for a callus-like growth and root inhibition of immature *Datura* embryos grown on a medium containing coconut milk. Rietsema *et al.* (1953) have obtained more detailed data on the effect of IAA on embryo growth *in vitro*, including histological responses of young and late heart-shaped embryos, young torpedoes and nearly mature ones. The elongation of cotyledons proved to be largely independent of the auxin concentration. Concentrations lower than 10^{-2} ppm did not affect the length. The hypocotyls were slightly more susceptible to IAA. With 1 ppm their growth was 40 per cent more than that of the control for the smallest embryos. With larger embryos, they looked abnormal, being swollen and brittle. Some inhibi-

tion was observed at 10^{-2} ppm IAA in the case of nearly mature and very small embryos but lower concentrations did not show any appreciable effect. In contrast to the cotyledons and the hypocotyls, the roots never developed at the beginning of the experiments, although the radicle was histologically differentiated. However, they developed *in vitro* only when the hypocotyl attained a length of at least 3 to 4 mm and at extremely low concentrations (10^{-5} to 10^{-7} ppm) but were completely inhibited at 0.1 ppm, 10^{-2} and 10^{-3} ppm.

In cultures of nearly mature bean embryos, Solacolu & Constantinesco (1936), Gauthéret (1937) and Gardiner (1940) report a swelling of the hypocotyl at auxin concentrations of 1 to 100 ppm and its eventual growth into a tumoroid unorganised mass of cells. Similar results have been reported by Hamner & Kraus (1937) with bean seedlings.

The influence of gibberellic acid and IAA on cotton embryos cultured *in vitro* for a 10-day period has been studied by Dure & Jensen (1957). The younger embryos did not undergo any cell division or elongation under any treatment although the cells of the cotyledons expanded by water uptake whereas slightly older ones showed some cell division. The only synergistic effect found in the IAA/GA culture was in water uptake.

The embryo growth *in vitro*, therefore, is inhibited by IAA at 10^{-2} ppm and higher. This is more so with regard to longitudinal growth. No visible effect is observed on lower terminal portion of the hypocotyl and complete lack of meristematic tissue precludes epicotyl formation. An increase in width of the cotyledons occurs, young embryos are more sensitive than the advanced ones and IAA acts as a growth stimulator at low concentrations.

Rappaport *et al.* (1950) report an irregular twisting and bending of *Datura* embryos with the use of NAA (0.5 to 10 ppm). Seeds of *Eranthis hiemalis* containing pear-shaped undifferentiated embryos at shedding, soaked in 0.1 per cent solution of 2,4-D, gave grades of syncotyly and pleiocotyly and twinning in nearly every case (Haccius, 1955).

Influence of Amino Acids

Embryos of *Datura* grown on enzymatic casein hydrolysate (Paris *et al.*, 1953) were often translucent especially when excised at a very young stage ; the cotyledons were diverging and the shape was very often abnormal. Frequently brown spots and anthocyanins were present in the post-heart stages. Embryos cultured at the bent torpedo or nearly mature stages showed normal growth. On *d*-glutamic acid, the embryos showed toxic effects. They were transparent, brown-spotted or showed anthocyanins. The cotyledons, if ever developed, were diverging. Modifications in the proportion of embryos increasing or decreasing the size of the cotyledons and the number of root primordia were caused by

some individual amino acids or small groups of them (Sanders & Burkholder, 1948).

Addition of casein hydrolysate to the culture medium caused a favourable effect on embryo differentiation. Adventive embryos originating from the nucellus of *Citrus* ovules inoculated along with the nucellar tissue on WM supplemented by 400 ppm casein hydrolysate (vitamin free, acid hydrolysed CH) produced abundant callus masses designated as 'pseudobulbils' from the proliferation of proembryos and the cells of the nucellus (Ranga Swamy, 1958). Enhanced growth was observed if kinetin was incorporated in the medium. In 2-month old cultures, fasciated seedlings were observed with all grades of abnormalities of the cotyledons and hypocotyl. In cultures of 'entire' ovules of the post-pollinated stages using WM with 5 per cent sucrose, or a medium containing tomato juice, considerable morphological variations in the development of the proembryos were observed, a few showing a tendency to produce extra-embryonal masses from the suspensor end (Ranga Swamy, 1959). However, such embryos underwent no differentiation either on coconut milk or malt extract.

Summary and Conclusions

Embryo development in artificial cultures may follow normal or abnormal paths in different circumstances. Sanders (1950) working with embryos of *Datura* noted several abnormalities: (1) Failure to grow or unusual differentiation, (2) Tissue swelling into undifferentiated masses, (3) Swollen, spreading cotyledons, (4) Multiple and fasciated embryos, (5) Premature differentiation of roots and plumules and (6) Irregular bud-like outgrowths showing unlimited power of producing buds. When normal embryonic differentiation is arrested and the apical meristem is affected, the renewed growth proceeds from several growing points. The degree of differentiation of the embryo at excision is a chief factor determining the type of growth produced in culture. Embryo culture experiments have also demonstrated the independence of growth developments in plumule, hypocotyl and radicle, their individual nutritional requirements being different quantitatively and qualitatively. It is not surprising that some parts of the embryo may become extensively modified in relation to the supply of nutrients. By varying the concentration of nutrients provided and by their absence in the medium, the development of embryos could be altered. The latent potentiality for division evokes a characteristic response in the embryo, 'the intrinsic behaviour of the embryo being susceptible to environmental alteration'. Growth in an artificial medium is dependent not only on the genetic make up of the embryo but also upon its stage of development and condition at the time of excision. The study of factors responsible for inducing abnormalities

in embryos in the culture media may contribute towards an understanding of those responsible for normal development.

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CYTOLOGY AND SYSTEMATICS OF INDIAN ORCHIDACEAE

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Orchids are a highly popular group of flowering plants and are universally accorded a coveted rank in nature from an artistic and aesthetic point of view for the extraordinary beauty and novelty of their flowers. Although the Orchidaceae are cosmopolitan in distribution having a total of about 15,000 species and varieties in the world flora, they attain their highest development in the humid tropical and subtropical forests of both the hemispheres.

Orchids in nature are very often the 'barometer' of climatic types, the latter in turn serving as limiting factors to their geographical distribution. Most of the orchids require 60 to 80 per cent relative humidity to enable the aerial roots to absorb optimum quantity of moisture from the atmosphere with the help of their sponge-like velamen. A minimum of 21°C (70°F) day temperature and 12.7°C (55°F) night temperature is necessary to carry on the vital processes of photosynthesis and respiration at satisfactory rates. These conditions limit the distribution of orchids noticeably in nature. Accordingly, millions of orchids grow in the rain forests of humid tropics the world around, particularly in Mexico, Bolivia, Peru, Venezuela, Central Africa, South Asia and Australasia.

During our field studies over the last five years, special attention has been paid to look for orchids in their wild home and to collect them for cultivation with adequate field notes on the locality, habitat and the colour and peculiarity of their blossoms.

Among the areas visited so far, it has been observed that orchids as epiphytes are very prolific in the moist tropical and sub-tropical evergreen forests occurring in the lower altitudes of the foot hills of the North-east Frontier Agency (N.E.F.A.) and in the Lakshimpur, Sibsagar, Darrang and Cachar districts as well as the Khasi and Jaintia Hills of Assam (India). Although the orchids can very well stand the tropical moist to dry climatic conditions occurring in the forests met within Goalpara, Kamrup, North Cachar, Nowgong and Darrang Districts of Assam, they show their optimum development in altitudes between 1340 to 1700 metres. They grow profusely in the Khasi and Jaintia Hills, in the Patkoi ranges in the Naga Hills, and Tirap Frontier Division of N.E.F.A. Orchids occur from time to time in altitudes ranging to 2,400 m. beyond which they become gradually rarer. Indeed, only a few epiphytes ascend higher and reach the wet temperate forests met with in the sub-Himalayan

ranges. On the other hand, the grasslands of Khasi and Jaintia Hills on way to Cherrapunjee, Rupa valley in Kameng Frontier Division, N.E.F.A., as also the sandy alluvium of Rebna and Gandhamardan Reserved Forests in Orissa support prolific development of terrestrial orchids.

While the epiphytic *Vanda,* including the blue *Vanda caerulea* of commerce, has been bagged from the Naga Hills and parts of N.E.F.A., *Vanda teres,* from which the teres vandas of Hawai are derived, are common place orchids in the plains of Assam. The old world genus *Dendrobium* with its evergreen and deciduous species together with species of *Cymbidium, Coelogyne, Aerides,* and *Rhyncostylis,* add colour and grandeur to the moss and fern clad trees of oaks, firs, rhododendrons etc. in the deep forest as well as to the damp mossy rocks on the banks of streams. These include *Dendrobium chrysanthum, D. fimbriatum, D. lituiflorum, D. moschatum* and *D. nobile, Cymbidium grandiflorum* with its drooping racemes of 5-12 very large sweet-scented, pale yellow-brown flowers, species of *Coelogyne* with their fragrant white flowers and variable confluent of orange-yellow markings on the lips, *Rhyncostylis retusa* and *Aerides multiflorum* with crimson-red pendulous racemes with sweet-scented flowers.

On the other hand, *Phaius tankerviliae* (Aiton) Bl. (=*P. wallichii* Hk.f.) is a tall terrestrial orchid with scapes several feet high producing large attractive blossoms which have been collected from different parts of Assam. The saprophytic orchid *Galeola falconeri* Hk.f. growing on humus and litter covered forest floor at 1300 m. at Sissini and Niusa in N.E.F.A. possesses brownish yellow flowers on scapes about 3 m. high.

Genera Represented

The survey for orchids has yielded 64 genera and 322 species, out of the 85 genera and 507 species reported by Hooker (1888-1890) for areas now included in the Eastern Circle of the Botanical Survey of India. Hooker (1888-1890), in his 'Flora of British India', described 1235 species belonging to 117 genera, arranged in a system of classification which recognises 5 tribes and 20 subtribes. But 18 of his genera include 350 species, out of the 507 species belonging to 85 genera as reported by him from Eastern India. Table I shows the relative position of genera with regard to number of species as recorded by Hooker, and by the Botanical Survey of India, Eastern Circle, collected up to date. An analysis of the species identified establishes 29 species as new records for Assam and N.E.F.A., 2 species for Eastern India, and 9 species as new records for India (Table II). Similarly, it appears that *Dendrobium, Habenaria, Eria, Coelogyne,* and *Bulbophyllum,* are the richest in species in Eastern India, many of them extending to Malaya in the south. On the other

hand *Sunipia* and *Ceratostylis* are rarer elements in our flora and have been collected only from Nongkhlaw and Digboi of Assam, respectively.

TABLE I

DOMINANT ORCHID GENERA AND THE NUMBER OF SPECIES IN EASTERN INDIA

Genus	Number of species recorded by Hooker (1888-1890) for Eastern India	Number of species recorded by Hooker (1888-1890) in Flora of British India	Genus	Number of species collected by Botanical Survey from Eastern India up to 1960
Dendrobium	61	158	*Dendrobium*	45
Habenaria	41	106	*Eria*	24
Eria	29	94	*Coelogyne*	23
Bulbophyllum	31	79	*Bulbophyllum*	18
Liparis	31	59	*Habenaria*	15
Coelogyne	24	55	*Liparis*	15
Saccolabium	19	43	*Saccolabium*	15
Calanthe	18	34	*Calanthe*	13
Oberonia	15	41	*Cymbidium*	12
Eulophia	14	26	*Oberonia*	12
Cirrhopetalum	13	34	*Cirrhopetalum*	8
Cymbidium	11	15	*Eulophia*	8
Goodyera	11	15	*Vanda*	7
Sarcochilus	8	34	*Sarcochilus*	6
Vanda	8	15	*Phaius*	6
Microstylis	6	22	*Pholidota*	5
Aerides	5	15	*Sarcanthus*	5
Cleisostoma	5	15	*Aerides*	5

Table I shows that these 18 genera comprise 350 species out of the 507 species belonging to 85 genera found in Eastern India. Similarly, the same 18 genera contain within them 860 species out of the 1235 species belonging to 117 genera recorded by Hooker from India, Burma, Malaya and Ceylon. An analysis of our collections from Eastern India shows that the above 18 genera also comprise 242 species out of 322 species belonging to 64 genera collected by Botanical Survey of India, Eastern Circle, up to March, 1960.

A thorough analysis of Vols. V and VI of "Flora of British India" reveals that out of the 117 genera recorded and described by Hooker, 23 genera are not found in India at all, 9 genera, although occur in the rest of India, have not been reported from the areas included in the Eastern Circle of the Botanical Survey of India (*see* Table III). Yet 3 more genera, viz. *Anthogonium*, *Biermannia* and *Phyllomphax*, subsequently described after the publication of "Flora of British India" have now been collected by us from different parts of the Eastern Circle.

Despite this richness of orchid flora of Eastern India as a whole and of Assam and its surroundings in particular, not much floristic work has

yet been done on this family after Hooker (1888-1890). King & Prantling's (1898) publication of the "Orchids of the Sikkim Himalayas" marks the second important landmark on the progress of our knowledge about Indian orchids. Although this work is very comprehensive in its nature, and many of the species and genera reported from Sikkim are also represented elsewhere in Eastern India, it can at best be treated as a regional flora only. Similarly, Hooker (1895), Duthie (1906), and Brühl (1926) have made further contributions to our knowledge of the orchids of India and Burma. After this only a few more notes have been published on orchids of Manipur and Sikkim by Ghose (1953), Deb (1957), Kingdon Ward (1948) and Mukerjee (1953), whereas Mitra's (1958) volume of 'Monocots of Eastern India' hardly adds anything new beyond the facts recorded by Hooker.

TABLE II

NEW RECORDS FOR INDIA

Name of species	Herbarium sheet number		Locality of collection
Bulbophyllum penicillium Par. & Reichb. f.	Panigrahi	21926	Jorain, Jowai, Assam
Calanthe diploxiphion Hk. f.	Panigrahi	8526	Palaspal, Orissa
Coelogyne carnea Hk. f.	Panigrahi	4998	Dumpep, K. & J. Hills, Assam
	Rolla	17757	Minguing, Siang Front. Div. N.E.F.A.
Dendrobium pendulum Roxb.	Deka	M99	Lushai Hills, Assam
D. chrysotoxum Lindl.	Panigrahi	15051	Niusa, N.E.F.A.
		19508	Kimin
Eulophia holochila Colf & Hendel.	Bor	22183	Assam
Geodorum citrinum Jackson	Panigrahi	4917	Cherapunji, Assam
Oberonia tahitensis Lindl.	Sharma	20316	Peak Forest, Khasi & Jaintia Hills, Assam
Sarcochilus hystrix Reichb.f.	Deka		Sairang, Khasi & Jaintia Hills, Assam
NEW RECORDS FOR EASTERN INDIA			
Cirrhopetalum acutiflorum Reichb. f.	Deka	10205	Dumpep, Khasi & Jaintia Hills, Assam
Liparis duthiei Hk. f.	Deka	12765	Sweet Falls, Khasi & Jaintia Hills, Assam

Schlechter (1926) has, however, proposed a new system of classification recognising four tribes and 80 subtribes for the 15,000 species of orchids in the world flora. Since Schlechter combines the importance of reproductive structures (*see* Bentham & Hooker, 1883 ; Lindley, 1830-1840) with the easily observable differences in the vegetative structures (*see*

Pfitzer, 1887) and his classification provides a very comprehensive treatment of all the genera and species, his system is now widely accepted and is considered as an improvement on those of Lindley and Bentham & Hooker. Certain species of *Cleisostoma, Microstylis, Pogonia* and *Saccolabium* of Hooker, are now referred to *Ascocentrum, Malaxis, Nervilia* and *Acampe* respectively by Schlechter and have been adopted as such by Holttum (1957). Similarly, the generic identity of *Habenaria, Peristylus* and *Platanthera,* is much in question.

The last 20 years' research on orchids has created new problems for taxonomic theory, which pose a real question as to the ultimate validity of a 'natural' classification based on morphological types. There is wide variation of vegetative, reproductive and floral characters in the orchids. Similar flowers are seen in widely different genera with dissimilar vegetative characters while dissimilar flowers are sometimes found within the same genus. In some of the polymorphic genera, there is almost as much variation between individuals as there is between species. Differences in the number of pollinia such as four in *Cattleya* and eight in *Laelia* are considered to be of generic rank in certain groups, despite the occurrence of both two and four pollinia in the genus *Eulophia.* Similarly, within the same species of *Habenaria,* the structure of the pollinium differs a great deal.

Dressler & Dodson (1960) have described Schlechter as "splitter", who followed narrow concepts for his classification emphasising differences rather than resemblances. Schlechter's system, according to them, was mainly analytical aimed at identification.

TABLE III

ORCHID GENERA

Not found in India	Not found in Eastern India
Acriopsis, Bromheadia, Claderia, Corysanthes, Cystorchis, Dendrochilum, Dipodium, Dossinia, Drymoda, Glomera, Grammatophyllum, Haemaria, Henosis, Hylophila, Ipsea, Microsaccus, Monomeria, Mystacidium, Neuwiedia, Platyclinis, Plocoglottis, Thecostele, Trichoglottis	*Corallorhiza, Cottonia, Diplocentrum, Disperis, Hemipilia, Josephia, Polystachia, Trias, Vanilla*

Considering, therefore, the artificial nature of the systems of classification and realising the necessity for the application of the international rules of botanical nomenclature to the family Orchidaceae, Dressler & Dodson (1960) have proposed a system of classification which recognises five tribes and 40 subtribes within 2 subfamilies (*see* Table IV). They claim

their system as 'synthetic' and the nomenclature used by them for these categories as in conformity with the rules (Lanjouw, 1956) including the ones added by the International Botanical Congress held at Montreal in 1959.

TABLE IV

CLASSIFICATION OF ORCHIDACEAE (DRESSLER & DODSON, 1960)

Subfamily Cypripedioideae
Tribe 1. Apostasieae
Tribe 2. Cypripedieae

Subfamily Orchidoideae
Tribe 3. Neottieae
Subtribe Limodorinae
Chloracinae
Rhizanthellinae
Pterostylidinae
. . . . . .
Neottiinae
. . . . . .
Diuridinae
Cryptostylidinae
Prasophyllinae
. . . . . .
Spiranthinae
a. *Tropidia* alliance
b. *Goodyera* alliance
c. *Spiranthes* alliance
d. *Cranichis* alliance
Tribe 4. Orchideae
Subtribe Epipogiinae
Orchidinae
Disinae
a. *Disa* alliance
b. *Satyrium* alliance
Coryciinae
Tribe 5. Epidendreae
Subtribe Vanillinae
Gastrodiinae

Tribe 5. Epidendreae (contd.)
Subtribe Pogoniinae
Sobraliinae
Thuniinae
Arethusinae
Bletiinae
Collabiinae
Coelogyninae
Epidendrinae
a. *Epidendrum* alliance
b. *Eria* alliance
c. *Polystachya* alliance
d. *Glomera* alliance
e. *Podochilus* alliance
f. *Arpophyllum* alliance
Pleurothallidinae
Adrorhizinae
Thelasiinae
Ridleyellinae
Liparidinae
Dendrobiinae
Genyorchidinae
Thecostelinae
. . . . . .
Cyrtopodiinae
Catasetinae
Cymbidiinae
Sarcanthinae
Stanhopeinae
Maxillariinae
a. *Zygopetalum* alliance
b. *Lycaste* alliance
c. *Maxillaria* alliance
Pachyphyllinae
Cryptocentrinae
Oncidiinae
a. *Oncidium* alliance
b. *Ornithocephalus* alliance
c. *Dichaea* alliance
Subtribes (uncertain position)
Grobyinae
Pachyplectrinae

Realising the value of experimental studies in elucidating problems of orthodox taxonomy, cytological investigations involving about 370 species belonging to 84 genera, mostly from temperate countries, have been made by different workers. Cytological studies on the old world genera, parti-

cularly from South Asia including the reports by Sharma & Chatterjee (1960), however, are confined barely to a score of genera (*see* Table V).

TABLE V

Plant	Haploid number of chromosomes	Diploid number of chromosomes
Paphiopedilum venustum		40×
P. fairieanum	13	
P. hirsutissimum	13	
P. insigne	16, 12, 13, 8, 9	26, 14
Dendrobium aggregatum		38
D. chrysotoxum		40
D. fimbriatum var. *oculatum*	19*	38
D. heterocarpum		38
D. moschatum		38 plus 3 fragments
D. nobile	19	38, 76
D. parishii		40
D. pierardii	19	
D. primulinum		38
Aerides affine	19	38, 40
Luisia sp.		40
Vanda parishii		38
V. tesselata		38
V. teres	19	38
Ascocentrum sp.		38
Sarcanthus sp.	18	
Doritis sp.	38	38
Phalaenopsis sp.		114, 40, 38
Calanthe		40
Rhyncostylis retusa		38
Renanthera coccinea		38
Cymbidium aloifolium		40
Oncidium lanceanum		26
Phalaenopsis mannii		38

* Preliminary observation by J. Joseph of this Department.
× Preliminary observation by S. K. Kataki of this Department.

An analysis of the cytological phenomenon in the family brings to light the following four trends in their evolution:

(*a*) All species within the same genus have the same chromosome numbers, viz. *Vanda*.

(*b*) Chromosome sets of different species within the genus differ by an additional chromosome type, viz. *Dendrobium*.

(*c*) Typical aneuploidy within the genus, viz. *Paphiopedilum*.

(*d*) Typical euploidy, viz. *Dactylorchis* with n=20 to n=120 ; *Platanthera bifolia* n=21 ; *P. obtusata* n=63 ; *Gastrostachys* (*Spiranthes*) *gracilis* n=15 ; *S. cernua* n=30.

It is seen that triploidy confers best in stiffness and keeping quality for cut flowers whereas pentaploidy acts in a reverse manner.

Cytological studies reveal that 23 genera are characterised by a haploid number n = 20, while the rest vary in their haploid number between 10 and 41, although in majority of the genera 'n' lies only between 16 and 24.

Table VI lists the variation in haploid chromosome numbers in the different groups of Schlechter's (1926) system:

TABLE VI

Name of tribe/group	Number of species		Different haploid numbers for the tribe or group, i.e. n	Generalised basic number for the group or tribe	Karyotype
	Total no. in World flora	Cytologically studied			
(1)	(2)	(3)	(4)	(5)	(6)
Diandrae	72	45	10, 11, 13-21, 29	12	Very large chromosomes
Basitonae	1063	78	10, 12, 14, 18-21, 30, 31, 40-42, 63	13	Very small chromosomes
Polychondrae	1894	55	11-13, 15-22	11	2 groups *_Goodyera_, _Spiranthes_: Very small chromosomes *_Listera_, _Epipactis_: Very large chromosomes
Acranthae	4152	77	14-18, 20, 21, 30, 40	9	Too small chromosomes (viz. _Dendrobium_, _Liparis_, etc.)
Sympodiales	3607	80	14, 16, 18-21, 24, 26, 28, 40	10	
Monopodiales	1132	35	19, 20, 26	3	Chromosomes compact, short but not minute

* Therefore, Polychondrae are polyphyletic and unnatural.

A survey of Table VI establishes the following general conclusions:

(*a*) The number of species cytologically investigated is too inadequate to draw valid phyletic conclusions, and more species, particularly the epiphytic ones in the rain forests of the tropics need intensive investigations.

(*b*) Although the generalised basic chromosome numbers for different groups appear to be quite distinct, a critical examination of column (4) in this Table does not unequivocally establish the basic number attributed to the respective groups.

(*c*) It is not the distinctiveness of chromosomes but the karyotypes of the group which appear to serve as isolating barriers between the various subfamilies and tribes in Schlechter's system (*see* Withner, 1959, and Fig. 1).

Yet the experience of horticulturists in successful hybridisation and introgression in the family, often between genera of different groups and tribes, and the discovery of plentiful natural hybrids in the field, lead one to presume that the evolutionary rate in orchids may have been too rapid for effective establishment of isolating barriers. It may also be that presence of isolating barriers may have a long term disadvantage in restricting the recombination types available for further adaptation. The great ease in synthesising intergeneric and multigeneric crosses within Sarcantheae, for example, offers splendid opportunities for obtaining material for analysing karyotypic differences between related orchid species and genera, an advantage not available elsewhere in the whole of the plant kingdom.

Yet the non-availability of any genetical data on the various species and genera of orchids despite extensive hybridisation, by professional horticulturists, though understandable, leaves a serious gap in comprehending the evolutionary trends within the family.

Accordingly, despite the phylogenetic inconsistencies within the 'natural' classifications of the family Orchidaceae, the time is not yet ripe for substituting it by a biosystematic classification based on cytotaxonomic findings.

Considering, therefore, the inadequacy of the knowledge in the systematics and taxonomy of the Indian Orchidaceae in sharp contrast to the richness of the orchid flora of Eastern India, the Government of India have established the National Orchidarium in Shillong, under the auspices of the Eastern Circle of the Botanical Survey of India. The scientific objective of the Orchidarium is to conduct a thorough systematic revision of the Indian Orchidaceae with a view to determine the number of different genera and species of orchids in our flora, as well as their ecological habitat and the range of distribution in the world flora as a whole. Hybridisation (both inter-specific and inter-generic) will be carried out, preceded and followed by cytological analysis. The cytology of the tropical orchid species is largely undetermined and there is practically no genetical data available on orchids, despite prolific hybridisation (both natural and artificial) in the family. It is, therefore, our principal objective to carry out a comprehensive cytological survey of the Indian species and genera and to plan a systematic hybridisation programme, for evolving new varieties and multigeneric crosses, not merely to unravel the phyletic relationships between the groups, but also for their introduction to the world of horticulture. This programme aims at exploiting the existence of natural self-sterility in the family which is characterised by obligate

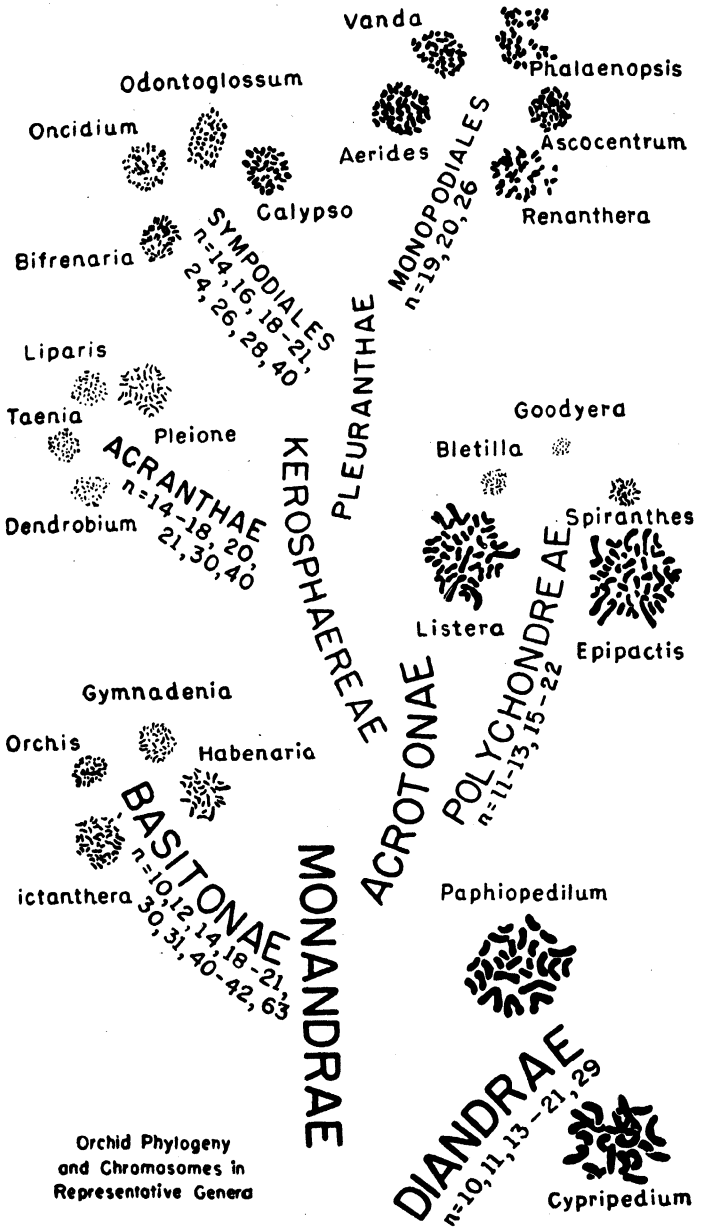


Fig. 1. Karyotypic evolution in Orchidaceae (*After* R. C. Duncan *in* Withner, 1959).

cross-breeding through the pollinating agents, such as, bees, wasps, flies, ants, butterflies and snails.

SUMMARY

Schlechter's (1926) system of classification of the family Orchidaceae comprising 15,000 species and 600 odd genera in the world flora is recognised as an improvement on those of Lindley (1830-40) and Bentham & Hooker (1883).

But Dressler & Dodson (1960) who consider Schlechter a "splitter", propose a new system of classification of orchids following international rules of botanical nomenclature and recognise 5 tribes and 40 subtribes within 2 subfamilies. Although orchids largely, as epiphytes, find their wild home in the tropical rain forests all over the world, it is rather strange that about one half of the total species belong to 20 to 30 genera, only a few of which are pantropical in distribution.

With a view to correlate the evolutionary diversity in vegetative morphology with the mechanism of speciation, cytotaxonomic investigations involving about 370 species belonging to 84 genera, mostly temperate, have been undertaken by various workers. These studies reveal aneuploidy, in contrast to euploidy, as the most widespread cytological phenomenon. This, coupled with obligate cross-breeding and prevalence of vegetative propagation, explains the relative ease of artificially synthesising viable multigeneric crosses, a most unusual feature met with only in the Orchidaceae.

This paper summarises our present knowledge of Indian Orchidaceae, particularly in relation to 322 species, collected by us and 1,200 specimens cultivated in the National Orchidarium, Shillong.

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CYTOLOGY AND ITS CONTRIBUTION TO FERN SYSTEMATICS

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About two hundred years ago Linnaeus (1753) listed only 14 genera and 182 species of ferns. At present 305 genera and over 10,000 species of ferns are recognised (Table 1), three quarters of which occur as natives in the tropics and subtropics. Their long fossil record extending up to the Devonian coupled with their extensive geographical distribution and variety of morphological lines of approach, have drawn the attention of the taxonomists. The phylogenetic chart (Figs. 1, 1A) outlines the varied taxonomic history of the group from Bower (1935) to Holttum (1949).

The work on Indian ferns by Beddome (1883, 1892) is a digest of the information on Indian ferns contained in all earlier publications made by Hooker, Clarke, Smith and Beddome up to 1892 and is, therefore, a very good handbook for reference recording informations and data up to the period. Beddome has recorded and described in it the occurrence of 98 genera and 750 species of ferns within the geographical limits of India, Ceylon and the Malayan Peninsula.

Unfortunately, however, study of the systematics of Indian ferns, as such, has been neglected after 1892. Mehra & Chowdhury (1958) in a review of the work done on Indian Pteridophytes in recent years have made reference to the work on systematics done by Hope (1903), Biswas (1939, 1942, 1943), Mehra (1939), Stewart (1938, 1945) and Alston (1946) and it is needless to repeat them here. All these publications deal with local floras only, sometimes dealing with a particular genus or family and use Beddome's outmoded classifications and obsolete nomenclature in general for listing their taxa.

Recently, Panigrahi (1960) has reported *Osmunda cinnamomea* L., *Vittaria ensiformis* Sw. var. *latifolia* Holtt. and *Araiostegia beddomei* (Hope) Copel. as new records for India and *Psilotum nudum* (L.) Beauv. as a new record for Eastern India.

A comparison of Copeland's (1947) system of classification with that of Beddome, would show the revolutionary changes brought about in the concept of systematics and taxonomy of the ferns during a period of fifty years. Within the limits of Beddome's comprehensive family Polypodiaceae *sensu lato* comprising 12 tribes, Copeland recognises 10 families coordinate in rank with each other. Further, the generic names used by Beddome, viz. *Nephrodium, Phegopteris, Niphobolus* etc. are now obsolete and the various species referred to *Pleopeltis* by Beddome are distributed by Copeland and other recent workers under *Lepisorus, Pleo-*

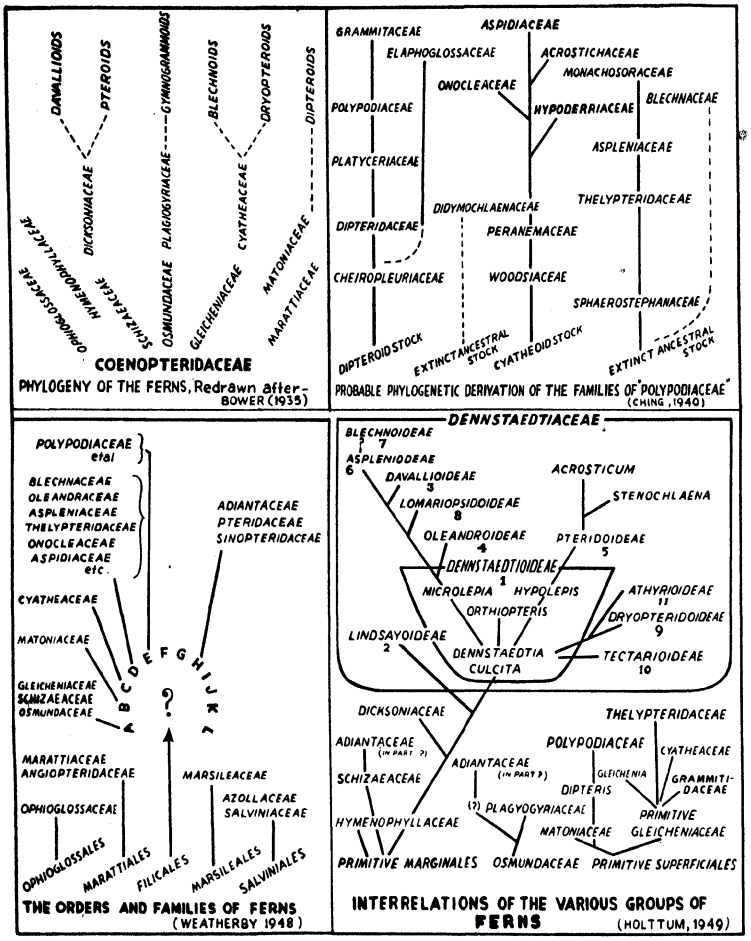


FIG. 1

peltis, Microsorium, Lemmaphyllum, Colysis, Crypsinus etc. (cf. Panigrahi & Patnaik, 1961b).

Despite the progress made in the treatment of systematics of ferns in recent years, there are considerable disagreements between competent authorities not merely in postulating the phylogenetic origin of different families, but even in the association of different genera within a family on the basis of "blood relationships" between them. Such differences in

the phylogenetic concepts between established authorities, although confusing to an amateur fern taxonomist, are understandable after instituting a comparison of Holttum's (1959) system (Table 2) with his own system proposed a decade earlier (1949) (Fig. 1). There is hardly anything

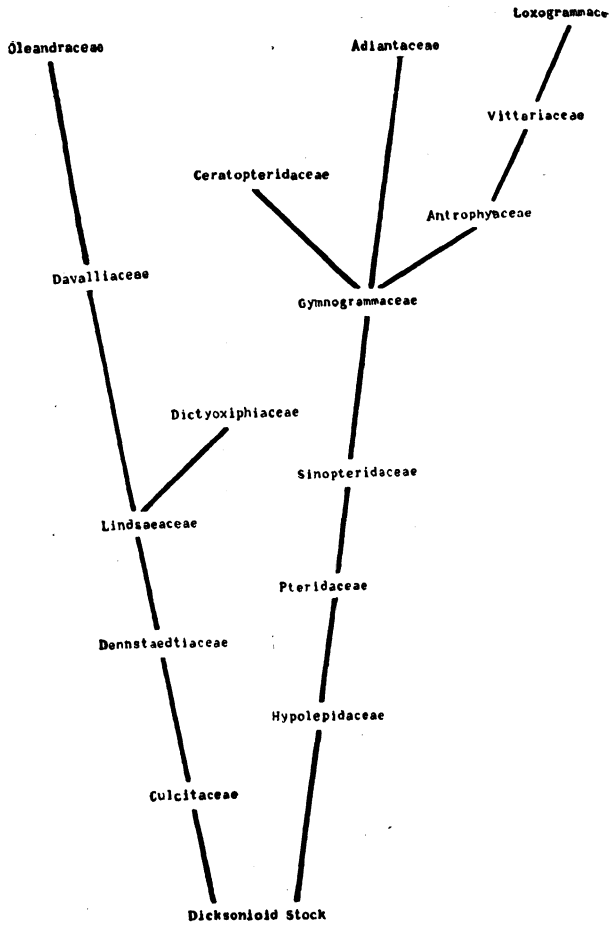


FIG. 1A. Probable phylogenetic derivation of the families of "Polypodiaceae" (*After* Ching, 1940).

common between his two systems and even he is doubtful whether to rank an association of genera like *Adiantum* etc. as constituting a family or groups. He splits up Copeland's Pteridaceae etc. into groups and arranges these groups alphabetically, presumably being uncertain regarding the phylogenetic sequence of the various groups.

Similarly, regarding recognition of the number of valid genera within a family, e.g. Gleicheniaceae, Holttum in 1949 and 1954 considered it as comprising only one genus, *Gleichenia*, whereas in 1959 he recognises *Gleichenia* and *Dicranopteris* as 2 distinct genera within the family Gleicheniaceae and considers Copeland's other genera as wrongly conceived. Similarly in 1954 he ascribed *Azolla* and *Salvinia* to two distinct families, Azollaceae and Salviniaceae, whereas in 1959 he accepts Copeland's system and assigns the two genera to one family.

Such disagreements as these drive the average fern worker in a herbarium into a dilemma and he finds it difficult to choose a standard acceptable system for arranging his fern herbarium. Realising that Hooker's system proposed between 1844 and 1864, after which the ferns of the Central National Herbarium, Sibpore (India), are arranged, is very much out of date, the writer has accepted Copeland's (1947) as a standard system, after which the very large collection of ferns from Orissa, Bihar, Assam and North-East Frontier Agency, comprising 2500 field numbers belonging to 350 species, have been arranged in the Regional Herbarium of the Botanical Survey of India, Shillong. Their enumeration with modern nomenclature, citing the names given by Beddome (1883, 1892), as synonyms, is being published elsewhere (Panigrahi, 1960a ; Panigrahi & Patnaik, 1961b ; Panigrahi & Chowdhury, 1961) with a view to help taxonomists working on Indian ferns. Acceptance of Copeland's system for arranging the fern herbarium has been done as a matter of convenience since Copeland's 'Genera Filicum' deals with all generic epithets proposed between 1753 and 1947 and assigns each of them a position in his system of classification. It has, therefore, very great utility for "pigeon-holing" a herbarium specimen into a family, whatever may be the generic and specific nomenclature used for identifying it in a herbarium. While Copeland's system is thus adopted for arranging herbarium materials, on the analogy of Bentham & Hooker's (1883) system for flowering plants of India, no opinion is being expressed here on the validity of the phylogenetic concepts put forward by Copeland. It may now be clear that despite the great contributions to fern taxonomy made by Christensen, Ching, Dikason, Copeland, Holttum and others over the last two decades, the fern systematics and taxonomy are now in a very fluid state due largely to the role of subjective judgement of the orthodox taxonomists. Consequently, the fern phylogeny is shrouded in mystery.

TABLE 1

Author	*Year*	*Reference*	*Genera*	*Remarks*
Linnaeus	1753	Species Plantarum	14	182 species
Swartz	1806	Synopsis Filicum	38	—
Presl	1845	Supp. Tent. Pterid	165	—
Hooker	1867	Synopsis Filicum	75	excluding Marsileaceae and Salviniaceae
Smith	1875	Historia Filicum	217	-do-
Fée	1850-'52	Genera Filicum	181	excluding *Cyathea, Dicksonia* etc.
Engler & Prantl	1898	Diels Nat. Pfl.2	141	—
Christensen	1906	Index Filicum	146	—
Christensen	1934	-do-	213	—
Christensen	1938	Manual of Pteridology	247	—
Ching	1940	Sunyatsenia	257	—
Copeland	1947	Genera Filicum	229	—
Holttum	1949	Biological Reviews	305	10,000 species

TABLE 2

Classification of Pteropsida (Holttum, 1959)

OPHIOGLOSSALES	*Remaining genera of ferns* (in groups alphabetically)	*Heterosporous ferns*
Ophioglossaceae	Adiantum group	Marsileaceae
	Asplenium group	Salviniaceae
MARATTIALES	Athyrium group	
Marattiaceae	Blechnum group	
	Davallia group	
	Dennstaedtia group	
FILICALES	Dicksonia group	
Osmundaceae	Dryopteris group	
Schizaeaceae	Lindsaea group	
Gleicheniaceae	Lomariopsis group	
Hymenophyllaceae	Nephrolepis group	
Matoniaceae	Plagiogyria group	
Cyatheaceae	Pteris group	
Polypodiaceae (*s. st.*)	Tectaria group	
Grammitidaceae	Thelypteris group	
	Vittaria group	

CYTOLOGY IN RELATION TO FERN TAXONOMY

The application of cytology (viz. determination of chromosome number of species and genera) in solving problems of fern taxonomy and systematics is a very recent endeavour. The line of enquiry initiated by Manton (1950) has been pursued by Britton (1953) and Wagner (1955) in America, Meyer (1952) in Germany, Mehra and Abraham in India and Brownlie (1957) in New Zealand. While it is virtually impossible to give a comprehensive review of the contributions of cytology to the systematics and taxonomy of the world's fern flora and since Panigrahi (1958) has reviewed the existing literature on cytology, ecology and phytogeography to interpret evolutionary tendencies in Pteridophyta in relation to Good's (1953) "Theory of tolerance", it will be useful to peruse the current trends of research on cytology in relation to the systematics of Indian ferns.

Mehra & Singh (1956) determined n=56 in *Hicriopteris glauca* (Thunb.) Ching and n=39 in *Dicranopteris linearis* (Burm.). Underwood collected from Darjeeling (2125 m.) and on account of the diversity of chromosome numbers (n=56 and n=39) without any correlation justified the segregation of these species to two different genera as proposed by Copeland (1947).

Mehra & Bir (1957) from their studies in the Aspleniaceae collected from Darjeeling found many diploid species in contrast to the occurrence of *Asplenium planicaule* and *A. ensiforme* as two tetraploid species and concluded that intraspecific polyploidy is absent among species of the genus and that polyploidy is restricted only to tetraploid level in Darjeeling area. Panigrahi (1954) has, however, established *A. aethiopicum* (Burm.) Bech, with n=216 from Madeira (and not n=144 as reported by Manton, 1950, p.283) as a polyploid complex and demonstrated intraspecific polyploidy in this "species" throughout its range of occurrence in Ceylon, Africa and Madeira.

Mehra & Khanna (1959) have recorded the chromosome numbers of the following Himalayan speices collected from Darjeeling: *Dennstaedtia appendiculata* (Wall.) J. Sm. and *D. scabra* each with n=66 are tetraploid species. Since, however, Manton & Sledge (1954) report n=64 or 65 from Ceylon, aneuploidy appears to exist within the genus. *Microlepia speluncae* (L.) Moore with n=43 is a diploid species in contrast to its tetraploid form with n=86 reported by Manton & Sledge (1954) from Ceylon. Whereas *M. rhomboidea* (Wall.) Pr. occurs both as diploid (n=43) and as an autotriploid (n=120?) with segmental interchanges, *M. pilosula* (Wall.) Prantl. is a tetraploid species with n=86 in the Darjeeling area. *Hypolepis punctata* (Thunb.) Mett. with n=ca 102, *Lindsaea cultrata* (Willd.) Sw. with n=150, *Sphenomeris chusana* (L.) Ching and n=39 in *Dicranopteris linearis* (Burm.) Underwood collected from Ceylon and Malaya by Manton & Sledge (1954) show the existence of different chromosomal types within them.

Again, *Araiostegia pulchra* (J. Sm.) Copel. and *A. pseudocystopteris* (Bedd.) Copel. each with n=40 differ from *Leucostegia immersa* Presl. with n=41. They, therefore, confirm the distinctive identity of the two genera on cytological grounds. Similarly, *Oleandra neriiformis* Car. and *O. wallichii* (Hk.) Pr. each with n=41 appear to have more in common with *Nephrolepis cordifolia* (L.) Pr. having n=41 rather than with *Davallia* with n=40. Hence Holttum's recognition of Oleandroideae and Davallioideae as two distinct subfamilies is supported on cytological grounds. But the derivation of Aspleniaceae with n=36 from Davalliaceae with n=40, as maintained by Holttum, finds no cytological confirmation at all.

Mehra & Verma (1957a) from their studies of *Onychium* (*O. auratum* and *O. japonicum*, each with n=29, and *O. contiguum* with 2n=n=58

apog.) support Copeland's inclusion of *Onychium* in the Pteridaceae and consider Holttum's derivation of the genus from *Todea* with n=22 as untenable.

Mehra & Singh (1957) studied the cytology of eight species of Hymenophyllaceae from Darjeeling and discovered irregular meiosis in *Hymenophyllum javanicum* Spr. (2n=108), *Trichomanes auriculatum* Bl. (2n=108), *T. insigne* (v. d. B.) Bedd. var. β, and var. γ (2n=108, 72 respectively). These studies as also those of Manton & Sledge (1954) establish the base numbers for the family as 7, 9, 11 and 13.

The cytological gap between *Dennstaedtia* (n=33) and *Microlepia* (n=43) which resemble each other fairly closely in gross morphology and anatomy, can be bridged by the discovery of x=11 in Hymenophyllaceae, *Microlepia* presumably arising by the loss of one chromosome from n=44.

Mehra & Verma (1957b) from their studies of *Athyrium* have confirmed the base number for the genus as n=40 and have successfully established the status of *Ctenitis boryana* (Willd.) Copeland with n=40 as *Athyrium boryanum* (Willd.) Tagawa, the genus *Ctenitis* having n=41.

Mehra & Bir (1958) from their studies in the cytology of the Blechnaceae determined n=34 both for *Blechnum orientale* and *Woodwardia radicans* and n=73 for *Stenochlaena palustris* and considering all the available evidences they support Copeland in placing *Stenochlaena* in the family Blechnaceae.

Ninan (1956d) determined the cytology of *Ceratopteris thalictroides* as n=77 and supported Copeland's (1947) segregation of the genus to a separate family Parkeriaceae.

Abraham & Ninan (1954) report 2n=more than 1260 in *Ophioglossum reticulatum* L. and Ninan (1956c) reports n=94 for *Helminthostachys zeylanica* (L.) Hook. and 2n=180 for *Botrychium lanuginosum* Wall. ex. Hook & Grev. and concludes x=15 for the Ophioglossaceae in general.

Ninan (1956a,b) reports n=78 for *Marattia fraxinea* and n=80 for *Angiopteris evecta* and confirms Manton's finding of x=13 for the family and n=22 for *Osmunda ragalis* L. from India.

Panigrahi & Patnaik* (1961a) report *Agalomorpha coronans* (Wall.) Copel. as having n=36 in contrast to n=37 in the three species of the

* Panigrahi & Patnaik (unpublished) have now determined the chromosome numbers of the following species other than the ones already reported by them (*see* Panigrahi & Patnaik, 1961c) : *Dipteris wallichii* (R. Br.) Moore (n=66) ; *Polypodium amoenum* Wall. (n=36, 2n=72) ; *P. lachnopus* Wall. (n=36) ; *P. microrhizoma* Clarke (n=36) ; *Goniophlebium argutum* (Wall.) J. Sm. (n=36) ; *Lepisorus machosphaerus* (Bak.) Ching (n=35); *L. heterolepis* (n=47, 2n=94) ; *L. episorus* (2n=46) ; *Neocheiropteris phyllomanes* (Christ) Ching (n=36, 2n=72) ; *Pyrrosia mollis* (Kze.) Ching (n=74) ; *P. nummularifolia* (Sw.) Ching (n=37, 2n=74) ; *P. stigmosa* (Sw.) Ching (2n=74) ; *P. beddomeana* (Gies.) Ching (2n=74) ; *Drynaria propinqua* (Wall.) J. Sm. (n=36, 2n=72) ; *D. quercifolia* (Linn.) J. Sm. (n=37) ; *Lemmaphyllum subrostratum* (C. Chr.) Ching (2n=72).

allied genus, *Drynaria* (c.f. Manton & Sledge, 1954) and provide cytological evidence for the recognition of two distinct genera.

Some of the most important conclusions arising from the studies in the cytotaxonomy of ferns are summarised in the following pages:

(1) The chromosome number determination shows great preponderance of polyploids in ferns (up to 60 per cent) ; many of the "classical species" involving different grades of polyploidy. A few of these are (Panigrahi 1954, Manton & Sledge, 1954):

Cyclosorus parasiticus	2n, 4n
Asplenium aethiopicum	4n, 8n, 12n
Aleuritopteris farinosa	2n, 4n, 3n apogamous
Dryopteris villarsii	2n, 4n

(2) Although tetraploids, hexaploids etc. to dodecaploids turn up in species after species in the tropics in contrast to hexaploid as the highest grade of polyploidy in the temperate latitudes, it is indeed strange that very few diploid species are being discovered in ferns ; the base numbers being conjectured in such cases from other cytological evidences. Studies in interspecific hybridisation in the four species complexes cited under (1) above point to the prevalence of allopolyploidy, rather than autopolyploidy, in different species complexes, irrespective of their differing evolutionary trends in comparative morphology. In them, it has arisen independently from species to species with almost monotonous regularity. For the synthesis of the polyploid complex, *Asplenium aethiopicum* with n=216 from Madeira, I had to postulate successive interspecific hybridisations between seven hypothetical diploid species, all of which are either extinct or await their discovery in the tropics of Asia and Africa (Panigrahi, 1954).

(3) The commonest generic chromosome numbers among the leptosporangiate ferns are n=29, 30, 36, 37, 40, 41, and 52. However, the recent discoveries of base numbers such as 7, 9, 11, and 13 in Osmundaceae, Marattiaceae, Gleicheniaceae, and Hymenophyllaceae (Manton, 1954 ; Mehra & Singh, 1957) and 11 and 13 in Polypodiaceae *sensu* Copeland (1947 ; *cf.* Panigrahi & Patnaik, 1961c) find their parallel with similar low base numbers characterising many families of flowering plants. This points to the essential similarity in cytological picture characterising two distinct groups of vascular plants: one, the oldest—Pteridophyta, and the other, the most recent—Angiosperms.

(4) Discovery of apogamy as an asexual method of propagation in 18 species which constitute about 10 per cent of the cytologically investigated species of ferns in the tropics as opposed to 5 per cent in Europe, is comparable with the phenomenon of apomixis in flowering plants. Considering the occurrence of very high grades of polyploidy in apogamous

taxa in general, together with the evidence of chromosome pairing (viz. formation of 29 bivalents and 87 univalents in spore mother cells produced by 16-celled sporangia and 145 bivalents in those of 8-celled sporangia) in the synthesised pentaploid F_1 hybrid ($2n=145$) between the tetraploid sexual species, *Aleuritopteris anceps* (Blanford) Panigrahi from Ceylon with $n=58$ and the triploid apogamous form, *A. farinosa* (Forsk.) Fée from Kenya with $n=87$, the hybrid origin of apogamy is not only suspected (Manton & Sledge, 1954) but is definitely established. A hypothetical diploid species, other than *A. grisea* (Blanford) Panigrahi, has been suggested as common parent to both *A. anceps* and *A. farinosa* (Panigrahi, 1954).

(5) The revision of the following genera and species complexes, to cite a few examples, has been suggested in view of the existence of multiple chromosome types or aneuploidy and/or monosomics and due to the occurrence of inconsistent base numbers within them (*see* Manton & Sledge, 1954 ; Mehra & Khanna, 1957 ; Mehra & Singh, 1956 ; Panigrahi & Patnaik, 1961c,d):

(*i*) *Thelypteris*	n = 31, 35, 36
(*ii*) *Lygodium japonicum*	n = 58
L. scandens	n = 30
L. circinatum	n = 29, 2n = 116
(*iii*) *Dicranopteris linearis* (= *Gleichenia dichotoma*)	n = 39, 78
Hicriopteris glauca (= *G. glauca*)	n = 56
(*iv*) *Lindsaea cultrata*	n = 150
L. cuneata	n = 42, 43
L. caudata	n = 82
L. pectinata	n = ca. 100
L. decomposita	n = 50, 100
(*v*) *Crypsinus*	n = 33, 35, 36
Lepisorus	n = 22, 35, 36, 46, 47, 74
L. pseudonudus	2n = 39

GENETICS IN RELATION TO FERN TAXONOMY

Although literature on ferns abounds with cytological data, not much genetical studies have been carried out in Pteridophytes after Döpp (1939) either owing to the apogamous nature or infertility of the few F_1 hybrids synthesised between the diploids and their allotetraploid species.

However, Walker's (1958) studies on the genetics of *Pteris quadri-*

aurita, P. multiaurita and *P. confusa* open a new line of investigation in ferns and will greatly be utilised in discovering hybrid swarms in nature. Walker has conclusively proved *P. otaria* of the fern taxonomists as a member of the hybrid swarm between *P. quadriaurita* and *P. multiaurita,* in which introgressive hybridisation has been the rule. He has created a new genus *Idiopteris* with n=27 from his studies in this species complex.

Panigrahi (1954) has established the inheritance of apogamy through the male parent by the pentaploid F_1 hybrid with 2n=145 involving the tetraploid sexual species *Aleuritopteris anceps* (Blanford) Panigrahi (n=58) and the triploid apogamous form, *A. farinosa* (Forsk.) Fée (n=87). Again, the appearance of golden yellow ceraceous covering on the under surface of fronds of this pentaploid hybrid between *A. anceps* with white powder and *A. farinosa* with sulphur yellow powder, provides a striking instance of operation of complementary factors. Because of the transmission of the complementary factor through the spermatozoid from the apogamous male parent and because of the irregular distribution of various tinges of the ceraceous coverings on either surface of fronds, Panigrahi (1954) has suggested the operation of a complex nuclear mechanism governing expression of colour in this species complex.

Similarly, Panigrahi & Manton (1958) observed various shades of anthocyanin pigmentation in the young fronds of tetraploid species of *Cyclosorus* (pink in *C. parasiticus,* pinkish-blue in *C. subpubescens,* bluish-brown in *C. dentatus* and almost blue in *C. jaculosus*) whereas the diploid *C. repandulus* was completely devoid of any anthocyanin pigments. They found that the triploid F_1 hybrids between the diploid as the female parent and the tetraploid as the male parent, developed anthocyanin pigments remarkably identical with the varying shades of anthocyanins characterising their respective male parents. Panigrahi (1954) has, therefore, suggested that inheritance of anthocyanin pigmentation in this species complex is governed by a polygenic system located in the nucleus.

The striking association of triploidy with mechanisms of vegetative reproduction has at times led to the erroneous view that it has the inherent property of encouraging them. The breeding experiments involving the respective species groups of the genera *Cyclosorus, Asplenium, Aleuritopteris* and *Dryopteris* suggest that creeping rhizome as a means of vegetative propagation behaves always as dominant over erect rhizomes and that it does not appear to be a consequence of hybridity but is generally inherited from either or both of the putative parents possessing it. Mere incidence of triploidy in species of *Aleuritopteris,* for example, possessing erect rhizomes, failed to induce the production of creeping rhizome in the synthesised F_1 hybrids between *A. grisea* and *A. anceps* (Panigrahi, 1954, 1960b).

Again, the relative incidence of "somatic heterosis" (Gustaffson, 1952) was noticed as a rule in all the 12 different F_1 interspecific hybrids within the genera *Cyclosorus, Asplenium, Aleuritopteris* and *Dryopteris* (*see* Panigrahi, 1954). The young sporelings in all cases showed great physiological activity ; there was an increased rate of cell divisions leading to vigorous vegetative growth (Rabideau *et al.*, 1950). Subsequently, it was followed by 'reproductive precocity' indicated by the earlier sporangiferous conditions of the F_1 hybrids with respect to either of their putative parents. For example, the triploid F_1 hybrids between *C. repandulus* (2n) and *C. dentatus* (4n) became sporangiferous in five and a half months from the date of crossing unlike any of their parents which attained reproductive maturity when one year old.

What, then, is the genetic basis of heterosis? It is assumed to be caused by a combination in F_1 of a large number of linked dominant genes conditioning an increased vigour. Fisher's (1918) postulate of superdominance in a combination of two alleles belonging to the same locus has been adopted by Hagberg (1953) who symbolises "superdominance" of genes by the formula Aa >AA >aa.

However, Panigrahi (1954) contends that the four triploid hybrids of *Cyclosorus* showing evident resemblance to their respective allotetraploid male parents in all of their morphological characters exhibit an unusual feature of "patrocliny" in plants and provide examples of "superdominance of genome", an adaptation of Fisher's (1918) superdominance of gene (allele) theory. In these cases, the unpaired chromosome set from the allotetraploid parent carries the genes having optimum effect in single dose and completely dominates over the two sets of homologous chromosomes in F_1 hybrids (Panigrahi & Manton, 1958). The complete recessiveness of *C. repandulus* chromosomes in triploid F_1 hybrids, intermediacy in morphological characters of the tetraploid F_1 hybrids, as also the "reproductive precocity", "patrocliny" and "somatic heterosis" of F_1 plants over both parents may be explained by the superdominance of genome action and is symbolised as follows, each letter representing a haploid set, n=36.

aa<	Aaa>	aaAA
C. repandulus	3N F_1 hybrid	allotetraploid parent
aaAA<	aaAB>	BBaa
C. subpubescens	4N F_1 hybrid	*C. sumatranus*

Thus, Pichi-Sermolli (1959) has critically evaluated all the available morphological, anatomical, cytological and genetical evidences from the sporophyte together with phyletic data from the gametophyte (Stokey, 1951, 1960), and divides Filicinae into six sub-classes, viz. Ophioglossi-

dae, Marattidae, Osmundidae, Filicidae, Marsileideae and Salviniideae. He recognises 14 orders and 32 families amongst the modern Filicidae (Table 3) which, therefore, reflects the current synthetic approach to solve baffling problems of fern phylogeny.

TABLE 3

	Orders	No. of families	Generalised haploid sets n	Remarks
1	Schizaeales	2	29, 30, 38, 77	
2	Pteridales	8	29, 30, 77	Copeland's Pteridaceae (in part from genera no. 31-63 plus Vittariaceae)
3	Dicksoniales	3	42, 43, 50, 52, 82, 100, 150	Includes *Pteridium*
4	Davalliales	2	40, 41	
5	Hymenophyllales	1	11, 13, 18, 21, 22, 26, 27, 28, 33, 34, 36	Copeland's recognition of more than 2 genera justified
6	Gleicheniales	1	20, 34, 39, 43, 56	Copeland's recognition of genera justified
7	Cyatheales	2	69, 70	
8	Loxsomales	1	Not determined	Primitive but isolated
9	Aspidiales	5	31, 34, 35, 36, 40, 41, 42	Copeland's Aspidiaceae split into 4 families
10	Blechnales	1	28, 32, 33, 34, 35, 73	
11	Matoniales	1	26	
12	Polypodiales	4	22, *ca* 33, 35, 36, 37, 46, 47 and 2n=39 in *Lepisorus pseudonudus* Ching	Holttum's Grammitidaceae maintained though cytology does not support it
13	Plagiogyriales	1	not determined	Isolated
14	Hymenophyllopsidales	1 (genus)	not determined	*"incertae sedis"*

Orders 2, 3, 5, 6, and 13 related to and derived from the Schizaeioid ancestors.
Orders 4, 7, 9, 10, 11, 12 related to and derived from Gleichenoid ancestors.

SUMMARY

A perusal of the various systems of classification involving 305 genera and 10,000 species of ferns based on comparative gross morphology and anatomy, reveals striking disagreements on the taxonomy and systematics of ferns.

Osmunda cinnamomea L., *Vittaria ensiformis* Sw. var. *latifolia* Holtt. and *Araiostegia beddomei* (Hope) Copel. as new records for India and *Psilotum nudum* (L.) Beauv. as new record for Eastern India, have been reported.

A review of the current trends of research in cytotaxonomy of Indian ferns has been made and taxonomic problems at the generic and specific levels arising out of cytological and genetical studies have been discussed.

Pichi-Sermolli's system of classification has been presented in a tabular form to indicate the modern synthetic approach to solve age-old problems of fern phylogeny.

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THE GAMETOPHYTE OF THE PSILOPHYTALES

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In the short period following the discovery of the Rhynie Psilophytales in 1913, many more early vascular plants have been discovered. Some of these appear to be of even greater interest because they are older and have a relatively complex structure. But up to date the descriptions of the four excellently petrified Rhynie forms, viz. *Rhynia gwynne vaughani*, *R. major*, *Horneophyton* (*Hornea*) *lignieri* and *Asteroxylon mackiei*, by Kidston & Lang (1917, 1920a,b, 1921a,b) continue to remain the most complete accounts of any psilophytes. We may even say that their descriptions are nearly as complete as they could be even if the plants were living today, except for cytological details, gametophytes and embryo-development. Cytological details, it must be admitted, are almost always absent in fossils but fossil gametophytes are now known for many plants. The possibility that their gametophytes may have been more "delicate" than the sporophyte (*see* Kidston & Lang, 1921a ; Bower, 1935) and the great reputation of the original investigators have perhaps provided a ready escape from the urge for a reinvestigation of the Rhynie material. But the gametophytes of the presumed archaic plants of the present day (e.g. Lycopodiales, *Ophioglossum* and more specially the Psilotales, which group alone among the living plants forms the nearest approach to the Palaeozoic Psilophytales[1]) can hardly be termed as delicate. Indeed the detailed investigations of Holloway (1917, 1921, 1939) and Bierhorst (1953, 1954a), published after the discovery of the Rhynie plants, have amply proved that the prothalli of both *Psilotum* and *Tmesipteris* are fairly robust (the largest incomplete specimens being about 2 cm × 0.2 cm), dichotomously branched, radially organised structures with a well developed cuticular covering. The homologous gametophyte of the former genus (*see* Holloway, 1939 ; Bierhorst, 1953) has even been shown to possess a vascular strand with a stele not unlike that present in the axes of its own sporophyte generation or those of *Rhynia* or *Horneophyton*. As such it is quite likely that the gametophytes of the Psilophytales also were at least as robust as those of the two living genera. In fact Fritsch (1945) has with good reasons suggested that "if embryos and gametophytes of Psilophytales are ever discovered, it would not be astonishing to find that the latter were of the same general

[1] The reasons for this view have been enumerated from time to time by Kidston & Lang (1917), Holloway (1917), Scott (1920, 1923), Sahni (1925), Zimmerman (1930), Seward (1931), Bower (1935), Brown (1935), Eames (1936), Fritsch (1945) and others.

nature as those of the Psilotales ; the gametophytes may even have been less reduced". Bower (1935) also suggested that the gametophytes of the earliest colonisers of land had probably a robust build, and Eames (1936) vaguely speculated that "if gametophyte and sporophyte were much alike in early land plants, some of the sterile fossil branching axes may perhaps be gametophytic!" Kidston & Lang failed to find them in the otherwise ideally preserved Rhynie material where even delicate algae (Kidston & Lang, 1921a ; Croft & George, 1959), fungi (Kidston & Lang, 1921b), crustaceans (Scourfield, 1926) and germinating spores with germ tubes (Lyon, 1957) have been found well preserved. Judging from the detailed accounts of Kidston & Lang, which now belong to the classical literature of Palaeobotany, it appears, however, inconceivable that the gametophytes could have escaped their watchful eyes. It, therefore, remains to be considered whether the gametophytes of at least some of the Rhynie Psilophytales had cylindrical axes with a central vascular strand as in present day *Psilotum*. No such gametophytes had been recognized at the time of the discovery of the Psilophytales and it was natural for Kidston & Lang to have presumed that all the vascular axes in the Rhynie chert belonged to the sporophytic generation.

Fresh Observations and New Interpretations

It was Holloway's account of the vascular gametophytes of *Psilotum* (Holloway, 1939) which led me to think that the gametophytes of the Rhynie plants could perhaps be discerned in the hitherto described material itself. Accordingly, I made a thorough search for such structures in the few slides of the Psilophytales available to me at Allahabad. These slides had been obtained many years ago from the Lomax Laboratories of Bolton, England, and one of them (Slide No. A.U./A2) showed structures which encouraged me to cut and examine additional material. I also studied the figures and descriptions of Kidston & Lang (1917, 1920a,b, 1921a,b) in relation to the accounts of the gametophytes of the Psilotales (Holloway, 1917, 1921, 1939 ; Darnell Smith, 1917 ; Lawson, 1917a,b ; Burfeq, 1938 ; Bierhorst, 1953, 1954a). Further, I examined some slides of the prothalli of the Psilotales kindly lent to me by Professor P. Maheshwari, who has in addition given me his never failing encouragement and valuable suggestions. I have also had the benefit of Professor O. A. Hoeg's (then Director of the Sahni Institute of Palaeobotany at Lucknow) advice and help in obtaining material and slides of the Rhynie chert from Glasgow. No less helpful was Professor John Walton's ready response in sending material and slides and later his kindness in allowing me facilities to work in his laboratory and to examine the Kidston and Holloway collections of the figured slides of Psilophytales and gametophytes of Psilotales. Professor T. M. Harris, whose kindly

interest in my work has always been a source of inspiration, gave me critical suggestions and allowed me to work in his laboratory and examine the collection of Rhynie slides in the Reading University. The late Mr. W. N. Edwards, formerly Keeper of the Geology Department of the British Museum (Natural History), London, and Mr. F. M. Wonnacott not only extended every facility to examine the slides of the Psilophytales in the British Museum but also gave helpful suggestions. In London, Professor F. M. Haines and Professor L. J. Audus kindly allowed me to examine the slides of *Rhynia* in the collections of the Queen Mary College and the Bedford College. Dr. K. R. Surange was good enough to get some of the material sectioned and photographed at the Sahni Institute of Palaeobotany. To all of these persons I am exceedingly grateful.

As a result of these studies, it now seems that the presumed absence of the gametophytes of the Rhynie Psilophytales is not because of the proverbial "imperfection of the geological record" but due to erroneous interpretation. The following points are significant:

(*a*) The close structural similarity between the axes of *R. gwynne vaughani* and the vascular gametophytes of *Psilotum*.

(*b*) The possibility that at least some of the "stomata", below which the "hemispherical projections" and "adventitious branches" of this species are reported to be produced, could be archegonia whose necks had been shed already.

(*c*) The developmental similarities between the "hemispherical projections" of *R. gwynne vaughani* and the young sporophytes of Psilotales.

(*d*) The clear demarcation between the tissues of the "hemispherical projections" and the parent axes.

(*e*) The structural similarities between the "hemispherical projections" or "adventitious branches" of *R. gwynne vaughani* and the young sporelings of the Psilotales.

(*f*) The occurrence of gametophyte-like non-vascular axes in *R. gwynne vaughani*.

(*g*) The absence of actual organic connection between the indubitable axes of *R. gwynne vaughani* and the sporangia assigned to it.

It thus appears that at least those axes of *R. gwynne vaughani* which bear the "hemispherical projections" or "adventitious branches" may well represent the gametophytes of either of the Psilophytales described from Rhynie. Some fresh observations on *R. gwynne vaughani* and various other points in favour of this hypothesis will be discussed in some detail below. The structure of the axes of *R. major* or of *Horneophyton* (*Hornea*) *lignieri* is also similar but their stems have been found in organic connection with sporangia and any positive evidence of the presence of archegonium-like structures, the peculiar "hemispherical projections"

and the "adventitious branches", is lacking. It is interesting to note that Merker (1958, 1959) has already pointed out that the rhizomes, more especially the prostrate tuberous "rhizomes" of *Horneophyton*, could be portions of the gametophyte and the aerial axes the multiple vascular sporophytes.

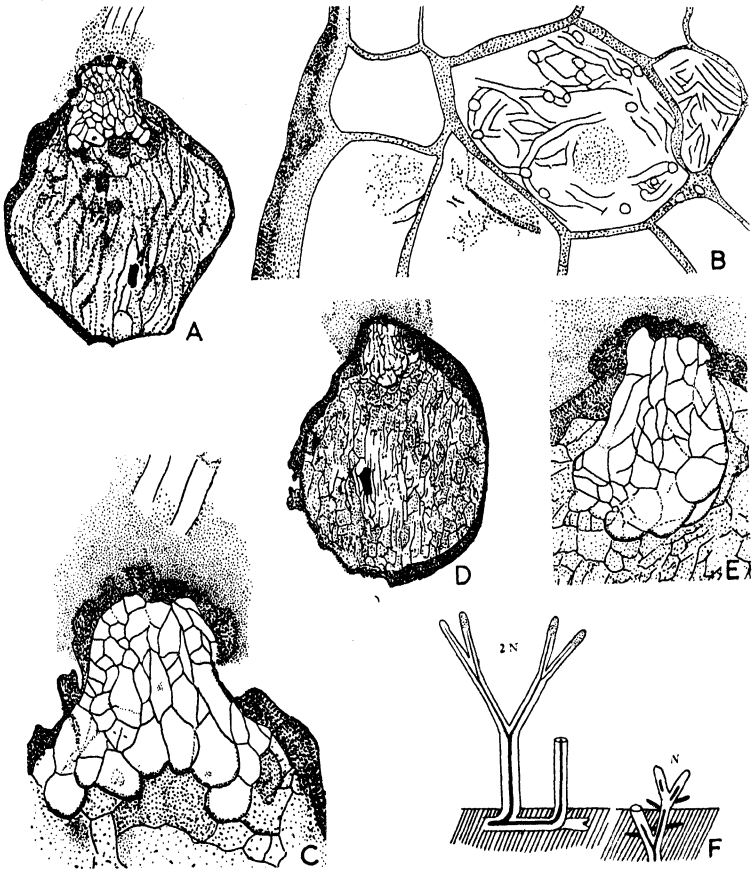


Fig. 1. *Rhynia gwynne vaughani*. *A*, axis showing "hemispherical projection." Slide No. A.U./A_2. × 55 (*see also* Fig. 10). *B*, cortical cells of axis showing fungal filaments. Slide No. A.U./A_2. × 290 (*see also* Fig. 5). *C*, portion of *A* more magnified to show haustorial cells at base. × 135 (*see also* Fig. 11). *D*, axis showing "hemispherical projection". Slide No. A.U./A_2. × 45 (*see also* Fig. 14). *E*, portion of *D* magnified to show haustorial cells (*see also* Fig. 15). × 135. *F*, representations of the sporophytic (*2 N*) and gametophytic (*N*) generations of a plant like *Rhynia* (redrawn and slightly modified after Fritsch, 1945).

The close structural similarity between the axes of *Rhynia gwynne vaughani* and the vascular gametophytes of *Psilotum*

It must be realised at the outset that the occurrence of a vascular strand in the dichotomously branched cylindrical prothalli of *Psilotum* at once suggests the possibility that at least a few of the similar but supposedly sporophytic axes of *R. gwynne vaughani* could equally well be attributed or at least suspected to belong to the gametophytic generation, because the fossil axes present an almost identical structure. The similarities between the two may be considered under the following heads:

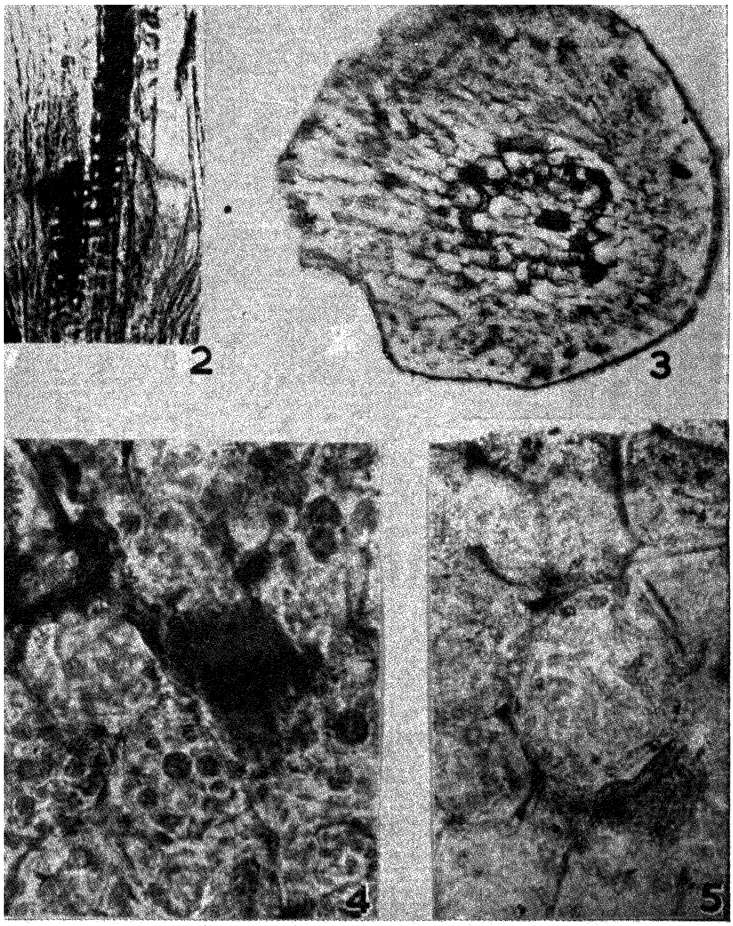
(*i*) Stele—The centrally placed cylindrical xylem strand in the axis of *R. gwynne vaughani*, like that of a vascular prothallus of *Psilotum* (*see* Holloway, 1939), is made up of annular tracheids (scalariform elements are also found in *Psilotum*) which are surrounded in both by a tissue of thin-walled tubular cells. These are filled with granular protoplasm in the living prothalli and are regarded as phloem elements although they do not show any sieve plates either in the fossil or the living axes. In the prothallus of *Psilotum*, the xylem also shows scalariform elements (according to Bierhorst, 1953, only scalariform reticulate elements are present); and although Kidston & Lang (1917) have not reported such tracheids in *Rhynia*, my Figure 2 clearly shows such thickenings in the xylem of an axis of *R. gwynne vaughani*.[2]

(*ii*) Endodermis—In the vascular prothalli of *Psilotum* a limiting endodermis is also present but no such structure has been reported in the axes of *R. gwynne vaughani*. However, two sections of the axes of this species show, around the thin-walled elements of its vascular strand, a single layer of cells whose walls appear to be slightly thicker (Fig. 3). This layer looks very much like an endodermis, but the typical Casparian thickenings or strips have not been found. Kidston & Lang (1920 a) have reported a similar endodermis in an axis of *Asteroxylon*.

(*iii*) Two zones of the cortex and mycorrhiza—Outside the stele, in the "aerial" as well as "rhizomatous" axes lies the cortex which is differentiated into two zones—an inner cortex with darker coloured cells and a few-layered outer cortex with larger and clearer cells. In the saprophytic prothalli of the living pteridophytes and also in the mycorrhizic roots and rhizomes of their sporophytes the cells of the inner zone are either more or

[2] It is interesting to note that Hansteen (1892) has figured and described scalariform thickenings on the walls of the medullary cells of *Sargassum*.

less uniformly filled with dense skeins or clumps of endophytic fungal hyphae or they contain brown vesicles where the hyphae are no longer recognizable (*see* Bruchmann, 1904, 1906, 1910 ; Campbell, 1911 ; Holloway, 1917). Only the epidermis and

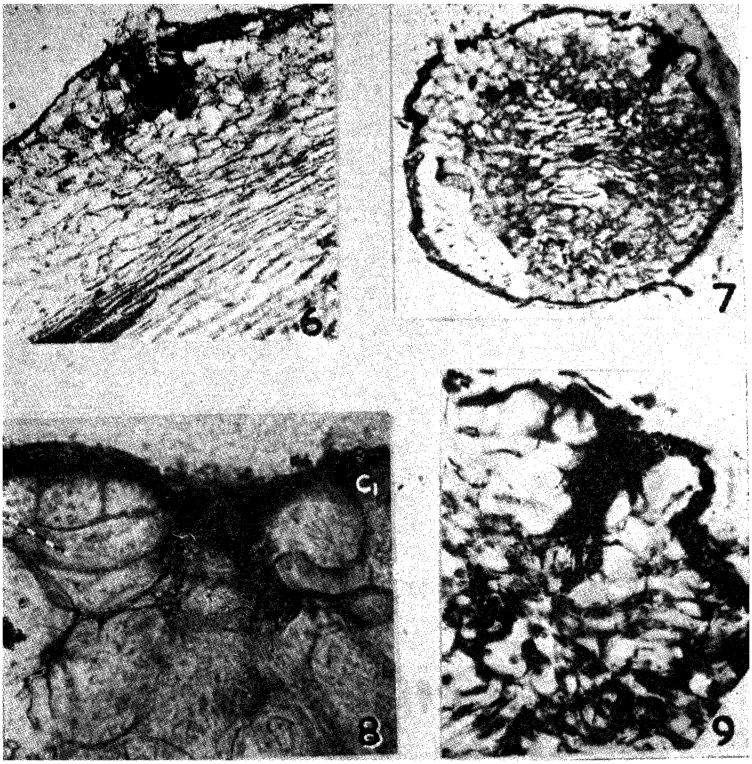


Figs. 2-5. *Rhynia gwynne vaughani*. Fig. 2. Xylem of axis showing scalariform thickenings. Glasgow University slide No. 278. × 220. Fig. 3. Axis showing endodermis-like sheath of thick-walled cells around stele. Slide No. A.U./A_2. × 65. Fig. 4. Cortical cells of axis showing fungal vesicles. Slide No. A.U./A_2. × 384. Fig. 5. Cortical cells of axis showing fungal filaments. Slide A.U./A_2. × 286.

3-4 layers of cells underlying it are relatively or completely free from these coils (*see* Holloway, 1917 ; Darnell Smith, 1917 ; Lawson, 1917a, b ; Bierhorst, 1953 ; Kidston & Lang, 1921a). The absence of a mycorrhizic fungus inside the cells of the axes of *R. gwynne vaughani* has, however, been very pointedly recorded by Kidston & Lang (1921a). They state that "no evidence of the presence of a fungus forming a mycorrhiza has been found although the saprophytic fungi in the peat are well preserved". They further point out that "there is no doubt that the active growth of various fungi with evident mycelium-bearing vesicles or resting spores was here (in *R. gwynne vaughani* and *R. major*) saprophytic". Nevertheless, they admit that "the distribution and appearance of the layer of cells with very persistent dark contents immediately below the outer cortex suggests, however, the possibility that this region might have contained a symbiotic organism" and that "in the case of *Rhynia* also the conclusion at present seems to be that proof of the existence of mycorrhiza is wanting though there are grounds for further enquiry into the question". They have themselves figured fungal hyphae and vesicles inside the cells of *R. gwynne vaughani* and also other Rhynie plants (Kidston & Lang, 1921b).

In sections of some axes of *R. gwynne vaughani* showing no apparent signs of decay, I also found well preserved thin-walled, narrow aseptate tubes ca 4 microns in diameter (Figs. 1B, 5), apparently anastomosing inside the lumen of cells. These are obviously the same intruding fungal hyphae which Kidston & Lang have described. The form and the anastomoses of these hyphae are similar to those of the mycorrhizic hyphae in the Psilotales and other plants (Janse, 1897 ; Bernatzky, 1899 ; Shibata, 1902 ; Burgeff, 1938). Some of the cells in *R. gwynne vaughani* show a number of rounded bodies or vesicles (Fig. 4) and similar bodies have also been figured by Kidston & Lang (1921b). These authors have, in addition, figured some rounded and probably badly preserved structures (Kidston & Lang, 1917), which they interpret as chloroplasts or starch grains. However, in the light of the occurrence of fungal hyphae and vesicles in some cells of these axes as discussed here and also on account of the presence of similar rounded vesicle-like bodies in the cells of the rhizomes and gametophytes of *Psilotum* (Janse, 1897 ; Bernatzky, 1899 ; Holloway, 1939 ; Burgeff, 1938), their fungal nature becomes more plausible.

The dark opaque contents of some cells of the inner cortex, especially those which lie immediately inside the outer cortex,



Figs. 6-9—*Rhynia gwynne vaughani*. Figs. 6,7. Archegonium-like structures with slightly projecting neck cells. Fig. 6, Glasgow University Slide No. 277. × 27. Fig. 7, Slide No. A.U./A_2. × 60. Fig. 8. A "stoma" with dark-coloured cells on the sides (C, C_1). Slide No. A.U./A_2. × 282. Fig. 9. Part of Figure 7 further magnified. × 170.

and the brownish colour of the inner cortex is again likely to be due to the formation of dense skeins of hyphae, vesicles or "Excretkorper" by the digestion of the fungal hyphae as in the Psilotales (*see* Holloway, 1917 ; Burgeff, 1938). A remarkable resemblance is seen between the dark bodies inside the cells of *Rhynia* and *Psilotum* gametophytes if we compare the illustrations of Kidston & Lang (1917) with those of Holloway (1939).

The differentiation between the inner and the outer cortex noted by Kidston & Lang (1917) may thus appear to be due to the abundance and the relative paucity or absence of fungal

filaments in the two zones as in the gametophytes[3] of *Tmesipteris* and *Psilotum* (Holloway, 1917). It is also interesting at this point to recall Kidston & Lang's (1917) original description:

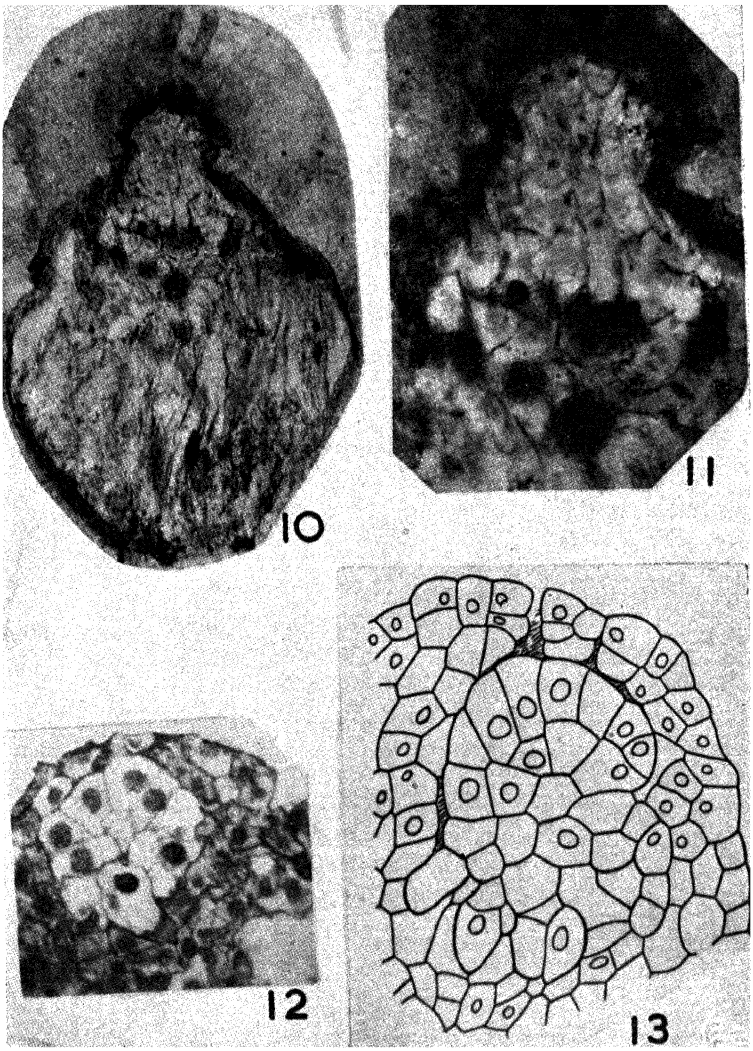
"The outer cortex consists of one to four layers of cells which are large relatively to the cells of the epidermis and the inner cortex. It is best marked in the lower and thicker portions of the aerial stems. The cells of the outer cortex generally appear clear and empty looking and are slightly longer than broad."

"The cells of the inner cortex are round in transverse section and were separated by fairly large intercellular spaces. They are longer than broad, this being more marked in passing from above downwards in the stems (cf. Pl. VII, Fig. 42 with Fig. 43). As preserved, the inner cortex often has a darker brown colour which is especially marked in the cells immediately below the outer cortex (Pl. V, Figs. 21, 22 and 24). In a few cases the cells of the inner cortex are filled with closely crowded bodies suggestive of chloroplasts or possibly starch (Pl. VI, Fig. 39). The inner cortex is specially liable to decay and stems are often found in which it has wholly disappeared while the stele and outer cortex remained."

As will be clear, the structure, thus described differs markedly from that of the photosynthetic aerial axes of *Tmesipteris*, *Psilotum* or *Lycopodium* where the outer zone of chlorenchyma has intercellular spaces between its cells and the inner zone has compact polygonal cells (without inter-cellular spaces). Therefore, on the analogy of these living stems it is difficult to understand the reversal in the position of the photosynthetic and the "hypodermal" tissues in *R. gwynne vaughani*. On the other hand, it tallies exactly with the structure of the cellular tissue in the vascular or the non-vascular gametophytes of *Psilotum, Lycopodium, Botrychium, Helminthostachys, Ophioglossum* etc.

Even in being specially liable to decay, the cells of the inner cortex of the fossil axes of *Rhynia* are remarkably similar to those of the gametophytes and sporophytic rhizomes of *Psilotum*. Bierhorst (1953) has reported empty "shells" of the outer cortex occurring in the soil along with *Psilotum* rhizomes and prothalli. The cells of these shells resist decay because their walls are highly cutinised. Probably a similar cutinisation resisted decay of the outer cortex of *Rhynia*.

[3] A similar anatomy is exhibited by the rhizomes of the Psilotales but, as will be clear later, the comparison is not close due to the absence in these of the "bulge"-like sporelings.



Figs. 10, 11. *Rhynia gwynne vaughani* and Figs. 12, 13—*Psilotum nudum*. Fig. 10. Axis showing lighter tissue of "hemispherical projection." Slide No. A.U./A_2. × 98. Fig. 11. Portion of Figure 10 magnified to show projecting haustorial cells at base. × 178. Fig. 12. Lighter tissue of a young sporophyte embedded in the gametophyte. × 160. (*After* Bierhorst, 1954a). Fig. 13. A young sporophyte showing haustorial cells at base. × 156. (*After* Holloway, 1939).

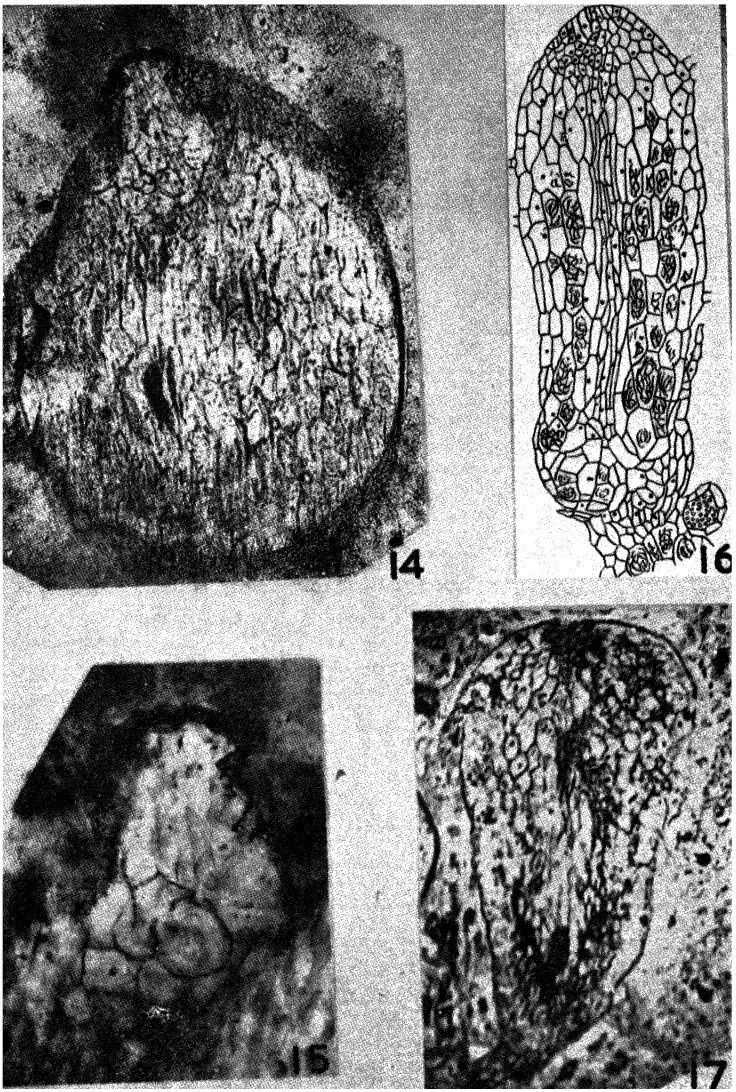
Moreover, if we regard the two cortical zones as made up of densely fungus-filled and relatively fungus free cells, it would also be in accord with the existence of the distinction (an inner and outer cortex) being "traceable throughout the plant" including the "rhizome" and even in the slender axes without a stele which can either be compared with cylindrical gametophytes or some underground sporophytic axes of *Psilotum* (*see* Bierhorst, 1954 b). The differentiation of the two zones is more marked in the basal parts of the aerial axes (Kidston & Lang, 1917).[4] On the other hand it could never have been so if the two zones represent a hypoderm (the cells of which should have thicker walls) and an assimilatory zone (which is not present in underground parts and is less marked at the base of aerial stems).

Even prior to this, endophytic fossil fungi of fairly indubitable mycorrhizic nature have been described by Weiss (1904), Osborne (1909), and Andrews & Lenz (1943), the last mentioned authors reporting them from beds as early as the Pennsylvanian. The present suggestion about the occurrence of mycorrhiza in these fossil axes would thus appear to be quite plausible in view of the typical structure of the fungal hyphae and the vesicles, and it would trace mycorrhizal associations down into the Devonian.

(*iv*) CUTICLE—The elongated cells of the epidermis of *R. gwynne vaughani* are covered with a fairly thick cuticle and a similar situation exists in the prothalli of the Psilotales (Lawson, 1917 a, b ; Darnell Smith, 1917 ; Kidston & Lang, 1917).

(*v*) INJURIES—The structural similarity between the prothalli and underground sporophytic axes of Psilotales and the fossil axes of *Rhynia* appears to be carried even to the occurrence of injuries and groups of necrosed cells in their superficial layers (Kidston & Lang, 1917, 1921a ; Darnell Smith, 1918 ; Bierhorst, 1953, 1954 a). The injuries in the living axes are regarded to be due to the growth of an unprotected apex through coarse soil while Kidston & Lang (1921a) have attributed the injuries in the axes of *R. gwynne vaughani* to the poisonous gases etc. of the presumed volcanic environment in which these plants are believed to have grown. It is also noteworthy that the injuries in the living gametophytes do not lead to the formation of any adventitious branches although they may occasionally lead to branching and the interruption of the vascular strand. As explained below it would, however, be clear from a comparison of the

[4] They have given a contrary account at another place (1921a), where they say that "the layer is marked in the stouter stems and not in the rhizomes".

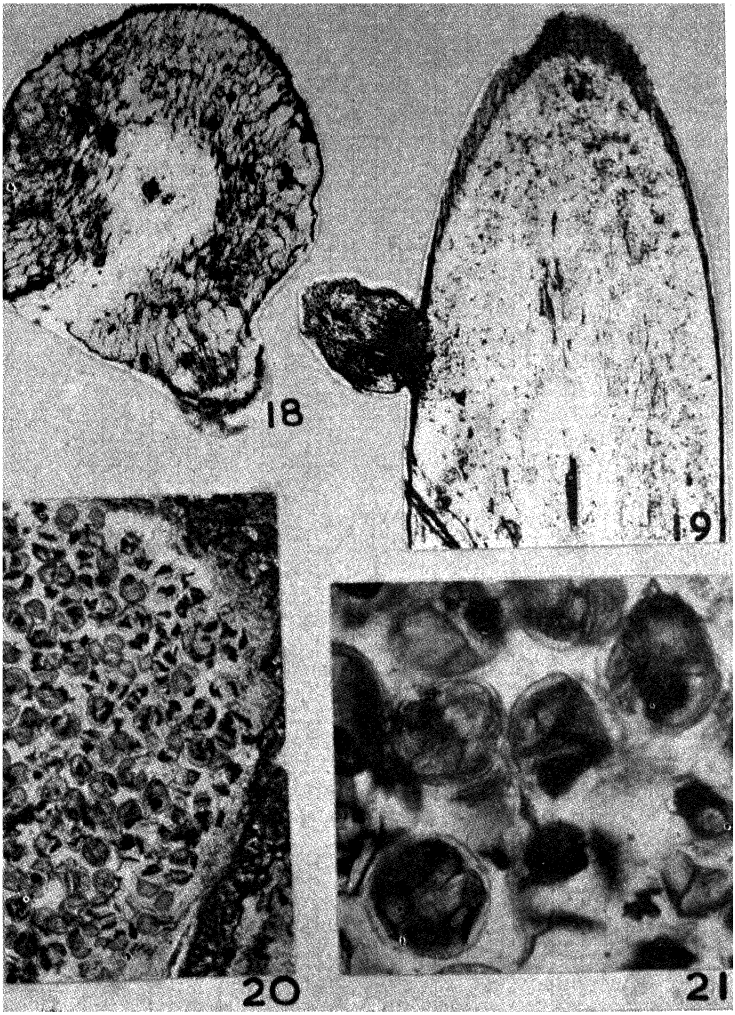


Figs. 14, 15 and 17—*Rhynia gwynne vaughani*, Fig. 16—*Psilotum nudum*. Fig. 14. Axis showing "hemispherical projection". Slide No. A.U./A_2. × 100. Fig. 15. Lighter coloured tissue of bulge further magnified to show rounded ends of haustorial cells at base. × 183. Fig. 16. Young detached sporeling. × 44 (*After* Holloway, 1939). Fig. 17. Detached adventitious branch. Kidston collection Slide No. 2395. × 34.

"hemispherical projections" and the "adventitious branches" in *R. gwynne vaughani* with the young developing sporophytes in *Psilotum* gametophytes that the injuries probably have no relation with the so called "adventitious branches" or "hemispherical projections". Despite the occurrence of necrosis and callus formation and despite the apparent resemblance between the actively dividing cells of callus and the growing tissue of the hemispherical bulges, to which Kidston & Lang (1921a) have drawn our attention, it is noteworthy that they have themselves clearly admitted that there is no constant association between necrosis and hemispherical bulges or necrosis and stomata.

The possible archegonial nature of at least some of the "stomata" in axes of *R. gwynne vaughani*

The presence of well developed stomata in the axes of *R. gwynne vaughani* and their absence in the prothalli of the Psilotales would appear to be a clear-cut distinction between the two. But in this connection we may first take note of the remarkable fact that the vascular gametophytes of *Psilotum* (and also those of *Tmesipteris*) develop archegonia in which all the projecting tiers of the neck cells except those of the basal tier are shed after fertilisation. The cells of the remaining basal tier then appear to be flush with the superficial cells of the gametophyte and become highly cutinized. In a section, then, it seems that often only two of them are present and but for the presence of the egg cell below they could be easily mistaken for the guard cells of a stoma (Holloway, 1917, 1921, 1939 ; Bierhorst, 1954 a). In the prothalli of *Ophioglossum, Helminthostachys* or Marattiaceae the neck itself is sunk and flush with the surface of the gametophyte. If supposing such prothalli got fossilized, an undivided or more particularly, a divided egg cell would not be easy to observe and the presence of the vascular strand in the axis would be another reason for their being confused with stomata (as it must have obviously been at the time when Kidston & Lang described the Rhynie Psilophytales—because no vascular gametophytes of the type had been described till then). If on the other hand, we could see them in surface view, their four-celled tops would at once distinguish them from true stomata provided the archegonial necks of these plants had four vertical rows of cells as in modern pteridophytes. Unfortunately, very few stomata have been observed in surface view. Out of the few stomata figured by Kidston & Lang (1917) in their Pl. VI, Figs. 33 and 34 (Fig. 37 was subsequently referred by these authors to *R. major*) show them in sectional views. None of them, however, shows a projecting neck or an egg cell although a dark structure under the "stoma" in Fig. 34 is quite



Figs. 18-21—*Rhynia gwynne vaughani*. Fig. 18. Axis showing "hemispherical projection". × 48. Fig. 19. Axis with "adventitious branch" showing dark coloured cells of abscission layer at base. Glasgow University Slide No. 278. × 26. Fig. 20. Sporangium attributed to *Rhynia gwynne vaughani* showing spores enclosed in mother cell membrane. Kidston Collection Slide No. 2424. × 126. Fig. 21. Part of Figure 20 magnified. × 525.

suggestive. Out of the only two stomata figured in surface view by these authors, one (Kidston & Lang, 1917, Pl. VI, Fig. 32) belongs to *R. major*. All the same the only remaining stoma of *R. gwynne vaughani* which is figured in surface view (Kidston & Lang, 1921, Pl. III, Fig. 9) definitely shows two guard cells. Nevertheless, the occurrence of true stomata cannot necessarily conflict with the suggested gametophytic nature for some of these axes because stomata-like pores are present even in the gametophytes of some of the Anthocerotales (Goebel, 1930 ; Bower, 1935) and there may still be other stoma-like structures below which alone all "adventitious branches" and "hemispherical projections" are developed.

In addition, a few "stomata" in sectional views have also been observed by me. Figure 8 shows a "stoma" with a small dark cell below the "guard cells" (the structure appears similar to that seen just below the "guard cells" in Kidston & Lang's Pl. VI, Fig. 34). Some of the epidermal and cortical cells by the side of the "guard cells" in this "stoma" also appear to have divided by periclinal divisions as in the formation of the "cushion" or the "calyptra" in *Tmesipteris* (Holloway, 1917, 1921) and *Psilotum* (Holloway, 1939). My Figures 6, 7 and 9 show some archegonium-like structures. It seems that these structures are situated on slight elevations or their necks are slightly projecting above the epidermis. Some of them show a clear canal-like passage leading into a widened venter-like base. These figures could also be very well compared with Holloway's (1921, 1939) figures.

Developmental similarities between "hemispherical projections" of *R. gwynne vaughani* and the young sporophytes of Psilotales

The suspicion about the gametophytic nature of at least some of the axes of *R. gwynne vaughani* is further strengthened by the formation and the development of the unique "hemispherical projections" below these "stomata" in *R. gwynne vaughani* and by the development of very similar spindle-like sporelings with hemispherical heads in the archegonia of *Psilotum, Lycopodium, Ophioglossum* etc. As the embryo of *Psilotum* or *Tmesipteris* enlarges, keeping pace with it "the surrounding prothallial cells rapidly subdivide, so that the embryo is enwrapped by a small-celled tissue which soon begins to project as an eminence from the side of the prothallus" (Holloway, 1917, 1921, 1939 ; Bierhorst, 1954a). Above the apex of the young sporophyte, the cells of the epidermis (superficial layer) of the gametophyte and those of its cells lying in immediate vicinity mainly partake in the formation of the calyptra, which is clearly in accord with the condition in *R. gwynne vaughani* and which could also explain the belief of Kidston & Lang (1917) that "the bulge is due to the periclinal division in the epidermis and outer cortical cells." Later,

when the enlarging sporophyte of Psilotales breaks open the calyptra enabling its hemispherical head to bulge out from between the ruptured gametophytic tissue (*see* Holloway, 1917, 1921, 1939), its structure again corresponds closely with the figures and description of Kidston & Lang (1917) of the developing "hemispherical projections" of *R. gwynne vaughani* wherein they say that "the outer wall and the cuticle cease to be marked over the projection ; this often exhibits a brown discolouration of its outer cells which are sometimes broken down".

Clear demarcation between the tissues of the "hemispherical projections" and the "parent axes"

In Kidston & Lang's (1917) Plate VIII, Figure 9 and my Figures 1A, C, D, E, 10, 11, 14 and 15 a clear demarcation is seen between the thinner-walled isodiametric lighter-coloured cells of the bulge and the darker-coloured cells of the parent axis, a feature which strongly supports the view advanced here because the cells of the uninfected young sporophyte of Psilotales also are clear and well demarcated from those of the gametophyte (Fig. 12 ; *see also* Bierhorst, 1954a). In spite of this clear differentiation between the two tissues, Kidston & Lang (1917) claim that they have satisfied themselves that "there is an unbroken continuity between the two", perhaps the embedded character of the foot and the dividing cells surrounding the cushion would also sometimes convey a similar notion, particularly if seen "preserved" in the fossil condition. Kidston & Lang's assertion that "the tissues at the base of the branch are continuous with the outer cortex and epidermis of the parent axis" is due to the formation of a calyptra. Even in the case of the young sporophytes of the Psilotales it appears to be continuous (*see* Holloway, 1917, 1921, 1938 ; Bierhorst, 1954a).

Structural similarities between "hemispherical projections" or "adventitious branches" of *R. gwynne vaughani* and young sporelings of Psilotales

(*i*) Similarity of shape and structure between "projections" and young sporelings and the production of rhizoids from their tops—The newly-formed "projection" in favourable sections shows a structure remarkably similar to that of the young sporophytes of Psilotales (compare my Figs. 11, 12, 14, 15, 18 and Figs. 1C, D with Holloway's, 1917, Figs. 58, 61 and 1921, Figs. 47A-D). Both types of structures produce rhizoids from their bases and apices. Kidston & Lang (1917) have themselves figured these clearly (Pl. V, Figs. 25 and 29 and Pl. VII, Figs. 52-54) and state "that the superficial cells of some of the small projections grow out as rhizoids similar to those on the rhizome" and this happens even when the "projections" are developed in otherwise

rhizoidless portions of the "aerial" axes (*see also* my Figs. 1A, C, 10, 11). All these figures can be suitably compared with Holloway's figures (1917, Figs. 58, 59 ; 1921, Fig. 69B ; 1939, Figs. 63, 64A, and 67A).

A feature not so far recorded but which is almost constantly present along with these "projections", is the accumulation of fine brown particles in the form of a brown haze (Figs. 10, 11, 14, 15) in the sections outside the apex of the "projection". Its nature is difficult to ascertain but it may be due to the growth of fungi or other organisms and a collection of humus in between the rhizoids opposite the "projection."

(*ii*) HAUSTORIAL CELLS—In Figures 1A, C, D, E and Figs. 10, 11, 14 and 15 the projections show on their basal sides, cells with rounded ends slightly projecting into the tissue of the parent axes like the haustorial cells of the foot of a young sporophyte which project into the tissue of the gametophyte in the Psilotales (*see* Lawson, 1917 ; Holloway, 1917, 1921, 1939 ; and Fig. 13).

(*iii*) "ADVENTITIOUS BRANCHES" AND DEVELOPING SPOROPHYTES—Kidston & Lang (1917) have further described that "many of the small projections were the seat of a further development of considerable interest, adventitious lateral branches being produced from them" but all these structures correspond closely with the developing sporophytes of the Psilotales as figured by Holloway (1921, 1939). The swollen negatively geotropic head and the narrowed base of attachment of the "adventitious branch" in *R. gwynne vaughani* and its ready detachment from the parent axis are again features finding their exact parallel in the parent gametophyte and the young developing sporophyte of the Psilotales. Even the branching of the "adventitious branch" while still attached to the parent axis (Kidston & Lang, 1917) has its corresponding stage in the Psilotales where too, in some cases, the young sporophyte has been recorded as branching while still attached to the parent gametophyte (*see* Holloway, 1921, 1939).

(*iv*) THE ABSENCE OF A VASCULAR CONNECTION WITH THE PARENT AXIS IN BOTH CASES—Kidston & Lang (1917) have described the absence of a vascular connection between the stele of the parent axis and the "adventitious branch", which is as it should be, if the "branch" belongs to a totally different generation as between the sporophyte and gametophyte of the Psilotales.

(*v*) PROBABLE FORMATION OF AN ABSCISSION LAYER—Again, like the sporophyte of the Psilotales, the "adventitious branch" also detaches itself at its narrow base (Kidston & Lang, 1917). The dark appearance of the tissue at this spot (*see* Fig. 19) and in Kidston & Lang's (1917) Figure 57 presumably indicates that this happened as a result of the formation of a "line of dehiscence" or an abscission layer by the "browning of the cell walls, along the line", as is reported by Holloway (1917) for the sporeling of *Tmesipteris*.

(*vi*) YOUNG DETACHED "ADVENTITIOUS BRANCHES" VS. FRESHLY DETACHED SPOROPHYTES—Young detached "adventitious branches" are also sometimes found (Kidston & Lang, 1917). The specimen figured by Kidston & Lang in Pl. X, Figure 72 shows a striking resemblance with a sporeling of *Psilotum* figured by Holloway (1939, Fig. 64A) in regard to its shape with its swollen head and a tapering base, and its structure with its growing apex having smaller cells and a central strand with elongated cells (*see also* my Figs. 16, 17). Such is the close correspondence between the two figures that a few cells at the base of the "adventitious" branch even appear to correspond to the cells of the gametophyte which have been torn off along with its young sporeling.

Kidston & Lang (1917) have suggested in this connection that "this was an important method of vegetatively propagating the plant" but if our present interpretations be correct then this was instead the formation of young sporophytes by the gametophytes.

(*vii*) SIMILARITY OF THE SCARS AFTER DETACHMENT—Kidston & Lang (1917) have further pointed out that the "scar left by the branch often became the seat of degenerative changes", and they found that "its position is then marked by a small patch of dead tissue" and that "the adjoining cells had frequently elongated at right angles to this". The scar left after the detachment of the sporeling in *Tmesipteris*, as described by Holloway (1917) is probably similar, where according to him: "On some prothalli a large cup-shaped prominence with an obviously lacerated rim was to be seen. This is where a young plantlet had been broken away, the cup-shaped prominence having been formed by the localised growth of the prothallial tissues around the embryo and their final rupture by the developing plantlet." And perhaps a similar thing happens in *Psilotum* (Holloway, 1939). The changes, if any, at the site of the foot after the detachment of the sporeling have, however, not been described so far for the prothalli of Psilotales, yet the necrosis that is reported to occur in the position of the scar in *R. gwynne vaughani* is probably what can be expected of a small pocket of cells of an abandoned foot which has remained anchored in the foreign tissue of a gametophyte after its purpose is over and after it has been abscissed from a sporeling in which the growth is confined to the apex. In any case it would be interesting to know if the foot undergoes a similar degeneration in the gametophytes of the two living genera as well.

OCCURRENCE OF GAMETOPHYTE-LIKE NON-VASCULAR AXES IN *R. gwynne vaughani*

Kidston & Lang (1917) have figured a transverse section of a small axis (Pl. X, Fig. 73) which does not show any vascular strand and according to them it is a section of an "adventitious branch". However, this

interpretation is not free from doubt and it could be assigned to a cylindrical prothallus with equal reason. In fact its being a section of an "adventitious branch" is less likely because along the median line in longitudinal sections of many almost equally developed "adventitious branches" (about their "adventitious" nature there can be little doubt in longitudinal sections) one can possibly always see either a vascular strand or a few elongated cells which are narrower than the surrounding ones (*see* Kidston & Lang, 1917, Pl. VII, Figs. 50, 51, 55-59, and 72), and a transverse section of such stems is, therefore, bound to show a central zone of xylem or cells of smaller diameter. So far as the author could make out the central cells in the non-vascular stem, are as wide as those at the periphery. On the other hand, one can see a well differentiated vascular strand in some other stems of the same species which appear to be even smaller (Kidston & Lang, 1917). The above facts also rule out the possibility of its being a section of the immature apical portion of a vascular axis (cf. apical portion in Kidston & Lang, 1920b, Pl. I, Figs. 2-5). The curious non-vascular axis can, therefore, be more plausibly interpreted as a gametophyte on the analogy of the non-vascular prothalli of *Psilotum* (Holloway, 1939) and *Tmesipteris* (Holloway, 1917) or of the non-vascular parts of the otherwise vascular gametophytes of *Psilotum* (Holloway, 1939). Bierhorst (1954 b) has no doubt figured certain non-vascular sporophytic axes in *Psilotum* but they develop no hemispherical projections.

Absence of actual organic connection between indubitable axes of *R. gwynne vaughani* and the five sporangia assigned to it

Besides the above positive facts (if we may call them so) of close resemblance between the axes of *R. gwynne vaughani* and the gametophytes of Psilotales, we may also note the negative evidence afforded by the absence of actual organic connection between indubitable axes of *R. gwynne vaughani* and the five sporangia believed to belong to that species (Kidston & Lang, 1920a). The reasons for assigning these sporangia to the smaller species appear to be their "much smaller size" and their occurrence among characteristic stems of *R. gwynne vaughani*. As regards the first point, their smaller size need not necessarily mean that they belong to the smaller species (the sporangia of *Asteroxylon* are much smaller than those of *R. major*) and it could even be that some of the sporangia in question are immature. At least one of them (Kidston & Lang, Slide 2424 ; *see also* my Figs. 20, 21) shows spores still enclosed within the mother cell membrane. Two others (Kidston & Lang, 1917, Pl. IX, Figs. 63, 63A ; 1929a, Pl. I, Figs. 6-8) are empty and, therefore, it cannot be ascertained if at all they contained any spores, mature or otherwise (shells of vegetative axes where all internal tissues have decayed

present similar appearance). So far as the second point is concerned, I can now say by confirming from the slides that it is not true for any one of the five sporangia ; Kidston Collection Slide No. 2424 containing one sporangium of *R. gwynne vaughani* exhibits in addition, the stems and rhizomes of *Asteroxylon* and also stems and sporangia of *Horneophyton*. Slide No. 2423 which contains another sporangium (Kidston & Lang, 1920a, Fig. 10) perhaps also shows axes of *R. major* and slide No. 2427 having a third sporangium (Kidston & Lang, 1920a, Pl. I, Fig. 8) contains pieces of the sporangial wall apparently of *R. major*. Moreover *R. major* sporangia are frequently found among the axes of *R. gwynne vaughani*.

Two of the five sporangia of *R. gwynne vaughani* (Kidston & Lang, 1920a) which show "neither a definite tapetal layer nor a characteristic thick walled epidermis" are regarded by Kidston & Lang as suggesting "a less differentiated (primitive) condition in which spores were formed within the end of a stem without being modified into a definite sporangium". But as stated above, one of these perhaps shows an undifferentiated wall because it is immature (it contains immature spores). In addition, it may be pointed out that both these sporangia are pear-shaped with a narrow base as in *Asteroxylon* where only the distal part has thickened epidermis (Slide 2428 containing the sporangium figured by Kidston & Lang in their Fig. 11 is actually labelled "Sporangium of *Asteroxylon*" and in pencil "*?Rhynia*"!

Discussion

It is clear from the above account that there is a close structural similarity between the gametophytes of Psilotales (more especially the vascular gametophytes of *Psilotum*) and the axes of *R. gwynne vaughani*, and also between the development and structure of "adventitious branches" in *R. gwynne vaughani* and the formation, development and structure of the young sporelings, in Psilotales. Indeed the resemblance between the fossil and the living structures is so close that it is hard to explain it on any other basis than by regarding even the fossil "parent axes" as gametophytic. This nature of the axes of *R. gwynne vaughani* explains *ipso facto* the nature of the curious "adventitious branches" which then satisfactorily fit in as young sporophytes. On the other hand, if we continue to regard them as "adventitious branches", they would be without parallel in any other psilophyte or other plants. Williams (1933) has compared them with certain growths in cut stems of *Lycopodium* but there is no proof of injury in *R. gwynne vaughani*. They can, therefore, be compared (Kidston & Lang, 1921a) only with certain pathological structures. But it is difficult to believe that the gases and other adverse conditions at Rhynie chose only the frailest among the contemporaneous

flora for trying their adverse effects and left the bigger forms (e.g. *R. major, Asteroxylon mackiei*) unaffected. Furthermore, it would be very surprising if in this plant a cancerous growth were to transform itself into an advantageous method of vegetative propagation. There is also the somewhat far-stretched view expressed by Brown (1935) that these "adventitious branches" may represent emergences of the nature of the spines in *Psilophyton* or *Drepanophycus* but even a cursory comparison of the development and structure of an "adventitious branch" clearly shows that they cannot be compared with such emergences.

It must, however, be admitted that the present evidence is mainly indirect. Direct proof can be supplied only by demonstrating well preserved sex organs (archegonia and antheridia) on these axes. The possible archegonial nature of at least some of the "stomata" and the occurrence of other archegonium-like structures is perhaps some evidence of the kind but at the present moment no clear antheridia have been observed. Further work is in progress.

The reported occurrence of two sizes of cutinised sporangia along with the remains of these plants in the Old Red Sandstone beds could suggest the occurrence of two distinct types of sporophytes of the same genus in the flora, but as already mentioned these might only represent developmental stages all belonging to *R. major*. This doubt is further strengthened by the fact that sporangia of *R. major* are recorded to occur among the axes of *R. gwynne vaughani* (Kidston & Lang, 1921b).

In fact, the distribution of the plant remains in the different layers of the Rhynie chert itself appears to be suggestive (Kidston & Lang, 1921b). Remains of *R. gwynne vaughani* which appear to have been fossilised *in situ* without undergoing any compression, are found at the bottom of the basal 15 inches (A″1) of the chert (only at one place they are found higher up but that layer could have been a transported one). Abundant fungi growing in the peat occur in close association with its axes and in this layer are also found algae and crustacea indicating more aquatic conditions. Above these are found in the same 15 inches of the basal region (A″1), undisturbed remains of the other Rhynie vascular plants, e.g. *Horneophyton* (*Hornea*) *lignieri, Rhynia major* and *Asteroxylon mackiei* (the beds above these show intervening layers of sand and could have all been deposited on transportation). If now a comparison is made between the mode of occurrence of *Rhynia gwynne vaughani* and the accounts of the growth of the prothalli of *Psilotum* (discussed in detail by Darnell Smith, 1917 ; Lawson, 1917 ; Holloway, 1939), and *Lycopodium* (Holloway, 1917), one feels tempted to imagine the former becoming petrified while growing under similar circumstances.

It would thus be clear from the arguments advanced here that the sporophytic nature of *Rhynia gwynne vaughani* is quite uncertain. In

fact the positive evidence provided by the occurrence of the curious spore-ling-like "hemispherical projections" and "adventitious branches" and their identical mode of development are clearly in favour of its being a gametophyte. The occurrence of other archegonium-like structures and the absence of any undoubted sporangia in organic connection with its axes give further support to the present hypothesis and at least for the axes bearing the unique "hemispherical projections" and the "adventitious branches" a gametophytic rather than sporophytic nature appears to be more plausible.

If *Rhynia gwynne vaughani* is a gametophyte, we may also attempt to reconstruct its entire form. As can be seen from the reconstruction of this species given by Kidston & Lang (1921a), it would be by far the largest known prothallus in a Pteridophyte (1-3 mm in diameter and about 18 cm in height) with well developed prostrate and erect branches. Compared to these, the vascular gametophytes of *Psilotum* (2 mm × 20 mm) or even the much larger cylindrical prothalli of *Ophioglossum vulgatum,* which are recorded to become about 6 cm long (Eames, 1936) are much smaller.

Indeed such a reconstruction appears as essentially indentical with the hypothetical ancestor figured by Fritsch (1945, Figs. 10, 12 ; *see also* my Fig. 1 F) and the only change that wo need to introduce is the insertion here and there of a few young sporophytes ("adventitious branches" or "hemispherical projections") in various stages of development in place of some of the archegonia. It is, however, difficult to say whether the erect portions of the axis in such a reconstruction were mainly aerial or underground. If, as it appears, some of the "stomata" on the axes proved to be ventilating pores, they would strongly suggest that these parts were subaerial. It may also be that only a part of the structure is gametophytic and, therefore, a reconstruction of the prothallus may have to be made afresh. In any case, from the almost identical structure of these probable gametophytic axes as compared to the other apparently sporophytic genera of the Psilophytales, e.g. *Asteroxylon mackiei* and *Rhynia major,* it seems, as was also predicted by Fritsch (1945), that the gametophytes of these plants were highly isomorphic, radially organised vascular structures (they would appear to resemble the sporophytes to a far greater extent than even the vascular gametophytes of *Psilotum* or the large cylindrical gametophytes of *Ophioglossum pendulum, Helminthostachys* or *Lycopodium billardieri* which also show a "distinct axial tissue consisting of considerably elongated fungus-free cells in the main axis and branches of the gametophytes" (*see* Holloway, 1939).

It may be argued that the presence of a vascular strand in such gametophytes as those of *Psilotum* is only an aberrant genetic development since Manton (1942) has proved that Holloway's large vascular gameto-

phytes, as well as some small non-vascular ones from Rangitoto Island are probably all diploid while his sporophytes from the same place appear to be tetraploid. She showed that even the tetraploid sporophytes had certain abnormalities of meiosis in their sporangia. Although at that time these anomalies were suspected to be due to the polyploid nature of the sporophytes yet, so far as the presence of the conducting strand in the gametophyte is concerned, she clearly states that demonstration of diploidy in a prothallus does not in itself provide an explanation of the occurrence of anomalous conducting tissue since many examples of diploid prothalli are known in the Pteridophyta, e.g. *Osmunda* (Manton, 1932), *Aspidium*, and *Woodwardia* (Lawton, 1932), and no morphological aberration need accompany a simple doubling of chromosomes if the parent plant is genetically normal. Recently, however, the meiotic irregularities of even the sporophyte have been shown to be metabolic in origin by Professor Barber (*see* Manton, 1950).

Of course, these probable gametophytes have still to be assigned to their particular sporophyte or sporophytes in the Rhynie flora. It would further appear that their generic designation will have to be changed if they ultimately turn out to be gametophytic unless we are able to assign them to *Rhynia major*.

In the end we may also briefly consider the impact of the probable final emergence of *R. gwynne vaughani* as the gametophyte of some of the Rhynie Psilophytales on the hitherto accepted ideas about the evolution and the relationships of the group. It has been claimed by some that the simple structure of the Psilophytales like *Rhynia* is the result of reduction (Scott, 1924 ; Eames, 1936) while others (Kidston & Lang, 1917, 1921a ; Kräusel, 1932 ; Bower, 1935 ; Hoeg, 1937) have suggested that it may not be so or, if at all, only to a very negligible extent. The unreduced nature of the homologous vascular gametophyte bearing spindle-like undifferentiated sporelings ("adventitious branches") would suggest that the plants in question are not only ancient and simple but also primitively so.

The undoubted close similarity between the structure of the axes of *R. gwynne vaughani* and the prothalli of Psilotales (specially the vascular prothalli of *Psilotum*) and the development of the "hemispherical projections" and the "adventitious branches" in the former and the similar development and structure of the sporelings in the latter, if it ultimately establishes *R. gwynne vaughani* as a gametophyte, would also bring the group much closer to the Psilotales. It would, in fact, carry the similarities between the two groups even to their gametophytes, embryogeny and life history. Like the scorpions of the animal kingdom, the "living fossils" included in the Psilotales seem to have retained an ancient type of structure right upto the present day.

SUMMARY

Merker (1958, 1959) has recently suggested that some portions of the Rhynie plants of Psilophytes may be interpreted as gametophytes. The author has been trying to elaborate and confirm a similar hypothesis for the last ten years or more. The new interpretation of the plant of *Rhynia gwynne vaughani* presented here is based on a re-examination of the original preparations of Kidston and Lang in the Glasgow University and numerous duplicates in the British Museum (Nat. Hist.) and other collections. It is suggested that axes of *R. gwynne vaughani* are more likely to be gametophytic and that their hemispherical projections and adventitious branches possibly represent young sporophytes developing on these gametophytes. The various similarities between *R. gwynne vaughani* and the gametophytes of some living and more especially the vascular gametophytes of *Psilotum* are discussed in detail. The sections of the supposed stomata, under which the hemispherical projections and adventitious branches develop, are compared with sections of archegonia after their neck has been shed off. Some structures which resemble archegonia are of particular interest. It is pointed out that the five sporangia which Kidston and Lang assigned to this species on the basis of association may not belong to it.

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SOME RECENT CONTRIBUTIONS TOWARDS OUR KNOWLEDGE OF THE GLOSSOPTERIS FLORA

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Almost up to the close of the Upper Carboniferous times the world appears to have been inhabited by a more or less uniform flora but about the end of the Carboniferous period the southern regions of the globe became deeply frozen. At this time the now cold or cold temperate regions of Europe and N. America were having a luxuriant vegetation in humid swamps. They later started drying and many of the present day tropical or sub-tropical regions of the south, e.g. Peninsular India, South and East Africa, Australia, South America etc. formed vast icy deserts. According to some authorities this was the greatest Ice Age that the world has ever witnessed. Later when the climate of these southern regions became milder they became covered by a vegetation called the *Glossopteris* flora, named after its most widespread genus of leaves. Everywhere the flora of the beds containing *Glossopteris* is so remarkably similar and at the same time so markedly different from the contemporaneous northern Carboniferous or Permian vegetation that it supplies one of the most cogent arguments in support of the Theory of Continental Drift which envisages a world split up not into the Eastern and Western blocks as at present but divided by a vast equatorial ocean (Tethys Sea) into a large southern land mass hypothetically called the Gondwanaland (after the Gond tribe of Central India) and some northern continents.

The barrier between the northern floras and the *Glossopteris* flora of the south was so strong that very few admixtures have been reported and, as Edwards (1955) clearly points out, even these must be viewed with utmost suspicion. The only well known northern plants which are reported to have trespassed into the Gondwanaland are described from Africa and Brazil (*see* Walton, 1929 ; Edwards, 1952) and the only southern elements which reportedly strayed into the northern regions are some *Glossopteris* leaves described from Russia (Amalitzky, 1897, 1901) and elsewhere (e.g. in Greenland ; *see* Harris, 1932). According to Edwards (1955) the Russian leaves are "completely worthless as records of *Glossopteris*". Moreover, there are now other reasons for regarding *Glossopteris* as a general leaf form which was perhaps possessed by diverse plants, and Mesozoic northern leaves like *G. crassinervis* Harris may have belonged to plants which were very different from the southern forms.

Despite the great abundance of *Glossopteris* leaves in the southern

regions, until recently so little was known about their structure and affinities that some botanists attributed them to ferns while others thought that they belonged to seed plants. However, a few contributions in recent years seem to clear the mist surrounding these plants although a great deal still remains unknown.

The Form Genus Glossopteris

The genus *Glossopteris* was erected by Brongniart (1828, 1828-1832) for some Indian and Australian Permo-Carboniferous, spathulate or tongue-shaped leaves (*glossa*=tongue). Brongniart's available type specimens show a clear, netted venation and a well differentiated midrib. Brongniart distinguished two species of the genus, viz. (*i*) *G. browniana* from India and Australia, and (*ii*) *G. angustifolia* from India. He recognised two varieties of the first species: *G. browniana* var. *australasica* from Hawkesbury River area in Australia and *G. browniana* var. *indica* from India. Many later authors recognize the two varieties as two distinct species.

Leaves of *Glossopteris* were subsequently found to be very common in the southern Permo-Carboniferous beds and a large number were described not only from India and Australia but also from Africa, S. America, Antarctica etc. The leaves are grouped under various species some of which are so very widely distributed as to occur in almost all the distant parts formerly comprising the Gondwanaland.

Stem or Rhizome of Glossopteris

There are at least five reports of attached *Glossopteris* leaves which are well known. In 1894 Etheridge described a small stem with rhomboidal leaf-base scars below and a small crown of *Glossopteris* leaves at the apex (Fig. 11). Zeiller (1896) described leaves of *Glossopteris* attached to axes called *Vertebraria*. According to Zeiller, the leaves are attached laterally, opposite to the transverse sides of the rectangular areas. Oldham (1897) confirmed Zeiller's discovery by describing a short *Vertebraria* axis with a crown of leaves attached in the manner of Etheridge's specimen (*see* Fig. 1B) ; his stem did not show any leaf bases. Another specimen with attached *Glossopteris* leaves has also been found in South Africa (Fig. 1C). As figured by Walton & Wilson (1932), it shows an apical group of *Glossopteris* leaves and at least two whorls of leaf bases, separated by well marked internodes. It does not show any rectangular areas which are characteristic of *Vertebraria* (Figs. 1D, 9). A more recent report of attached leaves of *Glossopteris* is by Dolianiti (1954) from South America (Fig. 8). Dolianiti's specimen shows clearly marked longitudinal and transverse grooves typical of the genus *Vertebraria* and four pairs of attached leaves out of which only one is complete. There

is an obvious dissimilarity between the axes with attached *Glossopteris* leaves showing the features of *Vertebraria* (as in specimens described by Zeiller, Oldham and Dolianiti), and those which show no rectangular areas. On account of this disparity some authors, e.g. Thomas (1952),

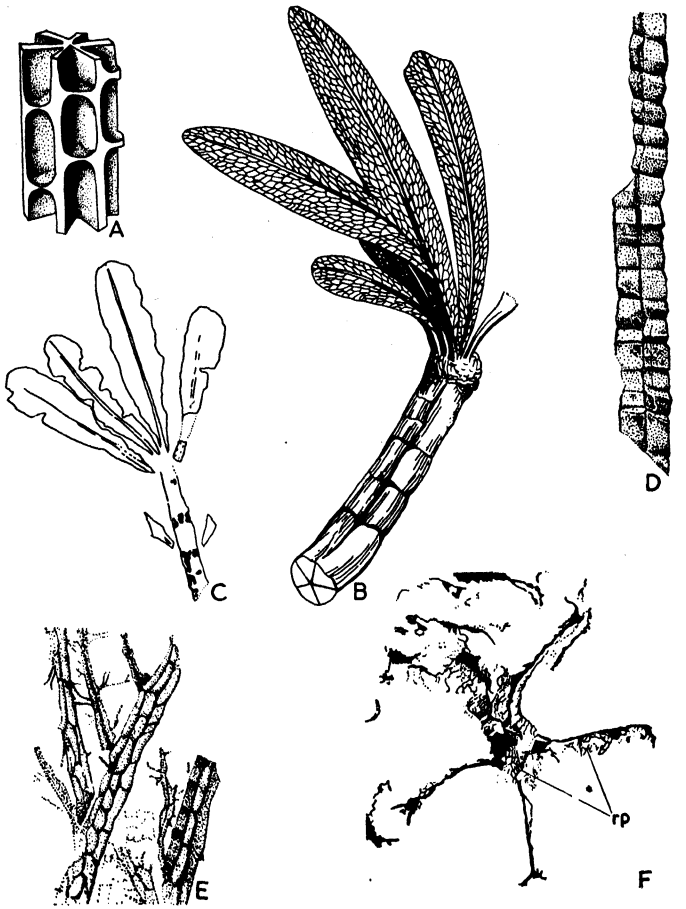


Fig. 1. *A*, reconstruction of the xylem of *Vertebraria* (*After* Walton & Wilson, 1932). *B*, reconstruction of a shoot of *Glossopteris* based on Oldham's specimen (*After* Edwards). *C*, *Glossopteris* axis with leaves (Molengraaff's specimen *after* Walton & Wilson, 1932). × ca ½. *D*. *Vertebraria*, Royle's genotype (*After* Pant, 1956). × ½. *E*, branched *Vertebraria* axes showing slender attached roots (*After* Pant, 1956). × ½. *F*, *Vertebraria* axis preserved almost vertical to the bedding plane, showing large rectangular parenchymatous cells (*rp*) flanking xylem rays ; some of these are clearly seen on the inner side of the mud-filled wedges between plates of xylem. Specimen No. 1001. Pant collection (*see also* Fig. 10). × 8.

had doubted the connection between *Vertebraria* and *Glossopteris* but the ground for such doubts is weakened by Dolianiti's subsequent discovery. The apparent differences between the two types of stems could perhaps be explained by regarding the features of *Vertebraria* as representing the stelar structure of the stem, its real surface features having been lost during preservation. This hypothesis is borne out by the work of Walton & Wilson (1932) and also by that of Plant (1956). According to these authors *Vertebraria* axes possessed radiating xylem plates which anastomosed here and there (Fig. 1A). The axis had virtually no pith and the spaces between the plates of xylem were filled by parenchymatous cells. Walton & Wilson showed that the thin coaly layer on the surface of the rectangular areas of a *Vertebraria* consists of secondary xylem and they put forward the theory that its broad parenchymatous rays had rotted and were subsequently filled with mud. Pant (1960) strongly supported Walton & Wilson's theory on the basis of an American *Stigmaria* where the broad rays have become similarly filled with rock matrix and appear like rectangular areas. However, Sen (1958) insists that the large parenchyma cells reported by Walton & Wilson and Pant are recognizable only towards the peripheral regions of the axes and that even the mud-filled rectangular blocks contain tracheids inside there rocky matrix. A vertically preserved *Vertebraria* in my collection shows parenchyma cells clearly on the inner side of the mud-filled wedges and along the sides of the xylem plates (*see* Figs. 1 F, 10) and I have already shown that sheets of parenchyma overlie the tracheids on the surface of whole rectangular blocks right from the centre to the periphery. In my otherwise excellently preserved material I could not find any tracheids in the rock matrix of the rectangular blocks and, therefore, I feel that Sen's tracheids perhaps represent displaced xylem. That the rectangular blocks represent the internal features of the axes is also supported by some specimens described by Plumstead (1958a). These are reported to show a fibrous surface and rectangular blocks in their split interior.

The secondary xylem of *Vertebraria* usually shows three-ranked bordered pits and narrow secondary rays (Walton & Wilson, 1932 ; Pant, 1956).

Vertebraria axes with attached slender roots have been known for a long time. The structure of a few such roots has been described by Pant (1958b) and even well preserved root tips with root caps and endogenous root primordia have been recognized.

Cuticular Structure of Leaves

Zeiller (1896) first described the cuticle of an African *Glossopteris* which he assigned to the species *G. indica*. The epidermal cells in his figure show thick, straight sides and the guard cells are apparently overlapped by a number of undifferentiated subsidiary cells (Fig. 2A). In 1923,

Sahni described the cuticle of an Indian leaf which he attributed to *G. angustifolia*. This leaf shows epidermal cells with sinuous walls on the upper side and less sinuous cells on the underside (Fig. 3D,E). The

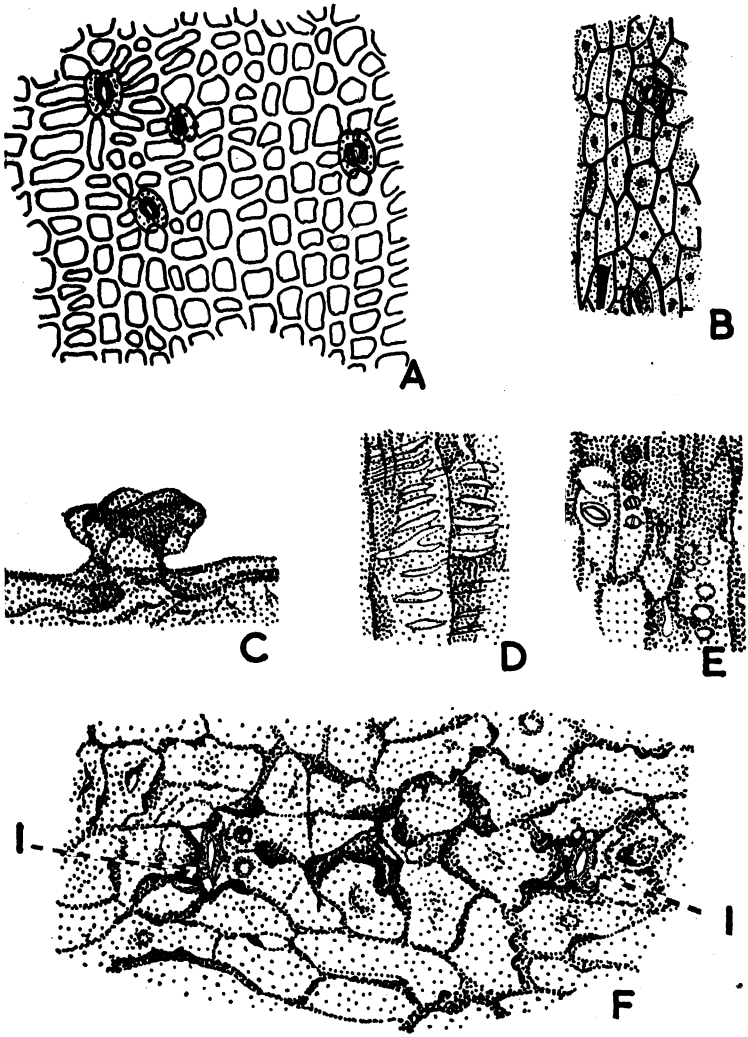


Fig. 2. *A, Glossopteris indica* (*After* Zeiller, 1896). *B-F, G. fibrosa* (*After* Pant, 1958a). *A*, cuticle of leaf showing stomata. × 190. *B*, midrib epidermis. × 110. *C*, stoma showing sunken guard cells. × 350. *D* and *E*, xylem from midrib of leaf. *D* × 700 ; *E* × 350. *F*, Lower epidermis showing lignin lamellae in guard cells. × 350.

stomata have a number of subsidiary cells which sometimes show marks of papillae. The cuticular structure described by these two authors is markedly dissimilar although as suggested by Pant (1959), it is possible that the thick-walled cells figured by Zeiller may be from over the midrib region (cf. Fig. 2A, B). I understand that long ago, in a thesis for the Ph.D. degree of the Lucknow University (India), Miss Chinna Virkki (now Mrs. C. Jacob) described the cuticles of a large number of *Glossop-*

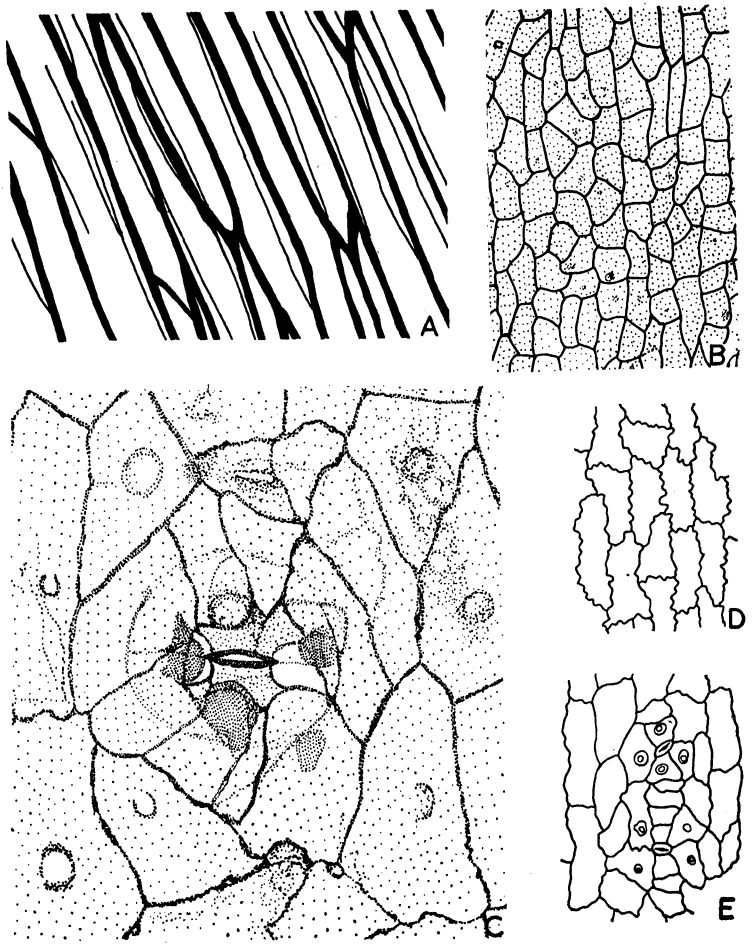


Fig. 3. *A-C, Glossopteris fibrosa* (*After* Pant, 1958a). *D* and *E, G. angustifolia* (*After* Sahni, 1923). *A*, details of venation of a typical leaf. × 14. *B*, upper cuticle. × 120. *C*, lower cuticle showing stoma. × 770. *D*, upper cuticle. × 110. *E*, lower cuticle with two stomata. × 110.

teris leaves but as the work has remained unpublished the details are unknown to me. Srivastava (1956) published an account of the cuticles of some Indian leaves which he assigned to a number of species of *Glossopteris, Gangamopteris* and *Palaeovittaria*. Later, Surange & Srivastava (1956) distinguished six groups roughly corresponding with genera among these species. The variety of fructifications which have been recently associated and attached to *Glossopteris* leaves also suggest that the present form genus includes leaves of a number of generic groups although they might all be related to each other.

Epidermal and Internal Structure of Leaves

Plant (1958a) described a few new species of *Glossopteris* based on structural details (*see* Figs. 3A,B, 4A-D). One of these, *G. fibrosa*, shows interstitial fibres (Fig. 3A) ; another, *G. hispida*, shows in addition to the interstitial fibres, multicellular simple hairs on the lower surface (Fig. 4A) ; and a third, *G. colpodes*, which has comparatively wider meshes is characterised chiefly by the absence of fibres and its sinuous walled epidermal cells on both surfaces of the leaf (Fig. 4C,D). All the three species show a midrib consisting of a number of anastomosing parallel vascular strands which pass out or send their branches as lateral veins into the lamina. The leaf bundles usually show scalariform tracheids but sometimes bordered pits are present in the xylem of the midrib (Fig. 2D,E). Pant has shown that the leaves of all the three species are dorsiventral. The lamina is flat on the upper side while the midrib and veins are raised on the underside. Both palisade and spongy parenchyma cells have been recognized. The lower epidermis shows haplocheilic stomata between the veins. The guard cells show gymnosperm type of lignin lamellae and are somewhat sunken and protected by overarching subsidiary cell papillae (Fig. 2C,F). Pant has also distinguished a related new genus of leaves, *Rhabdotaenia*, which was previously described under the name *Taeniopteris*.

Associated Reproductive Structures

From time to time a number of fructifications have been assigned to *Glossopteris* on the evidence of close association. Arber (1905) attributed some "sporangium like organs to *Glossopteris*" (Fig. 5E,F). These were believed to be borne on the lower surface of some scale leaves of *Glossopteris* (Fig. 5B). I examined Arber's specimens at the British Museum of Natural History and found no evidence of attachment although this type of sporangia are found closely associated with *Glossopteris* in widely separated parts of Gondwanaland, e.g. Australia (Arber, 1905 ; Walkom, 1928 ; Pant, 1958a), India (Pant & Nautiyal, 1960), Africa (Seward, 1907 ; du Toit, 1932 ; Pant, 1958a), Brazil (Lundqvist, 1919). Thomas

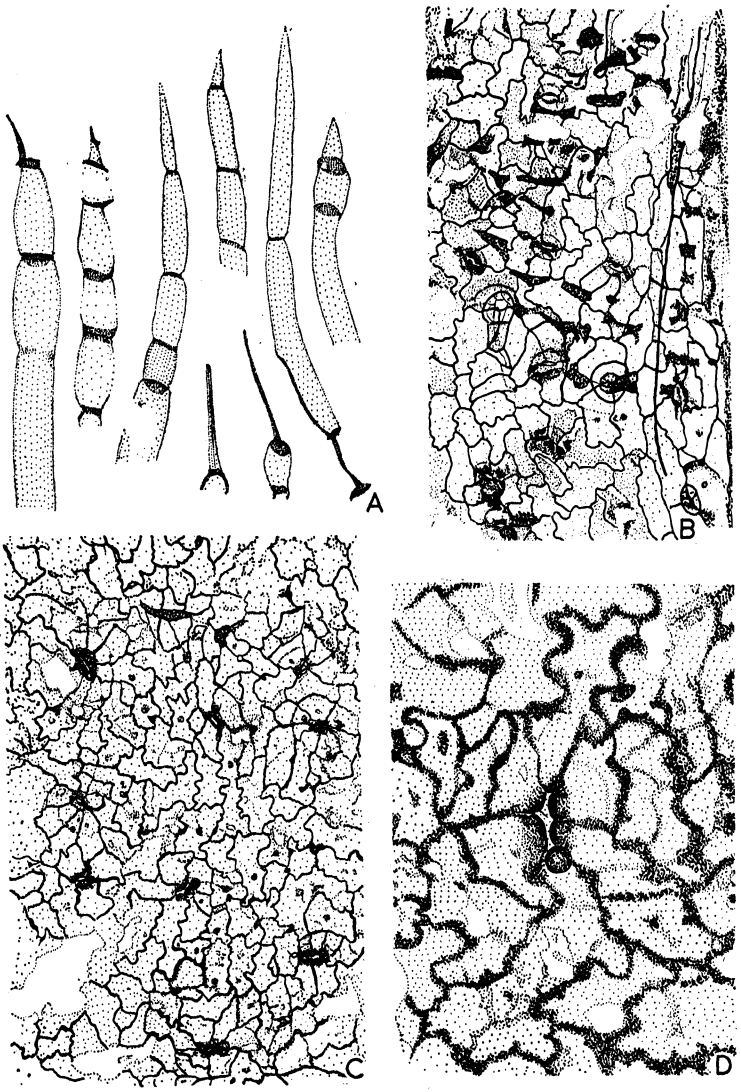


Fig. 4. *A* and *B*, *Glossopteris hispida*. *C* and *D*, *Glossopteris colpodes* (*After* Pant, 1958a). *A*, multicellular hairs from lower side of leaf. × 120. *B*, lower epidermis showing stomata and trichome bases (the dark elongated bodies overlapping epidermis are mesophyll cells). × 120. *C*, lower epidermis with stomata. × 120. *D*, stomata protected by overarching subsidiary cell papillae. × 384.

(1925) and Walkom (1928) thought that they might be ramenta but with the aid of some undehisced African and Australian specimens which were full of two winged spores I was able to demonstrate their sporangial nature. Some of these are borne on branched stalks (*see* Fig. 5G,H). Similar sporangia have been subsequently described from India by Pant & Nautiyal (1960) under the name *Arberiella* (Fig. 5C). In the British Museum collections I also found two undescribed concave discs with a large number of such sporangia attached on the concave side. Under the name *Eretmonia,* du Toit (1932) described some spoon-like, stalked fossil impressions in the shallow hollows of which, and also associated with them, he found sporangia of the same type (Fig. 5D). Du Toit believes that *Eretmonia* is the male fructification of *Glossopteris* and that it contains similar sporangia. Du Toit's *Eretmonia* looks rather like some lanceolate scales attributed to *Glossopteris* (Fig. 5A) seen upside down. Virkki (1937, 1946), Pant (1958a,b) and others have described *Striatites* type of pollen grains sticking in large numbers to the cuticles of *Glossopteris* from distant parts of Gondwanaland. Under the name *Nummulospermum,* Walkom (1921) described some Australian compressions of winged seeds which he assigns to *Glossopteris* on account of their close association in a number of localities in Australia.

Small compressed gymnospermous seeds showing a fibrous integument, a free nucellus and disaccate pollen grains inside their pollen chambers and micropylar canals have been recently described by Pant (1958a) from Africa and by Pant & Nautiyal (1960) from India (Fig. 6A-I). On account of their close association with *Glossopteris* and on the evidence of pollination it is possible that the leaves, sporangia and seeds are parts of the same plants.

Other associated fructifications attributed to *Glossopteris* are the species of *Ottokaria* described by Zeiller (1902) from India, White (1908) from S. America and Thomas (1921) from S. Africa. White has also described a structure, looking rather like his *Ottokaria ovalis,* as a fertile scale of *Glossopteris.*

Attached Fructifications

In a series of important papers Plumstead (1952, 1956a,b, 1958a) has described a number of very remarkable fructifications which were found by her countryman Le Roux. These are clearly attached to leaves of *Glossopteris, Gangamopteris* and *Palaeovittaria.* The abundant material described by Plumstead suggests that Zeiller's solitary specimen of *Ottokaria bengalensis,* whose slender stalk was traceable upto the midrib of a *Glossopteris* leaf, may have been actually connected with it. Plumstead's fructifications are apparently of diverse forms but their mode of

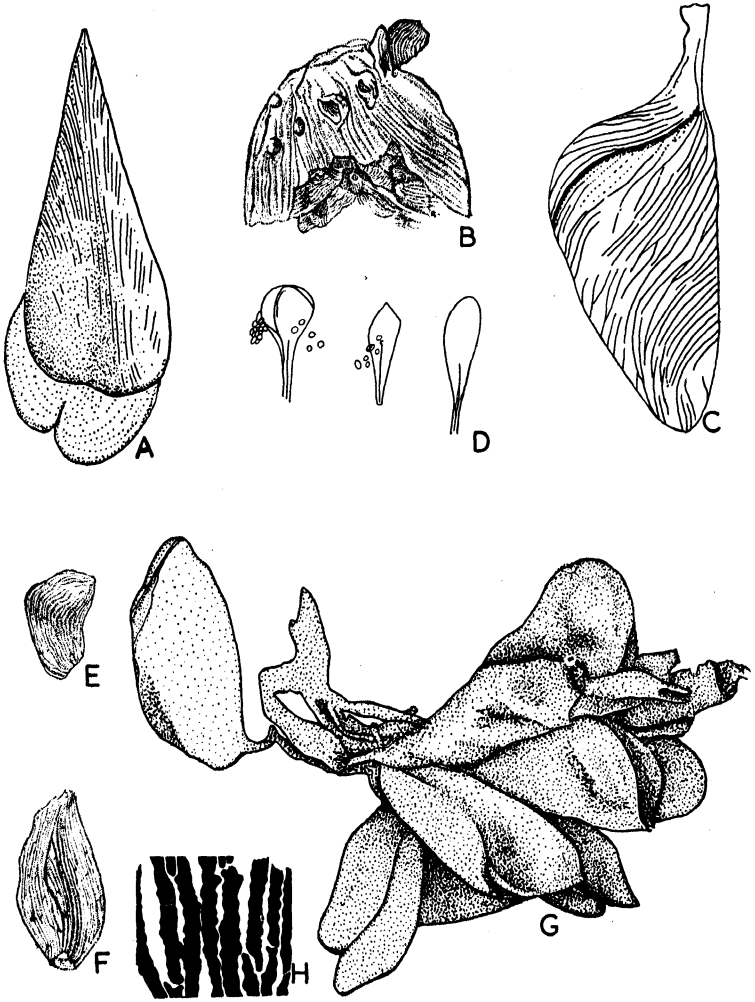


Fig. 5. *A*, lanceolate scales attributed to *Glossopteris*. (*After* Pant, 1958a). × 3. *B*, scale frond overlapping sporangia. (*After* Arber, 1905). × 13. *C*, sporangium of *Arberiella vulgaris*. (*After* Pant & Nautiyal, 1960). × 50. *D*, *Eretmonia natalensis*. (*After* du Toit, 1932). *E* and *F*, "Sporangium-like organs of *Glossopteris Browniana*". (*After* Arber, 1905). *E* × 13, *F* × 15. *G*, group of sporangia of *Arberiella africana* attached to a branching axis. (*After* Pant, 1958a). × 24. *H*, cells of sporangial wall of *A. africana*. (*After* Pant, 1958a). × 120.

attachment is essentially similar and somewhat resembles that of the fertile spike of *Ophioglossum* or the inflorescence of *Tilia*.

Although Plumstead found them attached, she reports the fructifications of *Glossopteris* under new generic names, viz. *Scutum, Hirsutum, Lanceolatus, Cistella, Pluma* and under Zeiller's genus *Ottokaria* (Fig. 7A-F). At least some of her fructifications are believed to be bisexual. Unfortunately, none of Plumstead's fructifications shows any trace of carbon and many of them are obscure. Her interpretations are no doubt ingenuous but ingenuity alone will not solve problems created by the lack of factual details. The wingless pollen grains reported by her were isolated by macerating the rock matrix scrapings from a number of specimens. The structures regarded as stigmatic scars could as well represent the micropyles or supramicropylar structures of seeds. The interpretation of the two counterparts of her supposed bisexual fructifications as the male and female halves of bivalved structures is doubtful, for if so there should at least be a thin layer of matrix between the two counterparts. Some of her fructifications, e.g., *Pluma,* could even be laterally compressed fructifications of the same type as the concave discs described by Pant (1958a).

Under the name *Lidgettonia,* Thomas (1958) has described another type of fertile *Glossopteris* which shows a number of shortly stalked peltate structures termed "cupules" (Fig. 7G). Leaves with these multiple fructifications seem to bear the same type of relationship with others having single fructifications, as *Ophioglossum palmatum* does to other species of that genus. Associated with *Lidgettonia,* Thomas found seeds and sporangia like those reported by Pant (1958a). He believes that these were attached to the peltate cupules.

Some other authors, e.g. Feistmantel (1886) and Sen (1955), have reported vague, rounded scars on leaves of *Glossopteris*. They regard them as sori or sori-like structures but such finds are valueless without the supporting evidence of any definite remains of spores and sporangia. Moreover, it appears that the preservation of some of these leaves, e.g. those described by Sen, is admittedly so poor that even the veins are obscure.

Habit of the Plants of Glossopteris

Most palaeobotanists believed that *Glossopteris* was probably a herb or at the best a small shrub (*see* Fig. 1B), but in a recent paper Plumstead (1958b) points to the possibility of the plants having been more robust. She suggests that the stems, *Vertebraria*-like or otherwise, could have been short shoots borne on rather strongly developed trunks. She is against *Vertebraria* being regarded a rhizome although the frequent occurrence of attached roots in some *Vertebraria* axes (*see* Pant 1956, 1958b ; Fig. 1E)

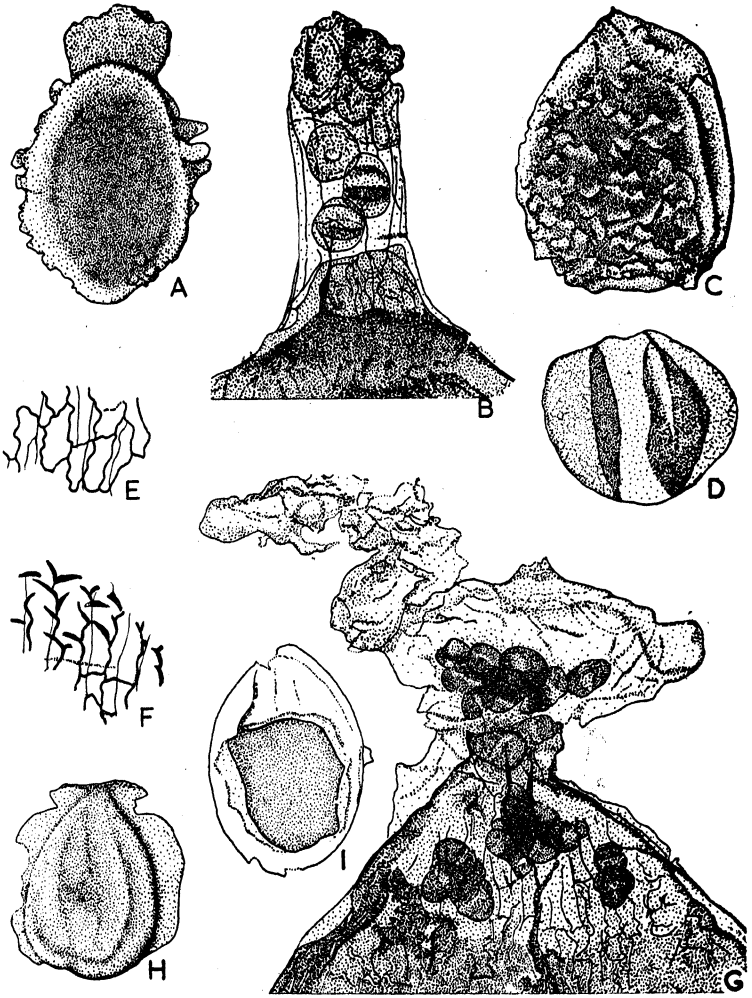


Fig. 6. *A, E, F* and *G, Stephanostoma crystallinum ; B, D, H* and *I, Pterygospermum raniganjense ; C, Platycardia bengalensis* (*After* Pant & Nautiyal, 1960). *A,* seed showing micropylar funnel and processes on the sides. × 22. *B,* micropylar tube with pollen grains. × 222. *C,* seed showing processes all over the surface. × 22. *D,* disaccate pollen grain from micropylar tube. × 836. *E* and *F,* sinuous-walled cells of nucellar cuticle overlapped by straight-walled cells of the inner cuticle of integument. × 110. *G,* macerated seed showing two-winged pollen grains inside funnel, micropylar canal and pollen chamber. × 110. *H,* seed showing wing. × 22. *I,* macerated seed showing megaspore membrane. × 22.

would support the close proximity during life of such portions of the axes to the ground.

Affinities of Glossopteris

Many persons have believed that *Glossopteris* was a fern. A point in support of this view is the occurrence of the supposed soral marks on leaves of *Glossopteris* but until someone is able to isolate spores and

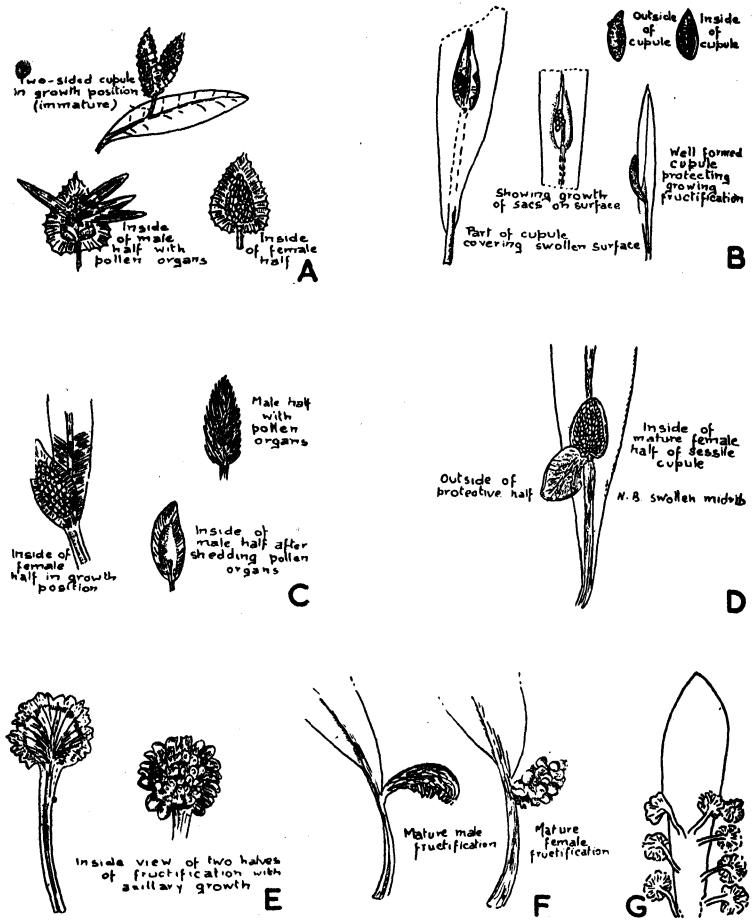


Fig. 7. Attached fructifications of *Glossopteris*. *A*, *Scutum*. *B*, *Lanceolatus*. *C*, *Hirsutum*. *D*, *Cistella*. *E*, *Ottokaria*. *F*, *Pluma*. *G*, *Lidgettonia*. (*A-F* × ½. *After* Plumstead, 1952, 1956a, b, 1958a. *G* × 4/5. *After* Thomas, 1958).

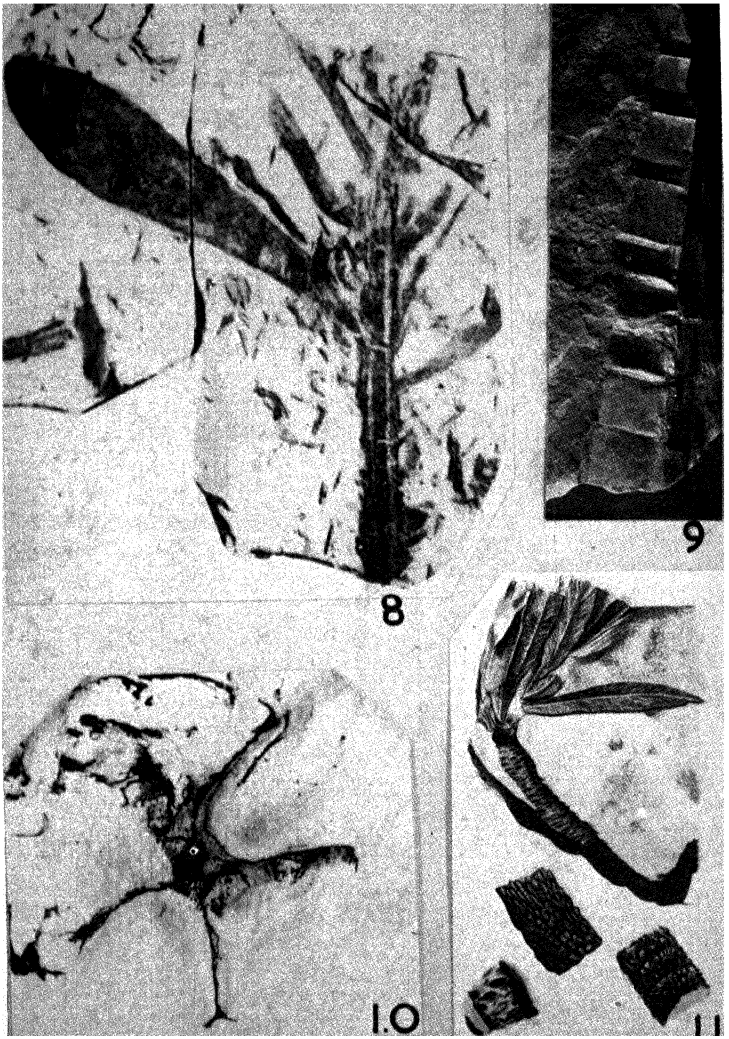
sporangia from such structures their actual nature will continue to remain extremely doubtful. The well preserved cuticle of most species of *Glossopteris* precludes the possibility that they belong to the ferns.

The wood of *Vertebraria* shows bordered pits which are found typically in the gymnosperms. Sometimes even the xylem of the leaves shows similar pits. The guard cells of the African species reported by Pant show lignin lamellae which again suggests that they might have been gymnospermous. This is further supported by the occurrence of gymnosperm-like two winged pollen grains scattered over the leaves of *Glossopteris*, and their close association with stalked sporangia containing these pollen grains and with seeds gymnospermously pollinated by them. The evidence of pollination suggests that the seeds and sporangia were parts of the same plants and if the associated leaves also belonged to the same plants it would certainly suggest that *Glossopteris* was a gymnosperm. Persistent association of this type in widely separated parts of the Gondwanaland provides strong circumstantial evidence in favour of this view. The various organs of *Caytonia* have been assigned to each other on almost similar grounds but in the past so many different fructifications have been attributed to *Glossopteris* on the evidence of association that any more additions to the list would be of no value till they are found in organic connection. Nevertheless, even the attached fertile parts described by Zeiller (1902), Plumstead (1952, 1956a,b, 1958a) and Thomas (1958) are so diverse that it would not be altogether impossible if all or many of the various associated organs also prove to be fructifications of *Glossopteris*.

Plumstead believes that the bisexual nature of the attached fructifications of *Glossopteris* points towards its angiospermous affinities. However, as I have already suggested, the very basis of this view, the bisexual nature of these organs, is largely conjectural and the solution of the problems created by her unique fructifications must await the discovery of better preserved material.

Summary

The paper is a brief review of a number of contributions made during the last few years which have considerably advanced our knowledge of the widespread southern fossil plant *Glossopteris* and some related genera. The more important of these researches include structural studies of the leaves, their supposed rhizome, *Vertebraria* and some associated seeds, sporangia and other fructifications attributed to *Glossopteris*. In addition, Plumstead and Thomas have described some unique fructifications actually attached to some fertile leaves of *Glossopteris* and *Gangamopteris* from Africa. The affinities of *Glossopteris* are discussed in the light of these researches.



Figs. 8-11. Fig. 8. Dolianiti's specimen showing *Glossopteris* leaves attached to *Vertebraria*. × ca 4/5. (*After* Dolianiti, 1954). Fig. 9. *Vertebraria indica* showing wider rectangles on the left due to flattening of mud-filled rays lying parallel to the bedding plane and narrower rectangles on the right which are formed by somewhat oblique flattening of rays. × 4/5. (specimen No. FY 6 in Oxford University Museum). Fig. 10. *Vertebraria* axis preserved almost vertical to bedding plane from Mhukuru Coalfield, Tanganyika, showing large parenchy-

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matous ray cells near centre and on the flanks of the rays of xylem. Specimen No. 1001, Pant collection. × 9. Fig. 11. Etheridge's specimen of stem showing rhomboidal leaf base scars on surface and a group of attached *Glossopteris* leaves. (*After* Etheridge, 1894).

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MORPHOLOGY OF ANGIOSPERM FLOWER[1]

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One thing that a student of botany would have learnt from his elementary studies is that it is very difficult to offer adequate definitions of biological terms denoting organs. The reason is that these terms connote *portions* of a living organism and do not in any way correspond to parts of a man-made machine. Broadly speaking, they represent *concepts* and hence can not be adequately defined. Those who have attempted to define them have done so for the sake of convenience of description only. If this limitation is remembered we may be spared of many a controversy involving axis and its appendages.

The flower is one such term. We have to picture its different forms for a full conception. Besides we cannot regard it separate from the shoot whose essential components or rather *portions* are the vegetative organs. Thus the cotyledons, leaves, bracts, sepals, petals, stamens, carpels, fruits, and the seeds are diverse manifestations of shoot development from its infancy to maturity. They are produced as separate discrete units. But one constant feature about the flower is that it is concerned with reproduction.

The morphology of the flower has been as difficult and controversial a subject as its definition. The so-called classical interpretation of the flower, which we owe to Goethe (1790 ; *see* Arber, 1946), is usually considered as a satisfactory explanation for its morphology. According to this the flower is regarded as *equivalent*[2] to a reduced vegetative shoot and the individual floral members as *equivalent* to foliage leaves.

Goethe's original conception which was purely morphological and not historical or phylogenetic has been misunderstood by subsequent authors, partly on account of his metaphorical language and partly because of lack of consistency in his account. For instance, sometimes, he is believed to have suggested the floral organs as *modified* or *metamorphosed* leaves, which implies that they have *evolved* from leaves. In his book "Versuch die Metamorphose der Pflanzen" he did not imply any thing of this type (*cf.* Arber, 1946). He regarded the flower as a contracted vegetative shoot and the vegetative shoot an expanded flower. He considered the sepals as contracted foliage leaves but also envisaged the foliage leaf as a

[1] Research contribution No. 31 from the School of Plant Morphology, Meerut College, Meerut.

[2] We have preferred this term to 'homologous' following Arber (1937) for this, unlike the latter term, depends neither upon the facts of ontogeny nor upon the changing concepts of phylogeny.

sepal. Reciprocal relationships of this type, as emphasized by Arber, have nothing to do with derivation. Besides, the idea of descent was not so prevalent in his time. What Goethe meant was that there exists a parallelism or equivalence between vegetative shoot and its parts on the one hand and flower and its parts on the other. In other words, both foliage leaves and floral organs have the same morphological nature, though they have not evolved from one another. The use of the term metamorphosis in the title of his book and also in the text is rather unfortunate. But this can be excused when we remember that Goethe was in no sense a professional botanist. He was a great poet-philosopher and only as a hobby he wrote this foundation-laying book on plant morphology.

THE VEGETATIVE SHOOT AND THE FLOWER—Let us briefly compare the floral shoot with the vegetative shoot. Though there are some features common to them, there is this fact that the so-called vegetative phase gradually changes into reproductive phase. For instance, in plants like *Gynandropsis* and *Cleome*, where the flowering and the vegetative regions are quite distinct, the foliage leaves in the transition region gradually become smaller and are ultimately replaced by minute and simple bracts. In others where the flowering region is not well marked off, a change in the phyllotaxy of the leaves may occur to anticipate flowering, as in *Calotropis*. Such transitional changes indicate a common basic scheme of organization underlying both vegetative and reproductive systems.

The features in which they resemble one another are:

1. The vegetative and the reproductive shoots of the same plant, as in certain grasses, often show the same general habit and mode of branching.
2. The structure of the axis in both is almost the same.
3. They arise in the axil of a foliage leaf or a bract.
4. The first leaves which they bear—prophylls in vegetative shoot and bracteoles in flower—show great similarity in form and structure.
5. Floral leaves, like foliage leaves, are 1-, 3- or multi-traced organs.

This indicates that up to the level of bracteoles or even sepals the similarities between the vegetative and reproductive shoots are well marked.

When we reach the inner floral whorls, some differences become apparent. These are:

1. While the foliage leaves always develop acropetally, the order may change in connection with floral organs, e.g. in Cruciferae, Umbelliferae and Compositae.
2. While the apex of the vegetative shoot is always conical, that of the floral shoot may be perigynous or epigynous.

3. While the vegetative shoot never ends in a foliage leaf, the floral shoot may sometime end in a carpel which is apparently terminal.
4. While the vegetative shoot normally has axillary buds in the axils of leaves, floral organs do not have anything in their axils.
5. The arrangement of foliage and floral leaves in the same plant is frequently different.

It will be seen that these differences are certainly more impressive than the similarities summarized above. In our understanding, however, it is easy to visualise that all these are associated and perhaps caused by the contracted or reduced nature of the flowering shoot in contrast to the vegetative shoot that grows indefinitely. On this assumption all these have been satisfactorily explained and the flower has been equated with a vegetative bud in which the internodes have not extended rather than with a mature shoot (Arber, 1937).

Some authors have not accepted this explanation. But on the whole this has to be admitted that it offers a coherent and consistent treatment of floral shoot and its variations in all classes of flowering plants. Hence its wide acceptance.

Among the floral organs the stamens and the carpels in particular are the most controversial, so much so that their interpretation has become almost synonymous with that of the flower. According to the classical interpretation the carpel is considered as an involutely and adaxially folded leafy structure bearing ovules on its margins. Many a competent authority believe that the value of the classical interpretation lies in offering a fairly consistent and comparatively more convincing interpretation of the carpel and its placentation.

Although the classical interpretation is based essentially on studies of comparative morphology, it has been adequately supported from studies of floral anatomy during recent years.

Recently some developmental morphologists also have tried to check this interpretation on the basis of ontogenetic studies. While some have evidence to consider the floral apex as an ontogenetic modification of the vegetative apex (*cf.* the Californian School headed by Professor A. S. Foster), others emphasize the differences between them as fundamental ones, thus subscribing to the *sui-generis* concept ; they further consider the two types of apices as irreducible units (*cf.* Gregoire's School).

ONTOGENETIC MODIFICATION—Those who regard the floral apex as an ontogenetic modification of the vegetative apex have also observed some differences in the organization and structure of the two types of apices. According to them these differences do not have much significance or consequence. They are believed to be associated with the peculiar mode of growth exhibited by them.

SUI-GENERIS VIEW—From a comparative study of the histogenesis of

the foliar and floral buds, Gregoire (1938) finds three fundamental differences between the two:

1. While the floral tip is entirely used in the formation of carpels and no residual apex is left over, the vegetative apex is never used in the production of leaves.
2. While the growing apex of a vegetative shoot shows clear differentiation into an outer tunica and a central corpus, no such differentiation exists in the apices of floral buds.
3. While differentiation of the procambium in the foliage leaf starts from the base of the leaf and extends both up and down (bidirectional), in the floral organs it extends from the receptacular stele and only proceeds up (acropetal).

From this evidence Gregoire concludes that there is nothing in common between the structure of the vegetative and floral apices after the formation of sepals and that these two should be regarded as two irreducible types. According to him petals, stamens and carpels are organs *sui-generis*. Plantefol (1948) also considers that there are no sporophylls in angiosperms and that the stamens and carpels are *sui-generis* emergences.

A careful perusal of the literature, published after Gregoire's monumental and valuable treatise, reveals that Gregoire's findings do not hold good for all cases and that the differences emphasized are not absolute. For instance, cases are on record where floral apices also show differentiation into tunica and corpus. Again, the differentiation of the procambium also varies considerably both in leaves and floral organs (*see* Lawalree, 1948), and floral apex does not always end in carpels. In our estimate, therefore, Gregoire was rather hasty in his conclusion. He depended too much on certain characters which are now generally considered of little constant value. In the same way Plantefol and his followers seem to attach much importance to the sequence of the origin of stamens and carpels.

SYNTHETIC APPROACH TO FLOWER MORPHOLOGY—Intimately connected with the problem of flower morphology are its various methods of study. The important methods are: (1) Comparative approach, (2) Anatomical approach, (3) Ontogenetic approach, (4) Palaeobotanical approach and (5) Teratological approach. These different approaches are all useful tools in the hands of floral morphologists. They lead him to his goal in much the same way as do the different religions or political ideologies practised by mankind. They represent different paths and focus attention on different facets of our objective. Consequently, each one of them reveals only partial truth and never the whole.

During recent years emphasis has been laid on a synthetic approach to the problem of the floral morphology. I wonder if the implications of this plea are clearly understood by those concerned with the problem. To some it seems to mean that the conclusions arrived at by one type of

approach should also be checked and verified by the other type.[1] For instance, it is argued by some developmental morphologists, that to be acceptable the classical interpretation of the flower and its parts that is based on comparative morphology and supported by floral anatomy, should receive support from ontogenetic studies as well. When they find such support not forthcoming they advocate the discard of the classical concept as an useless hypothesis (*see* Gregoire, 1938). In our estimate this is like testing the gospels of one religion or one political philosophy on the basis of another. It is common knowledge that such a treatment is bound to result in confusion and chaos. Consequently such an understanding of synthetic approach is basically wrong and will not lead us anywhere.

We believe that any conclusion arrived at in a certain field of study (based on sound reasoning) is valid within the limits of that field because this is conditioned by certain basic assumptions inherent in that method of approach. It can not be confirmed or rejected from the findings of another field of study. Investigations from different branches of study have brought out only partial truths on the morphology of the flower. These are inclusive rather than exclusive of one another. By synthetic approach we should, therefore, understand the piecing together in the best possible way of these partial truths obtained from different fields of activity with a view to have a glimpse of the greater truth or the whole reality. Whether one can achieve this is largely determined by ones' power of comprehension and synthesis.

Let us take some examples to illustrate this point. Troll's (1939) concept of the carpel as a peltate structure is not the same as the classical concept. In enunciating this he goes only so far as his ontogenetic findings take him. A classical morphologist, however, goes further and visualizes Troll's peltate carpels in terms of his basic 'type'.[2] Troll's concept, therefore, is more realistic but less idealistic and consequently less comprehensive than the classical concept. But it is not antagonistic to it ; it is rather inclusive and both the concepts can co-exist.

In a similar way two apparently different interpretations of angiosperm leaf have been offered in the form of partial shoot hypothesis and telomic hypothesis. These too are not antagonistic to one another but represent different facets of the leaf and hence we do not have to accept one and discard the other. Every one of them has some partial truth. While one of the interpretations is idealistic and based on comparative morphology, the other is historical and based on fossil record. They both have right to exist, even though students of leaf ontogeny may not find support for either of them.

[1] This may be all right when questions concerning relationships or taxonomic affinities are involved, but it is not so with morphological inferences.
[2] For a detailed consideration of 'type' *see* Arber (1950).

It is, therefore, not proper for investigators in one field of study to check and verify the conclusions and inferences based on studies in other fields. Every branch of study has some truth to offer and none the whole. Let this partial truth be sieved out and, whenever possible, synthesized with other partial truths towards a more complete and better realization of our objective. Such an attitude will eliminate controversies and conduce more harmonious relations between researchers in different fields. This will make us more tolerant and less dogmatic.

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ON THE CONCEPT OF CARPELLARY MARGINS[1]

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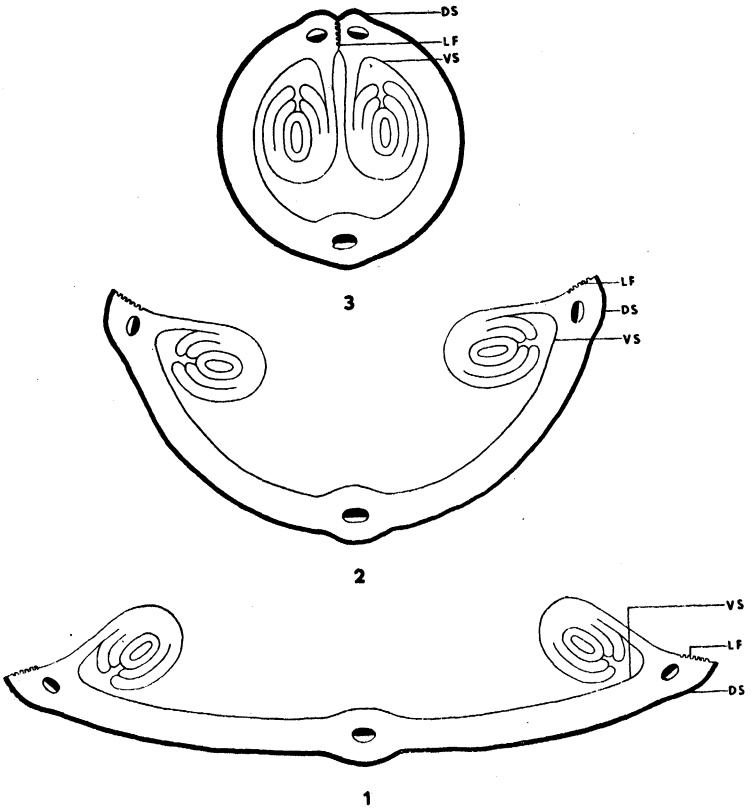
The classical concept of the carpel implies that the ovules are borne on carpellary margins. Other concepts also involve a direct or indirect reference to margins. But no attempt has so far been made to delimit the carpellary margins in any way. Consequently different authors have varied and vague notions about the size, magnitude and limits of carpellary margins. This has caused some confusion in discussions of carpel morphology.

It is not intended here to prescribe any well defined limits for carpellary margins ; this is neither possible nor desirable. But what needs emphasis is that the carpel in addition to its dorsal and ventral surfaces also has some thickness. In the foliage leaf this thickness usually tapers towards the margins which are, therefore, not very prominent. In a carpel, however, the margins are, as a rule better developed. Occasionally they have a more prominent vascular system than the mid rib regions (*cf.* Thomas, 1931 ; Hunt, 1937), a feature which is evidently connected with the ovule-bearing function of the carpel. The carpel thus has: (*i*) an *Outer face* (a part of the dorsal surface) ; (*ii*) an *Inner face* (a part of the ventral surface) that is usually fertile ; and (*iii*) a *Lateral face* which represents the thickness of the margins and is involved in their fusion whenever it occurs (Fig. 3). The occurrence of a more or less distinct lateral face, which is usually sterile, is an important structural feature that seems to have been completely ignored or misinterpreted so far in discussions of carpel morphology (*see* Puri, 1959). The orientation of the ventral bundles and ovules help us in distinguishing the lateral face from the ventral and the dorsal surfaces. While the ventral bundles have their xylem towards the ventral surface and phleom towards the dorsal surface, they are more or less *laterally* oriented with respect to this lateral face (Figs. 1-3). In the same way while the funiculi of the ovules stand at right angles to the ventral surface (and so to the dorsal), they lie more or less parallel to, or in line with, the lateral face (Figs. 1-3). These are important structural features which distinguish the lateral face.

If we accept this concept of carpellary margins then the data used to support the conduplicate concept of the carpel (Bailey & Swamy, 1951) can be satisfactorily explained on the basis of the classical concept. For instance, in *Degeneria* the areas that stand closely appressed together have

[1] Research contribution No. 32 from the School of Plant Morphology, Meerut College, Meerut.

been interpreted as representing the ventral surface of the carpellary leaf. The actual margins are believed to flare apart outward. If we examine the photographs reproduced by Bailey & Smith (1942) and subsequently by Bailey (1954) we find that the ventral bundles are flattened transversely to the surface in question (Fig. 4). In *Drimys* (Fig. 5) and *Cananga odorata* (Fig. 6), however, they are clearly transversely oriented. Besides,



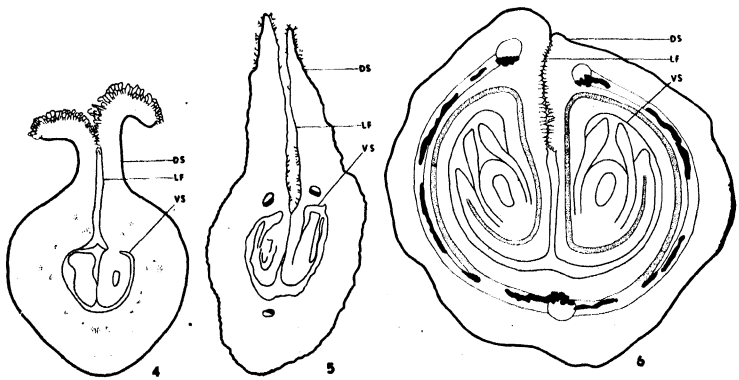
Figs. 1-3. (*DS* = Dorsal surface ; *LF* = Lateral face ; *VS* = Ventral surface). Schematic representation of our concept of carpellary margins. The lateral face (wavy) representing thickness of carpellary leaf can be easily distinguished with the help of orientation of ventral bundles and funiculi of the ovules.

the funiculi of the ovules are also in line with the surface. We are, therefore, inclined to suggest that this surface does not represent the ventral surface but it is actually the lateral face of the carpellary margins.

POSITION OF THE OVULES—Such a concept of the carpellary margins enables us to assess correctly the position of the ovules. In a majority of angiosperms they are borne on the ventral surface of the carpellary margins and are supplied with vascular traces from the ventral bundles or their fusion products, the placental strands.

In very few cases, as in *Nymphaea, Butomus,* etc., the ovules appear to arise from most of the ventral surface and this placentation is described as superficial or laminar. As far as the author is aware, the supporters of the classical concept have not been able to offer a suitable explanation of this phenomenon.

In some primitive Ranales also, e.g. *Drimys* (Bailey & Nast, 1943), *Degeneria* (Bailey & Smith, 1942), etc., the ovules have been described as superficial. This description is apparently based on the assumption that the surface by which the carpellary wall meets (here interpreted as lateral face) is the ventral surface, which as pointed out earlier is not well founded. We have reason to believe that the surface in question is actually the lateral face and hence the ovules in these are still marginal. There is, however, one point which deserves mention. In these cases and several others the vascular supply to the ovules is contributed partly by the dorsal bundles in addition to the ventral bundles. In *Cananga odorata* (Periasamy & Swamy, 1956) the ovules, though borne very near the ventral bundles, receive their vascular supply entirely from the dorsal bundle which is far away from their place of attachment (Fig. 6). This



Figs. 4-6. Fig. 4. T. S. of carpel of *Degeneria vitiensis* (*modified after* Bailey & Smith, 1942). Fig. 5. Same, *Drimys granadensis* (*modified after* Bailey & Nast, 1943). Fig. 6. Same, of *Cananga odorata* (*modified after* Periasamy & Swamy, 1956). By introducing the concept of lateral face (LF) of carpellary margin every one of these can be easily interpreted in terms of the classical concept.

instance prompts one to think that the bundles in such cases may be ineffective in determining the position of ovules.

Then there are some cases where the ovules are described to be borne on the dorsal suture instead of the ventral suture. The genus *Zygogynum* shows this situation. This condition has now been satisfactorily explained as having been brought about by the outgrowing of the ventral suture over the dorsal suture which has remained very much stunted (Bailey & Nast, 1943). In *Mesembryanthemum* (Buxbaum, 1951 ; Sharma, 1958), *Punica granatum* (Sinha & Joshi, 1959) and in some species of *Mouriria* (Morley, 1953) a similar thing seems to have happened but in the reverse direction.

THE THICKNESS AND EXTENT OF CARPELLARY MARGINS—The thickness and extent of carpellary margins varies considerably in different cases. In some the lateral surface of the margins may be very small and may not be clearly distinguishable. Ordinarily it is fairly prominent and in some cases it becomes extensive, flaring outward and resulting in a crest-shaped stigma (e.g. in *Drimys* and *Degeneria*). Such a condition may not necessarily be primitive as has been suggested. It may as well represent a specialized condition which, in course of time, did not meet as much success as the terminal stigma. Thus the crest-shaped stigma and the terminal stigma are respectively the results of the lateral and the vertical growth of the lateral face. We have no justification to believe that one stage has passed through the other in course of evolution.

How much of the ventral surface of the carpellary leaf is to be ascribed to the margins is another point that deserves some attention. This is a question that cannot be settled by arguments. In our understanding this region is ordinarily very small and is confined to the extreme inner edge of the carpellary margin. This is borne out by the fact that the two halves of a placenta usually occur so close to one another that they fuse together more or less indistinguishably. In extreme cases their ventral bundles may also fuse entirely or only partly.

In other cases the ventral surface representing the carpellary margins may be more or less extensive. In certain species of the Gentianaceae and Orobanchaceae and sometimes as an exceptional case in *Crataeva religiosa* (Puri, 1950), it is observed that the two halves of a placenta are separated from one another by extensive intervening regions. These separated half placentae have been considered by some as representing full placentae (Raghavan, 1939 ; Raghavan & Venkatasubban, 1941 ; Lindsey, 1940 ; Tiagi, 1951). The orientation of its ovules shows that this is not the case (Figs. 7-12). It has been clearly shown (*see* Puri, 1952) that each full placenta is a double-structure consisting of two halves more or less equally developed. The ovules on the two halves are oriented in opposite directions. In the cases under review, however, each supposed placenta has all the ovules oriented in one direction—towards the mid rib

of the carpel, while the adjacent one has them oriented in opposite direction. It is clear, therefore, that we are dealing here with half placentae separated apart from one another. Whatever may be the explanation of an ontogenist, a comparative morphologist would be inclined to explain this condition as having been brought about by excessive growth of the segments of carpellary margins occurring between two halves of a placenta. Whether this took place in ontogeny or in phylogeny is immaterial from his point of view.

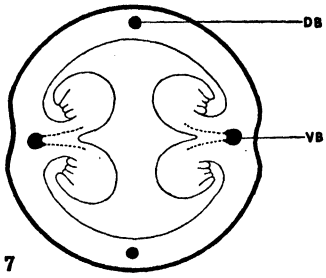
This tempts us to some further speculation. It is well known that the so-called superficial placentation or laminar placentation is a unique problem in carpel morphology. I have gazed at the condition in *Butomus, Nymphaea* etc. quite a number of times without ever having a logical understanding of it in terms of the normal condition. But this treatment of carpellary margins induces one to distinguish the ventral surface of the carpel into two categories: the carpellary margins and the rest of the lamina. While the former is potentially fertile and may bear ovules all along, the latter is sterile. If somehow the fertile ventral surface of the carpellary margins enlarges rather excessively and the rest of the ventral surface becomes suppressed except in the neighbourhood of the mid-rib, then a condition similar to superficial placentation will result. It may be that this type of placentation is derived in some such way.

While this suggestion is highly speculative, it is not entirely improbable. It is well known that in certain unifacial monocotyledonous leaves the ventral surface does not develop at all and the entire outer surface of the cylindrical leaf is dorsal or abaxial (*cf.* Thielke, 1948). In certain intermediate cases the ventral surface may develop to varying extents. It is quite likely that a similar behaviour is exhibited by certain carpels in which certain regions of the ventral surface grow much more than the rest.

Fusion of Carpellary Margins—The fusion of the carpellary margins may be ontogenetic or phylogenetic. In monocarpellary gynoecia it is usually effected through the coalescence of the lateral surfaces of the two margins. They may coalesce throughout or only a portion may be involved as in *Firmiana simplex.*

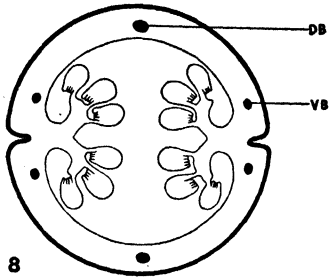
In compound gynoecia where the margins are variously modified the fusion—particularly in closed carpels—may involve a greater or lesser portion of the dorsal surface of the carpellary wall. In such cases it may be very difficult to delimit the different portions of the carpellary leaf. But there is little doubt that here also the ovules are borne on the ventral surface of the carpellary margins.

A word about fusion. When we use this word we do not mean that fusion of margin took place in ontogeny and that we can see it, although there are some cases where fusion is ontogenetic (*cf.* Baum, 1948). But in many cases there is no fusion at all in the literary sense of



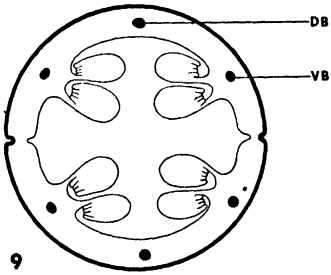
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L.CRISTATUM



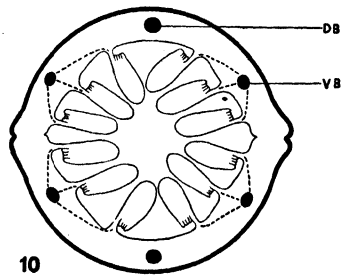
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E.RAMOSISSIMA



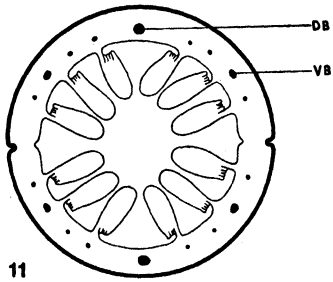
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S.PANICULATA



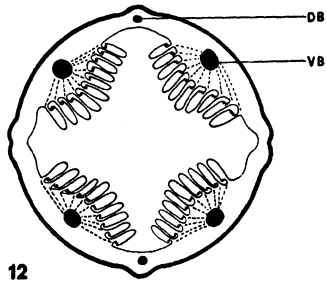
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G. PEDICELLATA



11

G. APRICA



12

G.CONTORTA

Figs. 7-12. T. S. of ovaries of certain genera (*Limnanthemum, Erythraea, Swertia* and *Gentiana*) of the Gentianaceae showing gradual separation of half placentae from their counter-parts. By imagining an excessive extension of the marginal regions, that are fertile, and non-extension of the mid rib regions, that are sterile, one can visualize how a condition of superficial placentation could have been obtained.

the term. All the margins grow as if fused together from the very beginning and no trace of fusion may be seen ; it is just inferred from a comparative study.

Such an understanding of the carpellary margins, in our estimate, does away with our having to believe in conduplicate folding of carpels and the superficial position of the ovules. Further, it restores faith in the classical concept of the carpel, as a convenient instrument of description for the entire group of Angiosperms.

I am thankful to my pupils, Mr. Radhey Shiam and Mr. Gopal Krishna for drawing the diagrams illustrating this article.

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THE GENETICS OF COLEUS

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Coleus blumei, the ordinary garden coleus, is characterized by a tremendous variation in leaf color and shape. It is unsurpassed in the variety of leaf colors it displays (Rife, 1948).

Variations in color are brought about by differences in the amount and distribution of anthocyanin, the shade and distribution of chlorophyll, and the interaction of these pigments. Some varieties show four zones of leaf coloration ; a white midbasal area, then a pink zone, beyond which is a belt of brown, which in turn is bordered by a green area on the leaf margin. The white area results from the absence of both chlorophyll and anthocyanin. Pink results from the presence of anthocyanin in a region where chlorophyll is absent. Anthocyanin plus chlorophyll results in brown, and chlorophyll alone produces green.

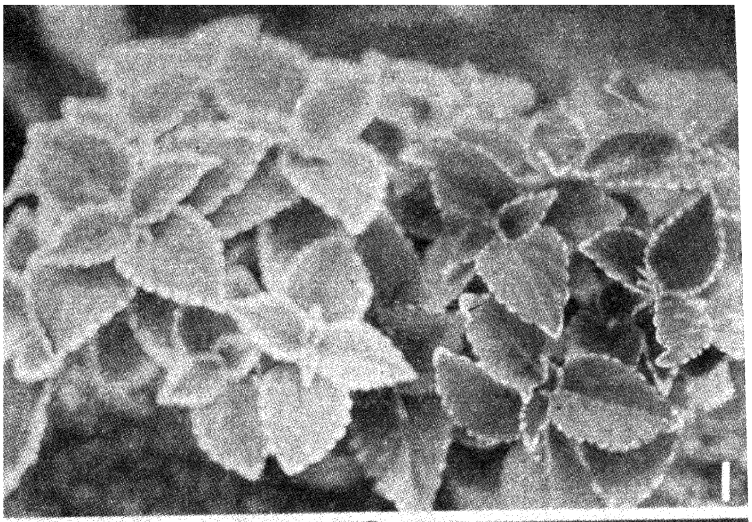
Many of the variations in color and shape of leaves are simply inherited. *Coleus* provides excellent classroom demonstration material for students in genetics. The variations are easy to see, and desirable clones can be propagated vegetatively.

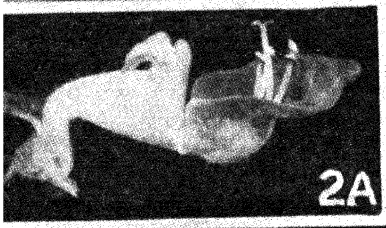
Inheritance of Leaf Colors—A total of 24 genes have been identified at 8 loci. Of these, 13 genes at 3 loci affect anthocyanin pigmentation, 6 genes at 3 loci determine variations in chlorophyll, and 5 genes at 2 loci affect leaf shape. Almost all types of simple inheritance, with the exception of those associated with sex, are to be found among these *Coleus* variations.

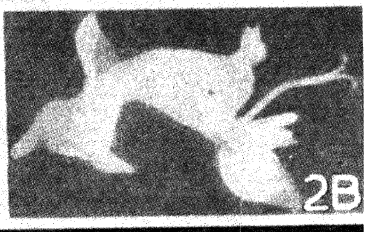
A common type of coloration in *Coleus*, known as pattern, is illustrated in Figure 1. The upper midportion of the leaf is dark, and the margin light. The dark pattern is due to anthocyanin in the upper epidermis in the middle of the leaf. The remainder of the leaf, including the lower surface is green. Examination of Figure 1 reveals that the plants on the right are darker than those on the left. Those on the right have brown patterns surrounded by a dark green margin, whereas those on the left are characterized by scarlet patterns surrounded by golden margins.

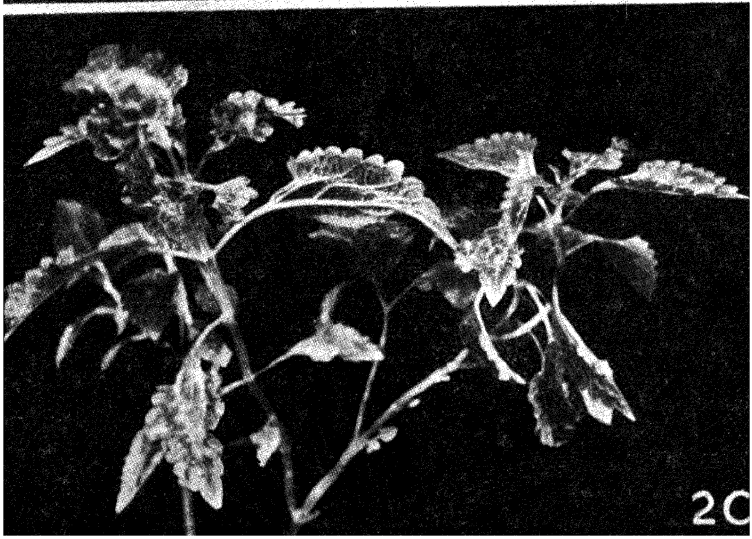
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Figs. 1—2c.—Fig. 1. *Coleus* with patterns. Those on the right have intense patterns, while those on the left have dilute patterns. Figs. 2a-c. Simply inherited variations in flowers and leaves. *a*. Male fertile flowers. *b*. Male sterile. *c*. Irregular leaves.


1


2A


2B


2C

These variations in color are caused by differences in shade of chlorophyll, the kind and distribution of anthocyanin being the same. Thus, anthocyanin over light green gives scarlet. Dark green phenotype is referred to as *intense* and light green as *dilute*. The difference between intense and dilute green results from a single pair of alleles, intense being dominant to dilute. The difference between intense and dilute green is readily apparent in plants having various amounts of anthocyanin, whether they be patterned, spotted, splotched or solid green.

The formation of a pattern results from a gene belonging to a series consisting of at least 9 multiple alleles. Five of these produce purple leaves, varying from each other in shade and amount. These purples result from anthocyanins distributed throughout the leaves and the sap. Each of these purples is dominant to pattern. Each of 3 alleles in this series results in solid green, one of which is dominant to pattern, another blends with pattern to form spotting, while the third is completely recessive to pattern. Two of these greens blend with purple to form intermediate shades known as gray, and the third is completely recessive to the purples. These same genes also have marked effects on the color of flowers and stem. These effects are summarized in Table 1.

TABLE 1

PHENOTYPE EFFECTS OF THE PURPLE ALLELIC SERIES

Gene	Symbol	Leaves	Stems
Even purple	P	Solid, even purple	Green, slight amount of brown
Uneven purple	P^L	Uneven distribution purple	Same as even
Raspberry	P^R	Uneven purple with black splotches	Spotted
Chocolate	P^C	Solid brown	Solid brown
Transient	P^T	Solid purple early leaves; later, green with brown near petiole	Brownish green
Dominant green	P^G	Solid green P^L P^G, Pp^G, gray P^R p^G, lilac P^T p^G, green, red edges	Solid green
Incompletely Dominant Green	p^s	Solid green Pp^s, uneven brown	Solid green
Recessive Green	p^g	Solid green Pp^g, purple	Solid green
Pattern	p	Pattern Recessive to all purples and p^G. p^sp—spotted	Solid green

A simple dominant gene results in solid purple color of the lower epidermis of the leaf. This is inherited quite independently of the other color variations. Thus, patterned plants may be of four phenotypes, intense or dilute, with green or purple epidermis.

Another dominant gene results in irregular splotches of purple in the leaves. This, too, is inherited independently.

Some of the most colorful varieties of coleus are characterized by lack of chlorophyll in the midportion of the leaves, sometimes referred to as an albino midrib. This variation is inherited as a simple recessive to the presence of chlorophyll throughout the entire leaf. Differences between solid green leaves and those characterized by the four zones of color referred to earlier result from two pairs of alleles, those responsible for green versus pattern plus those responsible for green versus albino midrib.

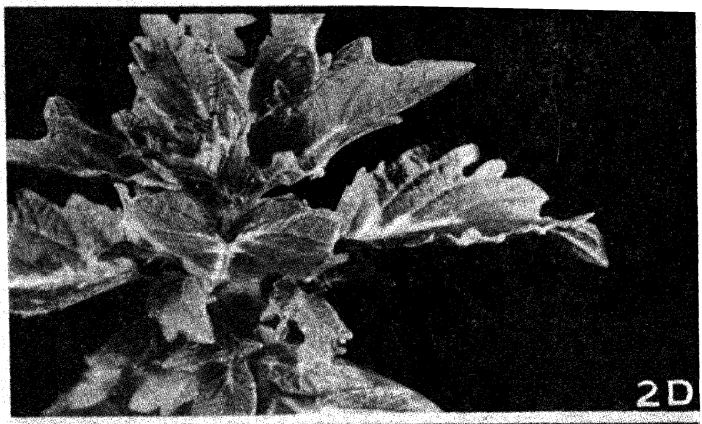
Each of two pairs of recessive genes results in complete albinism. Plants heterozygous for both pairs of recessive genes when self-fertilized produce offspring in the ratio of nine green to seven albino. The latter die soon after emergence, providing a good illustration of the action of lethal genes.

Inheritance of Leaf Shape and Associated Variations—Some clones of *Coleus* are characterized by deep lobes, as contrasted with the usual shallow lobes. Plants with deep-lobed leaves are usually male sterile, no anthers being present. The petals are smaller and have lighter color than those of male fertile plants (Figs. 2A,B). A simple dominant gene is responsible for deep lobes, male sterility, and the modification in size and color of petals. Another allele in the series produces deep lobes and male fertility. Three alleles are included in the series and react as follows:

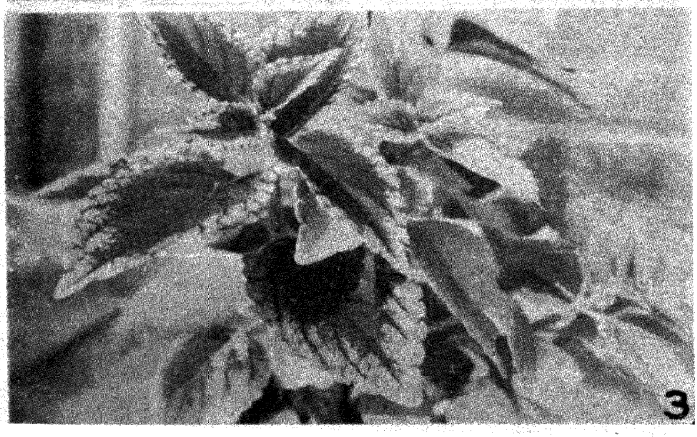
Gene	*Effects*
D	Deep-lobed leaves, male sterile, petals small and light
d^l	Deep-lobed leaves (but less so than D), male fertile
d	Shallow-lobed leaves, male fertile

Dominance is in the order indicated. Shallow-lobed male sterile plants have not been reported.

A simple dominant gene results in a leaf shape strikingly different from those encountered in most clones (Fig. 2C). Note that the leaves are distinguished by peculiarities throughout, the upper surfaces being depressed in the midportion, the margins being uneven, and the veins on the lower leaf surfaces having an irregular pattern of veination. The base of the leaf is also noticeably different from that of other *Coleus*. This peculiar leaf shape is called ***irregular***. *Coleus* plants possessing irregular leaves were formerly classed as a separate species, *Coleus laciniatus*. However, extensive genetic breeding trials have shown conclusively that the differences result from a single gene, irregular leaf shape being dominant to regular.


2D


2E


3

The dominant genes for deep lobing and irregular leaves interact to produce a bizarre shape. The phenotype of such leaves is termed "watermelon" because of their resemblance to the leaves of watermelons. They are characterized by very deep lobes, often with secondary lobes (Fig. 2E). Double heterozygous watermelon-leafed *Coleus*, when backcrossed with shallow-lobed regular-leafed plants produces the following phenotypes in approximately equal numbers: watermelon, deep-lobed regular, shallow-lobed irregular and shallow regular. The foregoing variations in leaf shape illustrate the interaction of two independent pairs of genes in the production of variation in a single trait.

Leaf color and shape are inherited quite independently of each other. Any particular color combination may occur in each of the four leaf shapes. Approximately 150 easily distinguishable phenotypes can be synthesized on the basis of recombinations of the genes we have listed.

Although the foregoing list includes only those variations which have been thoroughly investigated, there are still many other phenotypic variations whose modes of inheritance are suspected. These include crinkly leaf, and narrow leaf, each of which appears to be dominant. There are several types of splotching and spotting, including those due to chlorophyll deficiencies as well as to irregular distributions of anthocyanin. Much work remains to be done on their modes of inheritance.

Cytogenetics—Coleus blumei is characterized by 24 pairs of chromosomes. The most common indigenous species of coleus in India, *C. forshoklii*, possesses 14 pairs (Reddy, 1952) ; *C. barbatus*, a woody perennial indigenous to the Red Sea hills of the Sudan has 16 pairs (Hakim, 1953) ; 16 pairs are reported for *C. aromaticus*, and 24 pairs for *C. rehneltianus* (Scheel, 1939). Cytological investigations of *C. blumei* reveal secondary pairing and multivalent formation during meiosis, and suggests 6 as the basic chromosome number. If this be true, *C. blumei* is an octoploid species ; *C. rehneltianus* is also an octoploid species, with a basic number of 6. This in turn suggests that *C. barbatus* is a tetraploid species with a basic number of 8. *C. forshoklii* may thus be an amphidiploid species, having arisen from a cross between one species with a basic number of 8 and another with a basic number of 6.

The simple genetic ratios obtained in *Coleus blumei* are characteristic of diploid species and do not agree with the cytological evidence, the latter being indicative of octoploidy. Probably, *C. blumei* is an amphitetraploid, and the simple genetic variations have arisen from mutations occurring at a much later date.

←

Figs. 2d-3.—Fig. 2. Simply inherited variations in leaves. *d*. Deep lobes. *e*. "Watermelon" leaves. Fig. 3. Chimeras. Leaves in center have pattern, branches on right have solid green leaves, and leaf on lower left is splotched.

Tetraploid *C. blumei*, characterized by 96 chromosomes, has been synthesized by treatment of seed with colchicine. These produce viable seeds from which tetraploid ratios have been obtained. Tetraploids which are heterozygous for genes of the purple allelic series produce offspring in ratios agreeing closely with what would be expected on the basis of random chromatid segregation. This indicates that the purple locus is some distance from the centromere. Triploids have been synthesized by crossing tetraploids with diploids, but, as might be predicted, they are highly sterile.

Chimeras—Some clones of *Coleus* are much more stable than others, that is to say, chimeras occur rather frequently in some clones, and rarely in others. There is some indication that certain genes affect the mutability of others, comparable to the situation in maize. More remarkable still, two different chimeras (three phenotypically different tissues) occur repeatedly in some clones. This suggests somatic crossing over, but cannot be the explanation here, because this can happen only in case of blending inheritance. Figure 3 shows one of these three-way-chimeras.

Unfortunately, it has been impossible to get leaf tissue to bud, although it can be rooted. These chimeras ordinarily occur in somatic leaf tissue, and further progress in this type of research is dependent upon devising a methodology for transferring the mutant genes into germinal tissue.

Summary

Simply inherited variations are largely responsible for the striking array of leaf colors and shapes in garden coleus. *Coleus blumei*, the ordinary garden coleus is characterized by 24 pairs of chromosomes. Cytotaxonomic investigations suggest that it may be amphitetraploid. Chimeras occur frequently in some clones, rarely in others.

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SOME ASPECTS OF GENETICS OF POLYPLOIDS

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The genetics of polyploids is comparatively more complicated than those of diploids. So far as allopolyploids are concerned their genetical behaviour is essentially like that of diploids ; hence the name amphidiploid. But in autopolyploids, where the doses of genes increase with every ploidy, the genetics, especially the formation of gametes, genotypes and phenotypes, is a complicated phenomenon. In 1930's and 1940's this aspect of genetics was of only academic interest as most of the polyploids in nature were supposed to be of allopolyploid origin. But during the last few years evidences have accumulated and there is a shift in our thinking that what was once supposed to be an allopolyploid may not be actually so. The criteria by which autopolyploidy was judged were the occurrence of multivalents and some other secondary characters. But it is now being realised that laying too much stress on the presence of multivalents for determining auto- or allopolyploidy is not a safe criterion. It has been shown by Randolph & Giles (1951) that in an autotetraploid maize the frequency of quadrivalents was 9.5 in the C_1 generation which came down to between 5 to 7 quadrivalents per nucleus in the C_{10} generation. Such a rapid fall in the frequency of quadrivalents in only ten generations is a tendency which cannot be ignored. From this instance, species which originated as autopolyploids hundreds of sexual generations ago, would not show any multivalents today. Therefore, the presence of multivalents as an absolute criterion to determine autoploidy is wrong if seen in this context. Views are, therefore, now changing that many polyploids which show normal bivalent formation today need not necessarily be allopolyploids. It may be further pointed out that as early as 1936, Harland had suggested that initially homologous genes lying in the different chromosome sets will mutate in different directions and will gradually become so distinct as to be no longer allelic. Such a differentiation would eventually transform a polyploid into a diploid, in spite of altered chromosome number. Polyploidy is thus a mechanism, whereby, the gene number and the gene variety are increased. Autopolyploids, therefore, may behave with respect to certain loci as diploid after hundreds of years. The question which thus arises is that what is the safe criterion to judge auto- and alloploidy in a species. Here we have to fall back upon the genetics of the organism, because cytology does not help. An organism which gives tetrasomic segregation ratios instead of disomic can safely be looked upon as autopolyploid. Because of this it

is necessary to understand the segregation of genes in a polyploid species.

At present, however, this aspect of genetics is not receiving adequate attention by geneticists. Particularly in India even the fundamental difference between the genetics of diploids and polyploids is hardly taught at the Post-graduate level. In the following pages some of the important genetic behaviours of an autopolyploid species are described.

In the case of diploid, one locus can give rise to only three types of genotypes:

AA Aa aa

But in the case of an autotetraploid, for the same locus, the possible genotypes are:

AAAA	...	...	(Quadruplex)
AAAa	...	...	(Triplex)
AAaa	...	...	(Duplex)
Aaaa	...	...	(Simplex)
aaaa	...	...	(Nulliplex)

The quadruplex and nulliplex would obviously give only one type of gametes, AA and aa respectively. But the other three genotypes would give gametes of different types in different frequencies. On the basis of randomness, a triplex would give half the gametes of AA type and other half of Aa type. The duplex would give 1/6 gamete of AA type, 4/6 of Aa type and 1/6 of aa type. A simplex will give rise to gametes half of which would be Aa type and the other half of aa type.

The selfing of the triplex, duplex and simplex would, therefore, give rise to all dominant phenotypes ; 35 dominant, 1 recessive ; 27 dominant, 9 recessive phenotypes respectively. The dominants in these cases would be either pure dominants or a triplex, duplex and a simplex, which can be found out by their breeding behaviour and segregation ratios in the succeeding generations.

In the actual experiments, however, breeding results do not conform strictly to this frequency and mode of gamete formation. For instance, in the case of tetraploid *Datura* with respect to the colour of corolla, which is purple due to a single locus P, and white when its recessive allele p is present, a cross gave the following result (Blakeslee *et al.*, 1923):

	Purple		White	Observed	Expected
Triplex	PPPp	×	pppp	532 Purple 0 White	532 Purple 0 White
Duplex	PPpp	×	pppp	905 Purple 179 White On the basis of $\frac{5}{6}$ A: $\frac{1}{6}$a	903.3 Purple 180.7 White
Simplex	Pppp	×	pppp	292 Purple 266 White On the basis of $\frac{1}{2}$A: $\frac{1}{2}$a	279 Purple 279 White

In these crosses, with respect to the locus P, random assortment of the gene is taking place. But when we take another locus on the same plant which determines presence of spine on the fruit when dominant locus is present, and spineless smooth fruit when recessive allele is present, then the cross gives the following results:

	Spiny		*Spineless*	*Observed*	*Expected**
Triplex	AAAa	×	aaaa	257 Spiny 6 Spineless	263 Spiny 0 Spineless

* On the basis of random assortment of gene which gives ½AA and ½Aa.

The appearance of six nulliplex, i.e., spineless fruited plants not at all expected on the basis of random assortment of genes may be noted. Similarly, a duplex *Datura* plant for the same locus P when back crossed to the recessive parent, gave the following results:

			Observed	*Expected**
AAaa	×	aaaa	— 518 Spiny 137 Spineless	545.8 Spiny 109.2 Spineless

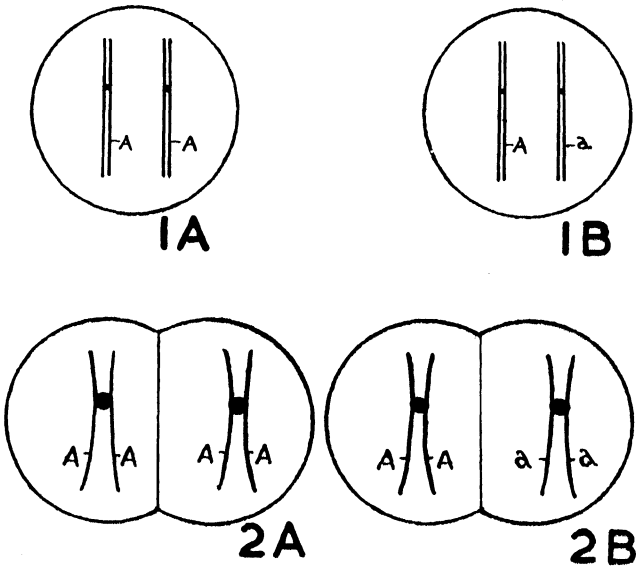
* On the basis of $\frac{5}{6}$A and $\frac{1}{6}$a gametes.

In this cross also the spineless fruited plant appeared far in excess than expected on the basis of random segregation of the gene. Similar examples have been reported in the case of polyploid plants like *Lythrum salicaria, Primula* and *Oxalis valdiviensis* (Fisher & Mather, 1943 ; Fyfe, 1950).

Indeed it is due to genetic studies made in *Lythrum salicaria* and *Oxalis valdiviensis* at the School of Genetics, Cambridge, by Fisher, his colleagues and students that it is now possible to understand clearly the mode of polysomic inheritance. Before him, the studies made by Barlow (1913) and East (1927, 1932) were very confusing and could not explain the ratios. While studying the inheritance of tri-styly in these plants, workers at the School of Genetics, Cambridge, found that a long-styled plant is a nulliplex, while intermediates can have any genotypes, ranging from quadruplex to simplex, but that the intermediate style genes are hypostatic to genes responsible for short style. The triplex mid when crossed with nulliplex long sometimes produced nulliplex long plant. This phenotype is unexpected just as the triplex spiny into nulliplex spineless *Datura* produced unexpectedly six spineless fruited plants.

It has been pointed out that a triplex will give half the gametes of AA type and another half of Aa type. These six combinations producing

3 AA and 1 Aa types of gametes are possible only when each of the four chromosomes splits into chromatids in the first division and at the second division they separate due to the division of centromere producing independent chromatids, any two of which can go to a gamete on a random basis ; but if the complete separation of the chromatids is delayed then at the end of the first division, the two daughter nuclei would come to possess a condition as illustrated in Figure 1. These two nuclei at the second division will give rise to a condition illustrated by Figure 2.

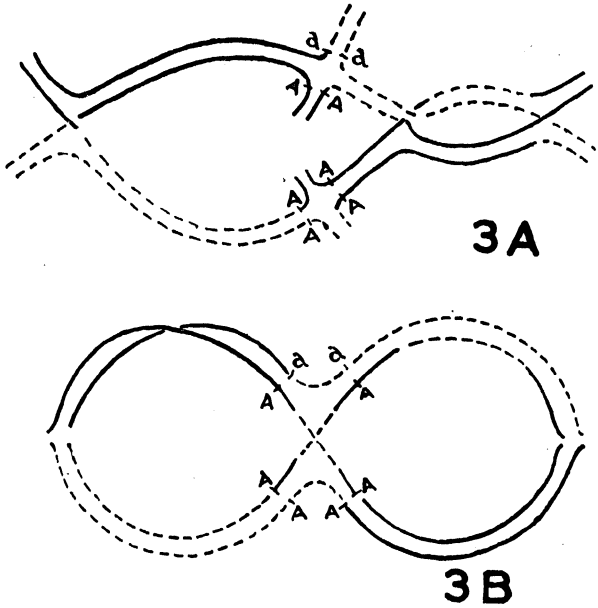


Figs. 1, 2. Explanation in text.

Thus instead of having 3 AA and 3 Aa gametes, this cell would give 3 AA : 1 aa. This type of situation is possible only when a quadrivalent is formed where both the chromatids from a single chromosome have the possibility to go to a single gamete as shown in the diagrammatic representation of a triplex quadrivalent at the diplotene and metaphase stages (Fig. 3a, 3b). This phenomenon is called chromatid segregation or double reduction.

In this diagram of the quadrivalent the locus P represents pink flower and p white flower in triplex *Datura*. Of course, because of dominance of P the triplex phenotypes will have all pink flowers. But in this quadrivalent is shown a cytological mechanism by which pp type of gametes can be formed as a result of chromatid segregation or double reduction. This type of gamete is not at all possible in a diploid. The farther away

a locus from the centromere, the greater is the chance of the occurrence of double reduction. If the locus is very near the centromere, it will always show chromosome segregation because of mechanical necessity.



Figs. 3A, B. Diplotene and metaphase stages respectively of a triplex quadrivalent showing chromatid segregation or double reduction (*After* Darlington, 1937).

As stated above, a triplex, duplex and simplex would give the following types of gametes if the locus is very near the centromere and chromosome segregation is taking place:

	Triplex	*Duplex*	*Simplex*
AA	1/2	1/6	
Aa	1/2	4/6	1/2
aa	—	1/6	1/2

But if the locus is not situated near the centromere, a proportion of double reduction will always occur giving rise to aa type of gametes in a triplex as a result of crossing over between the locus and the centromere, followed by nondisjunction of the sister genes at the first as well as at the second division of meiosis. If the coefficient of such a double reduction is

represented by α (alpha), then the frequency of gametes containing even one or two doses of A will be as follows: By chromosome segregation all the six gametes in the triplex have A. If double reduction occurs the gametes formed are four ; three of which are of A type and one of *a* type. Since in the same plant chromosome and chromatid segregation are occurring in the different pollen mother cells at the time of formation of different gametes, the total frequency of the gametes having A is $(1-\alpha) + 3/4\ \alpha$ and those containing *a* is $\alpha/4$ *or* $\frac{1}{4}\alpha$, where $1-\alpha$ represents the total number of A gametes formed by chromosome segregation. Therefore, for triplex, duplex and simplex the frequency of A and *a* types of gametes by both the processes ; i.e., chromosome and chromatid segregation would be:

	Triplex	*Duplex*	*Simplex*
AA	$\frac{2+\alpha}{4}$	$\frac{1+2\alpha}{6}$	$\frac{\alpha}{4}$
Aa	$\frac{1-\alpha}{2}$	$\frac{4-4\alpha}{6}$	$\frac{1-\alpha}{2}$
aa	$\frac{\alpha}{4}$	$\frac{1+2\alpha}{6}$	$\frac{2+\alpha}{4}$

Similarly the frequency of A and *a* types of gametes in a hexaploid organism by the processes of chromosome and chromatid segregation would be (Fisher & Mather, 1943 ; *see also* Catcheside, 1956):

Zygote	Gametes				Divisor
	m_3	Mm_2	M_2m	M_3	
m_6	1	—	—	—	1
$M\ m_5$	$3+\alpha$	$3-2\alpha$	α	—	6
M_2m_4	$3+3\alpha$	$9-5\alpha$	$3+\alpha$	α	15
M_3m_3	$1+3\alpha$	$9-3\alpha$	$9-3\alpha$	$1+3\alpha$	20
M_4m_2	α	$3+\alpha$	$9-5\alpha$	$3+3\alpha$	15
M_5m	—	α	$3-2\alpha$	$3+\alpha$	6
M_6	—	—	—	1	1

It has been pointed out above that by double reduction $\frac{\alpha}{4}$ aa gametes can be formed in a triplex. Therefore, the appearance of 6 spineless fruited plants in a cross triplex into nulliplex mentioned above can be explained as $\frac{\alpha}{4} = 6/263$; therefore, by cross multiplication $263\alpha = 24$; therefore, in percentage, $\alpha = \frac{24 \times 100}{263}$, or 9.2 percent ; i.e., one could have expected nulliplex plants upto this percentage in this cross. We are getting

much less than that showing thereby that only less frequently double reduction occurs. Similarly the high percentage of nulliplex plants in a duplex into nulliplex cross can be explained. It is as a result of these genetic studies that some disputed cases of polyploid species like *Solanum tuberosum* and *Lotus corniculatus* have been finally settled. These species give tetrasomic ratios for some loci and disomic for others. Therefore, they are now called segmental allopolyploids or auto-allopolyploids. Previously they were suspected to be allopolyploids on account of regular bivalent formation and some disomic ratios.

A convenient way of finding out the segregation ratio in autotetraploids is determined by the formula $\frac{n\ !}{(n-r)\ !\ r\ !}$ where n represents number of combinations of n genes taken r at a time. For chromosome segregation the gametes in a triplex DDDd would be of two types—DD and Dd. By this formula the frequency of DD and Dd types of gametes can be found out where n representing the gene D=3, out of which only 2 (i.e., r in the formula) can be taken at a time since each gamete is diploid. Thus the frequency of DD gamete is $\frac{3\ !}{1\ !\ 2\ !}$ =3DD. For the Dd type of gamete, generally this formula is re-written as $\frac{n!}{r\ !\ (n-r)\ !}$.

Then $\frac{3\ !}{1\ !\ (3-1)\ !}$ =6/2=3 Dd because r, that is, d is only one in this case. Thus the result is the same. There is another easier way of finding out the frequency of gametes but it is better to apply the formula. Now for chromatid segregation, the value of n will change from 3 to 6 ; i.e., 3 D chromosome would act as 6 independent chromatids and similarly 1 d chromosome as 2 independent chromatids. Therefore, the frequencies of DD, Dd, dd type of gametes would be:

$$\text{For DD } \frac{6\ !}{(6-2)\ !\ 2\ !} = \frac{6\times5\times4\times3\times2\times1}{4\times3\times2\times2} = \frac{720}{48} = 15 \text{ DD}$$

$$\text{for Dd } \frac{6\ !}{1\ !(6-1)\ !} = \text{for D and } \frac{2\ !}{1\ !\ (2-1)\ !} \text{ for d}$$

$$\text{Therefore } \frac{6(5\times4\times3\times2\times1)}{(5\times4\times3\times2\times1)} \times \frac{2\times1}{1\times1} = 6\text{D}\times2\text{d} = 12 \text{ Dd}$$

$$\text{for dd } \frac{2\ !}{2\ !\ (2-2)} = \frac{2\times1}{2\times1\times0} = 1 \text{ dd}$$

Once the frequencies of the three types of gametes formed have been found out, it is easy to determine the phenotypic ratios by checker board system as follows:

	15 DD	12 Dd	1 dd
15 DD	225 DDDD	180 DDDd	15 DDdd
12 Dd	180 DDDd	144 DDdd	12 Dddd
1 dd	15 DDdd	12 Dddd	1 dddd

Because of dominance of D, the phenotypes would be 225 + 180 + 144 + 180 + 15 + 12 + 15 + 12 = 783 D : 1 d.

The genotypes of the different types of D ; i.e., as to which plants are

triplex, duplex and simplex can be found out by their breeding behaviour in the succeeding generations.

Summary

The necessity for the study of the genetics of polyploids has been stressed to understand whether a polyploid species is an autopolyploid or allopolyploid. Examples have been cited where a polyploid species which was supposed to be of pure allopolyploid origin, gives tetrasomic ratio for certain loci, which is the characteristic of autopolyploids. Such species are, therefore, now considered as of auto-allopolyploid origin.

The mode of gamete formation of different types and their frequencies in a polyploid have been explained. The multiplicity of genotypes, possible in polyploid organisms, have been pointed out.

Segregation ratios when F_1 of different genotypes are backcrossed to their recessive parents have been explained with the help of data from breeding experiments. The gamete formation as a result of chromatid segregation or double reduction possible only in the case of polyploids, has also been reviewed with the help of diagrammatic representation of chromosome behaviour in a quadrivalent and genetic data from F_1 generation.

The formula that is generally used for determining the frequency of different types of gametes on the basis of chromosome and chromatid segregation has been explained and the significance of resulting unfamiliar F_2 ratios in polyploid organisms has been pointed out.

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CYTOTAXONOMICAL STUDIES IN THE GENUS ADIANTUM

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The genus *Adiantum* is distributed throughout the tropics of the Old and the New World. The majority of the species, however, grow in nature in Central and South America and a few are also found in the Indo-Malayan region. A classification of the different species found in India has been given by Blatter & d'Almeida (1922). On the basis of taxonomic characters these authors have placed the species in five groups. Group A named as 'Radicantes group' includes the species *A. philippense* and *A. caudatum*, both of which possess pinnate fronds and a rachis which roots at the apex. Group B called the 'Polysorous group' contains three species, *A. peruvianum*, *A. trapeziforme* and *A. formosum*. All these three species are characterised by fronds once or more pinnate and their sori are numerous, roundish, oblong or transversely kidney-shaped. Group C, the 'Oligosorous group', has only one species, *A. macrophyllum*, in which sori are more or less continuous on the side of the leaflets. Group D, the 'Capillus-veneris group', has seven species: *A. tenerum*, *A. capillus-veneris*, *A. aethiopicum*, *A. cuneatum*, *A. gracillimum*, *A. concinnum* and *A. collisii*. All these species are characterised by fronds which are at least bipinnate, segments fan-shaped with a stalk near the centre ; sori oblong or obversely kidney-shaped. The last group E is the 'Pedatum group' of which only one species *A. hispidulum*, is found in India.

In the present discussion only nine species belonging to groups A, B and D, have been included. They are: *A. capillus-veneris*, *A. caudatum*, *A. philippense*, *A. peruvianum*, *A. trapeziforme*, *A. tenerum*, *A. aethiopicum*, *A. cuneatum* and *A. concinnum*. Their detailed karyology, meiotic behaviour and morphological studies have been made. The investigation on this genus has been undertaken with a view to make detailed genetic studies. It is well known that genetic investigation in ferns is rather fragmentry. But inheritance studies will naturally take some time. In the meantime, therefore, the cytological data on the species mentioned above are being published.

For cytological studies squash preparations were made by routine methods with slight modifications as ferns are cytologically not as easy materials as higher plants. With some experience, however, they give as satisfactory preparations as the higher plants.

Mitotic Studies

The somatic chromosome number in the species investigated is 60, 90 or 120. The size of the chromosomes also ranges from long to medium. Some species have chromosomes with only primary constrictions, whereas others possess both primary and secondary constrictions. Broadly speaking, the chromosomes have been classified into three categories according to the position of the constrictions.

Category A includes chromosomes with primary and secondary constrictions.

Category B has chromosomes with only median primary constrictions.

Category C includes chromosomes with nearly sub-median to subterminal primary constrictions.

The karyological formulae based on the above criteria for the different species investigated are given in Table I.

TABLE I

S. No.	Name of Species	Type of chromosomes with their number in bracket
*1.	*A. cappilus-veneris*	B (8) C (22)
*2.	*A. caudatum*	B (17) C (13)
3.	*A. peruvianum*	A (7) B (13) C (10)
4.	*A. trapeziforme*	A (1) B (20) C (9)
*5.	*A. aethiopicum*	B (13) C (17)
6.	*A. philippense*	A (1) B (19) C (25)
*7.	*A. tenerum*	B (16) C (14)

* Species which did not show any chromosome with secondary constriction, may have nucleolus organising region at the terminal position with a very small trabant. These were not visible in aceto-carmine or orcein preparations.

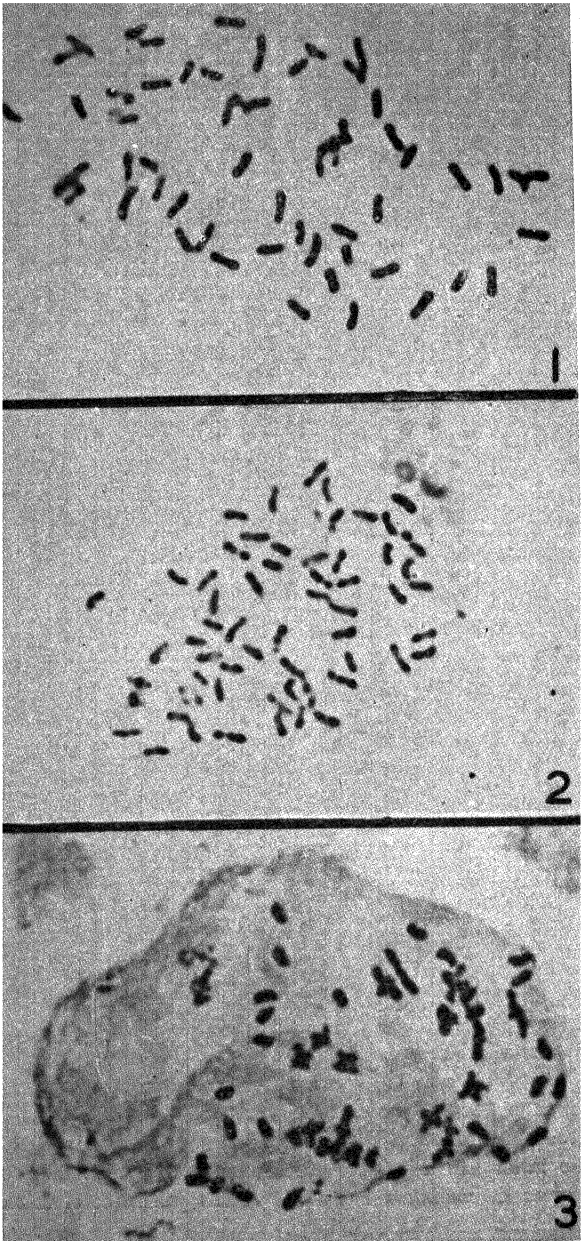
Somatic chromosome number of the different species so far determined is given in Table II. Grouping of species in this table is based on the basic chromosome number, unlike the grouping described at the beginning of this article, where it was based on taxonomic characters.

TABLE II

GROUP "A"				
	1. *A. pedatum*	x = 29		
	2. *A. diaphanum*	x = 29		
	3. *A. hispidulum*	x = 170-180?		
GROUP "B"				
	1. *A. capillus-veneris*			
	(European and Canadian specimens)	x = 30	2x = 60	
	2. (Indian specimen)*	x = 30	2x = 60	
	3. *A. caudatum*			
	(Specimen from India)*	x = 30	2x = 60	
	(Specimen from Ceylon)	x = 2x = 90		Apogamous
	(Specimen from Malaya)	x = 60	2x = 120	
	4. *A. philippense*			
	(Specimen from Ceylon)	x = 2x = 90		,,
	(Specimen from India*)	x = 2x = 90		,,
	5. *A. aethiopicum**	x = 30	2x = 60	
	6. *A. tenerum**	x = 30	2x = 60	
	7. *A. cuneatum**	x = 60	2x = 120	
	8. *A. trapeziforme**	x = 30	2x = 60	
	9. *A. peruvianum**	x = 30	2x = 60	
	10. *A. concinnum**	x = 90		

* The cytology of the species marked with asterisks has been worked out by the authors and the data about the remaining species in this table have been taken from Manton (1950) and Manton & Sledge (1954).

It is evident from Table I that the species *A. peruvianum* and *A. trapeziforme* of the Polysorous group are characterised by having some pairs of chromosomes with secondary constrictions (Fig. 2). The Capillus-veneris group has species whose chromosome number is 60, 90 or 120 but none of the chromosomes have secondary constrictions (Fig. 1). In the group A which includes the species *A. caudatum* and *A. philippense*, the latter has a pair of chromosomes with secondary constrictions, whereas *A. caudatum* has none. The significance of this chromosomal complement is discussed later.


1
2
3

MEIOSIS

Meiosis in the following species was found to be quite normal: *A. capillus-veneris, A. caudatum, A. trapeziforme, A. tenerum, A cuneatum* (tetraploid) and *A. aethiopicum.*

TABLE III*

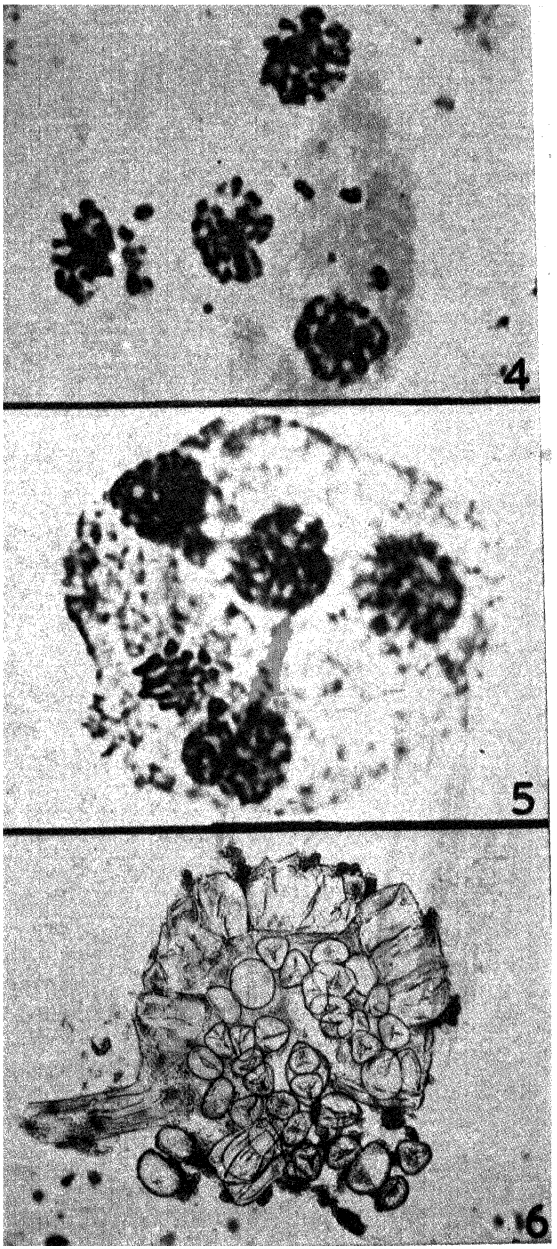
S. No.	Name of Species	Somatic Chromosome number	Univalents Mean	Rod bivalents Mean	Ring bivalents Mean	Total bivalents Mean
1.	*A. capillus-veneris*	60	—	16	14	30
2.	*A. caudatum*	60	—	15	15	30
3.	*A. trapeziforme*	60	—	12	18	30
4.	*A. aethiopicum*	60	—	23.7	6.3	30
5.	*A. tenerum*	60	—	18	12	30
6.	*A. peruvianum*	60	11.5	17.6	6.6	24.2
7.	*A. cuneatum*	120	—	25	35	60
8.	*A. philippense*					
	(16-celled sporangium)	90	19	23	12.5	35.5
	(8-celled sporangium)	90	—	69	21	90

* In *A. concinnum*, no detailed analysis on a statistical basis of the frequency of univalents and bivalents could be made, as very few spore mother cells at metaphase I were available.

In the remaining species, *A. peruvianum, A. philippense* and *A. concinnum*, meiosis was highly irregular (Figs. 3-5). There were a number of univalents at metaphase and at anaphase the chromosomes were distributed irregularly at the two poles giving rise to pentads and micronuclei (Fig. 5). This resulted in the formation of spores of different shapes and sizes as in *A. peruvianum* (Fig. 6) and also in *A. concinnum*. Most of the spores were abortive. In *A. peruvianum* occasionally spore mother cells with quadrivalents and double the original number of chromosomes were found. It may be noted that *A. peruvianum* is a sexually repro-

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Figs. 1-3. Fig. 1. Root tip squash of *A. tenerum* showing 60 chromosomes, none with secondary constriction. × 800. Fig. 2. Root tip squash of *A. peruvianum* showing 60 chromosomes, several chromosomes with secondary constrictions. × 1000. Fig. 3. Metaphase I of meiosis in *A. peruvianum* showing several univalents. × 800.


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ducing species, in which the occurrence of spore mother cells with double the normal number of chromosomes is interesting (Fig. 7). It is only in an apogamous species that such spore mother cells are found.

There are two types of sporangia in *A. philippense*. One type of sporangium has sixteen spore mother cells and the other contains only eight. The spore mother cells from the sixteen-celled sporangium are characterized by highly irregular meiosis with many univalents at metaphase and anaphase (Fig. 8). But meiosis is quite regular and there are no univalents in the spore mother cells from the eight-celled sporangium (Fig. 9). Spore mother cells from the former do not form viable spores, but spores from the latter are fertile. These spores do not contain the haploid number of chromosomes characteristic of the spores of sexual species, but have the same chromosome number (2n) as in the somatic tissue. They germinate to give rise to a prothallus which also has the 2n number of chromosomes. Any cell of this prothallus is capable of producing a new plant. *A. philippense* is, therefore, with 2x and x=90, a triploid and a typical apogamous species (Mehra, 1944).

The development of the gametophyte in species with regular meiosis is quite normal, namely the initial filamentous stage of the prothallus is followed by a cordate expanded prothallus bearing normal sex organs. But in *A. peruvianum* having irregular meiosis, the prothallus remains filamentous up to an advanced stage, but ultimately it becomes cordate and produces normal sex organs. In *A. philippense* spores from the eight-celled sporangium only produce the prothallus.

HYBRIDIZATION

The following interspecific crosses were made successfully: *A. capillus-veneris* × *A. peruvianum* and *A. peruvianum* × *A. capillus-veneris*.

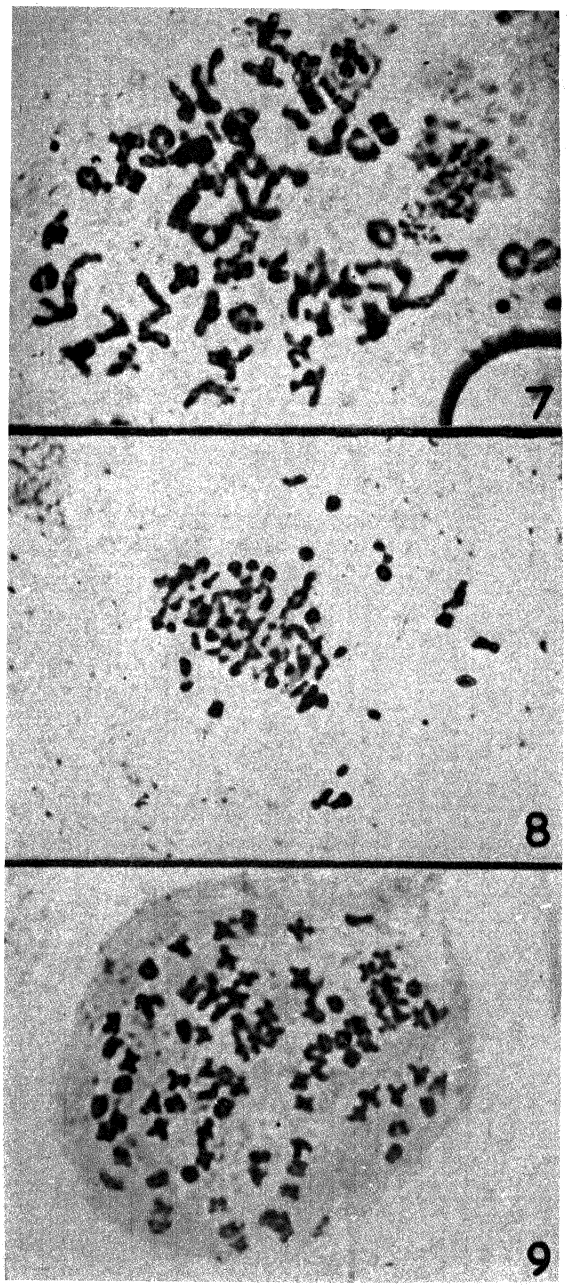
The vegetative features of the surviving hybrids *A. peruvianum* × *A. capillus-veneris* is intermediate between the two parents. The hybrid shows an erect rhizome, while in the case of selfed *A. capillus-veneris* the rhizome is of creeping type, and in *A. peruvianum* selfed, it is of erect type. So it seems possible that erect type of rhizome is dominant over creeping type.

Secondly, in the hybrid the pinnae of the leaf are fan-shaped as in *A. capillus-veneris*, with the margin entire to sub-entire, showing slight incision. In selfed *A. peruvianum* the sporophyte has deeply incised pinna,

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Figs. 4-6. Fig. 4. Anaphase II in *A. peruvianum* showing several laggards. × 800. Fig. 5. Telophase II in *A. peruvianum* showing the formation of pentads. × 800. Fig. 6. Spores of different shapes and sizes in a sporangium of *A. peruvianum*. × ca 250.


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8
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the shape of which is variable from ovate to obliquely ovate, but not of the fan-shaped type.. Leaf colour in hybrid is deep green like selfed *A. capillus-veneris*, while in selfed *A. peruvianum* the colour is slightly pale green. Here pale green colour seems to be recessive to deep green colour of *A. capillus-veneris*.

Discussion

A glance at the chromosome numbers shown in Table II indicates that most of the species have chromosome numbers which are multiples of 30. Therefore, 30 may be accepted as the basic chromosome number for the species included in the table. There are two species, namely *A. pedatum* (2x = 58) and *A. hispidulum* (2x = 170-180?) in which the basic chromosome number is 29 (Manton & Sledge, 1954). It is now well known how a change in the basic chromosome number can be brought about either due to addition of two other lower base numbers, by aneuploidy and by fragmentation of chromosomes etc. At present, however, the genus may be said to possess two basic chromosome numbers 29 and 30.

In a large number of cases it has been observed that species having the same chromosome number show conspicuous differences in the appearance of the karyotype. Generally, isobrachial chromosomes, that is, V-shaped chromosomes are looked upon as a generalized type. These give rise to J-shaped and other types of chromosomes. The ultimate result is that a generalised karyotype gives rise to specialized karyotypes containing chromosomes of unequal sizes. From this point of view *A. capillus-veneris*, *A. caudatum*, *A. aethiopicum* and *A. tenerum* all have isobrachial or V-type of chromosomes, as well as non-isobrachial chromosomes. In the species *A. peruvianum*, *A. trapeziforme* and *A. philippense* also there are V-type chromosomes as well as non-isobrachial types. Some chromosomes have two constrictions. In morphological features these species are different from the other species of group D. Although all these species have 30 as the basic chromosome number, still due to karyotype differences, morphological distinctions have appeared even at the diploid stage.

In addition to the detailed karyological analysis of the different species, data on meiotic studies and chiasma frequency of the species concerned have always been a favourite line of investigation of cytogeneticists.

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Figs. 7-9. Fig. 7. An aberrant spore mother cell with double the number of chromosomes in *A. peruvianum* showing complete pairing. No univalents but several quadrivalents are seen in this cell. × 2000. Fig. 8. Metaphase I in spore mother cell from 16-celled sporangium of *A. philippense* showing several univalents scattered around. × 800. Fig. 9. Metaphase I in spore mother cell from 8-celled sporangium of *A. philippense* showing normal 90 bivalents. × 800.

Meiotic studies can bring out such important points as to whether or not a particular taxon is of hybrid origin, based upon evidence from the pairing of chromosomes at meiosis. Chromosome behaviour in sterile hybrids at the time of spore formation may throw light on the parents of the given taxon. In the case of fertile ones, the chromosome number may be double of that of the parents, i.e. origin of hybrids through allopolyploidy. Evaluation of species, sub-species, or varieties can also be determined by meiotic studies.

The meiotic behaviour of the different diploid species differs in details. *A. capillus-veneris*, *A. caudatum*, *A. trapeziforme* and *A. tenerum* each form 30 bivalents but differ in the frequency of rod and ring bivalents. Rod bivalents can also be formed in haploids (*Scolopendrium vulgare*—Manton, 1950 ; also in haploids of higher plants—Riley & Chapman, 1957). High frequency of rod bivalents is, therefore, an indication of low affinity. It may, however, be mentioned that high frequency of rod bivalents may arise, not because of low homology of chromosome in gross structure, but due to differential arrangement of chromomeres in the chromosome complements. This may mean that a particular set of chromosomes possesses the same chromomeres or genes in totality, but in the sequence of arrangement of these chromomeres differences have arisen due to inversion or other aberrations. In such a situation, although the chromosomes are homologous, during pachytene stage identical chromomeres lie side by side only in small areas where Xta can be formed.

The diploid species *A. peruvianum* stands distinctly apart from other 60 chromosome species, as it has several univalents in each spore mother cell. This results in the formation of high percentage of abortive spores. Then the tetraploid species *A. cuneatum* has 60 bivalents in each spore mother cell. The question naturally arises as to whether this species has originated through auto- or allo-polyploidy. The regular meiosis, i.e. the absence of any multivalents precludes its autopolyploid origin. But there are cases where plants of autopolyploid origin do not show multivalents or very low frequency of multivalents after several sexual generations (Randolph & Giles, 1951). But if a tetraploid form possesses a whole array of phenotypic characters other than those observed in its immediate diploid form, its allopolyploid origin cannot be doubted, because in autopolyploids the characters of the diploid forms are only accentuated. If diploid *A. cuneatum* is discovered, then only the origin of tetraploid *A. cuneatum* can be solved.

The triploid *A. philippense* seems to be an allotriploid, as indicated by very high frequency of univalents in spore mother cells from 16-celled sporangium. Even the bivalents are mostly of rod type. Had it been of an autotriploid origin one would have expected high frequency of trivalents in spore mother cells. Up to 30 chromosomes remain as univalents. Obviously it is a hybrid between a diploid and tetraploid

species. The species *A. concinnum* (2x=180?), which has very irregular meiosis, produced very high percentage of non-viable spores. In all apogamous species the frequency of 16-celled sporangia is much more than 8-celled sporangia. Efforts are, therefore, being made to find out whether this hexaploid species behaves like apogamous triploid *A. philippense*.

A. caudatum (2x=60) forms regularly 30 bivalents but its triploid member reported from Ceylon by Manton & Sledge (1954) has highly irregular meiosis and is apogamous. But the tetraploid form is again sexual and is normal. Thus in this case there is a complete picture of sexual diploid, apogamous triploid and sexual tetraploid in the same species.

Apogamy in the Genus Adiantum—In the genus *Adiantum*, until now only 12 species have been cytologically investigated. Out of these apogamy has been reported in three species—*A. caudatum*, *A. philippense* and *A. hispidulum*. It is quite likely that when large number of species are investigated, several of them may show the apogamous mode of life history. Apogamous species may either be diploid, triploid or higher polyploids as shown in Table IV.

TABLE IV

POLYPLOIDY OF APOGAMOUS SPECIES IN FERNS (MANTON, 1950)

Grade of polyploidy	Species	Chromosome number
Diploid	*Pteris critica* L.	58
	Dryopteris borreri Newm	82
Triploid	*Pteris cretica* L.	90
	Dryopteris borreri	123
	D. remota	123
	D. atrata	123
	Cyrtomium falcatum	123
	C. fortunei	123
	C. caryotideum	123
	Asplenium monathes	108
Tetraploid	*Pteris cretica*	120
	Dryopteris borreri	164
Pentaploid	*Dryopteris borreri*	205

A perusal of the list in Table IV shows that most of the apogamous species are triploid. Even in the genus *Adiantum*, out of the three apogamous species so far reported two are triploids and one is hexaploid. It is well known that the triploids are generally sterile, due to highly irregular meiosis. The same situation exists in spore mother cells from 16-celled sporangia in the triploid apogamous species *A. philippense*. The species has evolved this modified form of life cycle in which due to anomaly at the pre-meiotic division at the 8-celled stage in the sporogenous cells, the chromosome number is doubled. Each chromosome has thus an exact counterpart and, therefore, the pairing is normal. The spores and prothallus come to possess the same chromosome numbers as the sporophytic plant and multiply by apogamous budding. Fertilization is bypassed, thus the ploidy of the species is not increased. It is, therefore, an adaptive function which the species exhibits for its existence and propagation.

The origin of such a mode of reproduction cannot be due to a sudden mutation of a gene in a pure line, otherwise both normal and mutant types (apogamous) could have been found in different frequencies in natural populations. No such example has so far been reported. A species is either apogamous or sexual. This clearly shows that the hybridization creates genic and other unbalance in the species, resulting in apogamy.

From this point of view the species *A. caudatum* is illuminating. As reported earlier, the diploid and tetraploid of this species are sexual but the triploid is apogamous. The origin of triploid forms could be conjectured as due to a cross between tetraploid and diploid. Autotriploids generally have trivalents and are to some extent fertile. The triploid *A. caudatum*, therefore, is of allopolyploid origin as it is completely sterile and no multivalents are found (Manton & Sledge, 1954). This triploid *A. caudatum*, therefore, strongly suggests that apogamy arises due to unbalance caused by hybridization. Occasional genic unbalance may arise in some species or even in some cells of a species. In the latter case as observed in the species *A. peruvianum*, aberrant spore mother cells are found in which the chromosome number is doubled and the pairing becomes regular but the normal spore mother cells have irregular meiosis. This situation in *A. peruvianum* is exactly comparable to that in apogamous triploid *A. caudatum* and *A. philippense*. The triploid hybrid nature of *A. philippense* has already been pointed out. Similarly, apogamous hexaploid *A. hispidulum* may have arisen by autopolyploidy of a triploid apogamous species.

Polyploidy—In every group of Pteridophyta investigated cytologically, a number of species are polyploid. Polyploid species appear to be as common in Pteridophytes as in Angiosperms.

In the genus *Adiantum*, although only a few species have been investi-

gated, a number of them are polyploid. Polyploid forms from both the basic numbers 29 and 30 are found in this genus. So far as the presence of base numbers 29 and 30 in this genus is concerned, it resembles *Lygodium* of the Schizaeaceae, a very primitive fern genus and the other two genera *Cheilanthes* and *Pellaea*, which belong to the same family Pteridaceae (Copeland, 1947), as *Adiantum*. Table II shows the role of polyploidy in speciation in this genus.

That most of these polyploid species are of allopolyploid origin is supported by observations on autopolyploid series in *Osmunda* (Manton, 1950) where all polyploid forms on account of irregular meiosis were unstable. The stable nature of these polyploids in *Adiantum* is, therefore, indicative of its allopolyploid origin.

Several morphological forms of diploid *A. caudatum* occur in different parts of Indo-Malayan peninsula. These forms, if crossed together and followed by chromosome doubling, would really be segmental allopolyploids at the tetraploid level. Similarly the probable hexaploid *A. concinnum* (2x = 180?) has morphological features of diploid *A. tenerum* and tetraploid *A. cuneatum*. Its origin from these two species as a result of crossing followed by chromosome doubling is not unlikely.

Next in importance to polyploidy in speciation in this genus are the chromosomal abberrations and gene mutations. All the diploid species of the group D have primarily a basic karyotype and still they differ greatly in morphological features. These differences then must have resulted by accumulation of structural rearrangements in chromosomes and gene mutations in each of the structurally rearranged karyotype. In the course of time such structurally differentiated forms would tend to evolve on different lines through gene mutations and acquire a separate specific status. The diploid forms of other groups may have similarly evolved.

Interrelationships of Species—An exact phylogenetic relationship between species can be drawn only when meiosis in interspecific hybrids is studied. It is with this end in view that hybridization work has been undertaken. Raising of hybrids in ferns is a very difficult job and the hybrids take three years to form the first sporangium. Until cytological data from interspecific hybrids is gathered, one has to content with the comparative karyology of the species.

Group A comprising of the species *A. philippense* and *A. caudatum* appears to be artificial. *A. caudatum* has been shown to possess generalised type of karyotype as other species of the Capillus-veneris group. *A. philippense*, on the other hand, has karyotype in which chromosomes of Capillus-veneris group and Polysorous group are present. Thus grouping of *A. caudatum* and *A. philippense*, one with a generalised karyotype and the other with a mixture of karyotypes, seems artificial. *A. caudatum* may, therefore, be included in Capillus-veneris group and the triploid *A.*

philippense should occupy a position in between Polysorous group and Capillus-veneris group. Other groups appear to be natural assemblages on the basis of karyotype.

SUMMARY

Cytological studies in the species *A. capillus-veneris, A. caudatum, A. philippense, A. peruvianum, A. trapeziforme, A. tenerum, A. aethiopicum, A. cuneatum* and *A. concinnum* have been made. The karyotypes of the species investigated possess chromosomes which are both isobrachial and non-isobrachial. The number of isobrachial and non-isobrachial chromosomes is different in different species. All the species except *A. philippense, A. cuneatum* and *A. concinnum* are diploid, the latter being triploid, tetraploid and hexaploid respectively.

Meiosis in all the diploid species except *A. peruvianum* was regular. *A. peruvianum* had irregular meiosis and consequently abortive spores. Another species, *A. concinnum* ($2x=180?$), is meiotically as irregular as *A. peruvianum*.

Successful hybridization involving the species *A. peruvianum* and *A. capillus-veneris* has been made.

The utility of karyotype studies in understanding the relationship of species has been discussed. The phenomenon of apogamy in this genus has been reviewed. Polyploidy and chromosomal aberrations appear to play an important role in speciation in this genus.

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RHIZOSPHERE MICROFLORAS

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The microflora of the root-soil interface, defined as rhizosphere (Hiltner, 1904), affords a very fascinating field of study. It is now well recognised that the root influence on the soil microflora arises chiefly out of the nutrient stimuli afforded by the metabolities exuded from the roots. In addition to the preformed essential energy materials exuding from the roots, the greater moisture held in the rhizosphere region and the presence of organic debris in the form of regularly sloughed off root cells make this zone more conducive for microbial growth. From out of the diverse, heterogenous soil microflora, a selective predominance takes place in the root region of growing plants. The rhizosphere thus becomes a metabolically active region wherein many interactions take place between the several groups of microorganisms under the influence of actively growing roots. Conspicuous changes effected in this specialized soil zone by plant roots are profuse growth of select groups of facultative and obligate, heterotrophic forms and the breaking of dormancy of many others, especially fungi. Variations in the rhizosphere microfloras depending upon the genus, species, and even strains of plants, age and phase of growth of plants, and soil conditions are spectacular. There is again a marked specificity in the rhizosphere effects of normal healthy plants and diseased ones whatever be the causal agent—bacteria, fungi or viruses. Yet another aspect of interest is the physiological behaviour patterns of the active rhizosphere microfloras and their repercussions on the nutrition and health of crop plants ; for instance, of significance is the conversion of essential elements from biologically unavailable forms into plant available forms by the microflora of the rhizosphere (Clark, 1949 ; Wadleigh, 1955), and the influence of the microbial metabolites in promoting or antagonizing spread of soil-borne phytopathogens in the root region. There are, therefore, many aspects of physiology of rhizosphere microfloras and those of the plants which need thorough study.

Rhizosphere analyses for qualitative and quantitative estimations of the microflora are generally carried out by employing the well known dilution plate technique of Brierley *et al.* (1927). In recent times, critical assay of root exudates has been made feasible by the elegant paper chromatography (Rovira, 1956 ; Bhuvaneswari, 1958), and by microbiological assay techniques using efficient test organisms such as X-ray mutants of *Neurospora* and strains of *Leuconostoc* and *Lactobacillus*. Other useful bioassay test organisms like *Aspergillus niger* van Tieghem for detect-

ing heavy metals in the rhizosphere soils and *Lemna minor* (Martin & Lavollay, 1958) for detecting halogens need special mention here. An essential prerequisite for this study is the collection of root exudate samples under reasonably aseptic conditions. The simple assembly used by Sulochana (1958) has proved useful in this respect.

In recent years, rhizosphere microfloras of many crop plants have been extensively studied in our laboratory by Agnihothrudu (1954) and Bhuvaneswari (1958). In general, the rhizosphere effects were higher during the vegetative growth period than during flowering and fruiting stages. It has also been shown that the rhizosphere effects were significantly less during winter months than during summer season. In most cases, species of *Aspergillus* predominated in the rhizospheres of crop plants during summer while during winter months *Penicillium* spp. occurred in profusion, and Aspergilli were considerably suppressed. Among the dicotyledonous plants studied, *Vigna catjang* Endl. showed the highest R/S ratios, and among the monocotyledonous food corps, it was *Sorghum vulgare* Pers. that induced the highest rhizosphere activity.

More interesting are the observations that plant varieties resistant and susceptible to certain soil-borne diseases reveal characteristically different patterns of rhizosphere microfloras (Lochhead *et al.*, 1940 ; Timonin, 1940 ; Harper, 1950). Working with *Cajanus cajan* (Linn.) Millsp., Agnihothrudu (1954) demonstrated that a sizeable actinomycetes population antagonistic to *Fusarium udum* Butler was present in the rhizosphere of resistant plants while the rhizosphere of the wilt susceptible variety was more conducive for the survival of the pathogen. A similar study was made to correlate the rhizosphere effects and root exudates of *Oryza sativa* L. with reference to the foot-rot disease caused by *Fusarium moniliforme* Sheld. (Bhuvaneswari, 1958). The healthy plants of the susceptible variety harboured in their rhizosphere greater numbers of physiologically more active groups of bacteria than the resistant variety studied. Among the nutritional groups of bacteria present in the rhizosphere, the susceptible variety had in its rhizosphere a greater percentage of organisms requiring amino acids than those that required amino acids plus growth factors. The resistant variety, on the other hand, appeared to stimulate abundant growth of autotrophic forms. Under artificial inoculation with *F. moniliforme*, the foot-rot diseased plants and plants showing 'bakanae' symptom (a gibberellic acid effect) showed different pictures of their rhizosphere effects. Foot-rot plants stimulated a greater percentage occurrence of physiologically more active forms of bacteria than those showing 'bakanae' symptoms. The point of interest here is that plants with foot-rot symptoms appear to exude a larger quantity of energy substances but not perhaps a variety of energy materials capable of supporting in larger numbers of forms in the several physiologically active groups, as classi-

fied by Lochhead & Chase (1943), than plants showing obvious 'bakanae' symptoms. This concept of studying the metabolism of infected plants using the rhizosphere microfloras as an index to their physiological grouping is a good one and warrants more elaborate experimentation.

This work was further followed up by analyses of the root exudates of resistant and susceptible rice varieties and it was reported that greater quantities of amino acids, except cystine, and sugars, but comparatively less of organic acids, were found in the root exudates of susceptible plants (Table I).

TABLE I

Root exudate pattern of rice varieties resistant and susceptible to foot-rot

	Resistant variety	Susceptible variety
AMINO ACIDS		
Cystine	+ + +	−
Aspartic acid	+	+ + +
Asparagine	+	+ + +
Glutamic acid	+	+ +
Tryptophan	−	+ +
Methionine	−	+ +
Valine	+	+ +
Norleucine	+	+ +
SUGARS		
Maltose	−	+ + +
d-Glucose	+	+ + +
d-Fructose	+	+ + +
d-Xylose	−	+
Unidentified	−	+
ORGANIC ACIDS		
Oxalic	+	+
Tartaric	+	−
Citric	+ + +	−

The resistant plants, on the other hand, exuded sizeable quantity of cystine, which appears to be a resistant factor and at least three organic acids, of which citric acid appears to be higher than oxalic and tartaric acids. As a logical continuation, the *in vitro* effect of the root exudates of resistant and susceptible rice plants on spore germination and growth

of the causal agent *Fusarium moniliforme* was then studied ; inhibition with exudates from resistant plants were recorded. This observation had further experimental proof in that the ability of *F. moniliforme* to survive in the rhizospheres of the susceptible plants was twenty-fold greater than in the rhizosphere of the resistant variety.

A somewhat analogous situation exists in the exudate pattern of organic acids in *Brassica juncea* H.f. & T. Considerable quantities of malic and citric acids were detected chromatographically in the root exudates of *B. juncea*. These organic acids eluted from the chromatograms were tested against the natural soil microflora in Petri plates. Citric and malic acids added singly or in combination with the other acids appreciably suppressed the bacterial numbers in both the control and the rhizosphere soils. The depressant effect of citric acid was greater than that of malic acid. The exudation of organic acids from roots seems to be a general feature of the many crop plants analyzed so far and its full import on rhizosphere microfloras, saprophytic or parasitic, has to be further examined carefully in light of the findings recorded in the case of *B. juncea* root exudate.

Yet another commercial crop plant, cotton, has been extensively studied in this laboratory with reference to root exudates and rhizosphere microfloras. Several strains of diploid *Gossypium arboreum* race *indicum* L. susceptible to *Fusarium vasinfectum* Atk. and amphidiploid, resistant *G. hirsutum* L. were chosen for this study (Sulochana, 1958). *Lactobacillus arabinosus* 17/5 and *Leuconostoc mesenteroides* P.60 were the two test organisms used for amino acid assays and X-ray mutants of *Neurospora crassa* and *N. sitophila* were used for vitamin assays of root exudates. Qualitatively, there were little differences in the number of amino acids recorded in the exudates of the various healthy diploid and amphidiploid strains. Quantitatively, however, taking into account all the amino acids estimated, the healthy diploid susceptible plants exuded a much higher quantity of amino acids as compared with the amphidiploids. Under pathogenesis, the wilting susceptible plants showed a considerable diminution in the total amino acids exuded (Table II). Obviously in the presence of the inoculum, it would be difficult to assess quantitatively whether the deficit in the root exudates was due to decreased synthesis by the host during pathogenesis or a decrease consequent on the depletion of these readily assimilable forms of nitrogen by the pathogen in the rhizosphere, as indeed, we have evidence to show that pathogenic fungi can grow in the rhizosphere with little recourse to external sources of food material. As regards the vitamin components of the cotton root exudates, even here the diploid plants exuded more than the amphidiploid plants and on pathogenesis there is considerable diminution in the case of diploid strains, whereas in the case of inoculated amphidiploids the decrease is but slight. These observations offer one of the first evidences to indicate that

exudation pattern appears to be gene controlled and worthy of more elaborate experimentation. Also this study further emphasizes the need to screen plasma membranes of genetic varieties for their semi-permeability qualities under controlled environmental conditions.

TABLE II

Amino acids in root exudates of diploid, susceptible and amphidiploid, resistant strains of cotton (Microbiological assay: Organisms—*Lactobacillus arabinosus* 17/5 and *Leuconostoc mesenteroides* P. 60)

Amino acid	Unplanted control soil	Diploid, susceptible strains of *G. arboreum* race *indicum* L.			Amphidiploid, resistant strains of *G. hirsutum* L.			
		K2	K5	6186.9	Co2	MCUI	MA5	Laxmi
	microgram in 250 ml.							
Threonine	0	0	0	0	0	300	0	0
Glycine	195	740	610	390	430	510	640	330
Leucine	0	270	280	410	370	380	420	350
Phenylalanine	0	1100*	400	600	300	600	0	750
Aspartic acid	0	1200*	0	1200	0	1300	600	0
Lysine	320	1480	1440	1440	640	320*	800	560*
Histidine	0	1200	1150	700	750	1500	0	650
Serine	1110	2400	1650	1910	1380	1920	1570	1930
Proline	1200	2240	1860	2200	1390	1880	1620	1880
Arginine	108	130	160	135	168	220	165	140
Cystine	0	345*	580*	576*	0	635	0	0
Total:								
Healthy	2933	11105	8130	10551	5428	9465	5815	6590
Inoculated	2933	5498	5930	4295	**	6625	6345	4075

* absent in the inoculated series
** not assayed

Growth of any plant is subject to the influence of environmental conditions. That these will indirectly have their repercussions on the rhizosphere microfloras seems a reasonable assumption and indeed, these have been noticed for cotton plants grown under different conditions of soil moisture and temperature. We have now evidence to show that changes in the root region can be induced so as to possibly produce new exudation complexes or specific mutual antagonisms between the microorganisms by aiding the production of specific inhibitor(s) of microbial metabolism by the plant or the selective production of antibiotics inimical to the growth of one another. One such evidence is the effect of foliar sprays of urea on *Oryza sativa* L., when a change occurs in the rhizosphere microfloras of the sprayed plants (Ramachandra-Reddy, 1959). Two to five sprayings of 0.1 M solution of urea with a detergent on the foliage of

a susceptible strain of rice resulted in a marked increase in the fungal numbers in the rhizosphere and a sharp decrease in the bacterial and actinomycetes population as compared with the rhizosphere microflora of unsprayed control plant. Apart from this quantitative effect, urea sprays seem to promote selectively the growth of a particular species of *Penicillium*. Two explanations seem probable for this behaviour: (*i*) preferential stimulation of *Penicillium* sp. in the rhizosphere and effective competition for available energy sources by this fast growing fungus resulting in delimited occurrence of actinomycetes and bacteria in the rhizosphere of urea sprayed plants, and (*ii*) inhibition of actinomycetes and bacteria by the possible production *in situ* of antibiotics by the *Penicillium* group.

A somewhat similar approach to the study of rhizosphere microfloras of one year old monoclonal selection of tea plants (*Camellia sinensis* Griff.) has been made by Venkata Ram (personal communication). His results indicate a general concurrence with the results of Ramachandra-Reddy (1959) in that the foliar spray of potassium chloride, magnesium sulphate and nitrogenous substances, organic and inorganic, did bring about a basic change in the pattern of the rhizosphere microflora. On the specific effect of urea there was, however, not much parallel between the rhizosphere effects of rice and tea plants, as the plant chosen for his study was a dicot and the soil conditions were totally different. Venkata Ram's results also indicate that sprays of various nitrogenous compounds containing comparable amounts of N_2, Na_2HPO_4 and KCl significantly increased the number of fungi in the rhizosphere over the control, whereas a significant reduction of fungal numbers was recorded with application of $MgSO_4$. Another point of interest emerging from this study was that more genera and species of fungi appeared in the rhizospheres of plants receiving different sprays over unsprayed plants growing under similar conditions. While it is known that roots of plants subjected to high temperature or light intensity (Rovira, 1959) or plants grown under desiccated soil moisture conditions (Katznelson *et al.*, 1954) exude large amounts of amino acids, such specific effects on the quality and quantity of the soil microfloras have not been so effectively demonstrated as in this foliar spray concept and rhizosphere microfloral changes.

It is now evident that many specific and aspecific metabolic accelerators or inhibitors are involved in the interactions between root exudates and rhizosphere microfloras. Inquiring into these aspects would be a fruitous-line of investigation. Should it be possible to selectively modify the rhizosphere microfloras of crop plants by growing them under controlled conditions or by giving foliar sprays, it would, indeed, point to a major advance in the biological control of certain soil-borne

SUMMARY

Qualitatively and numerically the rhizosphere effects of plants vary markedly. The main nutrient stimuli for such specific rhizosphere effects are afforded by the root exudates. Exudate patterns of rice, mustard and cotton roots and their effects on rhizosphere microfloras are described. More interesting are the observations that plant varieties resistant and susceptible to red gram wilt, cotton wilt and foot rot of rice reveal characteristically different rhizosphere effects. Latest development of considerable significance in this field is the effect of foliar sprays of nitrogenous compounds etc. on the alteration of their rhizosphere microfloras. Further study is advocated on the many specific and aspecific metabolic accelerators or inhibitors and their influence on the interactions between root exudates and rhizosphere microfloras.

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BIOASSAY PROBLEMS

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Living organisms respond in a characteristic way to the presence or absence in the substrate of substances that are essential for or inhibitory to their growth and metabolism.. Based on such observations accrued from the more fundamental studies on their growth and metabolic requirements, the use of certain organisms for assay of biologically active substances became a practical possibility in cases where objective and quantitative measurement of growth or inhibition of growth was feasible. The number of test organisms employed for bioassays is, however, low due to practical considerations. The principle of bioassay is based upon the proportional response of a deficient test organism to graded amounts of the substance, for which it is deficient, over a limited range of concentrations. The establishment of an effective range of concentrations depends upon the substance being assayed, the test organism and the basal medium. Some of the widely used test organisms include species and strains of algae, bacteria, yeasts, fungi and a few higher plants. A wide variety of organic and inorganic materials such as mineral elements, halogens, plant hormones, vitamins, amino acids, phytotoxins, antibiotics and fungicides occurring in simple or complex forms can now be microbiologically assayed in concentrations which are beyond the range of detection by known chemical tests. Besides the assay of known compounds, bioassay methods are of great use in studies of metabolic pathways of synthesis, isolation of growth factors and other active compounds (György, 1950).

A well known example is that of the isolation of biotin for the first time as a pure compound by Kögl & Tönnis (1936) using a biotin-deficient yeast strain. Also in tackling problems where availability as well as total amounts are of importance, as in the case of mineral deficiencies of soils, bioassay is as yet the best tool.

The following factors have contributed to the extensive use of microorganisms, especially bacteria and fungi, for assay purposes: simple technique and apparatus, sensitivity, specificity, stability, the short time required, and the small amount of sample needed requiring little or no purification or concentration of the active material. Only one element may be studied at a time by limiting that alone in an otherwise 'complete' medium. It is needless to mention here that a sound knowledge of the response of an organism to any particular substance is an essential prerequisite for a successful assay, and that all procedural details

in carrying out an assay, following standardization, must be strictly adhered to.

Bioassay of Mineral Elements

The response of fungi to nutrients is sensitive enough so that a number of bioassays for inorganic elements in soils have been developed to the point of practical utility. A radial growth assay for phosphorus with *Cunninghamella* sp. was devised by Mehlich *et al.* in 1934. The principal advantage of a bioassay method is that it may more nearly approximate a determination of the minerals actually available to a crop plant. Correlation studies have usually shown at least fair agreement between the growth of *Aspergillus niger* and growth of higher plants in soils deficient in phosphorus (Manil *et al.*, 1956), potassium and magnesium (Nicholas, 1950) and heavy metals like copper, molybdenum and zinc (Mulder, 1939, 1948 ; Gerretsen, 1948 ; Donald *et al.*, 1952b ; Nicholas, 1952 ; Saraswathi-Devi, 1958). However, the plant availability of Fe and Mn will be perhaps difficult to ascertain with this organism, because *A. niger* produces high amounts of organic acids, thereby making available for its growth quanta of Fe and Mn usually unavailable to higher plants.

Mulder (1939) was the first to use *Aspergillus niger* to detect copper and molybdenum in Dutch soils, and since then his strain of *A. niger*, recognised as *A. niger* 'M' Strain, has been shown to require in addition to generally accepted macronutrients, the micronutrients—copper, iron, gallium, molybdenum, manganese, zinc, and possibly vanadium and scandium—for normal growth and sporulation. Much of the success in *A. niger* bioassay work has been the result of effective purification of the basal medium freeing it from Cu, Fe, Mn, Zn and partially from Mo by the Al_2O_3 method (Donald *et al.*, 1952a) and by Nicholas' (1950) method of sulphide co-precipitation to remove Mo. Absence of any of these trace elements from the basal medium results in characteristic deficiency symptoms. The visual deficiency symptoms are markedly reduced growth and sporulation in Fe- and Zn-omitted cultures, and decreased growth and no sporulation where Cu, Mn, or Mo is deficient. In Mn deficient cultures, the sterile mat is much wrinkled and/or perforated. Growth is not reduced in Cu- and Mn-omitted cultures to the same extent as in the cases of Fe and Zn deficient cultures. Actually, the increase in growth and spore intensity from deficiency to sufficiency is directly proportional to increasing amounts of the element under test. Standard curves, though not rectilinear, have been established for Fe, Mo and Zn by Nicholas (1950) ; he also reported that use of mycelial mat weight in assessing Cu and Mn is not as sensitive as those mentioned previously.

Copper, however, has a specific effect on spore colour and is a reliable criterion. From the completely sterile white mat in Cu-omitted cultures, sporulation increases in amount and pigmentation through white, yellow, yellowish-brown, brown, brownish-black to the normal black with increasing concentration of Cu (Nicholas, 1952 ; Lakshmanan, unpublished). Strain MUBL 1 of *A. niger* is more sensitive to traces of Cu, and spore colour sets in even in the lowest concentrations after '—Cu' and gives a better gradation of colour intensity. This strain has been found to be less sensitive to traces of Mn, but more sensitive than 'M' strain to traces of Cu and Mo (Saraswathi-Devi, 1954). Under the conditions standardized, for the *A. niger* test, the essentiality of Fe and Zn for normal growth of nine species of *Fusarium* has been proved (Saraswathi-Devi, 1955). Studying the possible variation in heavy metal contents of soils from three cotton growing areas in the Madras State with definite wilt histories, using *A. niger* 'M' strain, it has been found that two 'wilt-sick' soils (Udamalpet and Palladam) had a lower amount of Zn than the third soil (Koilpatti) where the wilt disease has not been noticed (Saraswathi-Devi, 1956). It is to be noted that the same wilt-susceptible K2 variety of cotton (*Gossypium arboreum*) is grown, and the wilt pathogen *Fusarium vasinfectum* occurs, in all the three soils. Previous work has shown that Zn amendments to soil gave protection from wilt to cotton plants grown in them, by checking growth of the pathogen and promoting host vigour (Sulochana, 1952 ; Kalyanasundaram, 1954). Relatively high amounts of Zn (4 ppm) were also noted to inhibit toxin (fusaric acid) production by the pathogen (Kalyanasundaram & Saraswathi-Devi, 1955). It, therefore, seems possible that the higher Zn content of 'wilt-free' soil exerts an all-round influence in warding off the disease in that area.

It has been recently demonstrated by Martin & Lavollay (1958) that halogens, especially chlorine, can be effectively assayed using *Lemna minor*, and it has been concluded by them that halogen deficiency interferes with the utilization of Fe and Mn.

A microbiological determination of boron in soils was attempted by Gerretsen & De Hoop (1954) and it was established that boron is an essential microelement for *Azotobacter chroococcum*, the optimum boron content for normal development being *ca* 2 ppm in liquid cultures and 5 ppm in quartz-sand cultures. Sharp contrast is shown in pigmentation in cultures with and without boron. The authors have indicated that further assay work world be helpful in unravelling the complicated behaviour of boron in soils.

Working with *Anabaena cylindrica* Lemm., Allen & Arnon (1955) have established the essentiality of calcium and molybdenum for this blue-green alga. It is possible that this alga may be tried for assay of Ca and Mo.

Bioassay of Plant Hormones

Auxin assays can only be reliably carried out with auxin-deficient test objects. The most convenient of these is the dark-grown oat coleoptile from which the tip has been removed following the method developed by Went (1926) and modified by numerous workers. The 'deseeded' test, where the endosperm of the oat seed furnishing the precursor of auxin is removed, is three to five times as sensitive as the ordinary *Avena* test (Skoog, 1937). Other auxin assay methods include *Cephalaria* test (Söding, 1936), the curvature test with *Raphanus* hypocotyls (van Overbeek, 1933) and pea stem internodes (Went, 1934). For the assay of wound hormone, traumatic acid, the only method extensively used is the bean pod test of Wehnelt (1927), modified by Bonner & English (1938). In this test, the parenchymatous tissue of the pod beneath the unripe bean is the responsive material. A drop of juice of crushed tissue applied to this layer causes a small intumescence, i.e., elongation of parenchymatous cells. The height of this intumescence is proportional to the concentration of wound hormone.

Bioassay of Vitamins

Fungi and yeasts are widely used for assay of vitamins. One of the first quantitative bioassay methods to evince reasonable specificity was that tried by Schopfer (1935) using *Phycomyces blakesleeanus* for thiamine. Use has been made of *Saccharomyces cerevisiae* for biotin assay (Lilly & Leonian, 1944) and *S. carlsbergensis* for assay of pyridoxine (Snell, 1945) and pantothenic acid (Atkin *et al.*, 1944). Several biochemical mutants of *Neurospora crassa* and *N. sitophila* have been widely employed for assay of vitamins in food products and others, since Beadle (1944) first made use of a mutant of *N. crassa* to assay inositol. In this laboratory, using the respective deficient mutants of *Neurospora*, relative ability of Fusaria to synthesize the B-group vitamins have been investigated (Venkata Ram, unpublished). These X-ray mutants were used extensively for assay of vitamins in root exudates of cotton plants, resistant and susceptible to *Fusarium* wilt (Sulochana, 1958).

Bioassay of Amino Acids

Many strains of *Lactobacillus arabinosus*, *L. casei* and *Leuconostoc mesenteroides* have served as excellent assay organisms for estimation of amino acids. Root exudates of several strains of diploid and amphidiploid healthy cotton plants grown in wilt free and wilt sick cotton soils, and under pathogenesis, have been quantitatively assayed using *Lactobacillus arabinosus* 17/5 and *Leuconostoc mesenteroides* P. 60. This

study of amino acid composition of root exudates and their influence on the rhizosphere microflora have yielded much interesting data (Sulochana, 1958). These observations offer one of the first evidences to indicate that root exudate pattern appears to be a gene controlled phenomenon worthy of more elaborate experimentation.

Bioassay of Phytotoxins

Many fungal wilts are now known to be caused by phytotoxins elaborated by wilt pathogens. Such phytotoxins generally occur in complex forms in fractional quantities, difficult to separate in pure forms, and are recognised mostly by the physiological effects they produce in the host plants. Nevertheless, in many instances *in vitro* and *in vivo*, detection of phytotoxins is possible by the use of a suitable and sensitive test organism. A well known instance is the assay of fusaric acid produced by pathogenic fusaria using spores of *Ustilago* (Kobel, 1951), and by agar-cup technique where a soil bacterium serves as the test organism (Kalyanasundaram, 1955). Later work by Kalyanasundaram & Venkata Ram (1956) has proved conclusively the presence of fusaric acid *in vivo* in wilting cotton plants by a chromatography-*cum*-bioassay technique.

Bioassay of Antibiotics

Many species of bacteria have been in use for more than three decades now in the screening as well as the evaluation of antibiotics. *Sarcina lutea* has been in use for assaying antibiotic activity of penicillin, subtilin etc. (Salle, 1954). Three methods are commonly employed: (1) Oxford cylinder-plate method, (2) Agar cup-plate method, and (3) paper-disk method. For routine initial screening of soil microbes for checking on their ability to produce more potent antibiotics, these bioassay methods are extremely useful.

Bioassay of Fungicides

More recently, simple techniques based on inhibition of spore germination or growth inhibition zones in culture plates containing agar seeded with spores of *Aspergillus niger* are being successfully used to evaluate action of synthetic fungicides (Wain, 1959). By the same technique presence or absence of naturally occurring fungicidal compounds in crop plants can be studied with facility. This technique has immense possibilities in phytopathological investigations where more direct evidence could be readily obtained for the presence or absence of antagonistic factors with reference to any host-parasite complex. The significance of this approach need not be over-emphasized.

Microbiological assay methods are of great utility in studies on methods of isolation of new growth factors and other active compounds. Discovery of vitamins, some amino acids, and their synthesis and utilization have been made possible by studies using biochemical fungal mutants. From these studies also comes the realization that each step in the synthesis or utilization of a compound may be controlled or limited by a specific gene (Beadle, 1945 ; Bonner, 1946). The very recent report of Sayre & Mountain (1959) taking into consideration the onion seedling bloat as an index of nematode populations in soils, detecting the presence of as few as ten nematodes per pound of soil, certainly adds evidence to the immense potentialities of these elegant bioassay methods.

Summary

Living organisms exhibit specific responses to the presence or absence of substances essential for or inhibitory to their growth. Based on this property various biological assays are now in wide use for estimating a number of elements and compounds present in soils, plants, animal tissues, chemicals, plastics, etc. The number of test organisms employed are, however, limited owing to practical considerations, and these have been briefly discussed. Test organisms used for assay of mineral elements, plant hormones, vitamins, amino acids, phytotoxins, antibiotics, fungicides, and for indexing nematode populations in soils are described. Other practical uses of bioassay techniques are reviewed.

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RECENT EXPERIMENTAL EVIDENCES ON THE PARTIAL SHOOT THEORY OF THE LEAF

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The plant body in the angiosperms consists of an axis divided into the root and the shoot. The shoot is comprised of the stem and the leaf. The stem originates as a bud from which the leaves grow out. The stem, however, possesses a capacity for unlimited growth whereas the leaf lacks that ability. As to the nature of the leaf different theories have been advanced form time to time, one of which postulates the leaf as a partial shoot potentially capable of growing into a full shoot. Arber (1950) in her book "Natural Philosophy of Plant Form" has marshalled the various morphological evidences in an excellent manner in support of this theory. During recent years while working on the effects of growth regulating substances on leaves, certain new facts have been noticed, which lend further support to the partial shoot theory of the leaf.

The evidences pertain to various activities which are discussed below.

ROOTING CAPACITY OF LEAVES

(*a*) FORMATION OF ROOTS—Leaves of *Bryophyllum* and *Kalanchoe* have been used for propagating the species as they possess preformed buds in the notches of the margin of the lamina. Leaves of *Begonia* when injured give rise to adventitious buds which are used for vegetative propagation. Sometimes it has been erroneously mentioned in some of the elementary text books that the roots which come out from the attached buds of the *Bryophyllum* leaf are foliar roots. In fact these roots belong to the adventitious buds. When the rooting capacity of leaves is mentioned such roots do not come in the picture at all. Some leaves when isolated from the plants and kept with their cut ends dipped in water produce roots from the petioles. If the excised leaves are treated with a suitable concentration of any of the synthetic hormones, they produce roots from any part of the petiole and even from the veins. Several leaves have been induced to produce roots by the application of synthetic hormones (Gregory & Samantarai, 1950 ; Misra & Samantarai, 1953 ; Samantarai & Kabi, 1954 ; Samantarai & Sinha, 1954, 1956 ; Schwabe, 1958). Just like stem cuttings the leaf cuttings are also capable of forming roots and enable the leaves to live independently when grown in soil or culture solutions,

(*b*) ENDOGENOUS ORIGIN OF THE FOLIAR ROOTS—With the application of the synthetic hormones at the appropriate concentration, certain histological changes take place in the excised leaves which are very similar to those taking place in stems with regard to the origin of roots. The parenchymatous cells opposite the phloem and corresponding to the pericycle region of the stem become meristematic. Next they organize root primordia which grow out as roots. Vascular strands containing xylem and phloem are developed which connect with the xylem and the phloem of the petiole. This development is known as the endogenous origin of roots. The same state of affairs prevails in the stem. Gregory & Samantarai (1950), Samantarai & Kabi (1954) and Kabi (1960) have clearly shown the endogenous origin of adventitious roots from the petioles in a number of leaves.

(*c*) BIOCHEMICAL CHANGES—The biochemical reactions which are seen along with the formation of roots in stem cuttings are the same in the leaf cuttings. Stuart (1938) has shown the carbohydrate and nitrogen metabolism and their translocation in stem cuttings with the application of β-indolylacetic acid during root emergence. Gregory & Samantarai (1950) have shown in cuttings of dwarf french bean and ivy that hydrolysis of carbohydrates and proteins takes place in the lamina. The soluble products thus formed migrate towards the petiole where they accumulate for sometime. With the formation and development of the roots the soluble products very soon disappear showing their utilisation in the formation of roots. Therefore, it is seen that the biochemical changes almost synchronise with the stages of development of roots. Similar observation with regard to the organic acids has been made by Samantarai & Mohanty (unpublished). Chibnall (1954) working with isolated runner bean leaves has also come to the same conclusion with regard to protein metabolism.

BUD FORMING CAPACITY OF THE LEAF

(*a*) FORMATION OF BUDS—As has been already mentioned, the leaves of *Bryophyllum* and *Kalanchoe* possess pre-formed buds. Some others like that of *Begonia* produce buds when injured under suitable conditions. Isolated rooted leaves when planted in soil produce buds from the petioles.

(*b*) EXOGENOUS ORIGIN OF BUDS—The origin of these vegetative buds from the petioles is exogenous as in the stems (Kabi, 1960). A group of meristematic tissue organizes in the cortical region of the petiole. This differentiates into an apical meristem with a two-layered tunica surrounding a corpus. Later, leaf primordia develop from the leaf buttresses and remain surrounding the apical mound.

Secondary Growth in the Leaves

The next category of evidences are found in secondary growth of the leaves.

(*a*) Pattern of Secondary Growth—Ordinarily in the arborescent plants secondary growth is seen in the petioles of leaves. It has been found by Samantarai & Kabi (1957) and Samantarai & Nanda (unpublished) that simple and compound deciduous leaves as well as simple and compound leaves of evergreen plants show the same pattern of secondary growth as seen in their respective stems. In herbaceous dicot leaves where secondary growth is absent, it has been possible to induce secondary growth of the same pattern as seen in their respective stems. Samantarai & Kabi (1953, 1954a) and Kabi (1960) have shown that the type of secondary growth seen in the leaves of *Pogostemon* and *Tridax* is of normal type. In *Cephalandra* and *Ipomoea* where internal phloem is seen, the secondary growth also resembles that of their respective stems. Secondary growth in the leaves of *Chenopodium, Amaranthus* and *Boerhaavia* is of anomalous type as seen in their stems. Samantarai & Nanda (unpublished) have shown that in woody climbers the leaves produce secondary growth which does not attain the same stage as seen in the stems. However, such leaves could be induced to produce necessary secondary tissue to resemble the stem type by the application of hormones.

(*b*) The Course of Development of Secondary Growth—The secondary growth takes place through the cambium. If there is a rudimentary cambium present, it is activated, if not, new cambia—both fascicular and interfascicular—arise under the influence of applied hormones. Even accessary cambia are formed as in the case of *Chenopodium* and *Amaranthus*. The course of development of secondary tissue in the leaves is, therefore, same as in the stems.

Expansion of the Lamina

The fourth group of evidences is based on the growth of the lamina. Gregory & Samantarai (1950) showed that ivy leaves of different ages on isolation, although growing with their own induced root system for a period of two years, remained a size-series. Samantarai & Sinha (1956) have shown that rooted leaves of *Basella* outgrew the size of its counterparts in the stem several folds. Schwabe (1958) has also shown that *Kalanchoe* leaves grew to a very great extent after isolation and rooting. Kabi (1960) has also shown that the synthetic hormones stimulate the growth of veins and petioles and thus correspond to the caulocalines whereas substances like adenine and gibberellic acid bring about growth of the mesophyll cells thereby corresponding to the phyllocalines.

Although the lamina is devoid of any unlimited growth as seen in the shoot apex, still at times it grows much more than its size attained under attached conditions.

Self Maintenance

Self maintenance of a leaf as an independent entity is one of the greatest supports for the theory. Gregory & Samantarai (1950), Samantarai & Kabi (1945b) and Samantarai & Sinha (1956) have shown that the leaves of different species with their induced root system are capable of maintaining themselves. In fact, each of such leaves is a miniature plant performing all the physiological processes. The longevity of the rooted leaves is much more than that of the leaves attached to the plant.

Reproductive Capacity

Propagation of the species is one of the main functions of the stem. The leaf is capable of propagating the species by forming vegetative buds. When the leaves die out the adventitious buds which are developed from such leaves grow out as independent plants. Unpublished work done in this laboratory shows vegetative buds in the rooted leaves of *Ipomoea, Lantana, Heliotropium, Vitis, Daedlacanthus* and many others.

Conclusions

The account presented above shows the shoot nature of the leaf to some extent. So far as formation of secondary tissues, roots and buds is concerned, the leaf behaves like a full shoot. With regard to growth, the lamina shows a different type from the stem. Therefore, in this respect the leaf just approaches to possess the qualities of a shoot. Again, with regard to reproductive ability, the leaf is capable of reproducing the species vegetatively. Taking all these experimental evidences together it can be safely concluded that the leaf is a partial shoot potentially capable of attaining full shoot-hood.

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THE BOTANICAL EXPLORATION OF MAHABALESHWAR

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My interest in the flora of Mahabaleshwar has been of long standing. Ever since I took up the study of the flora of Bombay, I noticed that most of the books on the subject listed a fairly large number of *rare* or *very rare* plants found only in Mahabaleshwar ; at the time it did seem strange that we did not have a flora of Mahabaleshwar. Up to the present the only work that claims to be a sort of local flora for the area is that of Birdwood (1886, 1887, 1896, 1897).

About 1950 Dr S. A. E. Hakim asked my help for the study of the medicinal plants of Mahabaleshwar ; this was the beginning of my exploration of the plateau. In the course of my work I have been greatly helped first by Dr. Hakim himself, who often has acted as my host during my visits to the area, and Prof. P. V. Bole of St. Xavier's College ; several of my M. Sc. students have been of real help in the field.

DIFFICULTIES IN THE FIELD

The torrential non-stop rains of the monsoon have given us much trouble , often we have spent two full weeks always wet, and have had to struggle to keep our pressing papers and specimens from getting similarly wet.

In several spots on the Mahabaleshwar plateau there seem to be very numerous and apparently very hungry leeches ; the record of collection was one morning in August when I picked about 140 leeches from my person. The area near Lingmala is particularly bad for leeches ; Kate's Point is almost as bad.

Only on one occasion we have had trouble with dangerous animals ; fortunately for us the tiger we met one morning near the Lake had just had a full meal, and feeling rather sluggish left us alone ; but we did run!

During the monsoon the ground becomes very smooth and slippery ; some of my less experienced students have been much troubled with repeated falls in the field.

TOPOGRAPHICAL AND CLIMATIC NOTES

The geographical position of the meteorological observatory, which may be taken roughly as the middle of the plateau, is 17° 56'N. by 73° 40' E.

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The average altitude of the station above mean sea level is 1,350 m. (4,500 ft.), the highest point being Wilson Point at 1,410 m. (4,710 ft.).

Of the total annual rainfall, about 95 per cent belongs to the monsoon months, June to October. From the onset of the monsoon to about the end of August, there is scarcely a day without rain, often non-stop rain ; during September-October about one in four may be a clear day. The annual average rainfall recorded for the years 1881-1940 for Mahabaleshwar is 663.5 cm. (261.23 in.) ; the absolute maximum for any year is 1022 cm. (402.41 in.) recorded for 1896 ; the absolute minimum for the same period is 354.5 cm. (139.57 in.) of 1899. The average number of rainy days for the same period is 120.

The temperature is pleasant throughout the year, seldom going over 32°C in summer, or under 13°C in winter. The absolute maximum recorded for 1881-1940 is 33.1°C (91.6°F), the corresponding minimum being 9.2°C (48.7°F). The relative humidity of the atmosphere during daylight hours for the monsoon months is practically 100 per cent, for the rest of the year $\pm$ 40 per cent.

"The geology of the hills is simple, trap overlaid by a light capping of iron and clay. The trap shows in most ravines and in horizontal belts on the sides of the hill . . . The Mahabaleshwar trap is often columnar, and accompanied by crystallized quartz, apophyllite, stilbites, and scolecite found in cavities . . . The laterite ends on the Satara road $6\frac{1}{2}$ miles from the Frere Hall, on the Poona road $13\frac{1}{4}$ miles, and on the Mahad road $2\frac{1}{4}$ miles." (McConaghy).

Milestones in the History of Mahabaleshwar

The Mahabaleshwar Plateau, as distinct from Old Mahabaleshwar 5 km. NW of the bazaar, was 'discovered' by Peter Lodwick in 1827, when he climbed along what is now called the Lodwick Point from the Concan plains. The inscription at the base of the Lodwick monument records the fact thus: "The first European who set foot on these hills, he made known the salubrity of the climate and led to the establishment of the Mahabaleshwar Sanitarium, thus conferring an inestimable benefit on the Bombay Presidency . . . This Point, now by order of Government designated Lodwick Point in honour of his name, he reached in 1827, after hours of toil through the dense forest. . ."

The Mahabaleshwar Sanitarium was founded in 1828 by Sir John Malcolm, Governor of Bombay, who obtained possession of the area from the Maharaja of Satara in exchange for the village of Khandala near Wai. Soon Mahabaleshwar became not only a sanitarium, but also the summer seat of the Government of Bombay. This caused many dis-

tinguished officials to come to Mahabaleshwar for the 'season' ; among these must be mentioned Birdwood, who after several preliminary attempts at the Flora of Mahabaleshwar from 1886 onwards, finally in 1897 produced the definitive "A Catalogue of the Flora of Matheran and Mahabaleshwar". Cooke (1887, 1896), who later became the Director of the Botanical Survey of India for Western India, collected extensively in the area and wrote critical notes to complement Birdwood's Catalogue ; Lisboa, Gammie, Woodrow, Bhide and others also visited the area and made collections during the summer season. Perhaps the latest among such officials is Acland, who before he retired to England put in my hands an interesting manuscript of the flora of Mahabaleshwar as seen through a "layman's" eyes. As such visitors came only during the season, roughly during the hotter months of summer, we came to know the flora of the plateau rather well for the dry season ; the monsoon rains and leeches kept most visitors away until Blatter, who had retired from St. Xavier's College, Bombay, and stayed in Panchgani in 1924-1934, and our own parties from 1950 onwards, took up the complete exploration of the area throughout the year (Santapau, 1953).

About 1832 a number of Chinese and Malay prisoners, who had been languishing in the heat of Poona, were taken to Mahabaleshwar ; they were allowed to plant vegetables and other temperate plants for their own use and for sale in the market. During one season they are recorded to have collected 3500 lbs. of arrowroot from a wild plant, *Hitchenia caulina* Bak., which is very common in the area during the second half of the monsoon and early winter. The stay of the Chinese prisoners, which lasted until 1864, is commemorated in the 'Chinaman's Waterfall'. The Chinese greatly improved the station gardens, and it is in great measure owing to their industry that potatoes and English vegetables have ever since been so great a success. They also taught the inhabitants to make cane baskets and chairs. The terracing of the hill slopes in and around Mahabaleshwar is another development apparently introduced by the Chinese prisoners.

About the middle of the nineteenth century Sir Clements Markham was deputed by the British Government to make a study of the *Cinchona* trees in South America with a view to the establishment of plantations in India ; about 1860 Clements selected Mahabaleshwar and several other hill stations in India for trials ; the spot chosen in Mahabaleshwar was near Lingmala Falls, about 5 kms. from the bazaar ; the scheme failed repeatedly, and after having been kept going from 1865 to 1875 it was given up for Mahabaleshwar ; the land was then passed over to the Forest Department for the introduction of foreign plants into Bombay. Such use is still continuing today (for an account of the introduction of *Cinchona* in India, *see* Markham, 1862).

Present Position of Mahabaleshwar

Of late there has been some talk about setting up hill laboratories in various parts of India for the study of botanical problems that cannot be tested in the plains. Mahabaleshwar is an ideal spot in many respects. The conditions in the area seem to be favourable for the growing of many tropical and temperate plants. Further, there is already a good nucleus that in time may develop into a flourishing research station. The Lingmala forest land may continue to be used for testing the suitability of foreign trees in the climate of India. The Government of Maharashtra keeps a fully developed Rust Station, where strains of wheat are tested or new ones developed for their resistance to rust infection. Extensive private and public apiaries do serious work on honey production ; the Government keeps a full laboratory in which research is carried out on every aspect connected with honey production. Lately the Forest Department (1957) has established the Pratapsinh Park with the intention of keeping in a reserved area all the woody species of the district, and of using the same for doing introduction tests on garden or decorative plants ; the park has a building for a herbarium, in which it is hoped that the complete flora of the plateau will be preserved for the use of students and visiting scientists.

The Botany of the Mahabaleshwar Plateau

The forest is classed by Champion (1936) as 'Bombay Subtropical Evergreen'. Unfortunately the forest has been much affected by shifting cultivation and more or less constant lopping. "Where properly developed it is a dense evergreen wood of mixed species and low height generally not exceeding 25 ft., though locally up to 50 ft." (Champion, 1936). One of the forest's best areas is along the so-called Tiger Path ; on several occasions we have measured the trees and found them to be between 15 and 20 m. high.

Where the forest has been interfered with, cleared ground is soon colonized by *Pteridium aquilinum* Kuhn, followed by *Crotalaria retusa* Linn., *Lasiosiphon criocephalus* Decne., *Randia brandisii* Gamble, *Memecylon umbellatum* Burm., ultimately followed by *Syzygium cumini* Skeels, *Olea dioica* Roxb., *Actinodaphne,* etc. of the climax vegetation.

In Mahabaleshwar there are many species which are otherwise rare in Bombay ; they may be more or less endemic, or at least very restricted in their distribution. Such species may be common and abundant on the plateau. These are, e.g. *Hitchenia caulina* Bak., several ground and tree orchids, *Cryptocorine tortuosa* Blatt. & McC., *Paracaryum mala-*

baricum Clke., *P. lambertianum* Clke., *Ligustrum neilgherrense* var. *obovata* Clke., several species of *Strobilanthes*, of *Solanum*, a number of rare ferns, etc.

One of the more striking aspects of the forests of Mahabaleshwar is that many trees are literally loaded with mosses and lichens, and many trees show adventitious roots from the upper branches, a clear sign of the heavy monsoon of the place.

The Mahabaleshwar Forest Constituents

In the better parts of the forest, there are three clear levels: (*a*) the top about 15-20 m. high, (*b*) the middle 10-15 m., and (*c*) the lower 2-3 m. In the neighbourhood of the Yena Lake most of the larger trees have disappeared ; the forest in those parts is dominated by *Memecylon*, 6-10 m. high.

The Top Level of the Forest—The following trees are about the more common, and they are here listed in what we consider their order of abundance: *Syzygium cumini* Skeels, *Actinodaphne angustifolia* Nees, *Olea dioica* Roxb., *Glochidion hohenackeri* Bedd., *Ficus glomerata* Roxb., *Pygeum gardneri* Hook. f., *Nothapodytes foetida* Sleum., *Canthium dicoccum* Merr. and *Terminalia chebula* Retz.

Middle Layer in Tall Forest (or top layer in open forest)—More or less equally abundant are the following: *Memecylon umbellatum* Burm., *Lasiosiphon eriocephalus* Decne., *Ligustrum neilgherrense* var. *obovata* Clke., *Osyris wightiana* Wall. and *Callicarpa tomentosa* Murr.

Shrubby Constituents (often at edge of forest)—*Pavetta indica* Linn., one of the commonest, *Colebrookea oppositifolia* Smith, *Pogostemon plectranthoides* Desf., *Carvia callosa* Bremek. (=*Strobilanthes callosus* Nees), *Crotalaria retusa* Linn., *Psychotria truncata* Wall. (usually restricted to undergrowth of dense forest), *Embelia tsjariam-cottam* DC., *Solanum giganteum* Jacq. and *Thelepaepale ixiocephala* Bremek. (=*Strobilanthes ixiocephalus* Benth.).

Among the woody climbers of the area, the following deserve mention because of their abundance: *Elaeagnus conferta* Roxb., *Scutia circumcissa* Radlk., *Jasminum malabaricum* Wt., *Embelia viridiflora* Comm., *Piper hookeri* Miq., *Smilax zeylanica* Linn. and *Capparis longispina* Hook. f. & Th.

Herbaceous Constituents—The ground in dense forest is practically free from herbs, except at the edges. During the monsoon there are many herbs often with tuberous roots ; during the dry season we have noted one or two species of *Oplismenus* and masses of *Lepidagathis cuspidata* Nees ; several species of the Acanthaceae may carry over from the monsoon for several months.

The Monsoon Flora of Mahabaleshwar

The following paragraphs have been extracted from my diary written during an excursion in the middle of September, 1958.

"Sept. 14, 1958. The walls of houses, old walls, tree trunks etc. are covered mostly with *Lecanthus peduncularis,* masses of it all over the place ; in the grounds of our hotel noted plenty of *Dichrocephala ; Impatiens dalzellii* with yellow flowers is now one of the commonest plants on old walls, etc. *Begonia crenata* is in full strength all over, and about the most abundant species in flower at present. *Asystasia dalzelliana* is strikingly common and abundant under hedges. Some distance from our hotel we came across *Hitchenia caulina* in full bloom of pure cream-coloured flowers ; *Zingiber macrostachyum* was also noted but only as occasional. When we reached the flat or nearly flat rocky ground near the lake, we noted several plants as undoubtedly dominant: several species of a small *Eriocaulon,* also several species of *Utricularia, Anotis lancifolia, Ammannia* in masses in pools on the rocks ; at present we found very few grasses in flower or fruit. In sheltered spots we found some plants of *Heracleum concanense* with radiant and showy flowers ; the most striking plant of this morning is *Paracaryum malabaricum* with uniformly blue flowers of a light tinge ; these plants were gregarious near the lake."

"Sept. 16, 1958. (Fitzgerald Ghat). On vertical rocks under water falls or in the water course itself, we noted: *Rotala floribunda* in masses, the leaves being deep green ; where the water was just trickling down, *Impatiens acaulis* formed mats of colour for large areas on the rocks ; also *Sonerila scapigera* in large carpets, the underside of the leaves being deep purple. On rocks but away from falling water we noted many other plants, masses of grasses in leaf, several species of *Anotis, Exacum pumilum, Utricularia striatula, Senecio grahamii, Canscora diffusa, Smithia setulosa* and other *Smithia* species etc. *Impatiens pulcherrima* is very showy at the base of the rocks ; we also collected *Dicoelospermum ritchiei, Mucuna monosperma* etc."

The Commoner Monsoon Species

It is difficult to say which of the following plants is the commonest, or even whether one species is more common than the rest ; the plants here listed are certainly abundant in the monsoon. *Hitchenia caulina* Bak., *Habenaria heyneana* Lindl. ('The Tooth-brush Orchid'), *H. grandiflora* Lindl., *H. panchganiensis* Sant. & Kapad., *Remusatia vivipara* Schot., *Plectranthus stocksii* Hook.f., *Begonia crenata* Dryand., *Impatiens dalzellii* Hook.f. & Th., *I. balsamina* Linn., *I. pulcherrima* Dalz., *Utricularia caerulea* Linn., *U. striatula* Sm., *U. affinis* Wt., *Anotis lancifolia* Wall.,

father of plant physiology, who clearly enunciated the fundamental requirements and processes of nutrition and growth of plants in 1860. Sachs along with Knop (1861-62) introduced the method of water culture experiments for elucidating the mineral requirements of plants. As further investigations were carried out to obtain more precise knowledge, the method of sand culture was also introduced and various combinations of salts tried.

It was found that the essential requirements of plants for normal growth are suitable temperature, light, carbon dioxide, oxygen, water, different inorganic chemicals and an air supply in the soil which should have a suitable pH value. The stomata were found to be the structures through which the interchange of oxygen, carbon-dioxide and water vapour took place.

Since 1860 till today various combinations of salts have been suggested by different investigators to form a complete nutrient solution for plants. These experiments showed that apart from carbon, hydrogen and oxygen, plants require about six or seven major elements, viz. K, N, Ca, P, Mg, S and about five minor or trace elements, viz. Fe, B, Zn, Cu and Mn, for their nutrition (Crone, 1902 ; Tottingham, 1914 ; Shive, 1915 ; Hoagland, 1920 ; Richard & Templeman, 1936 ; Hamner, 1940).

I (*see* Sen Gupta, 1953) also did some experiments in sand culture on the effect of three levels of N, K, Ca and P on the growth of two species of jute, viz. *Corchorus capsularis* and *C. olitorius*.

Some of the outstanding results obtained by the culture of plants by hydroponics in the U.S.A. and Britain are the growth of tomato plants up to a height of 25 feet, a yield of 12 lbs of tomatoes per plant and of 200 tons to the acre.

While Gericke's original method of soilless culture was to grow plants in aqueous solution, this was followed by several other techniques in which sand, gravel, cinder and other aggregates have been tried with success at different centres of work in the U.S.A. Complex devices were brought to play ; the plants were grown in large glass houses and various devices were adopted for irrigation and for the aeration of roots. The different names by which this method has been called are: Hydroponics, Chemiculture, Tray Agriculture, Tank Farming, Soilless Culture, Gravel Culture and Sand Culture.

For various reasons the methods adopted in the U.S.A. are not applicable to India. For India any method of hydroponics should be simple in operation and inexpensive. The first serious attempt to grow plants without soil in India was made by Sholto Douglas (1951) in Kalimpong in 1946. He used troughs made of wood and mud plaster and worked out suitable nutrient mixtures.

To make the nutrient mixture less expensive, commercial chemicals rather than pure chemicals are used. One of the mixtures found suitable by Sholto Douglas is given below:

		Ratio: This quantity per 100 gallons of water on the day of application only.
Nitrate of soda ...	... 4½ oz.	
Superphosphate ...	... 3 ,,	
Calcium sulphate ...	... 2 ,,	
Potassium sulphate ...	... 1½ ,,	
Magnesium sulphate ...	... 2½ ,,	
Trace elements ...	... ½ dram.	

A trace element mixture (2 oz.) consisting of:

Zinc sulphate ...	... 3.0 dram.
Manganese chloride ...	... 9.0 ,,
Boric acid ...	... 7.0 ,,
Copper sulphate ...	... 3.0 ,,
Iron sulphate ...	... 10.0 ,,

Sholto Douglas' book also contains data on the hydroponics equivalent per acre against the agricultural average per acre for a number of crops, the average cost of cultivation in Kalimpong and the agricultural yields of rice, wheat, and cotton in a number of countries. The following crops are reported by Sholto Douglas to have been grown successfully at Kalimpong in soilless culture: tomato, peas, beet, broad bean, onion, lettuce, cabbage, cauliflower, oats, soyabeans, maize, cucumber, brinjal, lady's finger, cucurbita, wheat, kidney bean, rice, turnip, carrot, flax, chillies, sponge gourd, 'mung', pumpkin, sugarcane, *Pyrethrum* and *Chrysanthemum*.

The temperature and other climatic conditions are subtropical in Kalimpong, and it was thought necessary to work out a suitable method for tropical conditions. Since 1949 I have been doing some experiments on hydroponics, at the Experimental Garden of the Presidency College, Calcutta. The chief object was to find out a suitable technique for the plains of Bengal and to find out how far the large area of the flat roofs of the houses in Calcutta could be utilised for growing plants. Some attempts were then already being made to grow plants by hydroponics in a few centres in Calcutta.

Tanks made of cement were improvised. There were two sizes: Small—$2' \times 1\frac{1}{2}' \times 1'$ and Large—$5' \times 3' \times 1'$. I did not try mud plaster tanks as these were found to be damaged by the heavy rain of the monsoon season in Kalimpong, and were much less permanent and more difficult to work with than cemented tanks. The method used was to grow plants on sand and readily available aggregates like cinder, broken bricks, and stone chips of 0.5″ diameter. Each tank had aggregate up to a depth of 6″ at the base with a 4″ covering of sand, and the uppermost 2″ was uncovered (Fig. 1).

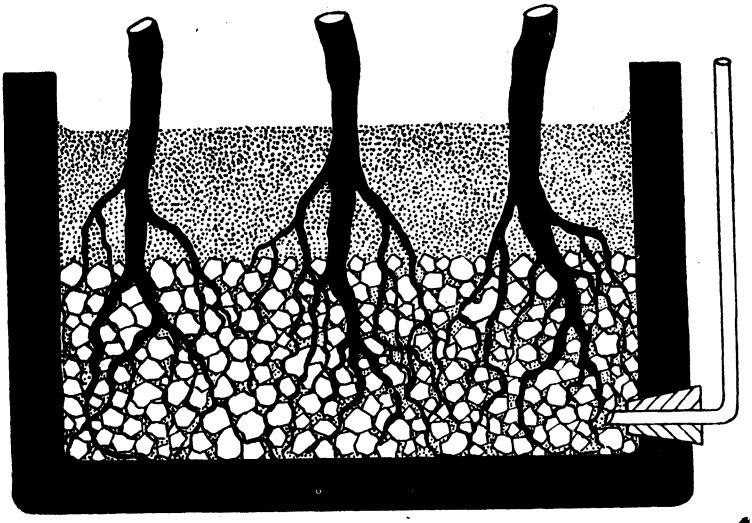


Fig. 1. Sectional view of a typical cemented tank.

Nutrients were added by dissolving tabloid mixtures in water and adding the solution to the tanks once in seven days during early and maturing stages and once in ten or fifteen days after the onset of flowers and fruits. The tabloids found suitable in Kalimpong were also found suitable by some of the experimenters in hydroponics in Calcutta. The chemical composition of the filtered water of Calcutta Corporation shows a monthly variation as shown from their records (Table I).

TABLE I

CHEMICAL COMPOSITION OF FILTERED WATER IN PARTS PER 100,000 DURING 1948-49 SHOWING MONTHLY VARIATIONS

Month	Chloride as Cl_2	Free ammonia	Albuminoid ammonia	Total hardness	Permanent hardness	Nitrate	Oxygen absorbed	pH
April	5.5	0.002	0.002	20.5	9.8	0.009	0.0615	8.0
May	8.3	0.002	0.002	22.2	11.0	0.019	0.083	8.04
June	4.4	0.002	0.002	18.8	9.6	0.030	0.068	8.3
July	1.1	0.002	0.003	11.2	5.4	0.0215	0.053	8.3
August	0.7	0.0016	0.002	9.1	4.4	0.015	0.033	8.3
September	0.5	0.001	0.002	9.4	4.5	0.009	0.026	8.22
October	0.45	Trace	0.002	9.5	4.5	0.005	0.057	8.2
November	0.6	,,	0.002	12.0	4.8	0.0024	0.072	8.2
December	0.8	,,	0.002	14.4	6.4	0.002	0.060	8.2
January	0.96	,,	0.001	17.2	8.9	0.007	0.052	8.2
February	0.85	,,	0.003	18.0	7.3	0.010	0.059	8.07
March	2.7	,,	0.0025	17.8	9.8	0.011	0.044	8.1

The temperature and pH values of solutions play a prominent part in the growth of plants. The monthly average maximum and minimum temperatures in Kalimpong are given in Table II, and the monthly average maximum and minimum temperatures and total rainfall in different months in Alipore, Calcutta, are given in Table III.

TABLE II

TEMPERATURE (°F) AT KALIMPONG

Year	Month	Maximum	Minimum
1946	October	79	58
	November	73	50
	December	66	49
1947	January	62	45
	February	66	50
	March	74	52
	April	80	54
	May	83	60
	June	81	63
	July	80	67
	August	81	68
	September	81	67

TABLE III

TEMPERATURE & RAINFALL IN CALCUTTA
(Alipore)

Month	Max. Temp. °F.	Min. Temp. °F.	Total rainfall in inches during the month
December 1948	78.19	57.61	Nil
January 1949	81.06	57.75	0.05
February ,,	85.29	64.20	0.03
March ,,	94.31	71.84	1.28
April ,,	91.75	75.77	6.00
May ,,	93.20	77.99	10.32
June ,,	93.86	79.67	8.93
July ,,	90.08	79.56	14.31
August ,,	89.88	79.48	7.31
September ,,	90.10	75.00	13.19
October ,,	91.20	76.80	3.60
November ,,	84.30	62.10	Nil

The monthly average temperatures were much higher in Calcutta than at Kalimpong. Records of the temperature of the solution with the containers were made at 7 A.M. and 2.30 P.M. The variation of temperature and pH in different months in the various types of tanks is shown in Tables IV and V. The temperature of the solutions during midday hours went up to 36° to 37°C during the months of April to July and was too high for the growth of plants. It was found desirable to

sow the summer season crops about the end of May so that the plants received occasional showers in early stages and grew well after the monsoon rains set in.

TABLE IV

VARIATION OF TEMPERATURE IN °C IN THE MEDIUM OF THE TANKS IN DIFFERENT MONTHS, TIMES OF THE DAY AND IN DIFFERENT MEDIA 1951-52

1951	MARCH		APRIL		MAY	
TIME	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.
Room Temp.	27	28.5	29	33	29	33.5
Brick Chips	26	29.5	29.5	34	29	34.5
Stone Chips	25.5	30	29	34	28.5	34.5
Cinders	25	29	29	34.5	29	34
1951	JUNE		JULY		AUGUST	
TIME	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.
Room Temp.	31	36	29.5	34.5	27.5	31.5
Brick Chips	30.8	36.5	29	35	26.8	32
Stone Chips	31	36	29.5	35	27	31.8
Cinders	30.5	37	28.8	34.5	27.5	31.5
1951	SEPTEMBER		OCTOBER		NOVEMBER	
TIME	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.
Room Temp.	28.5	31	26.5	29.5	24	27
Brick Chips	30.5	38.5	27	30.5	21.5	29.5
Stone Chips	30	37	27	30	22.5	30
Cinders	31	37.5	27.5	30.5	22	31
1951-52	DECEMBER		JANUARY		FEBRUARY	
TIME	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.	7 a.m.	2.30 p.m.
Room Temp.	17.5	24.5	22.5	24.5	25	26.5
Brick Chips	17	24	21	27	22.5	26.8
Stone Chips	19	25	21.5	27.5	23	27.5
Cinders	16.5	23.5	22	27.5	23	27.5

The pH values of tap water and solutions of different tanks also showed variations. They were on the alkaline side throughout the year and the alkalinity increased during April-July when the pH rose to 7.8. The pH value was in general higher in cinders than in the other aggregates.

TABLE V

VARIATION OF pH IN DIFFERENT MONTHS & MEDIA
1951-52

1951	March	April	May	June	July	August
Tap Water	7.2	7.6	7.4	7.5	7.5	7.2
Brick Chips	7.1	7.6	7.6	7.6	7.6	7.3
Stone Chips	7.0	7.6	7.8	7.6	7.7	7.2
Cinders	7.3	7.8	7.9	7.8	7.8	7.5
1951-52	**Sept.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**February**
Tap Water	7.2	7.0	7.0	7.0	7.1	7.1
Brick Chips	7.3	7.2	7.2	7.2	7.3	7.2
Stone Chips	7.2	7.2	7.2	7.0	7.0	7.2
Cinders	7.5	7.4	7.4	7.4	7.5	7.5

A large number of vegetable crops (Figs. 2-5) and some flowering and bulbous plants were tried. Some of them grew better in hydroponics than in soil, some grew better in soil, and others grew equally well in both.

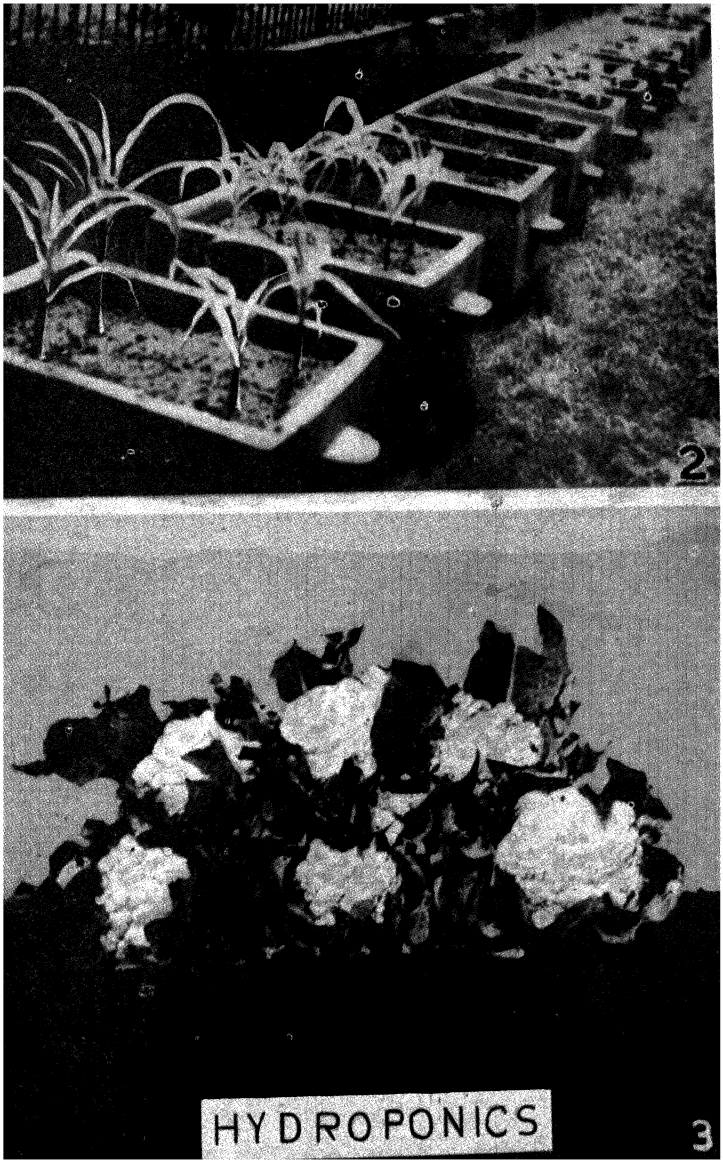
The crops which grew better in hydroponics were: chillies and bhendi (Lady's finger) among summer crops, and tomato, carrot, onion and turnip among the winter crops. Amongst the ornamentals, sweet sultan, *Calendula*, carnation, *Hymenatherum* and cornflower grew better in hydroponics.

Summer vegetable crops—Brinjal, 'Katwa data', 'Natey sag' and 'Lal sag', cucumber, gourd, sponge gourd and the winter crop potato (red round) grew better in soil than in hydroponics, and the summer vegetables radish, water melon, small bitter gourd, maize (Fig. 2) and the winter vegetables cauliflower and round 'Lau' grew equally well in both. The results are given in Table VI.

TABLE VI

GROWTH AND YIELD IN HYDROPONICS & SOIL

Crops	Better in Hydroponics	Better in Soil	Equally Well in Both
Summer Season	Chilli Lady's Finger Tomato	Brinjal Indian Spinach Cucumber Gourd Sponge Gourd	Radish Maize Small Bitter Gourd Water Melon
Winter Season	Turnip Onion Carrot	Potato (Red Round)	Cauliflower Round 'Lau'



Figs. 2-3.—Fig. 2. Small size cemented tanks with some maize plants. Fig. 3. Cauliflower grown in hydroponics.

Of the flowering plants, nasturtium, *Petunia* and balsam grew better in soil, and sweet pea, *Chrysanthemum*, hollyhock, *Phlox*, *Zinnia* and *Antirrhinum* grew equally well in both. All the bulbous plants tried, viz. *Eucharis*, *Cooperanthes*, *Zephyranthes*, *Pancratium longifolium* and *P. latifolium*, grew equally well in soil and in hydroponics. The results for flowering and bulbous plants are given in Table VII.

TABLE VII

GROWTH OF FLOWERING AND BULBOUS PLANTS IN HYDROPONICS AND SOIL

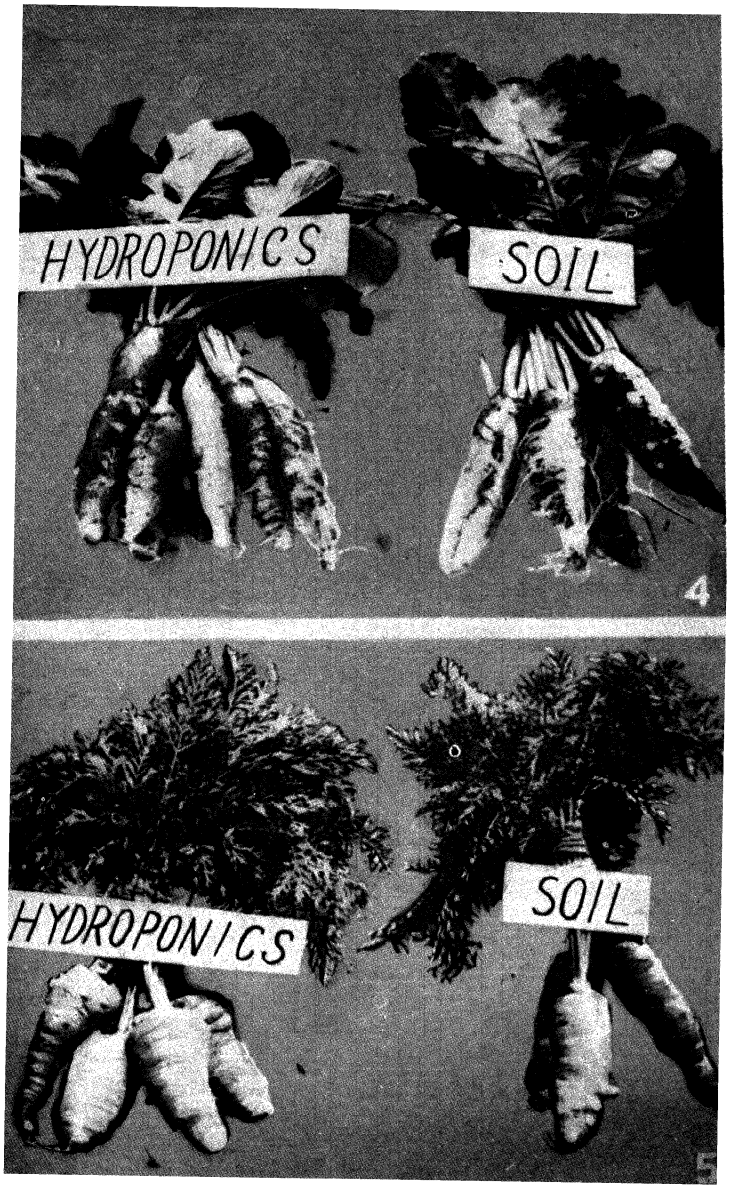
	Better in Hydroponics	Better in Soil	Equally Well in Both
Non-bulbous Plants	Sweet Sultan *Calendula* Carnation *Hymenatherum* Corn Flower	Nasturtium *Petunia* Balsam	Sweet Pea *Chrysanthemum* Hollyhock *Phlox* *Zinnia* *Antirrhinum* *Gerbera*
Bulbous Plants			*Eucharis* Lily *Cooperanthes* *Zephyranthes* *Pancratium longifolium* *Pancratium latifolium*

The average cost of cultivation of the crops in hydroponics by the above-mentioned methods is given in Table VIII.

TABLE VIII

COST (IN RS. AS.) OF CONTAINERS, AGGREGATES & NUTRIENT CHEMICALS

	Cost of Tanks	Cost of aggregates	Cost of sand	Total initial cost	Chemicals		
					1 month	3 months	6 months
Small Tank 2′ × 1½′ × 1′	Rs. 18/-	Broken bricks 3/-	1/-	22/-	-/1/9	-/5/-	-/10/-
		Stone chips 5/-	1/-	24/-	,,	,,	,,
		Cinder -/10/-	1/-	19/10/-	,,	,,	,,
Large Tank 5′ × 3′ × 1′	Rs. 35/-	Broken bricks 10/-	4/-	49/-	-/6/-	1/2/-	2/4/-
		Stone chips 16/-	4/-	55/-	,,	,,	,,
		Cinder 2/-	4/-	41/-	,,	,,	,,



Figs. 4-5.—Fig. 4. Radish plants grown in soil and hydroponics. Fig. 5. Carrot plants grown in soil and hydroponics.

The possibility of growing some of the vegetable crops by hydroponics on the roofs of houses in Calcutta, with four tanks of the size 5′ × 3′ × 1′ per house is seen from the following figures:

(*a*) Total number of houses (small & large) under the Calcutta Municipal area ... 1,23,000

(*b*) 4 tanks of 5′ × 3′ × 1′ size per house with 10 plants of tomato in each tank, each plant producing 5 lbs. ... ... 200 lbs per house

(*c*) Total for 10 per cent of the houses (viz. 12,300) @ 200 lbs per house ... 24,60,000 lbs.

i.e., 24.6 lac lbs. of tomato per season

Figures for some other vegetable crops are given in Table IX.

TABLE IX

FIGURES FOR THE YIELD OF OTHER CROPS IN 10 PER CENT OF THE TOTAL NUMBER OF HOUSES UNDER CALCUTTA MUNICIPAL AREA (12,300), EACH WITH 4 TANKS OF 5′ × 3′ × 1′ SIZE

SUMMER CROPS		WINTER CROPS	
CHILLIES (1 lb. per plant)	7,38,000 lbs.	CARROT (½ lb. per plant)	11,80,800 lbs.
15 plants per Tank	7 lac lbs.	48 plants per tank	11 lac lbs.
BHENDI (¾ lb. per plant)	5,43,500 lbs.	ONION (½ lb. per plant)	11,80,800 lbs.
15 plants per Tank	5 lac lbs.	48 plants per tank	11 lac lbs.

The results so far obtained show that if Calcutta becomes even partially hydroponics-minded, three winter crops (tomato, carrot and onion) and two summer crops (chillies and bhendi) can be successfully grown on roofs by hydroponics. By further experiments it may be possible to add other crops to the list. The same tank can be used for the summer crop followed by a winter crop.

In addition, the following common advantages of hydroponics may be kept in mind: higher yield, freedom from soil diseases, closer planting with larger number of plants in the same area, no dirt, no smell, no need of changing soil or addition of organic manure, quicker growth of plants, aeration of roots is simpler, and manual labour is very much minimised.

It is a simple method, and once it is under practice the average time to be spent will be about one hour per week for four tanks.

It will be a very pleasant practice for the leisure hours and will give the pleasure of watching plants grow and produce crops and should be found interesting by the young and old alike.

Summary

This paper deals with some experiments on hydroponics in Calcutta using cemented tanks. Plants were grown in three types of aggregates, broken bricks, cinder and stone chips. The variations of the pH in different media and temperature of the solution in different months and different time of the day have been noted.

The cost of culture of plants in hydroponics has been determined and the possibility of growing plants in soilless culture on the roofs of houses in Calcutta has been indicated.

The comparative growth of some vegetable crops, flowering plants and bulbous plants has been given.

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SUBSTRATE AND THE SOIL FUNGUS

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Soils offer a wide variety of organic substrates for colonization by micro-organisms. Remains of dead plants and animals are thrown back into soil, which are then attacked by various groups of micro-organisms and are ultimately broken down into simple substances. These chemical transformations are brought about by the production of a wide variety of enzymic systems. In the course of evolution certain micro-organisms have adapted themselves for colonizing particular substrates. Thus we speak of 'Sugar fungi', 'Cellulose fungi' etc. Apart from a suitable substrate, environmental factors like soil moisture content and aeration and also the competing microbial population influence the colonization of a substrate by a fungus. This results in a succession of colonizers until the substrate is exhausted. As the decaying substrate is exhausted, succession of these heterotrophic organisms comes to an end instead of leading to further change in the composition of the colonizing population, since exhaustion of substrate puts a stop to the active growth of these micro-organisms (Garrett, 1951). This is in contrast to the succession of autotrophic green plants above ground which results in a climax formation. Thus, habitats of micro-organisms in soil are discontinuous in space and time and these microbes have developed various means of survival during periods of starvation.

Burges (1939) suggested grouping soil fungi according to their nutritional requirements. He distinguished between 'Sugar fungi' and 'Humus fungi' to indicate the substances they readily utilize. Garrett (1951) extended his ideas and attempted to correlate certain characters developed by each group of fungi with the requirements for colonization of particular substrates. For instance, sugar fungi were stated to have high growth rates, heavy spore production and rapid rates of spore germination. When organic matter is thrown into soil, soluble substances like sugars are first utilized. There is an initial flare up of 'Sugar fungi' which are the pioneer colonizers. Competition is intense and high growth rates and heavy sporulation are distinct advantages. Production of antibiotic substances and resistance to such substances are also advantages as seen by the fact that dominant 'Sugar fungi' like *Fusarium* and *Trichoderma* are producers of antibiotic substances. Brian (1957) has convincingly shown that production of antibiotic substances by soil fungi plays a significant role in their ecology.

After the sugar and other soluble food materials in the substrate are

used up, fungi utilizing pectins, celluloses etc. develop and finally lignin decomposing fungi gain an upper hand when other food materials are used up. Lignin fungi are generally Basidiomycetes and in contrast to 'Sugar fungi' they are slow growing. Very often they produce mycelial aggregations and rhizomorphs. Production of such large structures becomes possible because competition is less and there is an abundance of food. Relatively few fungi have developed enzyme systems that can break down complex lignins.

Apart from the general soil saprophytes considered above there are many groups of fungi which have become adapted to specialized habitats, thus avoiding competition. Coprophilous or dung-inhabiting fungi, root parasites, mycorrhizal fungi and predaceous fungi can be cited as examples of such groups.

Coprophilous fungi utilize partly digested or modified substances found in dung. Their spores are resistant to the digestive juices of herbivorous animals and they find their way into dung from fodder, passing through the alimentary canal of animals.

Predaceous fungi capture and parasitize soil nematodes and they have developed various devices for capturing their prey. Some have sticky appendages which adhere to the wandering nematodes while others possess three-celled ring like structures which are sensitive to touch. As soon as a nematode enters the ring, the cells bulge inwards and become turgid thus holding the prey. These predaceous fungi send haustoria into their hosts and absorb nourishment.

Root-infecting fungi show a great variation in their capacity to compete with soil saprophytes and colonize dead organic substrates. Garrett (1956) visualizes that more primitive parasites are generally good saprophytic competitors. They are generally vigorous and fast growing on nutrient media. They have a wide host range and are destructive to their hosts, killing tissues rapidly. That they are primitive parasites is brought out by the fact that they are generally restricted in their parasitic phase by host resistance. Parasitism of *Pythium* and *Rhizoctonia* species is often confined to seedlings as they are restricted by the resistance of mature tissues. As parasitic specialization proceeds more harmonious relationship is established and sheltered parasitic phase within the host is prolonged. This seems to be an escape from highly competitive saprophytic life and there seems to be a gradual loss of characters that are assets for such a life. Garrett (1956) introduced the term 'competitive saprophytic ability' to indicate the capacity of a root-infecting fungus for active saprophytic colonization. There is plenty of direct and indirect evidence to support this hypothesis. Specialized parasites like *Phymatotrichum omnivorum* (Mitchell *et al.*, 1941), *Ophiobolus graminis* (Garrett, 1938 ; Fellows, 1941), *Fomes annosus* (Rishbeth, 1950) and *Armillaria mellea* (Leach, 1939) were shown to be incapable

of colonizing saprophytic substrates in the presence of competing microbial population. In contrast, *Fusarium culmorum* (Sadasivan, 1939), *Macrophomina phaseoli* (Zachariah, 1953) and *Fusarium oxysporum* var. *vasinfectum* (Subramanian, 1947) were shown to be capable of active saprophytic colonization.

Butler (1953) and Lucas (1955) have made a comparative study of 'competitive saprophytic ability' of four cereal root-rot fungi whose saprophytic behaviour could be predicted by the observations already made by other workers. Maizemeal-sand cultures of the test fungi were prepared and the cultures were mixed with soil in various proportions to give various levels of inoculum in soil. Autoclaved bits of wheat straw were buried in the mixtures and incubated for suitable periods. Percentage of straw bits colonized by the inoculant fungus at each level of inoculum was determined. A comparison of percentage colonization was taken to indicate their competitive saprophytic ability.

The above method was the only direct one available for the study of active saprophytic colonization by root-infecting fungi. It is time consuming and the nature of the substrate used is not well defined. The author had developed a simple laboratory method as a modification of the above to reduce the disadvantages inherent in it (Rao, 1959). Maizemeal-sand cultures of test fungi were prepared and incubated for four weeks. Inoculum of each test fungus was mixed with soil in the following proportions by weight: 98:2, 90:10, 75:25, 50:50, 25:75 and 10:90 of inoculum and soil respectively. Each mixture was spread in a Petri dish and a thin layer of cooled, sterile water-agar was poured on it. Inocula were cut from the mixture with no. 1 cork borer so that approximately equal quantities of inoculum were obtained.

A nutrient agar was used as a substrate for colonization. Dox + yeast agar, acidified to pH 4.0 was prepared and plates were poured. These plates were inoculated with portions from an inoculum-soil mixture and incubated for ten days at 22.5°C. Of the different fungi developed on plates, colonies of the inoculant fungus were recognized by its colony and spore characters. The extent of colony development out of the total of inoculated colonies was determined and the result was expressed as percentage of colony development. A comparison of the competitive saprophytic ability of twelve root-infecting fungi was made by this method. Large differences were noticed in the ability of different test fungi for active saprophytic colonization (Table 1). "Saprophytic colonization rating" was obtained for all the test fungi by calculating the mean percentage colonization for the whole range of inoculum-soil mixtures (Table 2).

Of the twelve fungi tested in this way, there were vigorous saprophytic colonizers on the one hand and specialized parasites like *Ophiobolus graminis* which were completely inhibited by only 2 per cent of

TABLE 1

SAPROPHYTIC COLONIZATION BY 'AGAR PLATE METHOD' AGAINST MICROFLORA OF KETTERING LOAM SOIL

	Per Cent Colonization			
Per cent inoculum	*Ophiobolus graminis*	*Fusarium culmorum*	*Curvularia ramosa*	*Helminthosporium sativum*
98	0	100	93	34
90	0	98	84	19
75	0	95	78	11
50	0	93	69	5
25	0	91	55	3
10	0	89	33	0

soil in the pure culture inoculum. All the Fusaria tested proved to be vigorous saprophytes. This is in accordance with the findings of Sadasivan and his associates at Madras. Garrett (1956) considers that although wilt producing Fusaria appear to be specialized parasites they are primitive and are confined to the dead xylem cells due to host resistance in living tissues.

TABLE 2

"SAPROPHYTIC COLONIZATION RATINGS"

Fungus	Colonization rating
Fusarium culmorum	93
F. avenaceum	77
F. oxysporum v. *cubense*	77
F. oxysporum v. *vasinfectum*	76
F. caeruleum	72
Curvularia ramosa	69
Verticillium dahliae	27
Macrophomina phaseoli	23
Helminthosporium sativum	12
Rhizoctonia solani	6
Fomes annosus	0
Ophiobolus graminis	0

SUMMARY

In the course of evolution soil fungi have adapted themselves for colonizing particular substrates or nutrients by developing characters that are required for successful colonization of such substrates. 'Sugar fungi' face heavy competition and they are successful by virtue of high growth rates, heavy spore production and production of antibiotic sub-

stances. 'Lignin fungi' are slow growing but produce a wide variety of enzymes that can breakdown complex lignins. Coprophilous fungi inhabiting dung, predaceous fungi feeding on nematodes, mycorrhizal fungi living in association with higher plants and root-infecting fungi parasitizing higher plants have been able to avoid competition by restricting themselves to specialized habitats. Primitive parasites are more successful than specialized parasites in colonizing saprophytic substrates because with parasitic specialization characters required for active saprophytic colonization are lost. On assessing the 'competitive saprophytic ability' of different root-infecting fungi it was found that more advanced parasites are less successful as active saprophytic colonizers.

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ORIGIN, DEVELOPMENT AND MORPHOLOGY OF TENDRILS OF VITACEAE

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The ontogenetic study of a plant organ is essential for a fuller understanding of its morphology. The problem of histogenesis of a tendril is a badly neglected area of organ development in plants. The tendrils of Vitaceae appeared to be an interesting object as certain morphological problems are involved in the shoot, apart from the importance of the histogenic study of its tendril.

Darwin (1875) considered the tendrils of Vitaceae as modified flower peduncles and, therefore, axial in nature. He considered the part of the tendril lying between the first scale and the base as the common peduncle, its normal branches as flower-tendrils and floriparous ones as subpeduncles. Sachs (1882) considered the tendrils of *Vitis* as metamorphosed flower stalks or inflorescences which are "produced (according to Nägeli & Schwendener) beneath the *Punctum vegetationis* of the mother shoot, opposite to the youngest leaf and somewhat later than it". Engler & Prantl (1897) mention that the tendrils are to be regarded as axillary organs which are morphologically equal to inflorescences. Regarding the monopodial or sympodial nature of the shoot, they mention that "the results of ontogenetic studies especially seem to stand in sharp contrast to and are incompatible with those of comparative morphology. Whereas almost all ontogenetic studies seek to establish that the cultivated Grape-vine (attention was directed almost exclusively to this alone) represents a monopodium, comparative morphology finds that the Grape-vine represents a sympodium". Braün & Eichler (quoted from Engler & Prantl, 1897) considered the vine as a sympodium and the tendrils as the sympodial internodes, projected laterally. On the other hand, according to Nägeli & Schwendener (quoted from Engler & Prantl, 1897) the vine is a monopodium and the tendrils are extra-axillary, ebracteate branches by themselves or the tendril is supposed to be equivalent to the axis of the vine, and is separated from it only by a kind of dichotomy. The general opinion that the tendril in Vitaceae represents the modified terminal shoot apex, and that the shoot axis is sympodial, formed by the successive growth of the axillary branches in the opposite leaf axil is stated in almost all the modern text-books of botany (Rendle, 1938 ; Lawrence, 1951 ; Dutta, 1952). Willis (1951) makes a similar statement but adds that there has been much argument upon the subject. Goebel (1900-1905), correctly stating the problem, mentions that the

history of development shows that the tendrils are not representatives of the terminal shoot apices, pushed to the side by the stronger growth of their uppermost axillary shoot, but either from the first they have the leaf-opposed position of the mature condition or they arise from the apex itself through its unequal division. Bugnon (1949 a, b, c) and Chadefaud (1949) have suggested a monopodial theory of the vine shoot on the study of phyllotaxy of its inflorescences and tendrils. Recent contributions in this direction have been by Bugnon (1953) and Moens (1956).

The present investigation was undertaken to study the following points:

(1) Whether it is possible to correlate and interpret the developmental structure of the tendril in the Vitaceae (as revealed in its ontogenetic study) in relation to the accepted belief (Darwin, 1875 ; Sachs, 1882 ; Engler & Prantl, 1897) of its modification of a flower-peduncle or an inflorescence.

(2) Whether the ontogenetic study supports the widely accepted belief (Rendle, 1938 ; Dutta, 1952 ; Lawrence, 1957) that the shoot axis in the Vitaceae is sympodial and its tendril is a modification of an entire shoot apex.

(3) Whether the ontogenetic study supports the view (Engler & Prantl, 1897) that the tendril arises due to the dichotomy or due to the unequal division of the shoot apex (Goebel, 1900-1905).

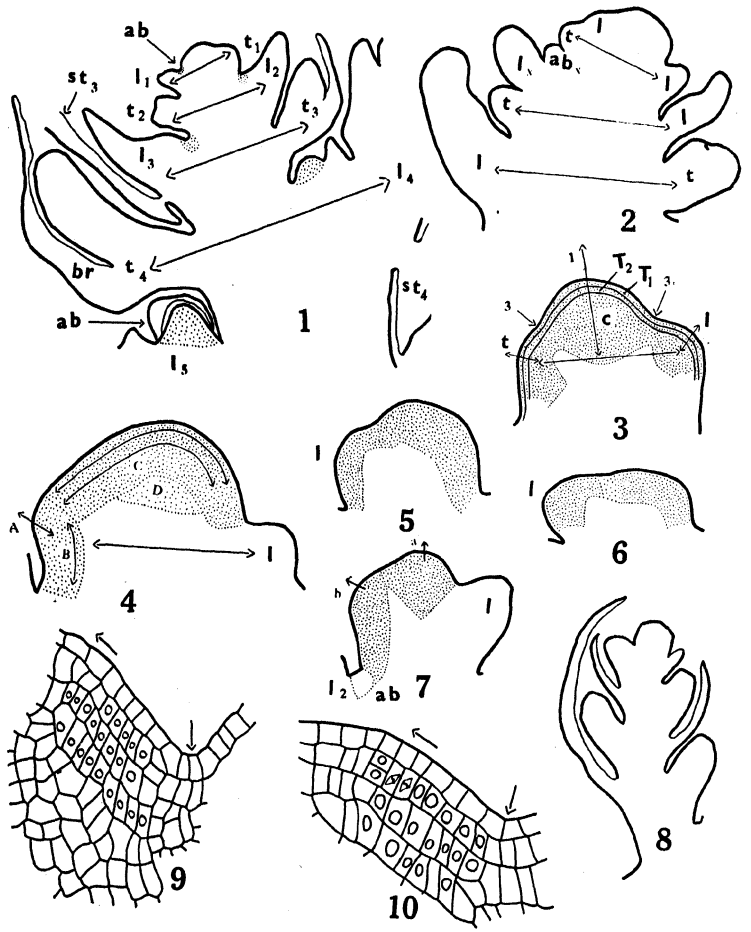
The foregoing aspects of the study have been investigated in the following species of Vitaceae: *Cissus rotundifolia* Vahl., *C. quadrangularis* Linn. (syn. *Vitis quadrangularis* W.C.), *Cissus* sp. (this species appears to be a variety of *C. quadrangularis ; see* Shah, 1958), *Cayratia carnosa* Gagnep. (syn. *Vitis trifolia* L.) and *C. auriculata* Gamble (syn. *Vitis auriculata* W.C.).

General Organography

Cissus sp.—It is a scandent tendril climber, branching vigorously during the monsoon. The unbranched tendril is alternate, distichous and opposite to every leaf (Fig. 1). It bears a small bract-like scale situated usually at one-third distance from the base. Sometimes the tendril becomes branched and bears flowers, resembling an inflorescence. Whenever a new branch either with or without flowers is present, it is associated with a new scale. The observations of Darwin (1875) that in *Cissus* there are no gradations between well developed tendrils and ordinary branches of flowers could not be corroborated in this study.

Cissus quadrangularis—The general arrangement, external form and characters of the tendril are similar to those of the species of *Cissus*.

Cissus rotundifolia—The tendril is simple, opposite to a leaf. Every third node does not bear any tendril, while its two successive ones,



Figs. 1-10.—(*ab*, axillary bud ; *abx*, axillary bud of a leaf having no tendril opposite it ; *br*, bract-like scale ; *C*, corpus ; *l*, leaf ; *lx*, leaf having no tendril opposite it ; *st*, stipule ; *t*, tendril ; T_1 & T_2, first and second tunica layers. Numerals indicate the number of the leaf or tendril primordium from the shoot apex). Fig. 1. *Cissus* sp., l.s. terminal bud. × 50. Figs. 2 and 4. (*A* shows direction of the growth of the tendril primordium ; *B*, obliquely oriented cell layers of the young tendril primordium ; *C*, dense staining area of the corpus showing the general cell alignments ; *D*, light staining area of the corpus). *Cayratia auriculata*, l.s. terminal bud and shoot apex. × 50. Fig. 3. (Arrow *1* indicates the direction of apical growth and arrow *3* points at concavity of the shoot apex. Arrows at *l* and *t* indicate the direction of vertical growth of leaf and tendril of the first node). *Cissus rotundifolia*, l.s. shoot apex. × 133. Figs. 5-7. (Arrows *a* and *b* show the direction of vertical growth of the respective apical regions.)

either above or below, bear tendrils opposite their leaves as in *Cayratia carnosa*.

Cayratia carnosa—The tendril is usually 4- to 5-branched (Fig. 8). Each branch has a small scale opposite to it. The branch tip is swollen into a tiny knob-like sensitive disc. For every two tendril-bearing nodes, a tendril-less one follows with great regularity (Fig. 2). A similar position of the tendrils is also reported in *Vitis vinifera* (Engler & Prantl, 1897). Some unusual forms between the tendril and the inflorescence were also observed.

Cayratia auriculata—The arrangement of the tendril is as in *C. carnosa* (Fig. 2). The tendril is usually soft, pubescent and branched. The scales are generally absent.

Shoot Apex

Cissus sp.—The shoot apex is dome-shaped, sometimes also appearing steep. Its outer surface becomes flat, depending upon the stage of the plastochron. There are two layers of tunica and an inner mass of corpus. T_1 invariably divides anticlinally and T_2 divides anticlinally and periclinally. The periclines in T_2 are not particularly confined to any plastochron changes. The corpus is a mass of meristematic cells without any evident cytohistological zonation, except during the phase of active initiation of appendages. At this stage, three zones are discernible: (*a*) a central zone consisting of the middle portion of the tunica and corpus, (*b*) a peripheral zone of flank meristem with slightly elongated cells appearing as stratified, and (*c*) an indistinct region of the rib meristem. The flanks of the apex are deeply stained probably because of their accelerated rate of meristematic activity. The activity of the rib meristem is not notable.

Cissus rotundifolia—The histological features of the shoot apex are similar to those of the *Cissus* sp. (Fig. 3). An interesting feature is the variation observed in the staining of the apical cells of the various apices. The cytohistological zonation is quite evident during the development of the tendril and leaf primordia. It can as well be stated that cytohistological zonation in *Cissus* does not appear to have any independent morphological status but is associated with the changing pattern of growth in the apex.

Cissus quadrangularis—The apical organization is similar to that of *Cissus* sp. (Figs. 5-7).

←

Cissus quadrangularis, l.s. shoot apex. × 133. Fig. 8. *Cayratia carnosa*, l.s. young developing branched tendril. × 50. Figs. 9, 10. (Horizontal arrow is towards the shoot apex and vertical arrow delimits the peripheral meristem.) *Cissus* sp., l.s. sector of shoot apex showing tendril initiation. × 380.

Cayratia carnosa—The apex has three tunica layers and an inner mass of corpus (Shah, 1960a). In some shoot apices, tunica and corpus cells were much vacuolated. Another noticeable feature is the absence of distinct light-staining central zone which is very commonly observed in most of the investigated angiosperm apices (Philipson, 1947 ; Gifford, 1950, 1954 ; Girolami, 1954). Another feature which contrasts with that of *Cissus* is the absence of any marked variation in the shoot apex form during plastochron periodicity as the primordia arise down the basal region of the apical dome.

Cayratia auriculata—The notable variation in this species is that the apex sometimes appears broader (Fig. 4) and its cells are comparatively smaller. There are three tunica layers.

Origin and Development of the Tendril

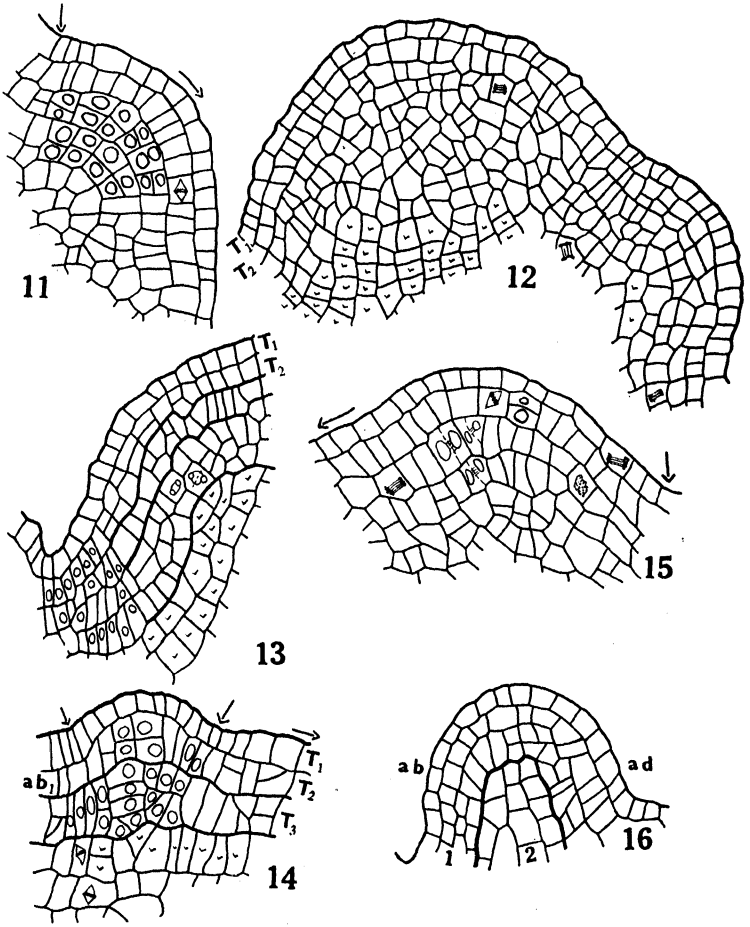
Cissus sp.: Initiation—The tendril and the leaf appear almost opposed on the adult shoot. But their initiation and development at the apex are not simultaneous. Usually the leaf develops first and its associated opposite tendril projects out considerably later (Fig. 1). Another notable feature is that the earlier primordia of leaf and tendril arise more or less at right angles to the longitudinal axis of the shoot.

The first visible sign in the initiation of the tendril is the stratified appearance of the flank meristem (Fig. 9), but the exact contribution of tunica and upper corpus in building up this stratification is not properly understood. But it appears that T_2 cells of the flank meristem play some part (Fig. 10). Sometimes no periclinal divisions occur in T_2 but are observed in the third subsurface layer (Fig. 11). Occasionally both the layers simultaneously divide periclinally. Periclinal divisions followed by anticlinal ones occur in the adjacent corpus cells which raise the tendril primordium. Further, immediate growth in height is due to periclinal divisions in T_2 and other layers and also due to some elongation of the centrally situated corpus cells (Figs. 15-17). T_1 divides only anticlinally differentiating into the protoderm. T_2 though discernible shows frequent periclines and hence is not discrete. In most of the tendrils of this stage, two zones are distinguished: *zone 1* shows frequent periclinal and anticlinal divisions, increasing the height and diameter of the tendril ; *zone 2* shows regular transverse divisions resembling rib meristem activity (Fig. 16).

The appearance of the stratified layers of the flank meristem, initiating the tendril primordium deserves some comment. These regularly arranged layers of cells appear column-like and resemble the "shell-zone", considered to be an early bud meristem in the initiation of the axillary bud (Esau, 1953).

The next stages in tendril development are its vascularization, initiation of the bract-like scale and further increase in length and diameter.

VASCULARIZATION—No provascular or procambium cells could be distinctly identified at the time of initiation of the tendril. The pro-



Figs. 11-16.—(*ab*, abaxial side ; ab_1, bud meristem ; *ad*, adaxial side ; T_1-T_3, tunica layers ; *v*, vacuolated cells). Figs. 11, 15, 16. (Heavy line delimits zones *1* and *2* in Fig. 16). *Cissus* sp., various stages in the initiation and development of the tendril. × 380. Fig. 12. *Cissus rotundifolia*, l.s. shoot apex with a young tendril primordium. × 380. Fig. 13. *Cissus rotundifolia*, l.s. sector of the shoot apex showing initiation of the tendril and bud meristem. × 380. Fig. 14. (Vertical arrows delimit the tendril primordium.) *Cayratia carnosa*, tendril initiation. × 300.

cambium is discernible when the tendril reaches 60-70 microns in height and 84 microns in diameter (Figs. 17, 18). Signs of vacuolation first appear in the lower surface cells of the abaxial side and centrally situated cells which will later constitute the future pith region. Between these regions an abaxial procambial strand is distinguished (Fig. 17). Procambium cells could be distinguished on the adaxial side of the tendril only at a later stage. The young tendril at this stage shows a ring of vascular meristem in its transection.

At the node the ring opens as a loop or an arc, the two ends of which join with the flanks of the opened axial ring. This ring of residual meristem first shows a single procambium strand (which is associated with the median trace of the scale). Then the two lateral procambium strands appear, followed by a number of them in the older tendrils. A single procambium trace thus becomes a composite one (Shah, 1960b).

INITIATION OF THE SCALE—The initiation of the bract-like scale occurs either in the first or second tendril, 130-133 microns high, invariably developing on the abaxial side (Figs. 19, 20). The scale-trace procambium appears precociously as in *Linum* and certain gymnosperms (Girolami, 1954). The initiation of the scale occurs by a periclinal division in one of the abaxial cells of T_2 which is followed by further divisions in the adjacent cells (Figs. 19-22). The vertical growth of the scale is due to apical and subapical initials while the lateral growth is due to marginal or submarginal initials.

FURTHER DEVELOPMENT OF THE TENDRIL—For some time, the rate of growth in the tendril is less than its opposed leaf primordium. The tendril after the seventh node from the apex elongates at a faster rate. The scale almost fully develops and matures at fifth or sixth node. Further growth in the length of the tendril mainly occurs in two ways. The subprotodermal layer and a small group of meristematic cells below the apex divide periclinally and otherwise. There is also a marked intercalary growth in the other part of the tendril. Tracheary elements are absent in the tendrils, 600 microns high, although they are present in its scale.

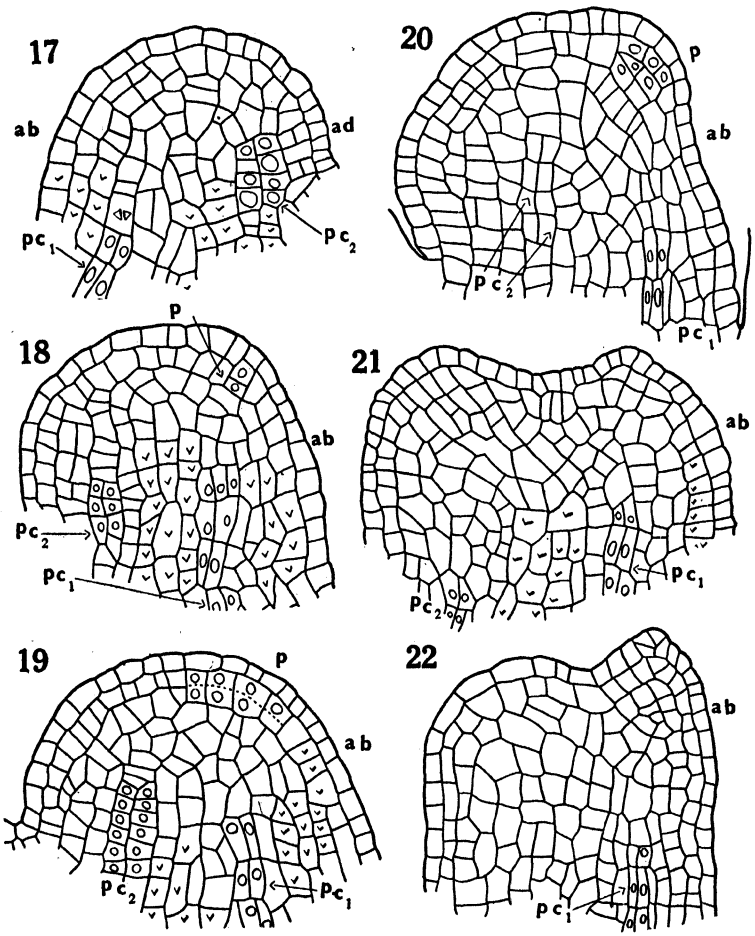
Cissus rotundifolia—The general pattern of origin and development of the tendril is similar to that of *Cissus* sp. (Figs. 12, 13). The earliest tendril primordium is delimited from the rest of the apex by a constriction or a narrow concavity (Fig. 3).

Transition forms between the tendril and the inflorescence are observed. When only inflorescences arise from the shoot apex in place of the tendril, the opposite leaves are very small or atrophied. When the tendril primordium has a transition form of the inflorescence, its apex is meristematic and deeply staining.

The vascular relation of the tendril with the axis is generally similar as in *Cissus* sp.

Cissus quadrangularis—The origin and development of the tendril in this species is as described in *Cissus* sp. (Figs. 5-7).

Cayratia carnosa—The origin and the general developmental features of the tendril are similar to that in *Cissus* (Figs. 14, 23, 24). The tendril generally arises from the basal part of the apex similar to the leaf. Either T_3 or T_2 and T_3 cells of basal sector of the flank meristem divide periclinally (Fig. 14). As far as could be observed, the corpus cells do not



Figs. 17-22.—(*ab*, abaxial side ; *ad*, adaxial side ; *p*, periclinal divisions ; pc_1 and pc_2, abaxial and adaxial procambium ; *v*, vacuolated cells). Various stages in the development of the tendril of *Cissus* sp. × 380.

participate in the tendril initiation. The periclines in T_2 are usually few, due to which the identity of T_2 layer remains usually distinct in the young primordia (Figs. 26, 27).

The tendril in *C. carnosa* is a branched one (Fig. 8). The successive branches and the scales alternate with one another. After the initiation of the first branch and the scale (Fig. 26), the successive branches and the scales arise in a regular order (Fig. 27). Each tendril usually produces four scales and branches and a scale-less terminal branch (Fig. 8). The general origin and development of the scale is similar to the one described for *Cissus* (Fig. 25). In the initiation of the branch, a few cells of the subsurface layers divide periclinally. Later, the inner cells divide making the branch primordium protrude out (Figs. 26, 27).

Cayratia auriculata—The flank meristem initiating the tendril is stratified as in *C. carnosa* and *Cissus*. The part of the flank meristem, initiating the tendril later becomes distinct owing to the characteristic orientation of cell layers. The upper cell layers of the apex are generally arranged parallel to the outer apical surface while those of the tendril are obliquely oriented (Fig. 4). The direction of the vertical growth of that sector also appears to be different.

The branch initiation occurs from the adaxial side of the primordium. The procambium of the abaxial side is present near the site where the histogenic activity for the initiation of the scale is evident. But its further development is aborted as the other tendrils of this terminal bud are without any scales.

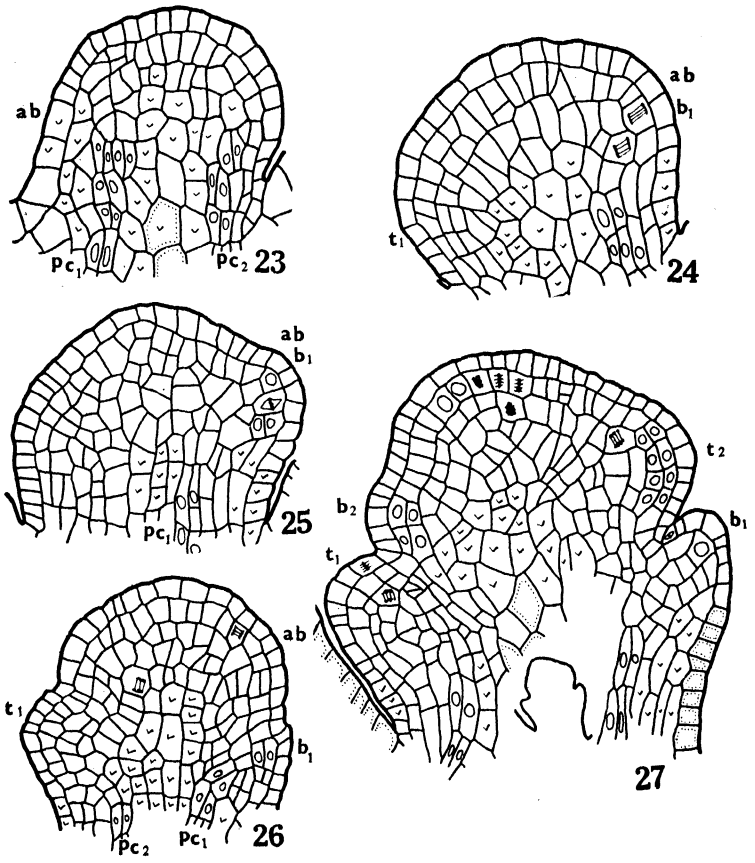
Discussion

The tendril in *Cayratia* is branched while it is usually unbranched in *Cissus*. In *Cayratia carnosa* the tendril branch is opposite to the scale except at the terminal end where the scale is absent. The scale is usually absent in *C. auriculata*. The unbranched tendril always has one scale.

It will be useful in the present study if certain facts of the tendril ontogeny are noted before discussing the morphology of the tendril. The tendril primordium invariably arises after the initiation of the foliar primordium on the opposite side. It is initiated from the stratified peripheral meristem which greatly simulates the typical "shell zone" area of the axillary bud. Moens (1956) also reported it in *Ampelopsis veitchie*. The mode of early development of the tendril is almost similar in all the investigated species. This fact is of great importance in deciding the morphology of the tendril. Usually T_2 layer or T_2 and T_3 where T_3 is present, with or without the outer layer of corpus, take part in the tendril initiation. What was before a broadened and slightly raised sector of the peripheral meristem, later becomes a dome-shaped tendril. No unequal division of the apex has been observed in tendril

development. The initiation of all the scales is similar to that of the foliar primordium.

A single trace bundle is observed for a young tendril, which becomes a composite one in the older nodes of the shoot axis. The composite trace consists of three vascular strands which later fuse in the lower region of the axial node. In *Vitis vinifera* the lateral branch trace and the tendril trace consist of three vascular bundles (De Bary, 1884). It



Figs. 23-27.—(*ab*, abaxial side ; b_1 and b_2, first and second bract-like scale primordia ; pc_1 and pc_2, abaxial and adaxial procambium ; t_1 and t_2, first and second branch primordia of the tendril ; *v*, vacuolated cells). Various developmental stages showing the initiation of the scales and branch-primordia of the tendril of *Cayratia carnosa*. Figs. 23, 26 and 27. × 300. Figs. 24, 25. × 380. Inset diagram of Fig. 27. × 50.

is of interest to note that the mode of "meeting" between the tendril trace and the axial stele closely resembles that of the axillary bud (Shah, 1960b).

The morphology of the tendril can now be discussed. It is suggested that if the floral (or inflorescence) apex is to be considered as an ontogenetic modification of the vegetative apex (Foster, 1939), the tendril should be considered as a modification of a lateral vegetative branch apex that may give rise to an inflorescence in the flowering season. The tendril in *Cayratia* is not usually modified into or replaced by inflorescence probably because of very characteristic behaviour of the "mixed bud" (Shah, 1960a). The facts of ontogeny mentioned in the foregoing section support this view. The meristem initiating the tendril can be compared with that of the axillary bud meristem. The only difference is that the former has extra-axillary position. If this view is accepted, the so-called tendril scales could be considered as modified or atrophied foliar appendages. In *Hieracium* the number of trace bundles is related to the size of the leaf, the smaller the bracts, the fewer the number of traces (Philipson, 1948). Hence the variation between the scale trace and the foliar trace in the shoot of *Cissus* and *Cayratia* should not be considered against the acceptance of this view. The tendril branches should be considered as extra-axillary branches of its main axis. This view also obviates the difficulty of correlating every part of the tendril with that of the inflorescence. The foliar nature of the scale is also supported by the reported presence of the axillary vegetative bud in the axil of one of the tendril scales in *C. carnosa* (Shah, 1960a). It has been noted that in a branched tendril of *C. carnosa,* the scale is opposite to the tendril branch except at the terminal end. In an unbranched tendril, only one scale is present. The absence of a branch opposite to this scale is because the apical primordium of the tendril is modified into a blind elongated axis after the initiation of this scale. Thus the tendril and the inflorescence of *Cissus* (and perhaps of other genera of Vitaceae) can be considered as interrelated morphological organs whose development from the extra-axillary bud meristem depends upon the vegetative and reproductive phases of the shoot.

As already mentioned in the introduction, there has been considerable difference of opinion regarding the nature of the shoot. According to Bugnon (1949a, 1953) there are three theories to explain the nature of the vine shoot.

On the basis of phyllotaxis, Chadefaud (1949) believed that there is a close relation between the tendril and the axillary bud of the leaf at the same node. The tendril represents a branch of the aborted prophyll of the bud, which rotates around the vine-shoot through 180° to have an opposite foliated position. The vine-shoot, therefore, he concluded, is a monopodium. As Bugnon (1953) remarked and the present ontogene-

tic study also shows, this theory often refers to facts which cannot be noticed.

Bugnon (1949 a, b, c) working on the phyllotactic pattern of *V. vinifera* and later after studying various species of Vitaceae concluded that the shoot is a monopodium (Bugnon, 1953). While discussing the various theories, he raised the following objections against considering the tendril as an extra-axillary bud: (*a*) The theory is only a statement of a simple ontogenetic observation, which is rarely observed in the dicotyledons. (*b*) The theory does not give any interpretation of the important parts of the tendril like the first bract-like scale B and the two branches or arms I and E (the internal and external arms) of the tendril.

The new theory proposed by Bugnon (1953) is as follows: (*a*) The shoot of the Vitaceae is a monopodium. (*b*) The axillary buds, inflorescences and tendrils are all lateral axes of the order N plus 1 if the supporting axis is of the order N. (*c*) At every inflorescence or tendril which is opposite-foliated three types of organs are distinguished: (*i*) The first bract B has the same value as a vegetative leaf borne by the axis. (*ii*) The two arms or branches, I and E are equivalent and have arisen as superposed branches from the axil of the bract B. (*iii*) The portion of the tendril between its base and bract B is a typical "Hypoclade" holding on its top the bract B and two axillary superposed branches I and E.

The present study on *Cissus* and *Cayratia* has shown that the shoot is monopodial. Bugnon (1953) discussing the structure of the shoot apex of *Parthenocissus quinquefolia* also concluded that the terminal apex is exactly comparable to an unquestionable monopodial axis. There are serious objections against his interpretation of the parts of the tendril. The present study has shown that in the unbranched and branched tendrils, the ontogeny of all the bract-like scales is similar and they should be considered as reduced leaves as already mentioned above. The contention of Bugnon (1953) that bract B has not the same value as the following bracts is not supported by the present study. In fact his categorical statement that B is absolutely constant is not proved as in *C. auriculata*. His other contention that from the ontogenetic point of view, B, I and E have a simultaneous and precocious formation is not supported by the present study. His observation that the two branches or arms I and E are constant is not correct. That only bract B is transformed into a vegetable leaf when such transformation occurs in the bracts of the tendril is also not supported by the reported presence of an axillary bud in the terminal scale of the tendril of *C. carnosa* (Shah, 1960a). The present ontogenetic study also does not support the view of Bugnon (1953) that the two arms I and E of the tendril arise as superposed branches from the axil of the bract B.

The interpretation of the shoot of Vitaceae based on the present study is as follows: The prevalent belief that the tendril of the Vitaceae represents

the modified terminal apex and the shoot axis is a sympodium formed by the successive growth of the axillary branches in the opposing leaf axils is totally rejected by the present ontogenetic study. Similarly, the dichopodium theory is also unacceptable as the apex does not divide to initiate the tendril. The vascular relation between the tendril and the shoot is also against this view.

Having considered the tendril as an extra-axillary bud, the shoot of the Vitaceae is obviously a monopode. Generally the vegetative apex is completely transformed into a reproductive one during the flowering season. This transformation is believed to depend upon one or more hormones whose nature is not completely known (Wardlaw, 1952). Boke (1947) reported three types of apical meristem in *Vinca*, one of which is a reproductive apex concerned with the production of both leaf and flower primordia. The apex (its meristematic complex) of *Cissus* and *Cayratia* (and also perhaps of other genera in Vitaceae) is an example where the vegetative and reproductive functions are simultaneously performed without a complete transformation from the former to the latter type. This situation can be visualised from two observations: (*a*) the existence of intermediate forms between the tendril and the inflorescence, and (*b*) the position of the inflorescence in place of the tendril. This presupposes presence or absence of one or more factors that will initiate a tendril, an inflorescence or an intermediate form between the two. The *modus operandi* for their action or effect is confined alternating to the right or left flank of the apical meristem and opposite to the foliar primordium. The histogenic activity started by them becomes distinctly pronounced after the initiation of the foliar primordium. In *Cayratia*, the morphogenetic factor (or factors) that initiates the tendril development is probably absent or inhibited at every third plastochron because of the peculiar behaviour of the mixed bud (Shah, 1960a). Morphologically the tendril or an inflorescence represents an extra-axillary and ebracteate lateral branch as in many Cruciferae, Compositae, Gramineae and other families.

Summary

The origin and development of the tendrils of some members of the Vitaceae, three species of *Cissus* and two of *Cayratia*, have been studied. The tendrils are either branched or unbranched. They are generally situated at the node opposite to the leaf. The apical meristem of the stem in *Cissus* consists of two tunica layers and an inner mass of corpus. The central zone is usually absent and the rib meristem is not well marked. The species of *Cayratia* differ in having three tunica layers. In *Cissus* the tendril initiation occurs by periclinal divisions in T_2 or/and the third subsurface layer. In *Cayratia* similar divisions occur in T_3 or T_2 and T_3 cells.

In the earliest stage a single abaxial procambium trace is observed. The vertical growth of the tendril primordium is due to the activity of a group of subapical meristematic cells and to the generalized intercalary growth.

The morphology of the tendril and the shoot is discussed. It is suggested that the tendril is a modified lateral branch whose development from the extra-axillary meristem depends upon the vegetative and the reproductive phases of the shoot. The sympodial as well as the dichopodium theories are not supported by the present ontogenetic study. An attempt is also made to interpret the various parts of the tendril.

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RECENT ADVANCES IN THE STUDY OF CHROMOSOME STRUCTURE

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Phenomenal advances have been made in recent years in the study of chromosome structure due to refined and critical techniques evolved. The important techniques evolved recently involve the use of oxyquinoline, aesculine, *p*-dichlorobenzene and α-bromo-naphthalene (Tjio & Levan, 1950 ; Sharma & Mookerjea, 1954). The usual drawback in following classical methods of fixation, was the difficulty in securing well scattered chromosome plates as also clarified constriction regions. In the recent pretreatment techniques, not only these difficulties have been overcome but at the same time the original time-consuming methods have been replaced by rapid schedules.

In all these schedules, the general procedure is to pretreat the tissue before fixation in a specific fluid at a particular temperature and for a particular period depending on the material. This process is followed by heating in a mixture of acid and dye (commonly orcein), succeeded by squashing in 1 per cent aceto-orcein solution. The entire process does not take more than three to four hours. The underlying principle of pretreatment is to change the viscosity balance of the cytoplasm, thus inhibiting spindle formation. Due to this change in viscosity, the cytoplasm becomes solidified and the chromosomes, being devoid of any binding stress, can be squashed by artificial pressure over a solidified background. The differential hydration and dehydration, caused in the chromosome segments, allow the constriction regions to be well clarified, thus making the morphology clear. Heating in the dye and acid mixture serves the triple purpose of precipitation of chromatin matter or fixation, dissolution of the middle lamella and staining. Fixation in any other fluid can safely be eliminated after the pretreatment.

A question may be raised regarding the utility or efficacy of the different pretreatment schedules. If the principle of action is the same in all, what is then the use of having so many fluids for chromosome studies? It may be noted that even following treatments in water and dilute isotonic solutions, viscosity change can be caused in the plasma, resulting ultimately in the scattering of chromosomes. In order to work out the effect of water (Sharma & Sen, 1954a), root tips were treated separately in distilled water, redistilled water and water distilled in a metallic retort. It has been recorded that except with redistilled water, effect has been noted with all the other forms. This indicates that any trace of a salt or metal

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can cause a disbalance in the viscosity of the plasma, thus resulting in the clarification of metaphase plates. It further demonstrates the subtle metabolic equilibrium that is being maintained within the cell. The reason underlying the fact that certain chemicals are effective while others are ineffective for the same species, is because the degree of viscosity disbalance required to bring about a disruption of the spindle may be different in different species of plants. Evidently different chemicals, due to their inherent chemical differences, can cause viscosity difference to varying extents. Certain species respond to specific chemicals only.

So far only aesculine and *p*-dichlorobenzene have been found to be widely applicable. The treatment with these has not only helped in clarifying chromosome morphology from the root tips but also from other organs, such as leaf, growing stem apex and endosperm. For endosperm and leaf, special fixation involving modification of Carnoy's fluid often becomes necessary after pretreatment. As a majority of these chemicals, like colchicine, can cause metaphase arrest as well, a large number of metaphase plates can be secured following a short period of treatment.

Just as in chromosome morphology, rapid advancement in the field of chromosome chemistry is also due to the refined techniques invented in recent years. The most important of these is the use of purified enzymes, viz. the nucleases. The application of the two nucleases—desoxyribonuclease and ribonuclease—is based on the principle that the digestion of DNA and RNA is brought about by them. Evidently with the use of specific staining for both types of nucleic acids before and after digestion, one can locate the specific sites of the occurrence of DNA and RNA in chromosomes.

Of the principal staining techniques meant for localizing these two types of nucleic acids, pyronin-methyl green schedule worked out by Brachet and later on modified by Kurnick (1952) and Leuchtenberger (1950) needs special mention. It is now known that one molecule of methyl green combines with ten phosphoric groups and DNA being highly polymerised stains specifically with methyl green. Evidently, the specific staining of RNA and DNA is due to the depolymerised and polymerised nature of these two types of nucleic acids.

Mirsky (1947, 1951) has evolved a technique of isolating chromosomes from tissues. The technique involved is very straightforward and simple. The first phase in this procedure is to break up the cells through a colloid mill and Waring mixer followed by straining through fine cloth and differential centrifugation for separation of chromosomes from other cellular constituents. Later on sodium chloride is added for separation of the outer mantle of chromosomes from the inner core.

For localising the phosphoric groups in chromosomes, the ingenious method of Gomori (1939) for the detection of phosphatase activity needs special mention.

In the field of chromosome morphology, the demonstration of the chromomeric nature of centromere (Lima-de-Faria, 1952) needs comment. It has been rightly claimed by some cytologists that the centromere of a metaphase chromosome is composed of four identical chromomeres, two in each chromonema joined by interchromomeric thread with each other and also by lateral threads with the arms of the chromosomes. The demonstration of this typical centromeric apparatus bids a complete stop to all the speculations so far held regarding this vital structure of the chromosomes. This clarification can be brought out by any of the pretreatment schedules mentioned previously.

Another important discovery is the existence of diffused centromere in *Luzula*, noted originally by Portuguese workers led by Camara, and later in other groups of lower plants as well (Camara, 1951 ; King, 1953). Such plants with diffuse centromeres provide ample scope for fragmentation helping in the process of speciation. The experimental demonstration of the fact that fragmented chromosomes can behave as independent ones has also been made possible with the aid of X-rays. This process provides a method for an increase of chromosome number without undergoing polyploidy. The primitive species, such as *Luzula purpurea*, have $2n=6$ large chromosomes, whereas the advanced ones have 24, 48 and 96 chromosomes in the body cells with a corresponding decrease in length of individual chromosomes, the total amount of chromatin remaining the same.

With regard to another segment of chromosomes concerned with nucleolar formation, interesting data have been collected. It has been shown that such secondary constriction segments differ from the rest of the heterochromatin with regard to their function, staining behaviour, susceptibility to different chemicals and response to pretreatment schedules. Following pretreatment methods, the secondary constriction segment becomes extremely exaggerated possibly indicating its despiralized state under conditions of treatment (Sharma & Sharma, 1958). Evidences show that these are definite chemically differentiated segments set apart in correlation to the ribonucleoprotein metabolism of the nucleus.

The extreme fibrillar nature of chromosomes brought out through electron microscopy by Kaufmann & De (1950) is especially commendable wherein it has been shown that the chromosomes may be composed of 128 or even 256 lamellar units. Evidently, the observations are going down to the molecular level and the original controversies regarding the bipartite or quadripartite nature of the chromosome have got little significance in the light of the recent researches. However, the fact which is most important to a cytologist is that whatever may be the degree of lamellation involved in the chromosome, it must behave as a binary unit during division. Though, no doubt, the lamellar nature is an observed

and undisputed fact, its implications in crossing over and fragmentation of chromosomes are yet to be explained. With regard to another aspect of study, i.e. the nature of chromosomes in the differential nuclei, much understanding has been obtained in the light of recent research. Through the works of Geitler (1953), Huskins (1948, 1949) and Huskins & Steinitz (1948a,b) it has been shown clearly that chromosomes in the adult nuclei divide endomitotically and as such they lie in a state comparable to the polytene condition in *Drosophila*. As chromosome reduplication is an essential necessity for gene action, the latter can conceivably be operating in the adult nuclei. The adult cells, because of their apparent nondividing condition, posed a very serious problem on this aspect in previous years. If differentiation is a gene controlled process, which evidently it is, it was problematic as to how adult nuclei in which differentiation is no doubt at its maximum do not show any chromosome division. The discovery of Huskins and others evidently helped to clarify this issue indicating further the genetic control of differentiation.

The nature of the endomitotic chromosomes in the adult nuclei was also brought out by Sharma & Datta (1956) and Sharma & Bhattacharya (1956). Further attempts were made to work out the causal factor of such partial chromosome division in the adult nuclei. As nucleic acid treatment (Sharma & Sen, 1954b) was found to induce division in the adult nuclei it was inferred that a deficiency in nucleic acid or any of its constituents is responsible for this partial division, specially as it is well established that nucleic acid is essential for gene protein reduplication. Sustained efforts in this direction have finally led to the solution of this problem (Sharma & Mookerjea, 1954), proving clearly that deficiency in the sugar moiety of the nucleic acid in the adult nuclei is responsible for decreased nucleic acid synthesis which ultimately is responsible for abnormal chromosome division. Once this deficiency is made up, the cells divide normally and enter into different later phases of mitosis.

The importance of nucleic acid in the maintenance of chromosome structure has further been shown by Sharma & Sen (1954b), Sharma & Mookerjea (1954), Sharma & Datta (1956) and Sharma & Bhattacharya (1956) in their work on the susceptibility of the chromosomes to breakage following chemical treatment. Taking into account the knowledge available on this subject it can be inferred that a chemical may cause derangement in any step of nucleic acid metabolism, ultimately leading to chromosome breaks by causing hindrance to gene reduplication at certain segments.

Though Meischer's analysis of nucleoprotein was done long back, proper researches on chromosome chemistry could be started only after the invention of Feulgen reaction. An idea that gradually developed due to the works of previous workers is that the chromosome is composed of a simple protein skeleton having a nucleic acid covering over it

(Gulick, 1941). In recent years, this conception has undergone a complete modification and the necessity of further researches in this direction is being felt and pursued at various centres (Mirsky, 1951 ; Kaufmann, 1952 ; Sharma & Roy, 1956 ; Ris, 1947 ; Sharma & Sharma, 1958). Through the chromosome isolation technique it has been shown that the fundamental chromosome skeleton is composed of a non-basic protein associated with DNA as well as RNA which is responsible for the individuality of the chromosome. A separable complex lies associated with the skeleton which is composed of DNA and a simple histone type of protein. The presence of RNA and non-basic protein in the chromosome is indeed a very important finding. The presence of all these four components in the chromosomes has been confirmed by Kaufmann (1952), Mazia (1941) and from our laboratory where enzyme digestion methods and acid extraction techniques have been resorted to. Our findings have, however, shown that generalization cannot be made from the study of *Drosophila* alone and plant chromosomes so far analysed differ in certain respects from animal chromosomes, specially of *Drosophila* (Sharma & Roy, 1956). The main thread of the plant chromosomes is composed of a non-basic protein which is rich in phosphatase activity. Discontinuous segments of basic protein lie over this ultimate fiber possibly associated with heterochromatin regions and the final envelope is the nucleic acid which forms the mantle of the chromosome. No trace of RNA in plant chromosomes has so far been obtained by us.

Recent work on the study of the structure and morphology of chromosomes in different groups of plants has clearly shown that the role of gene mutations was overestimated in the past. In several families of flowering plants, both dicotyledonous and monocotyledonous, allied species are often found to reveal differences in chromosome structure between themselves indicating the possibility of structural alterations playing a significant role in their evolution. Not much emphasis on structural alteration was laid in the past (Sharma & Sharma, 1959).

Studies have also revealed that karyotypes may differ within the same tissue of species which reproduce through vegetative means (Sharma & Sharma, 1957). In the absence of an effective method of sexual propagation, karyotypic variations which appear, are accumulated within the somatic tissue and are not eliminated in selection. As such, inconstancy of chromosome complements exists in such species and the regular normal number requires to be worked out from a study of the frequency and the one occurring in the highest frequency is to be taken as representing the normal karyotype. In the absence of sexual reproduction, indirect evidences have been put forward to show that such variations may enter into the growing tip of the daughter shoots and help in the production of new forms.

SUMMARY

Progress in the study of chromosomes within the last few years has mainly been due to refinements in techniques for their analysis. Most important of these are: (*a*) Pretreatment schedules for karyotype study, (*b*) Electron microscopy, (*c*) Enzymic digestion procedure and (*d*) Ultraviolet spectrophotometry.

In the field of the finer details of chromosome structure the extreme fibrillar or multilamellar nature as worked out through electron microscopy has completely replaced the older and simple chromonema concept. With regard to chemical nature, chromosomes are now known to be composed of RNA and DNA as well as basic and non-basic proteins. Plant chromosomes differ from those of animals in the fact that so far no RNA has been detected in plant chromosomes. Chromosome structure in the adult cells has been shown to be polytenic in nature and deficiency of nucleic acid and specially of its sugar moiety has been demonstrated to be responsible for this incomplete division. In the field of chromosome morphology, the demonstration of diffuse centromere and the clarification of normal centromeric structure is a distinct advancement. Karyotype study through refined methods has shown that species and strains differ with respect to minute details of chromosome morphology. It has been emphasized that the role of structural alteration of chromosomes in speciation was not properly assessed in the past.

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SPORE CONTENT OF THE ATMOSPHERE

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Study of the spore content of the atmosphere is of interest to workers in many fields. It is important in plant pathology because spores of many pathogens are dispersed by wind currents ; in medicine because of the plant spores acting as respiratory allergens and in industrial microboilogy because of deterioration of stored materials and spoilage of foodstuff by air-borne mould spores. As the interests of workers in these fields are quite varied, information on the air-borne spore and pollen flora (named 'Air Spora' by Gregory, 1952) has been gathered from different parts of the world. In this paper a brief review of some of these investigations, with special reference to those conducted in India, is given.

EARLY OBSERVATIONS

The existing knowledge on the composition of the air spora can be said to have started from 1830 when Ehrenberg first started publishing work on the nature of organisms present in the air. The results of his work extending over 17 years were presented in his treatise "Passat-Staub und Blut-Regen" published in 1849. The cholera epidemic of 1848 and the controversy over the question of spontaneous generation in the French Academy of Sciences which started in 1859, stimulated an early interest in the study of the air spora, which led to the publication of numerous accounts. Full accounts of these reports were given by Pasteur (1861), Robin (1871) and Tyndall (1887).

When Louis Pasteur demonstrated that spores of some microorganisms normally present in the air are capable of causing fermentation or putrefaction when introduced into sterile organic substances, it was speculated that other types of microbes in the air might be the cause of the so-called "zymotic" diseases, whose etiology was then unknown. Medical workers soon turned to a systematic microscopic examination of the air to see if there was any evidence of a connection between fluctuations in number and type of any organism occurring in the air and the outbreaks of such diseases as cholera, typhoid, malaria etc. The observations of Cunningham (1873) who undertook a systematic study of the microorganisms occurring in the air within two large jails (Alipore and Presidency) in Calcutta are of interest to those concerned with aerobiology in India. "The two jails were selected" by him "as localities for

observation, on the grounds that their inmates are subject to much more fixed and ascertainable conditions than any class of the outside population, and that their statistics of disease for particular periods are almost absolutely accurate" (Cunningham, 1873). He recorded his observations in great detail and presented beautiful drawings (colour plates of the fungus spores, pollen grains, bacteria and nematodes). According to Cunningham (1873) the "spores and other vegetable cells are constantly present in atmospheric dust and usually occur in considerable numbers ; the majority of them are living and capable of growth and development ; the amount of them present in the air appears to be independent of conditions of velocity and direction of wind ; and their numbers are not diminished by moisture. No connection can be traced between the numbers of bacteria, spores etc. present in the air and the occurrence of diarrhoea, dysentery, cholera, ague or dengue ; nor between the presence or abundance of any spcial form or forms of cells, and the prevalence of any of these diseases". Detailed work on these lines was also being pursued at the same time on the continent but observers there could not establish any connection between spores in the air and the "zymotic" diseases ; they suggested the existence of a rich and variable air spora. The role of some of these components in hay-fever was also recognised (Blackley, 1873).

Spore Trapping Techniques and Results

Using a continuous water aspirator, Pasteur (1860) sucked air through a nitrocellulose filter. Pouchet (1860) was the first to construct an aeroscope. In his aeroscope, by means of a water aspirator, a measured volume of air was drawn through a funnel-shaped tube on to a glass slide coated with an adhesive so that particles in that volume of air impinged on the slide. Maddox (1870) and Cunningham (1873) modified Pouchet's aeroscope by pivoting it in a horizontal position and adding a tail to keep it turned towards the wind acting as a wind wane. In the first quarter of the present century pure culture techniques were introduced. During the second quarter of this century, when a number of physicians reported successful results with preseasonal pollen-desensitization therapy, interest on the constituents of the air spora was again aroused. Several local surveys were started employing horizontal and vertical slides to know the changes in the daily concentrations of the various suspected allergens. During this period extensive studies on the dissemination of plant pathogens had also been started by plant pathologists in different parts of the world. The use of aeroplanes (Stakman *et al.*, 1923) and kites (Mehta, 1933) in the study of rust epiphytotics opened up a new possibility of studying the air spora of the upper strata

of the atmosphere. The Inter-divisional Committee on Aerobiology of the National Research Council of the United States of America, sorted out the extensive data gathered by these workers. Their publications, one on the techniques (Committee on apparatus in Aerobiology, 1941) and the other on the results (Moulton, 1942) give an excellent summary of this work.

Mehta (1933, 1952) studied the dissemination of cereal rusts in the plains of India using aeroscopes with vertically placed glass slides. Padmanabhan *et al.* (1953), by exposing greased slides in aeroscopes in paddy fields at Cuttack during the months of April to July in 1950, 1951 and 1952, estimated the number of air-borne conidia of *Helminthosporium oryzae*. Recently, Lakhanpal & Nair (1958) reported the results of a survey of atmospheric pollen at Lucknow conducted for a period of one year from October 1954, employing an aeroscope with the slide exposed in a slanting position. They found that the pollen content of the atmosphere was maximum during February to April, minimum during July and August and moderate during October to January. A total of 23,274 pollen grains was counted under 29 named and one 'Unidentified' categories, of which *Holoptelea* constituted 42.55 per cent. By exposing Petri dishes with potato dextrose agar, a census of fungal spore population in the atmosphere at Kanpur was conducted by Rajan *et al.* (1952) from July 1945 to June 1946, with a view to find out the frequency of the occurrence of cellulose-destroying fungi, at two heights (4 feet and 8 feet above the ground level) in the atmosphere. The Petri dishes were exposed for 5 minutes against the wind at 10.00 a.m. every Wednesday at these two heights. The plates were incubated for 7 days at 32°C and the number of colonies appearing were then counted. Thirty-eight species of fungi were recorded of which Aspergilli were the most common. These species were found commonly on various types of cotton textiles like water-proof canvas and cordage deteriorating under humid conditions of storage at Kanpur. The other prevailing types were *Alternaria, Curvularia, Fusarium, Pullularia, Helminthosporium* etc. Species of *Cladosporium, Rhizoctonia* and *Syncephalastrum* were few and sporadic. The statistical analysis of the data indicated that the variation between months and heights was found to be highly significant. The number of organisms found at 4 feet was less than those at 8 feet. They also observed a periodic rise and fall in the number of organisms simultaneously at both the elevations, the number being minimum between July to September and again in December and January. The first decrease was attributed to the settling of the spores due to rain and the second to the nocturnal condensation in the winter months. Saluja (1941) isolated five species of *Aspergillus* from the air in Lahore and indicated their seasonal variation. Recently, Konger & Baruah (1958) have reported that spores of *Mucor, Aspergillus, Penicillium*, and *Fusarium* were abun-

dant in and around potato plantations in upper Shillong while those of *Phytophthora* and *Thielaviopsis* occurred in much lower concentrations.

Employing the gravity slide and the Petri dish exposure methods, the predominant air-borne spore types in different parts of Europe and America have been determined by different workers. This work indicates that *Cladosporium* is the dominant constituent of the air in Great Britain and *Alternaria* is more important than other types in the U.S.A. Similar observations have been made by different workers elsewhere.

Although these methods have been extensively used, several attempts have been made to improve the methods of air sampling and develop simple devices for special purposes (Erdtman, 1937 ; Hawes *et al.*, 1942 ; Luckiesh *et al.*, 1946 ; Bourdillon *et al.*, 1948 ; Pady, 1954 ; Decker & Wilson, 1954 ; Richards, 1955 ; Ogawa & English, 1955 ; Orr *et al.*, 1956 ; Gregory, 1957 ; Stephen, 1957 ; Rishbeth, 1958).

Hirst Spore Trap

Gregory (1951) and Gregory & Stedman (1953) have determined the efficiency of some of these traps employed in routine aerobiological surveys in a wind-tunnel under controlled conditions of turbulence, and found that many of these traps are highly selective. From this work Gregory (1952) came to the conclusion that for efficient sampling of air, an apparatus is required which draws in a known volume of air without altering its spore content, removes all particles beyond a range of 2-100μ, and leaves them in a form in which they can be examined, counted and classified. He found that the 'Cascade Impactor' (May, 1945) satisfies all these requirements and is most suitable for spot sampling. For continuous sampling it was found to be unsuitable as the deposit becomes too dense and requires changing of slides too frequently. A new type of suction trap has been designed by Hirst (1952) in which this difficulty is overcome by the movement of the sticky surface at a constant rate past the orifice through which the air stream enters. The trace on the slide can be scanned under the microscope and the time of deposition of a spore determined with an error of about $\pm$ 1 hour. This trap is relatively non-selective for particles in the size range of plant spores, and has been shown to collect not less than 60 per cent of the total spores present in the air under normal field conditions. Thus, the introduction of this 'Automatic Volumetric Spore Trap' makes it possible to estimate accurately the concentrations of different kinds of spores and follow the diurnal and seasonal changes. It is also possible to correlate these variations with the changes in the weather conditions. The results obtained with this trap have shown the deficiencies of the earlier methods and brought to light many interesting features of the air spora. Some of these are: (1) The occurrence of basidiospores forming a significant

element of the air spora, especially during the early hours of summer mornings in England, sometimes reaching a maximum of about a million/m^3. of air (Gregory & Hirst, 1952 ; Hirst, 1953 ; Gregory & Sreeramulu, 1958). (2) Short period changes which are usually associated with changes in the weather (Hirst, 1953 ; Gregory & Sreeramulu, 1958 ; Gregory & Stedman, 1958) or other conditions (Sreeramulu, 1958*a*; Hirst, 1959). (3) The diurnal and seasonal periodicities (Hirst, 1953 ; Cammack, 1955 ; Gregory & Hirst, 1957 ; Gregory & Sreeramulu, 1958 ; Sreeramulu, 1959). (4) Recognition of the numerical preponderance of the different spore types and their relative contributions to the total air spora (Gregory & Hirst, 1957). (5) Recognition of some additional potential air-borne allergens (Gregory & Hirst, 1952 ; Gregory *et al.*, 1955). (6) Assessment of the total mass of spores inhaled by an adult (as the suction rate of the trap at 10 l/min. approximates to the volume of air inhaled by a normal adult) and information on the brief maximum concentrations to which allergic patients are exposed. The work of Hirst *et al.* (1955) on the apple scab epidemic, of Cammack (1958) on maize rust disease gradients, of Waggoner & Taylor (1958) and of Hill (1960) on downy mildew of tobacco and of Sreeramulu (Unpublished) on the dissemination of loose smut of barley, further indicates how this trap can be used in understanding the factors that contribute to the building up of an epiphytotic condition in any air-borne disease.

In India, the Hirst trap was used for the first time by Sreeramulu & Seshavataram (1959, 1960) to sample air two metres above the ground level in a paddy field from 21 September to 31 December, 1957. The diurnal and seasonal changes of 20 named and one miscellaneous groups were described. In normal dry weather, *Trichoconis padwickii*, *Trichoconis* type, *Nigrospora*, *Cladosporium*, *Cercospora* and pollen grains were found to have their peaks at or before noon, while those of *Neovossia*, *Helminthosporium oryzae*, *Helminthosporium* type and *Curvularia* in the afternoon before sunset. *Fusarium* type (Septate fusiforms), basidiospores, *Podaxis* and others were having their daily peak concentrations only during the night before dawn. Spore types like basidiospores, *Curvularia*, *Cercospora*, *Helminthosporium* type were present throughout the trapping period more or less in uniform concentrations. *Trichoconis padwickii*, *Trichoconis* type, *Nigrospora* and *Podaxis* occurred early in the season, while spores of *Helminthosporium oryzae*, *Cladosporium* and *Neovossia* were found in maximum concentrations only late in the season. Presence of certain spore types like the *Fusarium* type, hyaline spores and *Pleospora* type depended mainly on the weather conditions, especially rainfall. The relative importance of these 21 spore types was recorded and it was found that basidiospores and *Cladosporium* were the two major components contributing 24.95 and 29.60 per cent respectively to the total air spora. Although the trap was sited in the middle

of a paddy field surrounded by many fields with the paddy crop, spores of fungi pathogenic to the paddy crop occurred in low numbers, forming only about 10 per cent of the total air spora, among which *Trichoconis padwickii* and *Nigrospora* occurred in comparatively higher numbers.

To recognise the important components of the air spora inside cattle sheds in India, Sreeramulu (1960) sampled air inside a cattle shed in the Government Dairy Farm near Visakhapatnam with a Hirst trap kept with its orifice at a height of 1.5 metres above ground from January 5 to 30, 1959. Spores of *Cladosporium* and *Fusarium* (growing as saprophytes on straw) accounted for 48.3 and 27.0 per cent respectively of the total catch ; basidiospores and spores of Aspergilli contributing 8.3 and 3.2 per cent and fragments of hypae and 'Others' accounted for 3.4 and 9.8 per cent respectively.

Although the Hirst trap is extremely suitable for the study of the variations in the air spora, it has some limitations. It is not portable and it requires a source of power to drive the vacuum pump for sucking air into the trap. Therefore, Gregory (1954) designed a portable hand-operated, volumetric spore trap for use in places where power supply is not available. The results obtained with this trap (Gregory, 1954 ; Last, 1955) indicate that it is useful for sampling marked concentration gradients, especially in the lower 2-3 metres of the atmosphere and in circumstances where a sharp time differentiation is required. Brook (1959) modified this type of spore trap for sampling pastures. A 'simple 24-hour slide spore collector' which is much less expensive has been devised by Panzer *et al.* (1957). Recently, Pady (1959) described a 'continuous spore sampler' which resembles the Hirst trap in many respects.

With this method of air sampling, it is not possible to distinguish living spores from dead ones and also it is difficult to identify certain spore types as the identification has to be based entirely on the visual characters of the spores. The improvement of slit samplers (Pady, 1954) is helpful in estimating the number of viable spores. Unfortunately, at present no method exists which is efficient both for visual and cultural recognition. An ideal trap has yet to be designed and modification of the present methods is required for application to new problems. The results obtained so far with the Hirst trap indicate that this method of air sampling has many advantages and is most suitable for conducting general surveys of atmospheric spore content.

Air Spora in Relation to Locality

The information on the components of the air spora of the out-door air in inland regions collected so far from different parts of the world indicates how local conditions influence the different components to a

certain extent. The work of Gregory (1954) and Gregory & Sreeramulu (1958) provides further evidence on how the conditions in different environments alter the quantitative and qualitative nature of the air spora.

That the spore content of the air inside buildings is different from that of the out-door air has also been recognised. Recent studies of Gregory *et al.* (1953), Maunsell (1954), Richards (1954) and Di Menna (1955) indicate some of the features of the air spora inside buildings. Studies on the air spora of the farm buildings has also being taken up (Baruah ; cited from Hyde, 1957 ; Sreeramulu, 1960) in recent years, with a view to understanding the respiratory mycoses and allergic diseases of farmers and their cattle. Studies on the air spora of pastures with special reference to those that can cause facial eczema diseases in sheep are also being taken up (Brook, 1959).

The study of the spore content of the sea air was initiated with the pioneering work of Meier (1935) which was continued by Polunin (1951), Pady & Kapica (1953, 1955) and Pady & Kelly (1953, 1954) providing information on the spores over oceans at higher altitudes. Information on the air spora of the lower layers of the sea air has also been collected (Bisby, 1935 ; Erdtman, 1937 ; Rittenberg, 1939 ; Sreeramulu, 1958b).

Thus the recent studies, using some of the improved methods of air sampling, are bringing to light many interesting features of the ever changing air spora with characteristic populations occurring in the air inside buildings, in the out-door air in cities, agricultural fields, pastures and in marine regions. As already pointed out, the dependence of the concentrations of the different components of the air spora in a place and the diurnal and seasonal factors on the one hand and changes in the weather conditions on the other, prevent us from drawing any general conclusions on the normal air spora of a particular area. Long term surveys (like those that have been started at places like the Rothamsted Experimental Station in England by Professor P. H. Gregory and his associates and the Kansas State University in the U.S.A. by Professor S. M. Pady and his associates) are needed for understanding the normal features of the air spora of an area. Understanding of the phenomena of the occurrence of spore showers, vertical and horizontal gradients, is much more difficult with our present state of knowledge and they will remain as fascinating problems in aerobiology for some time to come.

Studies on Air Spora and their Significance in Plant Pathology

Ideally, assessment of the disease spread should be made after the deposition of air-borne spores on the host plants. However, because of practical difficulties, data obtained by trapping spores during their trans-

port are often used in studies concerned with the dissemination of plant diseases by air currents. The recent advances in air sampling techniques now enable plant pathologists to obtain data which can be used efficiently in the prediction and control of different air-borne diseases. Understanding the relationship between the discharge and dispersal of spores and the changes in weather conditions, and the use of this knowledge in perfecting the existing spraying programmes for their control are some of the aspects which are receiving the attention of many workers in this field.

Conclusions

Our knowledge of the composition of the air spora and the factors that influence the relative concentrations of the different components is in an early phase and the few aerobiological studies conducted in India indicate the existence of a rich air spora in different parts of the country. As Stakman & Harrar (1957) have remarked "it is easy to show that winds carry a heavy and diverse cargo of spores at certain times and places, but what they are, whence they come, whither they go, and what they do when they get there is still unknown in many important cases. There is need for ingenuity and inventiveness in learning what needs to be known" about the spore content of the atmosphere in many areas.

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ASPECTS AND PROSPECTS OF THE STUDY OF WOOD ANATOMY

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The study of wood anatomy, a somewhat specialised subject, has advanced in the recent times in various aspects due to the fundamental research of numerous investigators. It may be suggested that modern wood anatomy had its origin from the time of the first World War. However, there are evidences (Solereder, 1908) to show that some work along modern lines had been started earlier also. For instance, in Germany, long before the end of the last century, some of the more important timber yielding trees were studied intensively with respect to their anatomical structure. Sanio (1872) has made some generalizations regarding the variation in tracheid length in the conifers.

It appears that by far the greatest number of investigators in this field held official positions in the Government institutions throughout the world and it is not surprising, therefore, that investigations have been chiefly carried upon woods of economic or potentially economic importance. An outcome of this economic pressure has been the investigation of the timber resources of certain areas, especially those of the tropical regions.

A knowledge of anatomical details is of importance, however, when the question of substitutes for woods of known quality is considered. With the dwindling of the forest areas which appeared to contain an inexhaustible supply of the wood, there has arisen a need for more and more timber, which led to the appearance of more and yet more unknown timbers in the market. Woods usually of inferior quality and less costly were supplied in the place of the timber required. Genuine attempts have been made in the various Government institutions and laboratories, to find out the individual characteristics of all the new timbers that have come to use. From the study of wood anatomy, coupled with the researches on the physical and mechanical properties of a wood and a study of its defects and seasoning qualities, intelligent forecasts were made to some extent regarding the uses of the new woods. Investigations on wood anatomy in relation to mechanical strength can profitably be conducted upon common woods as is demonstrated by Clarke's (1933) work on Ash, *Fraxinus excelsior*. He has shown that for a given portion of summer wood in the ring, an increase in specific gravity is accompanied by an increase in mechanical strength, owing to the increased thickness of the fibre walls.

Another aspect of the subject which may be regarded as an outcome of economic conditions is the appearance of several keys designed to facili-

tate the identification of certain groups of woods. Of these mention may be made of the work on British hardwoods by Chalk & Rendle (1929) and that of Chowdhury (1932) on Indian Sleeper woods. Besides these, various publications have appeared in different countries summarising, as far as possible, the anatomical features of woods according to their families. Dadswell & Burnell (1932) described in detail 37 species of coloured woods of the genus *Eucalyptus* and provided a key for the separation of the woods into different species. Chalk & Davy (1932, 1933, 1935) and Chalk *et al.* (1939) made a systematic study of some forest trees and timbers of the British Empire (African timbers). Pearson & Brown (1932) have dealt with the Indian timbers and described about 300 timbers of commercial value. This work proved to be useful to the applied side of the subject ; a feature of great value in this book is the summary of the anatomical characters of the secondary xylem for each family. Metcalfe & Chalk's (1950) "Anatomy of the Dicotyledons", is a comprehensive compilation of the anatomical characters of the dicotyledons. Solereder's (1908) vast compilation has been rendered much less valuable since many of the descriptions borrowed from different investigators were limited to small stems of herbarium specimens. Recently, from the Forest Research Institute, Dehra Dun (India) one of a series of volumes on the anatomy of the Indian Woods has appeared. This may be of considerable value in providing information as to the possible range of structure among the allied species.

As has already been stated, work on wood anatomy was mainly confined to Government institutions throughout the world, such as: (1) Forest Products Research Laboratory, Princes, Risborough, (2) Jodrell Laboratory, Kew Gardens, Kew, (3) Commonwealth Forestry Institute, Oxford, (4) Division of Forest Products, C.S.I.R.O., Australia, (5) Forest Research Institute, Dehra Dun, etc. Fortunately there are now workers in several universities also in different countries. In India, apart from Madras and Andhra Universities, in no other university anatomical work on wood appears to have been taken up. Individuals in such institutions are, to a great extent, free to pursue any line of research which interests them, regardless of its possible economic significance. Recently, a number of papers dealing with different aspects of wood anatomy which are of greater importance from the point of view of pure botany have appeared. Various lines of phylogenetic specialisation have been established by a number of wood anatomists during the past three or four decades. In the words of Barghoorn (1954) "The interpretative, analytical and essentially phylogenetic view point strongly expressed by Bailey's anatomical and cytological contributions has resulted in a unique synthesis of knowledge regarding the evolution of vascular tissues in land plants. These studies firmly established the significance of the structure of the secondary xylem as an essential tool in solving problems of phylo-

geny in the angiosperms, and demonstrated that one of the most outstanding evolutionary documents to be found in the plant kingdom lies in the progressive modification of the secondary body." Eames (1911) and Sinnott & Bailey (1914) have established the advanced nature of the herbaceous habit over that of the arborescent habit in the angiosperms.

Jeffrey (1917), Bailey & Tupper (1918) and Frost (1930a, b) have pointed out that vessel elements with scalariform perforations are less specialised than those with simple perforations. Frost (1930a, b, 1931) has also traced the major lines of specialisation in the secondary xylem—the origin of vessel, evolution of the end wall and specialisation of the lateral wall of the vessel element.

Kribs (1937) affirmed that diffuse arrangement of wood parenchyma is more primitive than are the various aggregate arrangements, such as banded apotracheal and the various paratracheal types as vasicentric, aliform and confluent types.

Kribs (1935) and Barghoorn (1940, 1941a, b) have elaborated the primitive and advanced nature of the heterogeneous and homogeneous rays.

A type of investigation which illustrates the value of a knowledge of structure to the botanist, rather than to the wood technologist, is that in which the anatomy of the woods of a supposedly natural group is studied in relation to taxonomy. The idea of employing the internal structure of plants as an aid to their systematic classification originated very early. For example, stem structure is one of the criteria for distinguishing the major groups of angiosperms—the dicotyledons and monocotyledons. Solereder (1908) believed that the anatomical characters are often preferable for the determination and delimitation of a systematic group since they are more precise than external morphological characters. Wood anatomists are mainly concerned with the delimitation and position of the genera. Sometimes various species recognised on the characters of flower and fruit, appear from the study of the wood to be the varieties of a single species. It is, however, to be recognised that classifications based on anatomical characters alone would lead to weird results as viewed from the standpoint of our present concepts of classification. For instance, vessels and other wood structures appeared independently in unrelated groups and hence their presence in different groups does not imply any affinity. In other words, parallel and convergent evolution has occurred. Bailey & Howard (1941) have pointed out that the rates of phylogenetic change differ in various parts and organs of plants, and thus the development of one structure may be accelerated or retarded in comparison with the evolution of other parts of a plant. Furthermore, there is no constant relationship among these rates, for in some groups the flower forges ahead, while the wood lags ; whereas in other groups, the anatomical characters may develop at a more rapid rate. Consequently, in attempting a natural system of classification, consideration should be given to harmonize and

integrate evidence from all parts of the plant and from all branches of botany including anatomy, genetics, cytology, physiology, embryology, paleobotany etc. Wood anatomy is an essential auxiliary to taxonomy but is an auxiliary that botanists cannot afford to neglect. It is, for example, of special value in throwing light on the systematic position of anomalous genera and provides a valuable test of the homogeneity of a family. On the basis of wood anatomy there is much to recommend that the Magnoliales are a very primitive Order. On the other hand many members of the Amentiferae with simple perforation plates, ring-porous nature, often short, round and large vessel elements with opposite or alternate pittings, and with the presence of fibre tracheids, libriform fibres and homogeneous rays, are rather highly specialized from the viewpoint of wood anatomy (Tippo, 1938).

Wood anatomy forms a link between botany and forestry. The demand, in the modern times, for practical methods of identifying and investigating the physical properties of commercial timbers has necessitated the study of the wood of different species. The cellular structure of wood is one of the most important factors in determining its physical properties and a close correlation exists between the changes in structure and changes in technical properties of the wood. This type of work is being carried out in many of the institutions concerned with the forest products' research.

For a long time certain difficulties barred rapid progress of the study of wood anatomy ; for example, the difficulty of obtaining authentic material. Forest officers and Government departments helped to a great extent in the supply of proper specimens. However, it is a satisfactory method to use wood obtained from trees from which herbarium material has been taken. The method of collecting herbarium material with wood specimens is pursued as far as possible in the larger institutions. The International Association of Wood Anatomists (I.A.W.A.), which was formed to enable the advancement of knowledge of wood anatomy in all its aspects, has been helping in the exchange of authentic material for research. The publication of International Glossary of terms used in wood anatomy (1957), under the chairmanship of Dr. L. Chalk, was the result of joint effort by the committee of nomenclature, I.A.W.A.

Methods of describing the structure of woods appear to be inadequate as too little notice is taken of the variations which occur in the wood of a single species. There is considerable range of variation in structure of the secondary xylem in the bole of an individual tree. In view of the different types of variations met with in the secondary xylem, detailed accounts of the structure of wood should emphasize the average for the species under consideration and should be based on sufficiently large number of samples taken from different parts of the range of the species

and from varied habitats and also from the different parts of the same tree.

There is a supreme necessity in India for the formation of a body similar to the Timber Development Association of Britain forming a liaison between the producers and the users of wood, its primary task being the interpretation and dissemination of scientific knowledge of wood by its advice, publications, lectures and educational schemes.

In conclusion it may be stated that fundamental research treating wood as a plant product, and not merely as a potential commodity, may be encouraged without regard for its economic possibilities.

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VARIATION IN THE STRUCTURE OF WOOD IN SOME DICOTYLEDONOUS TREES

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Much of the work done during the last 50 years or so, on the problem of variation in wood structure, relates particularly to tracheid and fibre dimensions throughout the bole of a tree, but the results appear to be somewhat contradictory. Sanio (1872) put forward a few generalizations on tracheid length in conifers from his studies on *Pinus sylvestris* L. Of these the important ones are: (1) there is a regular increase in tracheid length from the centre of the tree outwards until a certain maximum is attained after which the length remains constant, and (2) that in any one ring there is a gradual increase in tracheid length up to a certain height in the tree, after which there is a decrease towards the top.

Numerous investigators have studied the problem of variation in the length of vertical elements of the secondary xylem from the following three different aspects: (1) from pith outward in successive growth rings at one level in the bole, (2) from base upwards in specific rings of the bole, and (3) in the early and late wood of an individual growth ring at one level. It appears to have been tacitly assumed that variation in fibre length in dicotyledons follows the same trend as that of the conifer tracheids.

Most of the work done was on the variation in tracheid length in some gymnosperms. In view of the comparative lack of definite information regarding vessel element and fibre length in angiosperms a detailed investigation of such variation was undertaken in three different types of woods: (1) a diffuse porous wood—*Acer pseudoplatanus* L., (2) a ring porous wood where the vascular cambium is not stratified—*Quercus robur* L., and a ring porous wood where the vascular cambium is stratified—*Robinia pseudoacacia* L. (Sundarasiva Rao, 1959).

Four transverse discs about three inches thick were cut from each tree at different levels. With the help of an eye piece micrometer, measurements of the vessel elements and libriform fibres were made at random from the maceration of the wood which was accomplished by Jeffrey's method. Fifty vessel elements and 50 libriform fibres were measured from each of the samples and the choice of these numbers was based on the recommendations of Rendle & Clarke (1934). Mean lengths of these elements are expressed in millimetres.

Experimental Results and Discussion

Variation from pith outwards—The vessel elements and libriform fibres increase in length in successive growth rings from the pith outwards at four different levels from which observations were made, with the exception of the vessel elements of *Robinia*. There was a rapid initial increase in length of the libriform fibres at any one level in all the three trees through approximately the first 20 growth rings. The vessel elements showed a similar increase except in *Robinia* where, at one level, little change in length occurred. In *Robinia* a sharp increase in fibre length is noticeable through the first 20 growth rings, where as in oak through the first 15 growth rings. In sycamore, however, this increase was more irregular, being greater throughout the first 30 rings.

After this initial increase fluctuations occurred although there was an overall, but much smaller, increase in length through the succeeding rings. In *Robinia* there is a remarkably little difference in length of the vessel elements in successive growth rings from pith outwards at any one level (Table I).

This uniform length may be related to the stratified arrangement of the fusiform initials of the vascular cambium. However, such an explanation is somewhat facile, for the libriform fibres which are equally products of the fusiform initials finally have a length of about 6 times that of the vessel elements.

Another point that is clear from the data is that the rate of increase in length of the elements of wood in successive rings from pith is more gradual in discs taken near the base of the bole than in those at higher levels. In all the three species studied, neither the vessel elements nor the libriform fibres attained a constant length in the later rings, except of course the vessel elements of *Robinia*. This may be due to the fact that the wood of all these trees lay within the period of increasing length, which was, as stated by Bailey (1923), to be approximately 60 years in the angiosperms.

The types of variation met with in the three different types of woods are related ultimately to the size of the cambial initials from which the wood has arisen. At a particular level the xylem cells, whatever their position on the radius, are all formed from the same initials, i.e. those which are formed when the vascular cambium became differentiated at a particular level in the tree. The initial rapid increase in the length of the libriform fibres and vessel elements is thought to be due to juvenile vigour, the fusiform initials enlarging rapidly and thus giving rise to larger derivatives. Although the rate of this enlargement slows down, it evidently continues for many years and in the material examined it is assumed that the increase had not ceased because the libriform fibres and the vessel elements became increasingly longer towards the peri-

TABLE I

Variation in length (in mm) of the vessel elements and libriform fibres in sycamore, oak and robinia in successive growth rings from pith outwards at different levels in the bole. (*D* = disc ; *Fib* = libriform fibre ; *VE* = vessel element).

Ring No.	D_I		D_{II}		D_{III}		D_{IV}	
	VE	Fib	VE	Fib	VE	Fib	VE	Fib
				SYCAMORE				
1	0.22	0.45	0.32	0.57	0.29	0.48	0.32	0.48
2	0.30	0.55	0.36	0.60	0.32	0.57	0.36	0.52
3	0.33	0.57	0.36	0.66	0.36	0.63	0.36	0.60
4	0.34	0.64	0.36	0.66	0.37	0.65	0.38	0.71
8	0.33	0.67	0.38	0.67	0.37	0.72	0.37	0.77
12	0.36	0.83	0.37	0.79	0.36	0.74	0.38	0.82
16	0.31	0.71	0.37	0.80	0.38	0.77	0.38	0.87
20	0.32	0.86	0.38	0.80	0.36	0.78	0.38	0.84
24	0.34	0.82	0.39	0.82	0.40	0.81	0.42	0.88
28	0.31	0.70	0.39	0.90	0.39	0.85	0.42	0.91
32	0.30	0.80	0.41	0.90	0.40	0.86	0.41	0.88
36	0.33	0.82	0.40	0.90	0.40	0.90	0.40	0.94
40	0.37	0.87	0.44	0.92	0.43	0.87	...	...
44	0.34	0.86	...	...	...	...	...	...
				OAK				
1	0.35	0.68	0.41	0.76	0.43	0.83	0.27	0.57
2	0.36	...	0.40	0.76	0.44	0.95	0.31	0.63
3	0.41	0.92	0.44	0.87	0.45	0.99	0.40	0.92
4	0.42	0.95	0.40	0.90	0.45	0.99	0.44	1.16
8	0.40	0.94	0.43	1.00	0.46	1.08	...	1.09
12	0.43	1.09	0.48	1.15	0.44	1.15	0.50	1.21
16	0.44	1.11	0.46	1.12	0.46	1.28	0.54	1.25
20	0.43	1.10	0.54	1.28	0.49	1.30	0.57	1.33
24	0.48	1.22	0.56	1.32	0.54	1.40	...	...
28	0.52	1.25	0.60	1.39	0.53	1.40	...	...
32	0.51	1.22	0.58	1.38	...	...	...	...
36	0.52	1.25	0.61	1.41	...	...	...	...
40	0.56	1.38	...	...	...	...	...	...
				ROBINIA				
1	0.25	0.49	0.18	0.61	0.20	0.42	0.22	0.55
2	0.22	0.66	0.19	0.66	0.20	0.63	0.23	0.59
3	0.25	0.70	0.18	0.75	0.21	0.63	0.22	0.61
4	0.20	0.79	0.19	0.81	0.21	0.77	0.21	0.80
8	0.20	0.98	0.19	0.94	0.22	0.90	0.22	0.90
12	0.20	0.99	0.19	1.02	0.22	1.01	0.22	0.95
16	0.20	1.03	0.20	1.09	0.21	1.08	0.20	0.98
20	0.20	1.05	0.20	1.09	0.21	1.11	0.20	1.00
24	0.20	1.08	0.19	1.08	0.21	1.11	0.21	1.08
28	0.20	1.08	0.19	1.10	0.21	1.11	0.21	1.11
32	0.20	1.08	0.20	1.10	0.21	1.12	0.20	1.14
36	0.19	1.08	0.20	1.13	0.22	1.14	0.21	1.14
40	0.20	1.09	0.20	1.14	0.21	1.17	0.21	1.13
44	0.20	1.08	0.20	1.13	0.21	1.20	0.21	1.15
48	0.20	1.08	0.20	1.16	0.22	1.19	...	...
52	0.20	1.08	0.20	1.16	0.22	1.19	...	...
56	0.20	1.10	0.20	1.17	...	...	...	...
60	0.20	1.11	...	...	...	...	...	...

Each figure in the above table represents the mean value in millimetres of fifty measurements.

phery. The vessel elements of *Robinia* formed an exception, for here a maximum size was reached in the second growth ring and after this the size remained constant. This has been related to the constant length of the fusiform initials which in stratified cambium, could not increase in length without disarrangement of the regular tiers of initials and a consequent loss of stratification. The libriform fibres of *Robinia*, however, evidently increase considerably in length during differentiation, their ends pushing between the other elements and thus the stratified arrangement is lost for the libriform fibres.

It might be argued that attention should also be given to the possibility that differentiation of the derivatives of the fusiform initials may not itself be constant and that a libriform fibre produced, in say the twentieth ring, may become longer than one produced in the second ring, even if the initial which produced both of them was to remain of the same size.

Variation upwards in the bole—In all the trees there is generally an increase in length of the vessel elements and the libriform fibres from the base of the bole to a certain height after which there is a decrease toward the top of the trunk (Table II). Judged from the data available the vessel elements and the libriform fibres attained their maximum length at a height

TABLE II

Variation in length (in mm) of the vessel elements and libriform fibres in specific growth rings upwards in the bole in sycamore, oak and robinia. (*Fib* = libriform fibre ; *VE* = vessel element).

Height from ground	Ring 1922		Ring 1936		Ring 1952	
	VE	*Fib*	*VE*	*Fib*	*VE*	*Fib*
			SYCAMORE			
1′-0″	0.338	0.651	0.335	0.818	0.371	0.874
7′-0″	0.368	0.695	0.388	0.834	0.433	0.902
13′-6″	0.347	0.689	0.363	0.782	0.411	0.911
20′-3″	0.339	0.596	0.351	0.867	0.410	0.885
			OAK			
	Ring 1925		Ring 1939		Ring 1955	
0′-6″	0.470	1.082	0.475	1.223	0.555	1.375
6′-0″	0.396	1.014	0.541	1.276	0.606	1.408
12′-0″	...	...	0.463	1.223	0.561	1.396
18′-0″	...	...	0.456	1.122	0.547	1.351
			ROBINIA			
	Ring 1922		Ring 1936		Ring 1952	
1′-6″	0.204	1.053	0.197	1.085	0.198	1.105
10′-9″	0.196	1.065	0.201	1.123	0.204	1.159
20′-0″	0.216	1.006	0.211	1.123	0.218	1.194
29′-3″	0.207	0.800	0.209	1.079	0.210	1.153

of 6 to 12 feet in sycamore and oak but in *Robinia* at about 20 feet. It was further observed that the height in the bole at which the maximum length of the elements of wood is attained varied, being higher in those growth rings nearer the bark than those nearer the pith.

Kribs (1928) in *Pinus banksiana* and Bisset & Dadswell (1949) in *Eucalyptus regnans,* have demonstrated that there is a high correlation between age from the pith and the length of the tracheids (or fibres) in higher levels of the tree. Thus the two factors, age of the growth ring and height in the tree, appear to be responsible for the type of variation in a specific growth ring at different levels.

In attempting to explain the differences in length of the elements of the secondary xylem in different parts of the bole, it is assumed that the actual length of an element like a tracheid reflects the length of the fusiform initial from which it arises. During the early life of the tree, when growth is most vigorous, it has already been suggested that any fusiform initial gradually becomes larger, until with slower growth of the tree it ceases to increase further in size. Further, during the period of greatest vigour of the tree, it might be supposed that successively larger fusiform initials would be laid down as new vascular cambium is formed, so that during this period, the fusiform initials nearest the apex of the part would be the longest and those nearest the base shortest, unless these had increased in size later with age. Consequently, longer elements would be produced nearer the top of the young tree in the first formed growth rings. Thus in any one growth ring the longitudinal elements would be longer, the nearer one approached the apex. After this period of maximum vigour is past, the fusiform initials subsequently formed would be shorter, hence the smaller length of the derivatives at the top of the bole in the older trees and the corresponding decrease in length of these elements in a growth ring at higher levels in the bole.

Variation within one growth ring—It was observed that the vessel elements and libriform fibres are shorter in the early wood of a growth ring than in the late wood. The relative difference in length of these elements between the early wood and late wood of a ring varied in the three trees (Table III). This difference was greater in the ring porous oak than in the diffuse porous sycamore. In *Robinia,* although the vascular cambium is stratified, the type of variation is essentially the same as observed in the other two.

The vessel elements in a growth ring of dicotyledons, especially those in which the secondary xylem is more specialised structurally, are supposed to have approximately the same length as the cambial initials from which they are derived (Bailey, 1920). The distinct difference in the length of vessel elements within a ring between the early and the late wood, as observed in the present investigation, may be due to shortening of the early formed vessel elements as a result of varying degrees of dis-

TABLE III

Variation in length (in mm) of the vessel elements and libriform fibres within one growth ring. (*Fib* = libriform fibre ; *VE* = vessel element)

	Growth ring	*VE*	*Fib*	
	1930	0.389	0.825	Late wood
S		0.358	0.731	
Y		0.350	0.725	
C		0.363	0.734	
A	1931	0.364	0.780	
M		0.356	0.795	
O		0.373	0.746	
R				
E	1932	0.365	0.746	Late wood
	1944	0.568	1.414	Late wood
O		0.388	1.128	
A	1945	0.474	1.249	
K		0.591	1.399	
	1946	0.410	1.113	Early wood
R	1942	0.204	1.075	Early wood
O		0.157	0.931	
B	1943	0.181	1.013	
I		0.191	1.087	
N				
I	1944	0.154	0.919	Early wood
A				

tension. The shortening that is taking place in the vessel elements of the early wood is more marked in the ring porous oak than in the diffuse porous sycamore.

The variation in length of the libriform fibres, on the other hand, appears to have been brought about by an altogether different factor. The proportionate increase in the length of the libriform fibres as between the early and late wood of a ring may be due to a strong intrusive growth taking place at either end of the derivatives of the vascular cambium during differentiation. These fibres show different degrees of elongation in the early and late wood of a ring in the three types of trees. In sycamore and oak an increase in length of about 120 to 260 per cent was observed in the last formed fibres of a ring as compared with that of the early formed fibres. Bisset & Dadswell (1950) reported an increase of 200 per cent. In *Robinia* this increase is about 600 to 800 per cent as against an increase of 100 to 300 per cent reported by Chalk *et al.* (1955) for woods with storeyed structure.

In angiosperms Bisset & Dadswell (1950) attributed the variation in length of the libriform fibres within a growing season to a time factor. They suggest that there is not sufficient time for considerable elongation of the developing fibres in the early part of the season when the cambial initials divide more actively. They presume, on the other hand, that more time is available for extension growth as the season progresses and the cambium is less active. It seems possible that conditions during the period of active growth and metabolism at the beginning of the growing season may be most suited for the early lignification of the differentiating fibres and that the process of elongation of the fibres may be carried further before lignification sets in as the season progresses, giving time for the formation of long and narrow fibres in the late wood.

It has often been recorded that there is a very sudden drop in fibre length from the last formed late wood of one ring to the first formed early wood of the succeeding ring (Bisset & Dadswell, 1950 ; Amos *et al.*, 1950 ; Chalk *et al.*, 1955). Such an abrupt decrease in the length of elements is also observed in all the three trees investigated in the project. This sudden decrease in my opinion appears to be independent of the length of the fusiform initials. This may be proved by an examination of the structure of the wood of *Robinia,* where the small late wood vessel elements and the strand parenchyma of the first formed early wood are stratified. It is evident that these stratified narrow vessel elements in the last formed late wood of one ring and strand parenchyma of the first formed early wood of the succeeding ring show remarkably uniform length. It seems reasonable to suggest that the length of the fusiform initials, as indicated by the length of these elements, remains the same at the region of transition from the late wood of one ring to the early wood of the succeeding ring. Further, Chalk *et al.* (1955) have shown that strand parenchyma is almost of uniform length throughout a growth ring. As the variation in length of the vessel elements and the libriform fibres from one ring to the other is essentially the same in the storeyed and non-storeyed woods, it seems reasonable to suppose that the cause may also be the same. This sudden decrease in length of the libriform fibres may, therefore, be due, as suggested earlier, to the earlier lignification and consequent cessation of elongation while that of the vessel elements may be explained by the shortening of those of the early wood in relation to lateral distension, notwithstanding the length of the fusiform initials.

In conclusion, it may be stated that the variation in length of the vessel elements and libriform fibres from pith outwards along one radius is associated with the activity of the same fusiform initials throughout, i.e. with the initials which originated at one time in the life of the tree. Variation in a single ring throughout the bole is, on the other hand, to be associated with the different fusiform initials, which have been formed over a long period in the life of the tree. Variation within a ring horizontally

may be due to the seasonal flow of hormones accompanying the opening of the buds and the resumption of the apical growth as well as to the environmental effects on the differentiating wood tissue.

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PHYSIOLOGY OF ANTHERS

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The development of the flower and its various parts is the result of a series of properly coordinated physiological and morphological processes that take place in the plant prior to flowering. There is intense activity in the flower bud at the time of its development. This increased activity is followed by a marked drop in the respiratory rate and a consequent increase in the dry weight/fresh weight ratio of the anther during meiosis. A similar respiratory drop is recorded during the division of the microspore nucleus following which there is again a rise in oxygen consumption followed by a decline until anthesis takes place (Erickson, 1947, 1948). In *Zea mays* metabolic activity in the tassel reaches a peak 7-12 days after reduction divisions. Drought conditions, even for a short while during meiosis, greatly affect pollen development, and according to Naylor (1952) some of the abnormalities attributed to high temperatures (*see* Randolph, 1932 ; Sax, 1937) can really be due to water shortage. If wilting occurs the entire development of the pollen may be inhibited. Dehydration of the anthers of *Tradescantia* causes inhibition of spindle formation in one or both meiotic divisions resulting in the production of diploid and tetraploid microspores (Giles, 1939). Carbohydrate and nitrogen deficiency also cause sterility of pollen (*see* Skazkin & Zavadskaya, 1957). These observations clearly indicate that even a slight change in the external or the internal environment of the anthers adversely affects the anthers and their contents.

Since the segregation of characters and the formation of microspores takes place in the anthers, the anthers occupy a very important place in the life of angiosperms. Moreover, the anthers and their component tissues are very susceptible to environmental and internal changes. An understanding of the physiology of anthers, especially the tapetum and the sporogenous tissue, therefore, becomes very important. The structure of the tapetum and its situation in the anther make it a tissue of considerable physiological significance. All food materials to the sporogenous tissue must pass through the tapetum which consists of large, active and richly cytoplasmic cells. The chief function of the tapetum is the production and transport of enzymes, hormones and nutritive materials which are utilized in microsporogenesis. The enzymatic and hormonal influences and the nutrition of the microspore mother cells are all achieved through the anther sap which is a diffusible secretion of the tapetum. The exact synchronization of meiosis in an anther

loculus is chemically controlled through the tapetal secretion (Linskens, 1959).

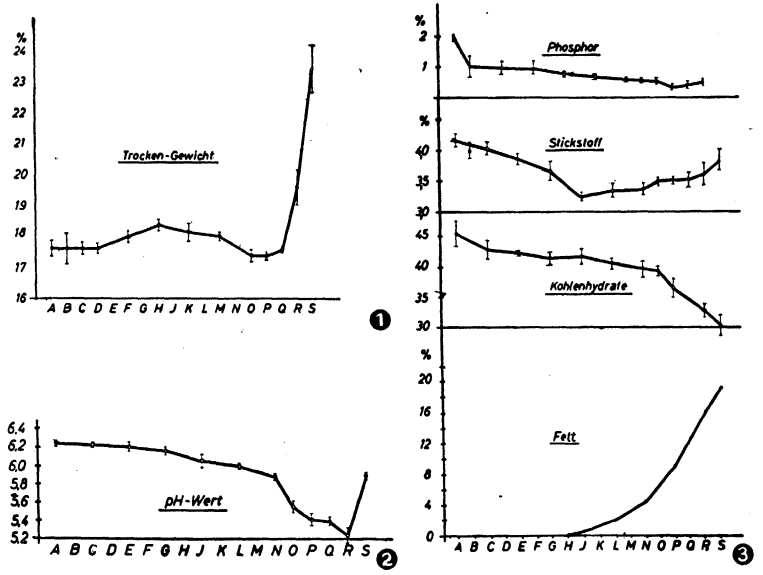
Any abnormalities of the tapetum will have an adverse effect on the development of the pollen (Vasil, 1957a, b). In *Lilium longiflorum* the tapetum may become multi-layered through some enzymatic disturbances (Steil, 1949). Some times when the pollen is sterile or due to some other disturbances (*see* Stein, 1932), the tapetum proliferates dividing the anther loculus into several chambers. In *Ranunculus acer* (Whyte, 1929) and *Gallium mollugo* (Fagerlind, 1937) premature degeneration of tapetal cells is associated with the death of microspore mother cells. In *Crepis acuminata* (Stebbins & Jenkins, 1939) pollen grains are not formed in the absence of nuclear divisions in tapetal cells which are typical of the genus *Crepis*. In certain hybrids of *Pyrus* (Maugini, 1952) and in *Allium cepa* (Kobabe, 1958) sterility of pollen is caused through disturbed or retarded activity of the tapetum which is believed to cause a disturbance in the hormone content of the anthers.

In recent years a new approach has been made to study the development of anthers by culturing excised anthers in test tubes under aseptic conditions (Gregory, 1940 ; Taylor, 1950 ; Sparrow *et al.*, 1955 ; Vasil, 1957b). This technique can also be used as a tool in solving some problems of experimental cytology, embryology and morphogenesis. Nutrient media containing coconut milk, kinetin, and gibberellic acid proved useful, but ribonucleic acid gave the most significant results with the anthers of *Allium cepa* (Fig. 5 ; Vasil, 1957b, 1959b). In this plant there was a 100 per cent survival of the mother cells up to the formation of 1-celled microspores and in *Rhoeo discolor* cultured anthers developed twice as fast as those in nature with the result that tetrads were formed within 24 hours instead of 48 hours (Vasil, 1959a, b).

Several workers have found that anthers fail to develop in cultures unless excised at or after a particular stage of development, usually pachytene or diplotene-diakinesis (Gregory, 1940 ; Sparrow *et al.*, 1955 ; Vasil, 1957b). Presumably certain substances are produced in the anthers or are transported to them from other regions of the plant at pachytene or at diplotene-diakinesis and anthers excised before the arrival of these substances degenerate within a short time. Gregory (1940) performed some grafting experiments to prove the translocation of certain chemical substances into the anther prior to meiosis. He cultured small branches of tomato plants bearing flower buds of different stages. In branches containing anthers in premeiotic stages no development was observed while normal development occurred in the buds of those branches in which meiosis had already been initiated. However, when branches bearing premeiotic anthers were grafted on to healthy flowering stocks, normal pollen developed in the former. These experiments clearly show that

meiosis is controlled by substances which arrive in the anthers (or are produced in them) at the beginning of meiosis.

Some investigators have noted that during meiosis, DNA or some other substance which gives a positive feulgen test is transferred from the tapetal cells to the microspore mother cells or their derivatives (Cooper, 1952). Taylor & McMaster (1954) have, however, criticised these observations and attributed such results to faulty fixation and artifacts caused



Figs. 1-3. (*A*, sporogenous tissue ; *B*, resting nucleus ; *C*, early prophase ; *D*, pachytene ; *E*, diplotene ; *F*, diakinesis ; *G*, metaphase I ; *H*, interphase ; *I*, metaphase II ; *K* and *L*, tetrads ; *M*, *N*, *O* and *P*, young pollen at different stages of development ; *Q*, *R* and *S*, mature pollen at different stages of development). Changes in the dry weight (Fig. 1), pH (Fig. 2) and phosphorus, nitrogen, carbohydrate and fat content (Fig. 3) in the anthers of *Lilium henryi* at different stages of development. (*After* Linskens, 1956).

by different fixatives. On the other hand, Linskens (1959) has confirmed that DNA does escape in the form of nucleotides and other degradation products from the degenerating tapetal cells and is absorbed by dividing microspore mother cells. This point still remains controversial for Takats (1959) has reiterated the earlier findings of Taylor & McMaster (1954) and has criticised the work of Linskens on the basis of his own spectrophotometric and radioautographic investigations (*see also* Remmel, 1959).

In the past five or six years autoradiographic and microphotometric techniques have been utilized for the study of developing anthers. Such studies have proved to be of considerable interest to cytologists, geneticists and embryologists. It has been found, for example, that DNA synthesis occurs only in the premeiotic microspore mother cells and that there is no synthesis of DNA during the whole of meiosis (Fig. 6 ; Taylor & McMaster, 1954 ; Moses & Taylor, 1955). After the completion of the meiotic divisions there is a marked drop in the DNA content of the nuclei which rises again before the mitosis of the microspore nucleus (Fig. 6). The final and the last synthesis of DNA occurs just before the division of the generative nucleus (the tube nucleus contains only about half the amount of DNA of the generative nucleus which explains its feeble staining reactions). Work done on this problem indicates that DNA synthesis occurs prior to each nuclear division so as to double the quantity before any visible doubling of chromosomes occurs. However, there is no evidence that it is DNA synthesis which triggers the division of nuclei although it is no doubt important for nuclear division in some other way. On the other hand, the evidence available on the basis of anther culture experiments suggests that it is RNA which is more important for the proper development of microspores than DNA (Vasil, 1959a, b). In 1953, Plaut had suggested that meiosis is dependent upon the supply of materials secreted by the cells which surround the microsporocytes or the spermatocytes. This tissue is nothing else but the tapetal tissue. In young anthers the cytoplasm of both the tapetal and the sporogenous cells is very rich in RNA. Mature pollen grains are also rich in RNA which is said to facilitate the synthesis of food materials during the growth of the pollen tubes. Further, the supply of RNA from the follicular cells (comparable to the tapetal cells) to the germ cells (comparable to the microspore mother cells) seems to be widespread in animals and it has been suggested that a large amount of RNA is the cause, or at least one of the causes, of the starting off of meiosis (Montalenti *et al.*, 1950).

Even if RNA has no direct role in cell division, its importance in the physiology of nuclear divisions can hardly be doubted (Brachet, 1950, 1954). Its intimate association with nuclear metabolism and structures is very well established (Brachet, 1954 ; Stern, 1956). How this association manifests itself in the course of mitosis (or meiosis) is a question which future experiments will surely answer. Some clues are provided by the histochemical demonstration of RNA in the spindle body (Brachet, 1950 ; Stich, 1954) and of variations in RNA content of chromosomes during mitosis (Ris, 1955 ; Jacobson & Webb, 1952). It has been suggested that during anaphase movement ribonucleoprotein is lost from the chromosomes to the spindle body (Ris, 1955 ; Jacobson & Webb, 1952). The physiological significance of this loss of RNA is not yet clear.

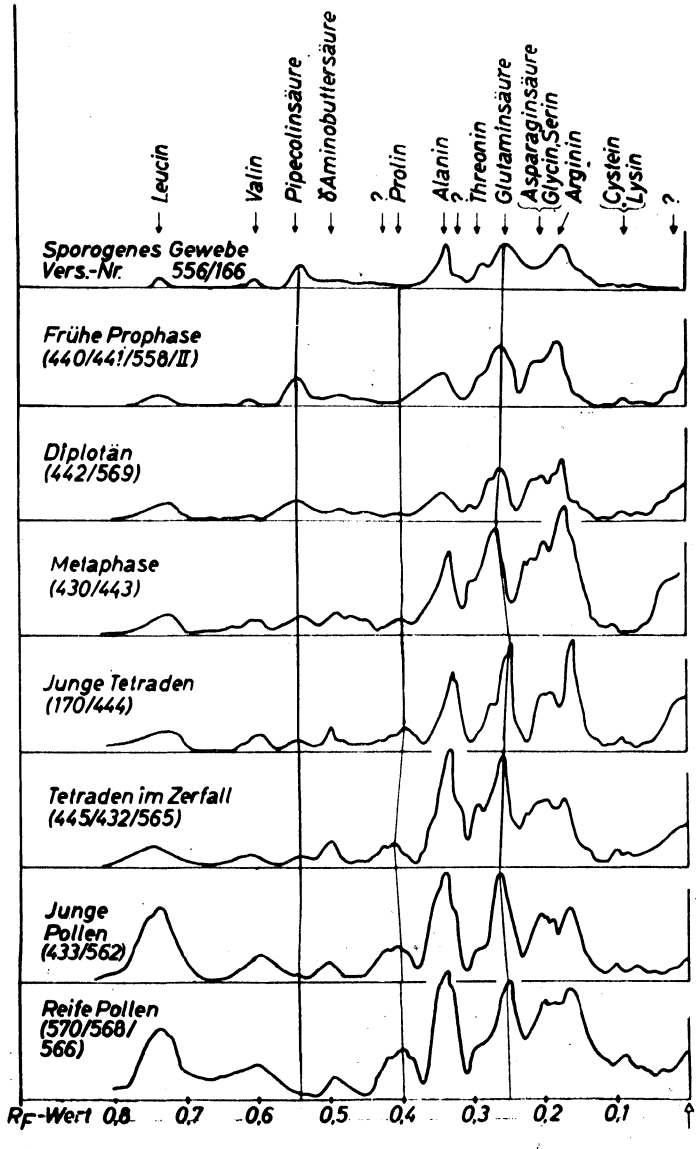


Fig. 4. Changes in the amino acid level of anthers of *Lilium henryi* at various stages of development (*After* Linskens, 1956).

Along with the synthesis of DNA and RNA, a large quantity of proteins is also synthesized in the microspore mother cells during premeiotic stages and in leptotene, but during meiosis there is little or no synthesis of protein. Protein synthesis occurs in the tapetal cells during meiosis but it is specially rapid during the maturation of the pollen grains. This is of significance, for the tapetal cells secrete wall material for the maturing pollen grains.

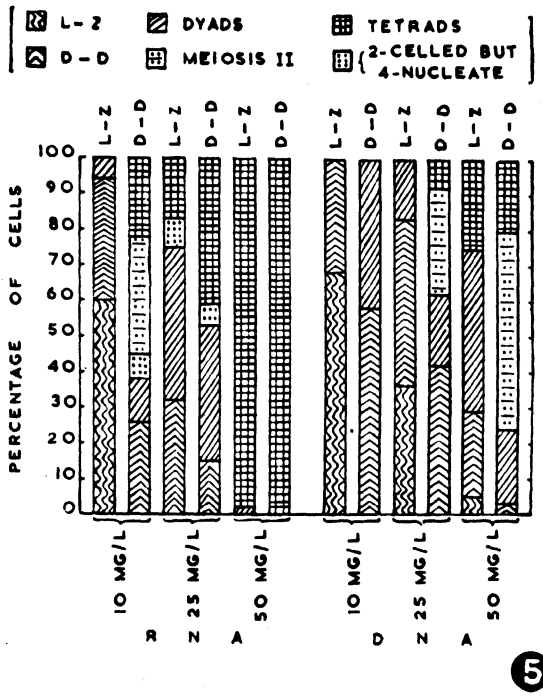
Linskens (1956) has refuted the supposition (*see* Taylor, 1950 ; Erickson, 1947, 1948) that for the determination of the meiotic stage of the anther a measurement of the bud or anther length alone would be sufficient. In *Allium cepa* it has been found that although bud length is closely related with the stage of development of the anther, one can not take this criterion alone for the determination of meiotic stages since the length of the anther and the bud of a particular stage varies, though slightly, from season to season (Vasil, 1959b). Apart from the variation in the size of the buds of *Lilium henryi*, Linskens (1956) has also shown that very marked and important changes occur in the dry weight of the anthers (Fig. 1), their pH (Fig. 2), and the content of phosphorus, nitrogen, carbohydrate, fat (Fig. 3) and amino acids (Fig. 4) during pollen formation and maturation.

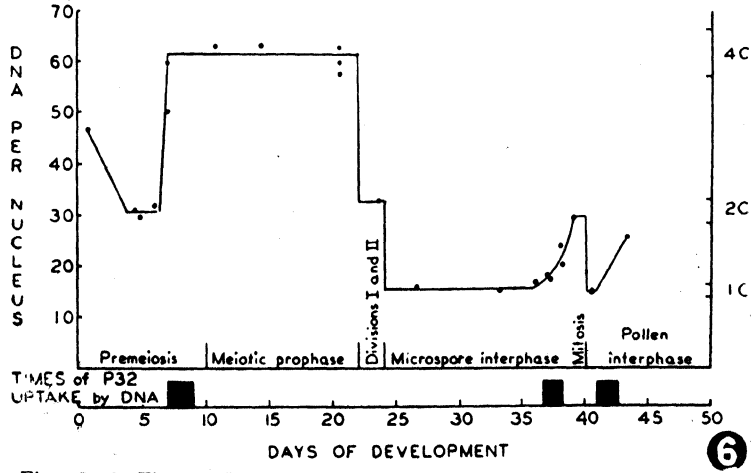
After the completion of meiosis certain materials appear in the tapetum which are of special significance in the formation of the exine. The most important of these is a cutin-like substance—the "sporopollenin" (*see* Frey-Wyssling, 1959 ; Sitte, 1959). Biourge, as early as 1892, had postulated a connection between the tapetum and the formation of the exine. Ubisch (1927) demonstrated the presence of a row of little granules closely appressed to the inner tangential wall (the wall facing the tetrads and young microspores) of the tapetum. These granules gradually form a continuous layer which is considered homologous to the exine since both arise at the same time and give the same chemical reaction. Later, Gorczynski (1934) found that the exine begins to be laid down first on the side of the pollen grains lying towards the tapetum and considered this to be the proof of the tapetal origin of the material forming the exine (*see also* Maheshwari, 1950). According to Cooper (1952) this material is synthesized in the cytoplasm of the tapetal cell. Taylor (1959) recently stated that "Synthesis of proteins occurs in the tapetal cells prior to each mitosis, but the most rapid synthesis occurs when the sporocytes are in late prophase and division stages. This assembly of proteins may be necessary for the secretory activity of the tapetum which occurs following

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tions of ribonucleic (RNA) and desoxyribonucleic (DNA) acids. (*After* Vasil, 1959a). Fig. 6. Mean amounts of DNA per nucleus at various stages of development of *Lilium longiflorum* anthers. (*After* Taylor & McMaster, 1954).





Figs. 5, 6—Fig. 5. Histogram showing development of excised anthers of *Allium cepa* on White'e modified medium supplemented with various concentra-

meiosis when the thick microspore walls are formed. When sulphate-35 was used as a label the late microsporocytes and especially the microspores of *Lilium* accumulate large quantities of the S^{35} in the walls during the period following meiosis. At the same time the tapetal cells bind the S^{35}, and the droplets of material that occur in the anther and along the inner walls of the tapetum are very radioactive while the inside of the microsporocytes and microspores have relatively little label (unpublished observations)". "The primary function of the tapetum, then, appears to be secretion of materials for wall formation on the microspores".

In addition to the exine-forming, cutin-like substances, the tapetum also produces some fatty and resinous substances which are more commonly known as the cement substances or "Pollenkitt" (for further references see Pankow, 1958). In *Galanthus nivalis,* Pankow (1958) has shown that the oily substance found on the surface of the pollen grain originates from the anther fluid which in turn is derived mainly from the tapetal cells. The pollen cement is more or less strongly viscous and sticky and its generally yellow color is due to various substances, especially carotinoids (Steffen, 1953 ; Linskens, 1955 ; Pankow, 1958). The pollen cement is found either *on* or *within* the exine. With the help of ultra-thin sections of the exine, examined under an electron microscope, Mühlethaler (1955/56) has shown that there are numerous canals within the exine which open towards the outside through pores. In these pores the cement substances are absorbed and the surplus spreads out evenly over the surface of the pollen and makes them sticky (Pankow, 1958). In such plants there is insect-pollination and the pollen gets stuck to the insects forming large pollen pellets. When the cement substances remain confined to the lacunose system of the exine and do not come out on its surface, the pollen remains dry and powdery. In this case pollination is affected through wind since the pollen is dry and does not stick to form clumps.

Anther physiology is thus an extremely promising and as yet an almost untouched field of research.

Summary

The development of anthers is marked by a series of physiological and morphological changes which are localised mainly in the developing microspore mother cells (or later the microspores) and the tapetal tissue. All available data suggests that the tapetal tissue has a very powerful influence on the normal development of microspores. Studies on the culture of excised anthers have shown that addition of RNA to the nutrient medium helps in a quicker and better development of microspores without any accompanying increase in the incidence of abnormalities. The initiation of meiotic divisions in the microspore mother cells seems to be controlled by some substance/s which arrive/s in the

anthers (or is/are produced in the anthers themselves) in the preleptotene stage, but nucleic acids (or their components) seem almost certainly to be involved. Significant changes in the dry weight, pH and the content of phosphorus, nitrogen, fat and amino acids take place in the anthers during their course of development.

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NATURAL HYBRIDIZATION IN PLANTS WITH SPECIAL REFERENCE TO ARGEMONE

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Natural hybridization between distinct species is considered a rare phenomenon. Had it not been rare, species would have been difficult to delimit. Isolation mechanisms of various kinds operate in nature and normally keep species from interbreeding. However, sometimes species are known to hybridize in nature. During the last 30 years, such instances of natural hybridization and their biological potential have received considerable attention not only in plants but also in some animals like snails, fishes, toads, moths and birds. Anderson (1949), Heiser (1949b), Allan (1949) and Stebbins (1959) have reviewed literature on natural hybridization in plants. Stebbins has also discussed the role of hybridization in evolution.

Broadly speaking, two categories of hybrids can be recognized from the point of view of fertility: (i) The so-called "mules" which are quite sterile and, therefore, of little consequence unless they are provided with means of apomictic propagation or become fertile by amphidiploidy ; (ii) Recombination hybrids which are more or less fertile and, therefore, by backcrossing, give rise to hybrid derivatives of various kinds. It is hybrids of the latter kind that may become involved in the process termed introgressive hybridization or introgression first recognized by Anderson (1949, 1953). By this term is meant the transfer of genes from one species to another by means of hybridization and backcrossing.

Certain well known and carefully analyzed instances of natural hybridization and introgression are found within the genera *Tradescantia, Iris, Helianthus, Quercus, Salix* and *Aquilegia* among angiosperms, and *Pinus* and *Juniperus* among gymnosperms. Ferns are also known to hybridize in nature but data on non-vascular plants—algae, fungi, mosses and liverworts—are still too scanty to evaluate.

METHODS OF ANALYSIS

There are several methods available for the detection of natural hybridization which can be used alone or together. The morphological method is the oldest but still the basic starting point for the recognition of hybrids. A geographical or ecological study may reveal hybridization in nature. The genetical method of artificially crossing species in the experimental garden is of course an invaluable method of detecting hybridization, and

combined with cytological investigations, it gives even more valuable information.

Besides the above mentioned methods, two others, namely, biometrical and biochemical methods have been developed recently to detect, and in the former method, also to measure hybridization and introgression of one species into another.

In the Index Frequency method first developed by Anderson (1936) for *Tradescantia* hybrids, a number of markedly different qualitative characters of the two hybridizing species are compared and assigned subjective values. The sum of the score of each character of one plant is the index value of the plant. The index values of several plants in a population are plotted in a histogram. The histograms for the two species do not overlap and are found at opposite extremes of a curve, but the histograms of various hybrid swarms may fall anywhere between these two depending upon the amount of hybridization and backcrossing. Even herbarium material can be used for this purpose, provided it is in large quantity.

The biochemical method consists of analysing and studying the components in parent species and the hybrids thereof. Mirov (1956) analyzed the turpentine of *Pinus contorta* Dougl. and *P. banksiana* Lamb. and hybrids between the two species. The F_1 generation possessed terpenes in which the bicyclic terpenes of *banksiana* dominated over the simpler monocyclic terpene, phellandrene, of *contorta*. Trees in a natural hybrid swarm also tended towards a predominance of the *banksiana* bicyclic terpenes.

Recently, Turner & Alston (1959) have run chromatograms of anthocyanins in the flowers of *Baptisia* (Leguminosae). The pigments are yellow flavonols, probably kaempferol. While the petal extracts from the parental species show clear and distinctive pattern of spots, the hybrid derivatives between the species show recombination of chemical components of their parents as evidenced by the redistribution of spots on the chromatograms. The degree of recombination depends upon the degree of hybridity and backcrossing. Some hybrid derivatives are more like one parent and others like the other parent, and still others are intermediate.

Ecology of Natural Hybrids

There is an interesting relationship of habitat to hybridization. In the New Zealand flora, Cockayne (1923) reported far more numerous hybrids in man-induced associations. There was an outburst of crossing where primitive vegetation had been disturbed by forest fires, lumbering or clearing for laying railroad tracks. Even primitive man, in the past, seems to have contributed to such a process. Stebbins (1959) believes that there

was a great burst of evolution following alterations in natural plant cover by man.

Of course, besides biotic factors, natural geodynamic forces like flowing water and receding glaciers have also contributed to natural hybridization.

In the Mississippi delta in Louisiana (U.S.A.) there occur two distinct species of *Iris*, *I. fulva* and *I. hexagona* var. *giganticaerulea*. In 1931, Small & Alexander, using only classical taxonomic methods described about 80 species of *Iris* from the Gulf states alone. Detailed morphological, genetical and cytological evidence, however, showed that a great majority of these so-called species were simply hybrid derivatives of the two above mentioned species. *I. fulva* grows in the clayey soils on the banks of deltaic streams and on flat lands on the far sides of the deltaic ridges. *I. hexagona* var. *giganticaerulea* on the other hand is found only in low lands in rich, mucky clay with a high water content bordering on marsh. Hybrid swarms occur only in intermediate situations.

Tradescantia subaspera grows in shade at the base of cliffs in rich deep soil, while *T. canaliculata* grows in full sunlight above the cliffs in thin rocky soil. Although the two hybridize readily by natural agencies in the experimental garden, hybrids are not usually met with in nature except where intermediate habitats are created by erosion of the face of the cliffs.

The above are just two examples to show that species do not usually hybridize in nature unless the normal ecological balance is disturbed in some way. Such disturbances not only create opportunities for hybridization but what is more important, they help to create diverse intermediate habitats which are necessary for the successful establishment of the variously intermediate hybrid derivatives.

Another aspect of natural hybridization is the competition between the parent species and the hybrids. In this competition, the hybrids are usually at a disadvantage. Firstly, because their perpetuation will depend so much on the availability of intermediate habitats. Further, the number of hybrids thrown into competition with the parents would usually be small. The equilibrium of competition is maintained if the two parent species are adapted to different habitat conditions. Such an adaptation is an important factor in keeping species distinct despite hybridization and introgression.

Introgression may help adaptation of a species to new environments. According to Heiser (1949a), *Helianthus annuus* has invaded California by absorbing genes from *H. bolanderi* already adapted to that climate. Introgression has also occurred in a reverse direction resulting in a weedy ecotype of *H. bolanderi* adapted to valley conditions. The possible role of introgression in the formation of weeds has been suggested by Anderson (1948).

NATURAL HYBRIDIZATION IN ARGEMONE

Argemone mexicana L. is a common introduced weed, which occurs on roadsides and in waste places throughout the plains of India. Recently, Venkatesh (1960) reported another species from Delhi, *A. ochroleuca* Sweet sub-sp. *ochroleuca,* not so far recorded from this country. *Argemone mexicana* is a native of the West Indies and probably also of parts of Central America and Florida. *A. ochroleuca* ssp. *ochroleuca,* which is often confused with *A. mexicana,* is widespread in Mexico which is probably its native home. Ownbey (personal communication), who has monographed the genus for North America and the West Indies (1958) states that in America the habitat and distribution of these two species differ and, therefore, although they cross readily in the experimental garden, he has not detected any putative hybrids between the two species in nature.

In the natural populations of the two above mentioned species that I have studied at Delhi, hybrids were noticed. The first of these two populations was small, with about 15 individuals of both species in approximately equal numbers. They grew in a highly disturbed roadside area where building material had been dumped for some time and then removed. Seedlings of plants from this area were transplanted and grown under uniform conditions of the garden. Both the species remained distinct and out of about 60 individuals thus raised two were found to be hybrids. This year I studied a much larger natural population near Okhla, in the neighbourhood of Delhi. The population grew in a roadside habitat and on bunds delimiting patches of cultivated and barren land. The population consisted of hundreds of individuals. Here and there individuals of each species grew together. But at several places individuals of both the species grew closely intermixed. In two instances, pairs of individuals of the two species grew so intimately together as to appear arising from a common base. From these circumstances, one is inclined to believe that the two species do not differ from each other in their habitat requirements and hence they are not likely to be ecologically isolated from each other. Six hybrid individuals were spotted. In every case the hybrid individual grew close to individuals of one or the other parent species and in one case near individuals of both the species.

Plants of both the species are highly self-compatible. Although at Delhi *A. mexicana* commences flowering a little earlier than *A. ochroleuca,* yet there is considerable overlapping in the flowering season of the two species. In the course of artificially crossing the two species in the experimental garden, it was noticed that a kind of bee regularly visited the flowers of *A. ochroleuca* during early hours of the morning to collect pollen. It is also usually seen that in both the species, even a day or two before the buds open, anthers are already dehisced and pollen stick to the stigma of

the same bud. Therefore, the flowers seem to be usually self-pollinated in both species. One might have expected more natural hybrids if cross-pollination were the rule.

All the hybrids studied were sterile and did not set any fruit or seed. The sterility is explainable on the basis of difference in the ploidy levels of the parental species, *A. mexicana* having 2n=28 and *A. ochroleuca* ssp. *ochroleuca* with 2n=56. This fact would imply that there is little chance of formation of hybrid swarms or of introgression of the two species. Further, since the plants are annuals with no means of vegetative propagation, each time a hybrid arises, it has been produced anew by naturally hybridizing individuals of the two parent species.

It is believed that seeds of *A. mexicana,* which is now widespread throughout the tropical and subtropical regions of the world, were carried in the first instance from port to port in ship's ballast. In its native area it is a common weed around sea port towns and it is supposed that this has promoted the incidental transport of its seeds along the sea ways to other parts of the world. Probably *A. ochroleuca* ssp. *ochroleuca* has come to India by similar methods due to the activities of man.

The example of *Argemone* described above illustrates how two species whose distributions do not overlap have been brought together to share the same habitat, very far from their native home, by anthropogenic factors. The two species being readily crossable, they hybridize by natural means in their adventive habitats.

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ORIGIN OF MAIZE

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Indian corn or maize ranks high among the most important crop plants in the world. Based upon the culture of maize the very ancient civilizations of Peru, Central America and Mexico were founded and corn has played a prominent part in the religious beliefs and ceremonies of these ancient peoples. As such, the problem of the origin of maize had excited and continues to excite the interest of botanists, cytologists, geneticists, archaeologists and ethnologists alike. Several hypotheses have been formulated and much speculation has been made on the problem since the time of Saint-Hilaire (1829), who was about the earliest to become interested and to put forward the theory of origin of maize from pod corn (*see* Mangelsdorf & Reeves, 1939, 1959 ; Mangelsdorf, 1947 ; Weatherwax & Randolph, 1955 ; Randolph, 1959).

In 1954, Barghoorn *et al.* made the very significant discovery of fossil pollen of corn from Mexico City which points to the existence of the corn plant in the valley of Mexico about 80,000 years ago, antidating the most primitive type of agriculture in the Western Hemisphere. In 1949, Mangelsdorf & Smith described prehistoric corn cobs recovered from Bat Cave in New Mexico. These specimens have been considered to be 5,600 years old on the basis of radiocarbon determinations of associated charcoal by Libby (*see* Mangelsdorf, 1958). Evidences of the above kind suggesting existence of corn in ancient times in any other part of the world are not available so far.

Apart from such evidences and in absence of any known wild progenitor of maize the only recourse is a study of the plant itself and its relatives. The evidence obtained from the field of cytology and genetics of the Maydeae is the most recent and is perhaps useful in evaluating the various hypotheses about the merits of which there is no unanimity at present. So far, cytogenetical evidence examined has come from the American relatives of maize. In the past few years the author and his pupils have been paying attention to the study of the oriental Maydeae, and the bearing of the observations made is discussed in this article.

MAIZE AND ITS RELATIVES

Before actually discussing the various theories, it is necessary to consider the botanical aspects and available information on the cytogenetics of the wild relatives of maize to enable a clearer understanding of

the problem. Data obtained on the oriental Maydeae by us are also included.

Maize or *Zea mays* is placed in the Maydeae, one of the sub-tribes 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* all of which are native to the region extending from India and Burma through East Indies into Australia and the Polynesian Islands. There are three American genera: *Zea, Euchlaena* and *Tripsacum*. Botanically the Maydeae "are characterised by the presence of unisexual spikelets, the staminate and pistillate spikelets placed in separate portions of the same inflorescence, the staminate above ; sometimes the spikelets placed in totally separate inflorescences (**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 the 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 being crowded on much thickened axis" (Henrad, 1931).

The available information on the chromosome number, chromosome morphology and crossability relationships of the Maydeae is summarised in Table I.

Theories on the Origin of Maize

In the light of the above information the various theories put forward on the origin of maize may now be considered. The several hypotheses may be classified as those based on (I) American origin and (II) Asiatic origin. Hypotheses of the first category are: (*A*) The pod corn theory, (*B*) Theories involving teosinte (*Euchlaena*) as a progenitor or as one of the probable parents, and (*C*) Theory of origin of maize by divergent evolution.

I. *A. Pod corn theory*—In 1829, Saint-Hilaire received from Brazil a peculiar variety of corn which he described as *Zea mays* var. *tunicata*.* He suggested that cultivated corn evolved from this. Later this theory was supported by Sturtevant (1899), one of the earliest American authorities on corn. In recent times Mangelsdorf & Reeves (1939, 1959) and Mangelsdorf (1947, 1948) have incorporated this idea into their theory (known as the tripartite theory) on the origin and evolution of maize. According to this theory: "(*a*) cultivated maize originated from a wild form of pod corn which was once and perhaps still is indigenous in the low lands of South America, (*b*) that teosinte, the closest relative of maize

* Inserted by me.

TABLE I

Summary of the available information on the cytology and crossability relationships in the Maydeae

Species	Chromosome number		Chromosome morphology					Nature of polyploidy	Meiotic behaviour††	B-Chromosomes	Crossability
			Length in microns		Differentiation into Eu- and Heterochromatic segments at pachytene	Knobs on pachytene chromosomes	Centromere position	Nucleolus organizer				
	n	2n	Karyotype	Pachytene							
* *Zea mays*	10	20	5-2.5	80-40	Non-differentiated	Mostly intercalary. 22 loci known	Mostly submedian	Satellite terminates the S.A. of ch. 6		10 ii	present 1-34	Crosses readily with teosinte; with difficulty with *Tripsacum*
* *Euchlaena mexicana*	10	20	-do-	-do-	-do-	Terminal knobs on both the arms of 9 of the 10 chromosomes	-do-	-do-		10 ii	Present	Crosses readily with corn
* *E. perennis*	20	40	—	—	-do-	—	—	Two Sat-pairs	Auto-	7-9 iv, rest ii	—	Crosses with tetraploid corn
* *Tripsacum floridanum*	18	36	—	Average length of chromosome less than $\frac{1}{2}$ as long as corn	Differentiated	Terminal or essentially terminal	More with subterminal	One of the short chs. median Nucleolus organizer median	—	18 ii	present in *Tripsacum*	—

* *T. australe*	18	36	—	—	—	No knobs	—	—	—	18 ii	—	—
* *T. maizar*	18	36	—	—	—	None or few knobs	—	—	—	18 ii	—	—
* *T. zopilotens*	18	36	—	—	—	Many knobs terminal	—	—	—	18 ii	—	—
* *T. dactyloides*	18	36	—	—	—	as in *T. floridanum*	—	—	—	18 ii	—	Crosses with corn with difficulty
* *T. dactyloides*	36	72	—	—	—	—	—	—	Allo-	iv & ii	—	Crosses with maize with difficulty
* *T. laxum*	36	72	—	—	—	—	—	—	Allo-	iv & ii	—	—
* *T. latifolium*	36	72	—	—	—	—	—	—	Allo-	iv & ii	—	—
* *T. pilosum*	36	72	—	—	—	—	—	—	Allo-	iv & ii	—	—
† *Coix aquatica*	5	10	15-9	112-70	Differentiated	Small terminal knobs present on the arms of all chromosomes	Three median & 2 sub-median	Satellite terminal in Median chromosome	—	5 ii natural populations show translocations & polysomy	present 1-2	Attempts to cross with maize failed
‡ *C. polionei*	5	10	—	—	Differentiated	—	—	—	—	5 ii	—	—
† *C. lachrymajobi*	10	20	6.2-2.8	85-35	Differentiated as well as non-differentiated	Terminal & intercalary	Mostly median & sub-median	Satellite terminal in median chromosome	Allo (?)	10 ii	—	Attempts to cross with maize failed
‡ *C. gigantea*	10	—	—	—	—	—	—	—	—	—	—	—

TABLE I (Contd.)

Summary of the available information on the cytology and crossability relationships in the Maydeae

Species	Chromosome number		Chromosome morphology						Nature of polyploidy	Meiotic behaviour ††	B-Chromosomes	Crossability
			Length in microns		Differentiation into Eu- and Heterochromatic segments at pachytene	Knobs on pachytene chromosomes	Centromere position	Nucleolus oranizer				
	n	2n	Karyotype	Pachytene								
‡ *C. gigantea*	20	—	—	—	—	—	—	—	—	iv & ii	—	—
§ *Polytoca barbata*	10	20	—	—	—	—	—	—	—	—	—	—
‡ *Polytoca macrophylla*	20	—	—	—	—	—	—	—	—	—	—	—
§ *Sclerachne punctata*	10	20	—	—	—	—	—	—	—	—	—	—
‖ *Chionachne koenigii*	10	20	—	—	—	—	—	—	—	—	—	
‖ *C. semiteris*	10	20	—	—	—	—	—	—	—	—	—	

* *See* Weatherwax & Randolph (1955).
† Unpublished information gathered in the author's laboratory.
‡ *See* Nirodi (1955).
§ *See* Mangelsdorf & Reeves (1939).
‖ *See* Darlington & Wylie (1955)
†† iv = Quadrivalents.
ii = Bivalents.

is a recent product of the natural hybridization of *Zea* and *Tripsacum* which occurred after cultivated maize had been introduced by man into Central America, (*c*) that new types of maize originated directly or indirectly from this cross and exhibiting admixture with *Tripsacum* comprise the majority of Central and North American varieties" (Mangelsdorf, 1947). This places the origin of maize in South America. Recently Weatherwax & Randolph (1955) and Randolph (1959) opined that the evidence derived from paleobotany and archaeology indicates a Central American origin and that natural hybridization between corn and *Tripsacum* as required by the tripartite theory is improbable. A third difficulty pointed out by them for the acceptance of pod corn as the progenitor is the fact that pod corn differs from normal maize by a single dominant gene *Tu*, located on the short arm of chromosome 4, governing the development of a readily disarticulating rachis and the production of prominent glumes enclosing the sheaths. They hold that pod corn may merely be taken as an aberrant genetic type along with teopod, corngrass and various tassel seed forms which are similar but distinctive dominant mutants. Further work has been carried out by Mangelsdorf (1958) who by appropriate crossings between pod corn and pop corn succeeded in synthesizing an ear, which resembled the primitive bat cave corn in having the male and female spikelets on the same inflorescence as in *Tripsacum*. Again in 1959 Mangelsdorf & Reeves reiterated their theory and held that evolution of maize to the modern state took place as a consequence of hybridization between teosinte and corn. They also stated that it is possible that corn might have originated at different places in the Western Hemisphere (including South America) thus overcoming the objection about its exclusively South American origin.

I. *B. Theories involving teosinte as the wild progenitor or as one of the putative parents*—

(*i*) *Theory that teosinte is the wild progenitor of maize :*

Teosinte is among the most similar of the wild relatives of maize in its habit, in having the male and female inflorescences separated out in different positions on the plant and in the chromosome morphology. Further more it readily crosses with maize to such an extent that some have accepted it as the wild corn from which modern maize evolved by means of large scale mutations or gene changes. According to this theory the transformation of the teosinte ear to corn ear is supposed to have come about by fusion of several lateral spikes into a central one. This theory was first put forward by Ascherson (1877, 1880) and supported by Schumann (1904) and more recently by Langham (1940). A very serious objection to this theory is the difficulty encountered in the visualisation of the steps required for the transformation of the ear of teosinte into that of corn. On this point Mangelsdorf (1947) writes: "In a transition

from teosinte to maize the single spikelets characteristic of teosinte must become the paired spikelets characteristic of maize and the distichous spike of teosinte must become the polystichous spike of maize. . . The prominent horny glumes of teosinte must be reduced to inconspicuous scales and this involves gene changes on atleast five different chromosomes. The readily disarticulating rachis of teosinte must be transformed to the rigid cob of maize, and genes or groups of genes on at least three different chromosomes participate in this process. The entire pistillate inflorescence must become strongly compacted and, although the linkage relationships of the genes for compaction have not been determined, it is evident from observations made on several maize-teosinte hybrids that this character too, involves several genes or groups of genes". At present this theory is generally held as unacceptable.

(*ii*) *Theory of hybrid origin of maize involving teosinte as one of the parents :*

In 1893, Harshberger came across a plant which he named as *Zea canina* and considered it to be wild corn. Later in 1896 when he learnt that it was a hybrid of teosinte and corn, he proposed the theory of hybrid origin of maize, according to which corn had originated from a hybrid between teosinte and some unknown wild grass. In 1912, Collins supported this theory and considered that the unknown grass is a member of the Andropogoneae. This theory, however, is no longer seriously considered by present day students of corn phylogeny (Weatherwax & Randolph, 1955 ; Randolph, 1959).

Teosinte is also involved in the tripartite theory of Mangelsdorf & Reeves (1939) and this has already been dealt with earlier in this paper.

I. *C. Theory of origin of maize by divergent evolution*—In 1906, Montgomery suggested that maize and teosinte are distinct co-ordinate genera developed from a common ancestor now extinct. In later years Weatherwax (1918, 1919, 1935, 1950) elaborated Montgomery's hypothesis to include the three American genera, *Euchlaena, Tripsacum* and *Zea*. He considered that they are basically alike in their structural pattern and that the visible differences among them are due to differential abortion of organs during development. He holds that these three genera stem from a common ancestor which probably gave rise also to some of the Andropogoneae. Regarding the hypothetical ancestor of corn he writes: "On one of the branches of this family tree a little nearer to teosinte than to *Tripsacum*, there was a wild plant which was to become the ancestor of corn". He considers it to be now extinct. Weatherwax & Randolph (1955) propose that the wild ancestor of corn was probably a perennial plant with something of the habit of the more caulescent species of *Tripsacum*. This includes male and female spikelets borne in

almost completely separated inflorescences, with an incompletely eight-rowed ear having the grains covered with glumes and lemmas as in most other grasses, and a cob which does not break into pieces at maturity, as do those of *Tripsacum* and teosinte.

This theory also has not met with universal acceptance. Kempton (1919) thinks that the fundamental differences between *Zea, Tripsacum* and *Euchlaena* have been overlooked and too much emphasis has been placed on the fact that the genera are alike in the supression of the sex-organs, while Collins (1931) considers that the theory fails to take into account the close relationship of *Zea* and *Euchlaena* as indicated by their interfertility, by their close agreement in number, size, shape and behaviour of the chromosomes, and the fact that they are hosts to the same parasites and diseases. Reeves & Mangelsdorf (1959) point out that there is no evidence for the existence of an original common ancestor, now extinct.

II. *Asiatic origin of maize*—In 1945, Anderson proposed the theory that maize originated in South East Asia, probably in Assam (India), as an amphidiploid of a hybrid between a five chromosome species of *Coix* and a five chromosome species of *Sorghum*. The idea that corn existed in Asia previous to 1492 (i.e. in the pre-Columbian age) is not new and was raised several times during the present century. Collins (1909) reported the occurrence of a new type of maize with waxy endosperm in Western China, under conditions which made it seem likely that it had been there for a very long time (*see* Weatherwax & Randolph, 1955). Corn was classed as an Asiatic plant in the 16th century Herbals, and also by Bonafous (1836). More recently, Stonor & Anderson (1949) described some peculiar varieties of corn grown by primitive people in a remote area of the hill country of Assam and Northern Burma. They have pleaded for an open minded approach to the subject.

It may be stated here that the theory of Asiatic origin has not met with any support. There is no evidence, be it historical, paleontological or archaeological which indicates that maize was known in Asia in pre-Columbian times. If maize arose as indicated by Anderson it should be an amphidiploid and its ten chromosomes should be made up of two sets of five chromosomes each. There is no genetic evidence in favour of such an assumption. If corn is an amphidiploid, duplicate genes should be prevalent but actually only 14 cases of duplicate genes, 2 of triplicate and 1 of quadruplicate genes are listed by Emerson *et al.* (1935). These can, however, be accounted as resulting from intrachromosomal duplications. The evidence from cytology is equally against the suggested Asiatic origin. *Sorghum intrans* is the only five chromosome species in which the chromosomes have been examined at the pachytene stage (Garber, 1947). They are found to be fundamentally different from the corn chromosomes. They are knobless and their length range is

different from the corn chromosomes. Their centromeres are indistinguishable and the haploid set contains two nucleolus-organizing chromosomes. Further, none of the five chromosome species of *Sorghum* is known to be native to South East Asia. Likewise, the chromosome morphology of five-chromosome species of *Coix* (*C. aquatica*) examined by us lends no support to the theory of Asiatic origin by Anderson. The pachytene chromosomes of *C. aquatica* are the longest in the tribe, being about 1.6 times those of corn. They show a highly differentiated structure with median and intercalary segments of heterochromatin. They are mostly median and all arms of the chromosomes bear small terminal knobs, intercalary knobs characteristically found in maize chromosomes being absent (*see* Table I). About 1,200 controlled pollinations have been made between *Coix aquatica* and maize, using the former as the female parent in the Experimental Field Station attached to the Department of Botany, Andhra University, Waltair. No success has been achieved so far. From what has been stated above it can be seen that the five-chromosome species of *Coix* shows no similarities in the chromosome morphology with corn and also its crossability with maize is poor if not nil. On these grounds it is doubtful whether it can be regarded as a putative parent of the maize progenitor.

A consideration of the various hypotheses thus shows that the problem of origin of maize in relation to its wild progenitor is not decisively solved and there has been no general agreement about it. From the information available at present, the case for an American origin of maize seems to be established. It may, however, be stated that more work on the oriental Maydeae is necessary, as cytological observations made by us on the morphology of the pachytene chromosomes in *Coix lachryma-jobi* (10-chromosome *Coix*) reveal resemblances between them and those of maize, not shown by *C. aquatica*. Studies on the other oriental Maydeae such as *Polytoca* and *Chionachne* are also in progress in our laboratory.

Summary

The problem of the origin of maize is fascinating and several theories have been proposed and much speculation has been made to explain the same. The several theories may be classified as those based on either an American or an Asiatic origin.

The pod corn theory and the tripartite theory, theories involving teosinte as a wild progenitor or as a putative parent, and the theory of the origin of maize by divergent evolution, all belong to the first category, while the theory of the Asiatic origin of maize as an amphidiploid of a hybrid between a five chromosome species of *Sorghum* and a five chromosome species of *Coix* belongs to the second category.

None of these theories is universally accepted. Further, the theory of the Asiatic origin of maize (as suggested by Anderson) has been examined in the light of cytological and cytogenetical information obtained from a study by us of a five chromosome species of *Coix, C. aquatica.* The information obtained on *C. aquatica* does not lend support to the Asiatic origin of maize. On the basis of information available from different fields of botany—paleobotany, archaeology, cytology and genetics—there is a general acceptance of the American origin of maize.

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APPENDIX I

List of Delegates

1. Dr S. C. Agarwala (University of Lucknow).
2. Professor K. S. Bhargava (University of Gorakhpur).
3. Dr D. Chatterjee (Sibpur Botanic Garden, Howrah).
4. Dr M. S. Chennaveeraiah (Karnatak University).
5. Dr N. Das (Gauhati University).
6. Dr T. V. Desikachary (University of Madras).
7. Mr V. Garcia (visiting Argentine botanist at the University of Delhi).
8. Dr B. M. Johri—*Organizer* (University of Delhi).
9. Dr T. N. Khoshoo (University of Punjab).
10. Professor T. S. Mahabale (University of Poona).
11. Dr J. K. Maheshwari (Central National Herbarium, Botanical Survey of India).
12. Professor P. Maheshwari—*Director* (University of Delhi).
13. Dr L. P. Mall (University of Saugor).
14. Dr G. Misra (Utkal University).
15. Dr M. Nagaraj (University of Mysore).
16. Dr A. N. Namboodiri (University of Kerala).
17. Dr H. S. Narayana (University of Rajasthan).
18. Dr S. Narayanaswami (Vallabh Vidyapeeth).
19. Dr B. Padhi (Utkal University).
20. Dr G. Panigrahi (Botanical Survey of India).
21. Dr D. D. Pant (University of Allahabad).
22. Professor V. Puri (Agra University).
23. Dr D. C. Rife (U. S. Embassy, New Delhi).
24. Professor R. P. Roy (Patna University).
25. Professor T. S. Sadasivan (University of Madras).
26. Professor B. Samantarai (Utkal University).
27. Father H. Santapau (University of Bombay).
28. Dr J. C. Sen Gupta (Botanical Survey of India).
29. Dr A. Seshagiri Rao (Sri Venkateswara University).
30. Dr J. J. Shah (Gujarat University).
31. Dr A. K. Sharma (Calcutta University).
32. Dr T. Sreeramulu (Andhra University).
33. Dr B. Sundarasiva Rao (Andhra University).
34. Dr I. K. Vasil (University of Delhi).
35. Dr C. S. Venkatesh (University of Delhi).
36. Professor J. Venkateswarlu (Andhra University).

APPENDIX II

PROGRAMME

Summer School of Botany—Darjeeling (June 2 to 15, 1960)

June 2
Thursday

9.30 A.M.	Inauguration by Dr B. C. Roy, Chief Minister, West Bengal Coffee
11.30 A.M.—12.30 P.M.	"Contacts between embryology, physiology and genetics—I"—P. Maheshwari
3.00 P.M.—4.30 P.M. *Chairman :* T. S. Sadasivan	"Substrate and the soil fungus"—A Seshagiri Rao "Aerobiology and dissemination of plant diseases"—T. Sreeramulu "Virus disease problems in India"—K. S. Bhargava
After-dinner talk *Chairman :* J. C. Sen Gupta	"The botanical exploration of Mahabaleshwar"—H. Santapau

June 3
Friday

9.30 A.M.—10.30 A.M.	"Rhizosphere microfloras"—T. S. Sadasivan Coffee
11.30 A.M.—1.00 P.M. *Chairman :* K. S. Bhargava	"Heterokaryosis in fungi"—B. Padhi "Biology of phytoplankton"—T. V. Desikachary "Spore content of the atmosphere"—T. Sreeramulu
2.30 P.M.	Excursion—Lloyd Botanic Garden
After-dinner talk ***Chairman :*** V. Puri	"Spring flora of Chicago"—M. Nagaraj

June 4
Saturday

9.30 A.M.—10.30 A.M.	"Soilless culture of plants"—J. C. Sen Gupta Coffee
11.00 A.M.—1.00 P.M. *Chairman :* J. C. Sen Gupta	"The role of micronutrients in plants"—S. C. Agarwala "Hormones in relation to flowering behaviour in rice"—G. Misra "Studies on the effect of auxins on flowering and fruiting of pineapple"—N. Das
3.00 P.M.—5.00 P.M. *Chairman :* B. Samantarai	"Recent experimental evidences on the partial shoot theory of the leaf"—B. Samantarai "Seed germination and its significance"—L. P. Mall "Photoperiodic behaviour in some varieties of rice grown in India"—G. Misra
After-dinner talk *Chairman :* H. Santapau	"Various aspects of the flora of Eastern India"—G. Panigrahi

June 5
Sunday

9.00 A.M.	Excursion—Senchal Lake

June 6
Monday

8.45 A.M.	Excursion—Lloyd Botanic Garden and Happy Valley Tea Estate
3.00 P.M.—4.30 P.M. *Chairman :* V. Puri	"Nutrition of the embryo sac"—B. M. Johri "Embryology of the Combretaceae"—M. Nagaraj "Stipules of some members of the Vitaceae"—J. J. Shah

June 7
Tuesday

9.30 A.M.—10.30 A.M.	"Cytotaxonomical studies in the genus *Adiantum*"—R. P. Roy Coffee

11.00 A.M.—12.30 P.M. *Chairman :* R. P. Roy	"Recent advances in the study of chromosome structure"—A. K. Sharma "Cytogenetical evolution in the gymnosperms—karyotype"—T. N. Khoshoo
3.00 P.M.—4.00 P.M. *Chairman :* T. S. Mahabale	"The gametophyte of the Psilophytales"—D. D. Pant "Variation in the structure of wood in some dicotyledonous trees"—B. Sundarasiva Rao
June 8	
Wednesday	
9.30 A.M.—10.30 A.M.	"Bioassay problems"—T. S. Sadasivan Coffee
11.00 A.M.—12.30 P.M. *Chairman :* T. S. Mahabale	"Morphology of angiosperm flower"—V. Puri "Some recent contributions towards our knowledge of the Glossopteris flora"—D. D. Pant
3.00 P.M.—4.30 P.M.	Group discussions on the improvement of botany syllabii in Indian Universities
June 9	
Thursday	
9.30 A.M.—10.30 A.M.	"Botanical nomenclature"—H. Santapau
11.00 A.M.—12.30 P.M.	"The genetics of *Coleus*"—D. C. Rife "Electron microscope studies on algae"—T. V. Desikachary
3.00 P.M.—4.30 P.M. *Chairman :* J. Venkateswarlu	"Some aspects of the cytotaxonomy of *Aegilops*"—M. S. Chennaveeraiah "Cytology and systematics of Indian Orchidaceae"—G. Panigrahi "Natural hybridization in plants with special reference to *Argemone*"—C. S. Venkatesh
After-dinner talk *Chairman :* D. Chatterjee	"Flora of Darjeeling and Sikkim" (Kodacolor cine film)—A. C. Gupta

June 10 Friday	
9.30 A.M.—10.30 A.M.	"Contacts between embryology, physiology and genetics—II"— P. Maheshwari Coffee
11.00 A.M.—1.00 P.M. *Chairman :* B. Samantarai	"Physiology of anthers"—I. K. Vasil "Controlled growth of ovary and ovule"—B. M. Johri "Morphological variations in growth and differentiation of embryos *in vitro*"—S. Narayanaswami
3.00 P.M.—4.30 P.M. *Chairman :* B. M. Johri	"Modern aids to plant taxonomy"—H. Santapau "Seed structure in the Aizoaceae"—H. S. Narayana "Origin, development and morphology of tendrils of Vitaceae"—J. J. Shah
After-dinner talk *Chairman :* H. Santapau	"Botanical gardens in the U.K. and Europe"—B. M. Johri
June 11 Saturday	
9.30 A.M.—10.30 A.M.	"Origin of maize"—J. Venkateswarlu Coffee
11.00 A.M.—1.00 P.M. *Chairman :* J. Venkateswarlu	"Some aspects of genetics of polyploids"—R. P. Roy "Cytology and its contribution to fern systematics"—G. Panigrahi "Genome analysis in wheat"—M. S. Chennaveeraiah
June 12 Sunday	
6.30 P.M.	Governor's At Home at Raj Bhawan, Darjeeling
After-dinner talk *Chairman :* H. Santapau	"Flora of the Andamans" (Kodacolor cine film)—T. S. Mahabale
June 13 Monday	
9.30 A.M.—10.30 A.M.	"Floristic patterns of Indian vegetation"—D. Chatterjee Coffee

11.00 A.M.—1.00 P.M. *Chairman :* H. Santapau	"Trends of evolution in the grass family"—S. Narayanaswami "An outline of Argentine Phytogeography"—V. Garcia "Habitats, plants and their indicator value"—L. P. Mall
3.00 P.M.—4.30 P.M. *Chairman :* D. Chatterjee	"On the concept of carpellary margins"—V. Puri "Cleistogamy in angiosperms"—J. K. Maheshwari "Aspects and prospects of the study of wood anatomy"—B. Sundarasiva Rao
After-dinner talk *Chairman :* H. Santapau	"Phytogeography of palms"—T. S. Mahabale "International Botanical Congress, Montreal"—V. Puri
June 14 Tuesday	
9.30 A.M.—10.30 A.M.	"Observations on the prothalli of some Indian ferns"—T. S. Mahabale Coffee
11.00 A.M.—1.00 P.M. *Chairman :* H. Santapau	"Buds in some Indian ferns"—T. S. Mahabale "Role of botany in pharmacognosy"—A. N. Namboodiri "Studies on the naturalized flora of India"—J. K. Maheshwari
3.00 P.M.—5.00 P.M.	Discussion on "Promotion of teaching and research in botany"
After-dinner talk *Chairman : J. C. Sen Gupta*	"Cinchona" etc.—Documentary films by courtesy of the Government of West Bengal
June 15 Wednesday	
9.00 A.M.—10.30 A.M.	Discussion on "Promotion of teaching and research in botany" Coffee
11.00 A.M.—1.00 P.M.	Discussion on "Promotion of teaching and research in botany"
3.00 P.M.—5.00 P.M.	Visit to Darjeeling Government College
7.30 P.M.	Farewell Dinner

APPENDIX III

PROMOTION OF TEACHING AND RESEARCH IN BOTANY

On June 14 and 15, 1960, several hours were devoted to a discussion on the "Promotion of teaching and research in botany". The following took part in the discussion: Dr D. Chatterjee, Dr T. V. Desikachary, Dr B. M. Johri, Professor T. S. Mahabale, Professor P. Maheshwari, Dr. D. D. Pant, Professor V. Puri, Professor R. P. Roy, Professor B. Samantarai, Father H. Santapau, Dr J. C. Sen Gupta, Dr I. K. Vasil, Dr C. S. Venkatesh and Professor J. Venkateswarlu.

On the basis of these discussions a brief report was prepared by Professor P. Maheshwari, Director, and was circulated to all the delegates. This report is reproduced below.

INTRODUCTION

The object of the meetings held on the last two days of the Summer School was to discuss the means for improving the teaching of botany and promoting research in this subject in India. Consideration was given to improvement of the syllabuses by pruning less important portions so as to make room for the newer knowledge in this subject. How to implement these changes came next. Finally, to make the teaching more effective, it was decided to ask for some added facilities and a better teacher-pupil ratio. These and several other points are discussed in more detail in the following pages.

SYLLABUSES

Syllabuses must be revised from time to time owing to the following factors:

(1) the growing mass of botanical knowledge,
(2) the trends of current research,
(3) provision of a sound botanical training,
(4) chances of employment and present needs of the country.

The extent of botanical knowledge today is much greater than it was a couple of decades ago. It seemed satisfactory then to spend most of the time on morphology and systematic botany. Today when rapid advances are being made in plant physiology, genetics and microbiology, the position is different and adequate time has to be found for these subjects, even if this has to be achieved by some pruning of others.

Post-graduate Classes : The following tentative distribution of papers is suggested for the M.Sc. classes:

		Marks
A.	(1) Algæ, Fungi, Bacteria, Viruses, Lichens and Plant Pathology	100
	(2) Bryophytes, Pteridophytes, Gymnosperms ...	100
	(3) Angiosperms: Anatomy, Embryology and Taxonomy	100
	(4) Practical	200
		500
B.	(1) Cytology, Genetics and Economic Botany ...	100
	(2) Physiology	100
	(3) Ecology ; History of Botany *or* Essay	100
	(4) Practical	200
		500
C.	Special Paper *or* Dissertation	150

The above plan lays equal emphasis on the descriptive and the experimental aspects of the subject but even under Section A the functional approach should be given adequate importance. The emphasis must naturally be on a clear understanding of the fundamentals rather than the memorization of unnecessary details which are sure to be forgotten as soon as the examination is over.

It might be said that the student going for the Master's degree should not be allowed to get away with just a superficial study of the subject. Under the arrangement suggested above, this danger is decreased by his having to prepare in the second year for a Special Paper or Dissertation whichever he chooses. Since he surely cannot specialise in all branches of botany, he may be permitted to take one branch for intensive study (to be chosen according to the facilities available in any particular laboratory) and begin the work for this in the summer vacation immediately after the first year of the course. It is here that he will develop his powers of a critical appraisal of the literature on a subject, of putting down his own observations briefly and clearly, and lastly of drawing conclusions from them.

Under-graduate Classes : Concerning under-graduate classes, at the Delhi University the better students are admitted to the Honours Course. This is of the same duration (3 years) as the Pass Course but the Honours students are given more individual attention and intensive training. It is these students who are later admitted to the M.Sc. course although the B.Sc. Pass students are also eligible if their performance is of a high

standard. In the experience of the Science Faculty of the Delhi University this arrangement has proved very satisfactory and it was approved by the Conference of Biology Teachers held at Delhi in 1959. This pattern could perhaps be followed at other places if considered practicable.

Choice of Subjects at B.Sc. Level : A point needing special mention is that Botany students are often handicapped in their post-graduate work because of an inadequate knowledge of the Physical Sciences. It is, therefore, desirable that other combinations should be permissible besides Botany, Zoology and Chemistry. The three subjects may be selected out of a group of four or five including Chemistry, Physics and Mathematics-Statistics. For example, a student taking up Botany as the Main subject may take Chemistry and Zoology *or* Chemistry and Physics as his subsidiary subjects depending on his aptitude and the branch in which he wants to specialise at the Ph.D. level. A student intending to take up research in plant physiology would naturally choose the second of the two alternatives. On the other hand, for one who wants to teach biology at the school or to Intermediate or Pre-medical students, the first may be preferable.

Admissions : It is important of course that only such students be admitted to the University who are likely to profit from it. Some teachers pointed out that undue pressure was exercised on them for admitting students of an inferior calibre. Admissions, it was agreed, should be solely by merit.

Doctoral and Post-doctoral Scholars

It is a pity that so many students are still going abroad just to get a doctor's degree. For such work reasonable facilities already exist in many of our own Universities and these ought to be further improved instead of sending young people abroad just for a degree. They should really visit foreign countries only for learning special techniques for which we lack the desired facilities in our laboratories. It should be the constant endeavour of the Government and the University Grants Commission to reduce this annual exodus of Indian students and to augment our own resources. It also needs to be pointed out that many of the students who are now going abroad are of a second or even third grade type and some screening is necessary before they are permitted to leave the country.

While our standards for the Ph.D. are generally comparable with those of foreign Universities, every Ph.D. student should be asked to learn an additional foreign language besides acquiring a better acquaintance with English itself. He should also not be allowed to become a narrow specialist. The American system provides for instruction and periodical tests even at the Ph.D. level. This method can of course be modified to suit our conditions ; a good deal can be done by arranging regular seminars

and group discussions and by asking every Ph.D. student to present his data or conclusions before his teachers and co-workers twice a year.

The UGC provides some facilities for exchange of scholars and teachers. These need to be properly planned and strengthened. To make the best use of our limited scientific resources there should also be provision for arranging more extension lectures by distinguished scientists, Indian or foreign. The hurried tours of scientists who give a lecture here and a lecture there are of comparatively little value. Instead, there should be a way of retaining a scholar for a week or ten days so that he gives a course of lectures to the post-graduate students, holds seminars and colloquia, and discusses with them new techniques of research in his special field.

Examinations

It has been emphasized many times by University teachers that the excessive emphasis laid on the annual examination is responsible for many of the evils in our educational system. Today the end and object of teaching is the inevitable examination. This evil can be greatly minimised if 50 per cent of the marks were set apart for home examinations and class records throughout the year. It may be objected that even in the same University standards will vary from College to College and there will be a competition between teachers to pass undeserving students. There may be some truth in it. But even so there seems to be no difficulty in beginning this with the M.Sc. classes in residential Universities where there is only one department of teaching and there are no Colleges. In the light of the experience thus gained the new system could be extended later to the under-graduate classes.

The Teachers

No matter how good the syllabus or the examination system, no real improvement can be affected unless there are good teachers. In fact while the syllabus can be a help to the ordinary teacher, it is only a handicap to the top man who should be free to awaken the mind of the student to new adventures of thought and enquiry by his own unbounded enthusiasm and sincerity.

How to find such teachers and retain them is a question that requires serious consideration. Since this is part of a larger problem facing the country as a whole, it seems unnecessary to discuss it in detail. However, two things need to be pointed out. One is the hours of work per week expected of a science teacher. Although some members said that they had to do as much as 24 periods per week, the average for a University lecturer seems to be about 18. Even this is excessive, for in addition

to normal teaching, a botany teacher must go on excursions, make collections of specimens, and take part in the building up of a museum, herbarium and a garden. In addition he must of course contribute his own share to the advancement of knowledge. To make this possible his hours of work should be reduced and there should be a better teacher-pupil ratio.

The second point to which attention must be called is the great disparity between the salaries of the administrators and scientists in India. As a result, most college professors look wistfully to the Principalship of the College and University Professors in turn look to the Vice-Chancellor's post. This is quite wrong. A competent professor should be able to have his reward right where he is. For this it is necessary to increase the number of National Professorships and institute several Research Professorships in each University. Those people, who are especially distinguished in research ought to be able to pursue their work without being burdened with administrative duties.

It is understood that the Government is planning to give special increments to deserving people after examining their cases individually. This should naturally be extended to those serving in Colleges and Universities.

There should also be a provision for personal Professorships and Readerships so that the brightest of our young men do not apply here and there and move about from place to place in search of higher emoluments irrespective of the interests of their work.

To avoid any misunderstanding it may be stressed that while scientists can and sometimes should be taken on to administrative jobs, this ought not to be considered as promotion to a higher sphere of activity but a way of introducing into the administration a new element which is capable of doing useful work. Science is increasingly becoming an instrument of both national and international policy and scientists will naturally be called upon to a greater and greater extent to share the administrative work of the country ; however, those who want to continue with research and teaching ought to be able to get their appreciation where they are.

Our Low Output of Research

There is a consensus of opinion that, like other scientists, botanists in India hardly function to their full capacity. Some produce only about 33 per cent of their maximum potential and even the best and ablest are probably limited to about 50 per cent. This would be tragic in any country but is specially so in India where both food and money are scarce. The question is what is wrong and how can it be remedied?

One reason certainly is the very heavy demand made upon the time of the professors and heads of departments in the discharge of their administrative duties. In fact most of the research in University departments is

done by the Ph.D. students and by junior members of the staff not yet encumbered with meetings, management, a wife and children. We estimate that in any large department at least three hours of the Professor's time is devoted every day to correspondence, scrutinising quotations, placing orders, signing import licence forms and petty vouchers, preparing reports and time tables and answering audit objections. Rules drafted out of mistrust make it necessary that quotations must be invited even for buying half a ream of paper or a battery. While the block grants for the library and laboratory made by the University Grants Commission are most welcome, there has been no corresponding increase in the machinery for a proper utilization of the money. In addition there are increasing calls upon University teachers for service on committees of the ICAR, CSIR, UGC and many sister Universities. Some of the professors are also editing important journals on a purely honorary basis.

It should be pointed out that the more competent a man is, the more burdensome is the volume of administrative work he carries upon his shoulders, and however honest and hard-working he might be, he often suffers from a sense of frustration for not being able to get some quiet time for his own study.

This problem of reducing the load from the shoulders of the senior staff is one which in our opinion calls for serious thought on the part of all Universities and institutions. One remedy is of course the provision of increased clerical assistance. But a good deal more is wanted sometimes. When a botany department is specially large and has already distinguished itself by the quality and volume of its publications and the doctoral theses it has produced, it should be possible to increase the number of professorships and readerships and create a special post of an administrative secretary in the grade of say Rs. 250-25-560.

Certain Improved Facilities

While appreciating the increased grants made to libraries and laboratories during recent years it must be pointed out that we are still far behind other advanced countries and there is much difficulty in getting the desired apparatus owing to import restrictions. Sometimes months pass before a reply is received from the Controller of Imports. Even photographic goods, so essential for biological work, have been difficult to obtain at controlled rates.

An essential requirement for a botany department is a good botanical garden and a herbarium and there are Universities in the West like Munich and Cambridge which spend almost as much on their botanical garden alone as on the botanical laboratory. In comparison, the botanical gardens attached to our Universities are insignificant. Excepting perhaps only the Banaras University, the total area is seldom more than one or

two acres and there are just a couple of illiterate 'malis' to look after it. An area of about ten acres is required for a reasonably good botanical garden and this should be put in charge of a Curator with a knowledge of the botanical names of plants and the methods of growing them. Where it is not possible to find a plot of ten acres, it might be a good idea to place the entire University campus under the Botany Department so that instead of growing whole rows of certain trees a number of different types may be grown and properly labelled so as to serve a decorative as well as an educative function.

Most Botany Departments also lack a museum and this is true even of the largest Universities like Calcutta, Bombay, Madras and Delhi. For good teaching in Botany, a museum, garden and herbarium are essential adjuncts to the laboratory and it is a pity that so far only half-hearted attention has been paid to this and even in such a large country we have nothing even remotely comparable to the grand museums of Natural History at New York, Chicago and London, and the museums of Economic Botany in the Kew Gardens. Besides their obvious utility for students of botany, the museums have a great educative value for the general public.

Every University Botany Department should also have at least a herbarium of local plants but it is hardly possible to expect the overworked lecturers to build up such a collection by their own efforts. Wherever the herbaria already exist a Curator should be provided to maintain and enlarge the collections and this facility should be gradually extended to all Universities which show an interest in building up a herbarium and have a systematic botanist on their staff.

Workshops and Instrumentation

One of the weaknesses of Indian science is that our students (and even teachers) understand little of the apparatus they use, and repairing a faulty machine or devising a new make-shift is of course even more difficult. The only way to remedy this is to have a good central workshop in each University to be administered in rotation by the different heads of the science departments. This is not only to see that all departments receive equal attention but also to ensure that every head of a laboratory himself maintains some contact with instrumentation.

A central workshop in itself would not be adequate, however. In addition every large botanical laboratory should also have a small workshop of its own with a staff of two mechanics. Besides being useful to the teachers and research workers, contact with these men would naturally help the B.Sc., M.Sc., and Ph.D. students to become more "instrument-minded". In fact all post-graduate students should be encouraged to spend a few hours per week in the departmental workshop.

Technical and Laboratory Assistants

At present these posts are held mostly by untrained persons who pick up things here and there by experience. We are of the view that trained laboratory workers are an essential part of a university organization and those who adopt this career should not feel that the rewards are not comparable with what might be expected from similar work in other spheres.

As to the proportion of technicians to be employed in a laboratory, while the student or the younger teacher will gain enormously by having to carry out for himself such work as glass-blowing, photography, and the making of microscopic preparations, adequate technical assistance should be available to all senior members of the staff. Not only will this augment the number of trained technicians which the country will require in increasing numbers but it will enable a scientist to produce more work than he is able to do at present.

Pure and Applied Research: Wanted A National Biological Laboratory

While everyone agreed that in teaching botany proper emphasis must be laid on the applications of botanical knowledge in agriculture and plant husbandry, the feeling was strong that the government's attention was directed mainly towards applied research. University professors were frequently advised to find out a new medicinal herb or a method of making two blades of grass grow instead of one. While the usefulness of applied research is certainly there, there are already many organizations like the ICAR, IARI, and various agricultural stations and departments to look after this—frequently also separate institutes for important crops like rice, potato, maize, coconut, tea, coffee, and so on. It is good that their number is increasing, but what about the fundamental aspects of the subject for which there is only the most meagre support at present? In the USSR and Germany there are several well-equipped institutes for research in Plant Physiology, Genetics, Mycology, Plant Pathology, Microbiology etc., but there is nothing comparable in India. It seems necessary to make a beginning by having at least one National Biological Laboratory properly equipped and staffed so that students and teachers could go there and spend a vacation or half-year to learn new techniques. In addition the University botanical laboratories need to be strengthened by providing more equipment and increasing the number of senior teachers. Without this the training centres of our would-be scientists would languish and we shall merely be doing imitation research instead of advancing the frontiers of knowledge in any significant manner.

It is hoped that the founding of a National Biological Laboratory would be followed at a later date by other laboratories and institutes in different

regions of the country, dealing with specialized branches of botany and zoology. The National Biological Laboratory could, however, serve as a fountain head and a correlating agency for the laboratories to come. While there are so many laboratories in the country for physical sciences, it is surprising that there is no comparable institution for the entire field of biology!

The Indian Botanic Garden

The Indian Botanic Garden at Sibpur, Calcutta, is perhaps the largest in Asia. The gardens at Bogor (Indonesia) and Peradeniya (Ceylon) are also well known for their beauty and wealth of tropical plants, but these are smaller. For nearly two hundred years the Sibpur garden has been a centre of botanical and horticultural research. Some of the pioneer work on the flora of India, Burma and Pakistan was done at this place. The garden was also responsible for the introduction of many economic plants like cinchona and rubber. It has at present a collection of more than 12,000 living plants from all over the world including many ferns, orchids, succulents and aquatics ; and such plants which cannot thrive here can usually be grown in the Lloyd Botanic Gardens, Darjeeling. Both these gardens have been a most valuable source of plant material for teaching and research to Indian botanists and it is a pity that they are inadequately financed. The recurring grants for their maintenance should be greatly enhanced and at both Calcutta and Darjeeling there should be arrangements for cheap residential accommodation so that students and teachers can stay there for some time and study living plants instead of just spirit-preserved or dried material taken in a hurry to their head-quarters. At each place there should be laboratory facilities for at least 20 workers.

The Calcutta and Darjeeling gardens should be considered a national treasure and opinion was unanimous that their administration should be on an all-India rather than a provincial basis.

Excursion Grants ; Hill, Marine and Freshwater Biological Stations

One of the strongest reasons why our teaching in botany often remains rather dull is that neither teachers nor students have a chance to see or show what they are talking about. While botanists in England, Germany and France have organized repeated tours to distant countries including Africa, Indonesia, and South America, Indian botanists have little chance to see the rich variety of plants even in their own country. The Excursion grants of University botanical laboratories average only about Rs. 1,000 per year excepting only the Panjab University which allows the

expenditure of a sum nearly five times as much. The result it that neither teachers nor students have any regular opportunities to study the flora of the Himalayas, sea-shores, and lakes.

It is urgent that the grant under this head should be multiplied many times and a jeep or pick-up made available to botanical laboratories for collections within a forty or fifty mile radius of the University. At least in the larger towns this can no longer be considered a luxury for almost no natural vegetation or crops can be seen for a distance of several miles. The public conveyances are of little value because they do not go to jungles, nor stop at desired places. Further, all the members of a party can hardly expect to climb on to the same bus.

Although the Himalayas are a vast repository of herbs and trees of various kinds, it is no longer enough merely to see plants and collect them. They need to be studied in the living condition. For this it is desirable to have properly maintained hill gardens and laboratories where teachers and students could go and work for several weeks. There is already a good garden at Darjeeling but no laboratory. In the Western Himalayas, Mussoorie is favourably located owing to its proximity to the Forest Research Institute at Dehradun but it has so far neither a garden nor a laboratory. In South India, Mahabaleshwar and Kodaikanal are suitable. Detailed plans can be drawn up if the proposal is accepted in principle by the Government.

Approximately 95 per cent of all biologists are engaged in terrestrial biology and studies on marine organisms are often considered of secondary importance. However, it is now being increasingly realised that the flora of the sea is also of great importance, sometimes on its own account and sometimes in an indirect manner as food for fish. Students of botany should, therefore, have a chance to see something of what they read about sea-weeds and phytoplankton. For this the facilities at Mandapam and Okha need to be enlarged and new stations opened near the Chilka Lake (Orissa) and Dal Lake (Srinagar). At all the four places there should be residential facilities and laboratories for teachers and students.

At present most of the summer vacation is just wasted by our students. If at least those working for the Master's degree could be sent to the hill and marine laboratories, not only will their time be better utilized but they will learn a good deal which they otherwise miss.

International Cooperation

Considerable time was devoted to a discussion of exchange of specimens with botanists in other countries and it was felt that the present restrictions on movement of living plants and propagative organs were so severe as to result in death of a large proportion of material. The botanists pre-

sent were conscious of the need of quarantine measures when plants are imported in bulk for agricultural or horticultural purposes but a way has to be found of releasing the consignments as expeditiously as possible.

There is also another category of import of plants for purely experimental purposes. Such import of seeds and cuttings is on a very small scale only and since the exchange is between one botanist and another, no stringent quarantine seems necessary. Botanists are expected to send only disease-free material and a certificate from a Professor or similar authority from the country of origin should perhaps be adequate for the purpose.

There was also a prolonged discussion about permitting foreign botanists to collect materials in India and to take the collections with them for further study in their country. The opinion was clear that when once a case had been examined by the Ministry of External Affairs and permission given, a botanist should be free to make collections except in areas which are also not open to Indian nationals. The collections are his property but he may be requested to return one labelled set to the Indian National Herbarium. It can create only international ill will instead of good will if a group of botanists who have spent much money in bringing their own equipment and in planning their travels are forced to leave behind what they have collected with their own labour and money. Further, an unlabelled set of plants without full notes is of little value to anyone.

Many of the botanists present at Darjeeling recalled with great pleasure and gratefulness their experience in foreign countries when they were freely permitted to collect and bring what they wanted. Sometimes these were herbarium or spirit specimens, sometimes fossils, and sometimes microscopic slides for research. Wherever possible we should naturally reciprocate warmheartedly, for science is international and if our plants receive a detailed study by competent persons elsewhere it is also ultimately to our own benefit. For many groups of plants we still do not have any specialists in this country and have to depend on foreign help for their determination.

It must be made clear that the taking of plants for commercial purposes does not fall under this category and the Government will naturally see that there is no abuse of the facilities offered by it for genuine scientific studies.

Publication of a Science Newsletter

Some persons suggested that in a large country like India with more than 40 Universities and various colleges and institutes it would not be surprising if there was some duplication of work due to ignorance of the activities of other scientists. For this a science newsletter resembling the

CSIR bulletin would be useful. This should give news about the addresses and activities of scientists, their visits to foreign countries or return to India from abroad, and the problems of research pursued by them. The newsletter should appear once every month and its scope might later be enlarged if considered desirable.

Summer Schools, Exchange Professorships and International Conferences

All the botanists present at Darjeeling appreciated the opportunity that was given to them to meet and carry on scientific discussions with their colleagues in an informal atmosphere and the cool and pleasant surroundings of Darjeeling. As a suggestion for future summer schools it was mentioned that the decision ought to be communicated to the participants five or six months in advance so that they can prepare their papers with more thoroughness.

It was also suggested that facilities should be available to such Indian botanists who have already done significant research to go abroad on study leave once every five years. It was pointed out that while young students were going abroad in large numbers often for work which could or should be done right in the country, older people had fewer opportunities of learning new techniques by working in foreign Universities.

As the number of specialists in different branches of botany is by no means large, an effective way to utilize their services more fruitfully is to institute exchange professorships whereby a professor or scientist of one place can be invited to give a course of six or seven lectures at another. It was also felt that it is not enough to have foreign scientists come to this country and spend a large amount of time and money in visiting places of cultural interest and in going from one end of the country to another, staying for a day or two at each place. They should be attached to the same laboratory for a period of at least a week so that we can learn something from them instead of merely "showing them round".

Finally, attention was also called to the need for better representation of Indian botanists at International Conferences which are of great value not only because of what they offer in the way of the reading of papers and taking part in discussions but because of the facilities they provide for personal contacts.

The meeting closed with an appreciation of the present opportunity which was most stimulating and a suggestion that the summer school ought to be a recurrent feature every year and certainly not less than once every two years.