

THE PLACE OF ANGIOSPERM EMBRYOLOGY IN RESEARCH AND TEACHING*

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In the history of Angiosperm Embryology there have been three distinct periods : the *first* in which the chief aim was to unravel the fundamental facts regarding the development of the pollen and embryo-sac, and the processes of fertilisation and seed formation ; the *second* in which interest centred largely round a study of comparative embryology and an evaluation of the data thus obtained for the improvement of the existing systems of classification ; and the *third* and most recent in which Embryology has become an experimental science like Physiology and Cytology, where one tries to study such problems as the storage of pollen and its germination, the receptivity of the stigma, fertilisation and fruit-setting, etc., and the optimum conditions required for them.

DESCRIPTIVE EMBRYOLOGY

It is not necessary to spend much time on the first of these, *i.e.*, Descriptive Embryology, as most of the facts relating to the course of development of pollen, embryo-sac, endosperm and embryo had become clear towards the close of the last century through the efforts of Amici, Schleiden, Hofmeister, Strasburger, Treub, Guignard, Nawaschin and others, and are now a commonplace in all textbooks of botany. A very good summary of this work was given by Coulter and Chamberlain in the year 1903 and it was followed later by the publication of Schnarf's (1929) "Embryologie der Angiospermen", which is at present the most important and exhaustive treatise on this subject. Although little that is fundamentally new has probably been discovered since then, many errors and misinterpretations made by previous workers have been corrected and a mass of valuable information has been added regarding certain details concerned with the formation of the male gametes, the types of embryo-sac development, the cytology of fertilisation, the origin and function of endosperm haustoria and the development of the embryo. Work of this type is still in progress but the results will not be proportionate to the time spent unless a worker devotes his attention to just one aspect of the life-history in which he is most proficient and studies this in as many plants as possible. It is in this way that Finn in the Ukraine and Wulff in Germany and recently also some workers in the U.S.A. have been able to discover a number of important facts on the structure and development of the male gametes and Souèges in France on the development of the embryo in a large number of angiospermous families.

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PHYLOGENETIC EMBRYOLOGY OR EMBRYOLOGY IN RELATION TO
SYSTEMATIC BOTANY

In the second period, which may be said to have commenced with the beginning of this century, embryology began to be used as an aid in the improvement of our systems of classification, the most important contributions in this line having come from Sweden (Stockholm, Uppsala and Lund), Germany (Bonn, Berlin and Vienna), and the U.S.A. (Chicago, Baltimore and California). A great impetus was given to such studies by the publication of Schnarf's excellent handbook entitled "Vergleichende Embryologie der Angiospermen" in which the author has summarised the existing state of our knowledge of the embryology of each family and added a number of valuable suggestions and comments at the end of each order. Most of the embryological work done in India has followed the publication of this book, to whose author we owe a debt of gratitude which cannot be expressed adequately in words. Although I alone among the Indian embryologists have had the privilege of working with Prof. Schnarf, yet all have gained considerable inspiration and insight into this difficult field through the medium of his publications.

As the value of embryology in questions relating to systematic botany does not appear to be sufficiently appreciated in this country by those who are engaged in other lines of study, it is necessary to consider this aspect in some detail.

It is a matter of common knowledge that on the basis of external morphology of the vegetative organs a genetical relationship may sometimes be inferred to exist between plants which belong to widely separated groups (*Equisetum* and *Casuarina*; *Ephedra* and certain *Asclepiads*; some cacti and *Euphorbias*). Taxonomists therefore take recourse to the flower as it is a more conservative organ than the stem and leaf. But, if we assume phyletic trends in the external morphology of the flower, why not in the internal structures, for these must be still more conservative (being less amenable to environmental influences) and therefore of special value in judging the proper position of certain doubtful groups? I am told that the zoologist would ordinarily refuse to assign an unknown animal to its systematic position until he has had an opportunity of examining its internal organs. No one can doubt that the same should be done with plants also, and if anything has prevented *us* from applying the anatomical and embryological method on a large scale, it is only the greater labour involved in it. The work *has* however to be undertaken now on a larger scale than ever as the systematist has taken us almost as far as he could towards our goal of a natural system of classification and can hardly make much headway without our help and co-operation.

Before proceeding further I must now enumerate such characters in the embryology of an angiosperm which are usually considered to be of major value in delimiting the larger plant groups:—

1. *Anther tapetum*.—Whether it is of the glandular or the amœboid type.

2. *Quadripartition of the microspore mother-cell*.—Whether it takes place by furrowing or by the formation of cell-plates.

3. *Development and organisation of the male gametophyte*.—Number and position of the germ pores and furrows; adornments of the exine; place of formation of the generative cell; number and shape of the nuclei in the pollen grain at the time of its discharge from the anther.

4. *Development and structure of the ovule*.—Number of integuments and the alterations in structure which they undergo during the formation of the seed; presence or absence of vascular bundles in the integuments; shape of the micropyle, whether it is formed by the inner integument or the outer or both; the presence or absence of an obturator.

5. *Form and extent of the nucellus*.—Whether it is broad and massive or thin and ephemeral; presence or absence of a hypostase; the place of origin of the integument or integuments, whether close to the apex of the nucellus (as in the Rubiaceæ) or near its base (as in the Orchidaceæ); persistence or disappearance of the nucellus in the seed.

6. *Origin and extent of the sporogenous tissue in the ovule*.—Nature of the archesporium, whether it is one-celled or many-celled; presence or absence of wall layers; the presence or absence of periclinal divisions in the cells of the nucellar epidermis.

7. *Megasporogenesis and development of the embryo-sac*.—i.e., to which of the following main types or its modifications does it correspond: Normal, *Oenothera*, *Allium*, *Peperomia*, *Fritillaria*, *Adoxa*, *Plumbago*, *Plumbagella*, etc.?

8. *Form and organisation of the mature embryo-sac*.—Shape of the embryo-sac and the number and distribution of its nuclei; an early disappearance or otherwise of the synergids and antipodal cells; increase in number of antipodal cells, if any; formation of haustoria, if any, from some part of the embryo-sac.

9. *Fertilisation*.—The path of entry of the pollen tube; the interval between pollination and fertilisation; any tendency towards a branching of the pollen tubes during their course to the ovule.

10. *Endosperm*.—Whether it is of the nuclear, cellular or Helobiales type, and direction of laying down of the first wall in such cases where it is cellular; presence or absence of endosperm haustoria and the manner in which they are formed if present; nature of food reserves in endosperm cells.

11. *Embryo*.—Relation of the proembryonal cells to the body regions of the embryo; form and organisation of the mature embryo; presence or absence of suspensor haustoria.

12. *Certain abnormalities of development*.—Apomixis, polyembryony, parthenogenesis, etc.

While these are the most important characters usually taken into account in systematic studies, there are many others which it is

difficult to put down in writing. Indeed, as a very competent embryologist (Mauritzon, 1939) recently remarked, the resemblances and differences in the embryological characters of the members of a family are sometimes of such a fine type, that they can neither be brought out in words nor even in a drawing but can only be appreciated under the microscope. He nevertheless considers them to be of distinct value in delimiting the smaller groups and in determining their interrelationships with one another.

Let us now take some specific instances where embryology has rendered an important service in the determination of the proper position of some difficult families or in giving a new orientation to our ideas of their affinities.

The relationships of the family *Empetraceæ* exercised the minds of systematists for a long time and it has been placed by some authorities in the Monochlamydeæ, and by others in the Sapindales or the Celastrales. Samuelsson's work (1913) has definitely shown however that its proper place is with the Bicornes, a group which is characterised by the following well-marked embryological features:—

1. Absence of a fibrous layer in the anthers.
2. Presence of a glandular tapetum which does not become amœboid.
3. Pollen grains remaining together in tetrads.
4. Ovule with a single integument and a thin ephemeral nucellus which completely disappears in later stages so that the embryo-sac lies in direct contact with the integumentary tapetum.
5. Absence of parietal cells in the ovule, the hypodermal archesporial cell functioning directly as the megaspore mother-cell.
6. Embryo-sac of the monosporic eight-nucleate type with small ephemeral antipodals.
7. A hollow and fluted style which connects the lumen of the ovary with the outside and along which the pollen tubes make their way into the ovary.
8. Endosperm cellular, the first two divisions being transverse and giving rise to a row of four cells placed above one another.
9. The formation of endosperm haustoria at both ends of the embryo-sac, micropylar as well as chalazal.
10. A single-layered seed-coat formed from the outermost layer of the integument, the remaining layers becoming absorbed during the growth of the embryo-sac and embryo.

All these are perfectly standard stages in Erican embryology, a combination of which is not known to occur in any other order except the Bicornes. The *Empetraceæ* show a close correspondence in all respects, while the Sapindales and Celastrales differ from them (Bicornes) in so many ways that Samuelsson may be said to have established his point of view fully and completely. Hutchinson's assignment of the *Empetraceæ* to the Celastrales is therefore considered

by Schnarf (1933, p. 283) to be due to nothing but a "ganz besonderer Verständnislosigkeit".

On the other hand, the Lennoaceæ, which have sometimes been placed in the Bicornes (Hutchinson, 1926) certainly do not belong here. In the very first place, the equality in number of their stamens and corolla-lobes (contrasted with the obdiplostemony of the Ericales), the alternation of the parts, the adnation of the filaments to the corolla and the dehiscence of the anthers by longitudinal slits, form a weighty objection against this view. Add to these the fact that the Lennoaceæ have a short and solid style, a normally developed endothecium, pollen grains separate from each other, and a seed-coat which is more than one-layered. Svensson (1923) and Copeland (1935) therefore correctly consider the assignment of the Lennoaceæ to the Bicornes as quite untenable on embryological as well as other grounds and suggest that they might more reasonably be placed among the Tubiflorales as a separate suborder occupying a primitive position.

Let us now pass on to another group, the Cactaceæ. F. Vaupel (1925), in the latest edition of *Engler-Prantl's Pflanzenfamilien*, writes that there is hardly a family in the plant kingdom, the allocation of which has allowed so much scope to individual tastes as this. Wettstein placed it in the Centrospermales and Engler-Prantl in a separate order Opuntiales near the Passifloraceæ. Hutchinson has erected the order Cactales and placed it closest to the Cucurbitales.

The views of the great Viennese systematist have received very definite confirmation in this respect from the works of two embryologists; Mauritzon (1934) and Neumann (1935). Although additional work on this family would be welcome, the following features seem to be well established:—

1. A secretory tapetum of parietal origin.
2. Division of pollen mother-cells of simultaneous type.
3. Pollen grains tri-nucleate at the time of shedding.
4. Ovules campylotropous with strongly curved and massive nucelli.
5. Two integuments; and the swollen lips of the inner, which alone forms the micropyle, protruding out to approach the funiculus.
6. A hypodermal archesporial cell which cuts off a wall cell.
7. A nucellar cap formed by periclinal divisions of the cells of the nucellar epidermis.
8. Embryo-sac of the Normal type.

Several other characters, for which a reference may be made to Frl. Neumann's original paper, point to the conclusion that the Cactaceæ belong to the Centrospermales and form a sort of bridge between the Aizoaceæ and Portulacaceæ. An interesting point, which has probably been overlooked by several workers but is nevertheless of considerable importance is the presence, in the chalazal part of the ovule, of a "Hohlraum" or "Luftspalt" between the two integuments and sometimes also between the inner integument and the nucellus.

This is quite distinctive of a number of other families belonging to the Centrospermales and its occurrence in the Cactaceæ is therefore of great significance.

On the other hand, a comparison of the embryology of the Cactaceæ with that of the Passifloraceæ offers so little by way of resemblance that any close relationship between them appears to be most unlikely.

Take again the Onagraceæ, in which the genus *Trapa* has long been considered to occupy a somewhat anomalous position. All the plants of this family so far investigated show a monosporic four-nucleate embryo-sac, *Trapa* alone being an exception with an eight-nucleate embryo-sac and a well-developed suspensor haustorium. From the embryologist's standpoint this strongly supports the case for a removal of this genus to a separate family—a course which has now been adopted by some systematists by erecting the family Hydrocaryaceæ for its reception. Regarding the relationships of the Onagraceæ with other families of the order Myrtales, it seems very likely that it has been derived from the Lythraceæ (Tischler, 1917) in which the ephemeral antipodals show the way to a complete omission of the chalazal part of the embryo-sac, leading to the four-nucleate condition of the Onagraceæ. I understand that this view is not challenged by systematists.

In Prof. Schnarf's laboratory at Vienna, some very important work has been done on the embryology of the Liliaceæ and Amaryllidaceæ, which has a great bearing on the interrelationships of the various sub-families and tribes included under these large and difficult families.

Taking the sub-family Lilioideæ, Engler (1888) divided it into the tribes Tulipeæ and Scilleæ. Schnarf (1929) stressed the sharp embryological differences between them, and in the second edition of the "Natürlichen Pflanzenfamilien" Engler and Prantl (1931) removed the Scilleæ from the association giving it the status of an independent sub-family, the Scilloideæ, so that the name Lilioideæ is now synonymous with the former Tulipeæ. Frl. Rosalie Wunderlich, a pupil of Prof. Schnarf, has again (1937) stressed the great contrast between the two (see table below) and even hinted at the desirability of separating them into two distinct families. She rightly points out that in more than one respect the Lilioideæ appear to be a derived group while the Scilloideæ are more primitive; the latter should therefore be placed *before* the former and not after as it has been done by Engler and Prantl.

Dr. Wunderlich further adds that the Scilloideæ themselves fall into two tribes: one with the Helobiales type of endosperm (*Ornithogalum*, *Muscari* and *Puschkinea*) which she calls the *Ornithogalum* group and the other with the *Nuclear* type including the genera *Hyacinthus*, *Scilla*, *Camassia* and *Galtonia*.

<i>Scilloideæ</i>	<i>Lilioideæ</i>
1. Parietal cell always present in ovule	Parietal cell absent
2. Embryo-sac of <i>Normal</i> or sometimes <i>Allium</i> type	Embryo-sac of <i>Fritillaria</i> type

*Scilloideae**Lilioideae*

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| 3. Endosperm of <i>Nuclear</i> or <i>Helobiales</i> type | Endosperm of <i>Nuclear</i> type |
| 4. Embryo large, occupying almost the entire length of the seed | Embryo small (<i>Tulipa</i> , <i>Lilium</i> , <i>Fritillaria</i> , <i>Erythronium</i> , etc.) and occupies only a small space in the seed |
| 5. Generative cell small and slender, not easily stainable with acetocarmine | Generative cell large and broadly spindle-shaped, staining easily with acetocarmine |
| 6. Male nuclei \pm spherical | Male nuclei \pm elongated |
| 7. Chromosome no. variable | Chromosome no. usually 12 |
| 8. Raphides present | Raphides absent |
| 9. Septal nectaries present | Septal nectaries absent |

The systematic position of the Moringaceae has long been a matter of some doubt. The astonishing observations of Rutgers (1923) on the embryo-sac and embryo of *Moringa oleifera* only increased this element of uncertainty. He reported in this plant the absence of a parietal cell in the ovule and the presence of a five-nucleate embryo-sac and a free nuclear embryo. My pupil, Prof. V. Puri of Meerut (1940), has shown that a parietal cell is *present*, the embryo-sac is of the normal *eight-nucleate* type and what Rutgers considered to be a free nuclear embryo is merely a group of some *endosperm nuclei* at the micropylar end of the sac, the fertilised egg having escaped his notice altogether! The resemblances which the Moringaceae show to the Capparidaceae in embryology and carpel morphology make it seem fairly certain that their correct position is in the order Rhœadales and the place assigned to this family by Hutchinson—between the Capparidaceae and Tovariaceae—is therefore justified.

Although provisionally placed with the Rosales, there has always been some doubt regarding the interrelationships of the Podostomaceae with the other families of this order. The extensive work on the embryology of the Crassulaceae and Saxifragaceae done a few years ago by Mauritzon (1933) has however brought out certain features which make it almost certain that the Podostomaceae are much reduced apetalous derivatives of the Crassulaceae. The peculiar structure of the ovules of the former appears to be brought about merely as the result of a continuation of the reduction already seen in the Crassulaceae and *Crassula aquatica*, in particular, whose mode of life is somewhat similar to that of the Podostomaceae and which has the most reduced endosperm in the Crassulaceae, may well form a transitional stage leading to the complete suppression of this tissue in the Podostomaceae. A striking agreement between the two families is the presence of a highly developed suspensor haustorium, a feature which in Mauritzon's (1939, p. 38) opinion offers "such an eloquent proof" of their relationship "as to convert many doubters",

It is possible to add numerous other instances where embryology has rendered signal service to systematic botany, but considerations of time and space forbid me from citing them here. I believe that a stage has now arrived when we should try to have an embryological formula for each family as a supplement to the well-known floral formula so commonly used by systematists. To make my meaning clear I give below the embryological formula of the family Alismaceæ with which I have been particularly familiar as the result of the work done on it by my pupil Dr. B. M. Johri of Agra and later by Balwant Singh and myself (Maheshwari and Singh, 1943) at Dacca :—

ANTH.-TAP. (amœboid) ; DIV. OF P.M.C. (succ.) ; P. (3-nucl.) ; OVULE (2-integ. ; anat.) ; PAR. CELL (absent) ; E.S. (*Allium* T.) ; END. (*He.* or *Nu.* T.) ; EMB. (*Sag.* T.).

Put in plain English this means that the anther tapetum is of the amœboid type ; the divisions of the pollen mother-cells are successive ; the pollen grains are 3-nucleate at the time of shedding ; the ovule is anatropous and has two integuments ; no wall cell is cut off by the primary archesporial cell which functions directly as the megaspore mother-cell ; the embryo-sac development is of the *Allium* type ; the endosperm is of the *Nuclear* or *Helobiales* type ; and the embryo is of the *Sagittaria* type.

It may be possible by means of further abbreviations to include this information in a still shorter space and to make other improvements so as to devise a symbolisation which will be internationally acceptable. I suggest that this point may be discussed, by those who are interested, in the pages of one of our monthly journals like *Current Science*.

The embryologist would however be glad to admit that he lays no claim to erect a phylogenetic scheme of his own. Indeed there are some very definite limitations to the embryological method, for, owing to parallel, convergent and regressive evolution, similar embryological characters may often be found in widely separated groups and if a system of classification were to be set up on such considerations alone, some rather fantastic results are bound to ensue. But, with the main lines of phylogenetic classification already chalked out by the systematist, it is possible for the embryologist, the cytologist and the anatomist to use this as a background and to help him in making it more perfect. A natural system has to be discovered (for it is already there) and not invented. In order to do this we have to do real detective work and take the aid of every branch of botany. Once a group has been assigned to its true place, every character that is studied will only serve to strengthen its position. On the other hand, if there are any discrepancies, they will be brought out in a more glaring fashion by the study of its internal structures (as these are less influenced by the environment) than the external.

I understand that in some of the big herbaria of the U.S.A. steps are being taken to have along with the dried specimen a preparation or two showing the structural features of its wood. This is a laudable effort, but it ought to be extended still further so as to include

in each case about half a dozen preparations of the pollen, ovule and seed as well.

APPLIED AND EXPERIMENTAL EMBRYOLOGY

Now we come to Applied Embryology which has evolved, for the most part, only during the last two decades or so. It is hardly possible to do any justice here to this subject, for although still in its infancy, it already has such a voluminous literature as to defy any attempt to review it in a few pages. I shall therefore satisfy myself merely by indicating some of the main lines on which work is being carried on in this field.

Before any improvement of our crop plants can be undertaken through breeding methods, it is necessary to have in *each case* a thorough understanding of the behaviour of the flower throughout its development and the setting of the fruit. Of the greatest importance in this connection are :—a study of the viability of the pollen and the optimum conditions for its storage and germination ; the receptivity of the stigma ; the rate of pollen tube growth under different conditions of temperature and humidity ; the interval between pollination and fertilisation and how it can be influenced by external conditions ; and the quantity of pollen necessary for a proper fruit set. Considerable work of this nature is being done in America and Russia, regarding the immense value of which in our breeding programmes there can be no question.

It is said that the Arabs put aside the pollen of the date palm from year to year so as to ensure a supply of dates even in the possible event of the male flowers failing to develop or the female flowers developing precociously. If we succeed in devising suitable conditions of temperature and humidity for storing the pollen of other cultivated plants, which normally is not so long-lived, we may be able to cross two varieties which flower on widely different dates or which are separated by considerable distances from each other. In the latter case it may be possible to transport the pollen by air from one place to another. We may hopefully envisage the possibility of opening one or more "pollen banks" in each country, where pollen of almost every important plant of economic value will be stored under optimum conditions and supplied to recognised workers, gratis or on a moderate charge.

Sterility and unfruitfulness are often caused by a very slow growth of the pollen tube. The flowers do not remain attached to the plant for an indefinite period and unless fertilisation takes place within a reasonable time, varying with the species under consideration, abscission takes place at the base of the style and fruit setting is consequently prevented. Premature as well as delayed pollination have the same result and we therefore need full information regarding all of our fruit trees and crop plants on the rate of pollen tube growth and the time when the stigma is most receptive. The optimum conditions for the germination of pollen also need to be investigated more fully. Tischler's (1910) discovery that much of the pollen of certain species of *Cassia* occurring at Buitenzorg fails to develop without an

outside supply of diastase illustrates the need of such work from various points of view.

Another aspect of applied embryology is a study of the possibility of obtaining a fruit set without the generally associated seed formation. There are a number of our edible fruits where the pericarp is the chief edible portion and the presence of seeds is neither necessary nor desirable. It was found possible in several cases to do away with the fertilisation of the egg cell and give the ovary the necessary stimulus for further development by the application of pollen extracts. This led to a chemical analysis of the latter which in turn opened the way for the induction of artificial parthenocarpy through the use of growth hormones (for literature see Maheshwari, 1940; Gustafson, 1942).

A very important paper was published in 1928 by C. A. Jørgensen, which showed the way to the induction of parthenogenesis in flowering plants. He pollinated the stigmas of *Solanum nigrum* with pollen from some other species of this genus. Most of the fruits were seedless but a few were found to have formed 2 to 8 seeds which gave rise to haploid plants of *Solanum nigrum*. An embryological study showed that in certain cases the foreign pollen had germinated successfully and the pollen tubes had also reached the embryo-sacs, but the male nucleus which enters the egg cell eventually disintegrates and disappears. The egg cell thus develops by itself into the embryo, stimulated no doubt by the entry of the male nucleus. The plants produced from the resulting seeds are therefore haploids resembling the maternal parent. In other cases, apparently, the male gamete alone may give rise to the embryo as inferred from the characters of the offspring which resembles the paternal parent but as far as I am aware the cytological and embryological processes leading to this condition are still unknown. Jørgensen's work opened the way towards the artificial production of haploids in a number of species and races. Although weak and valueless in themselves, they are of great utility in giving us an insight into the genetic constitution of the parent variety and for the production of homozygous diploids by a subsequent doubling of the chromosomes.

The effects of X-rays, colchicine treatment and exposure to extreme temperatures on the normal course of development are other aspects of recent cytoembryological research, which it is impossible to deal with here. It need only be said that while a fair amount of work has been done on the manner in which they influence ordinary mitotic and meiotic divisions, we are still very much in the dark about their effect on megasporogenesis, fertilisation and the development of other ovular structures like the endosperm and the embryo.*

THE PLACE OF EMBRYOLOGY IN BOTANICAL TEACHING

At the end of this very short and imperfect sketch of the aims and scope of embryological research we may now consider another aspect of the subject, e.g., its place in botanical teaching. That a study of embryology demands a more thorough training in microtechnique

* The important work, which is being done by Blakeslee and others on the artificial culture of excised embryos, will be reviewed elsewhere.

than is usually needed for other branches of plant science is a fact well known to everybody and it is perhaps for this reason that while much time is spent in our class-rooms on vegetative anatomy, in several universities little or nothing is shown to the students concerning angiosperm life-histories. A great opportunity is lost thereby of training them in the art of observation, reconstruction and critical interpretation. While granting that it is the advent of the microtome, as an instrument of precision in making serial sections, that has done so much for the recent progress in this science, it is not to be imagined that an elaborate scheme of fixing, dehydration, infiltration, imbedding, cutting, and staining is necessary in all cases. Much can be seen and shown by simpler methods. All stages of microsporogenesis and the maturation of pollen grains can be observed by making smears of suitable materials like *Tradescantia*, *Gloriosa*, etc., stained with either gentian violet or Feulgen (for technique see Darlington and La Cour, 1942). It is possible to mount the pollen grains of *Ottelia* and *Hydrilla* in acetocarmine and make a preparation, showing the vegetative and generative (or sperm) cells, even under the low power, in less than 5 minutes. Anthers of even herbarium specimens are often quite usable for such purposes (see Leitner, 1938). Some years ago Dr. Wulff and I (Maheshwari and Wulff, 1937) gave a schedule for making permanent mounts of pollen tubes to show the division of the generative cell and the organisation of the male cells. The common garden species of *Impatiens* is very good for this purpose as the pollen grains germinate readily and show the desired stages in only half an hour's time. Some of the Pontederiaceæ like *Monochoria* and perhaps several other plants may be equally suitable for the purpose. With the use of vital stains such slide cultures of pollen tubes may be used for studying the movement of the cytoplasm and the male gametes.

An observation of the stages in megasporogenesis and embryo-sac formation involves greater difficulties as the cells concerned are encased in several layers of other cells belonging to the nucellus and integuments, but Hillary (1940) has recently developed a technique by which he has been able to follow the development of the embryo-sac of *Lilium longiflorum* right from the megaspore mother cell up to the time of fertilisation and beyond, without cutting any sections. The ovules are here taken out from the ovary and the tissue around them removed as far as possible. Then they are fixed, washed, and stained with Feulgen's reagent in small vials or tubes. From the SO_2 water they are transferred to a drop of acetic acid placed on a slide and crushed under a coverslip. The author presents photomicrographs made from such preparations, which show the nuclei and chromosomes standing out quite distinctly in the colourless cytoplasm of the embryo-sac.

In his work on *Notonia grandiflora*, Ganesan (1939) used a somewhat similar method in order to select material of the right age for a study of the reduction divisions in the ovule. In this case an ovule is dissected out and mounted in a drop of acetocarmine mixed with an equal quantity of 1% safranin in 50% alcohol. By gentle and gradual increase of pressure on the coverglass, the nucellus is now freed from the

thick integument and in about half an hour's time the megaspore mother-cell nucleus is adequately stained for the purpose. By this method the author was able to exercise some judgment at the time of fixing the material and save much labour which would otherwise have been wasted if the cutting had been done at random. Poddubnaja-Arnoldi's (1938) "rapid method of embryological investigations" is essentially similar except that she recommends a mixture of acetocarmine and glycerine. She was thus able to follow the development in some plants upto the first stages in the formation of the embryo.

Suitable material for watching the process of fertilisation without recourse to section cutting has not yet been found* but one may try for this purpose such plants as *Torenia* and *Utricularia* in which the nucellus degenerates early and the upper part of the embryo-sac protrudes out of the micropyle so that it is naked and therefore more readily observable. The styles and stigmas of *Portulaca*, *Ottelia* or *Monochoria* should also be examined in order to follow the course of the pollen tube from the stigma to the ovule. A treatment with lactophenol and cotton blue often facilitates such observation.

That certain interesting features of endosperm morphology can be brought out more clearly from whole mounts of suitably dissected material than from sections, is shown by the work of Kausik (1939) on *Grevillea*. Dr. Kausik discovered in this plant a curious "worm-like" structure, which he calls the "vermiform appendage", formed by the chalazal part of the endosperm. This was missed by earlier observers since they used only sections which naturally fail to give any complete or intelligible picture of this tortuous organ.

There is perhaps no way of studying the development of the embryo except by cutting thin sections of the ovule but favourable material may yet be discovered in which the technique so successfully used by Buchholz (1938) in the study of conifer embryogeny will be found adaptable for at least some stages of this process. Also, in certain cases the seed coat may be so transparent (B. G. L. Swamy tells me that this is the case in many orchids) that it is possible to see the embryo in whole mounts of the seed without recourse to sectioning.

Let me explain why I am so keen that students should cut sections and make whole mounts or try other methods so as to see the entire process of development of the gametophytes and embryo in angiosperms as an essential part of any course in botany. This is because there are few other spheres of botanical study which offer a similar variety of technical problems or opportunities for the development of a critical attitude which is the most important quality that a young worker must learn to imbibe. I hope to be excused for citing here the case of a student who, having just taken the Master's degree, placed before me with great satisfaction a set of slides in which he claimed to have seen "all" stages of the development of the embryo-sac—1-nucleate, 2-nucleate, 3-nucleate, 4-nucleate, 5-nucleate, 6-nucleate,

* *Monotropa*, which is said to be very favourable for this purpose, is unfortunately not available in this country except in the hills and other inaccessible places, and the plant is not amenable to cultivation owing to its saprophytic habit.

7-nucleate and 8-nucleate. When the preparations were scrutinised it was found that all the sections were of mature embryo-sacs, but as the nuclei were spread apart into several sections, they were counted as they came, some here and others there, and imagined to be *stages in development* of an embryo-sac. Again, scores of students get away through a university course with the impression, gained from a study of book figures, that the integuments are lateral processes developing from the right and left sides of the nucellus, although a cross-section of the ovule or a whole mount of the same would have easily convinced them that it is not so.

I should add that it is not merely the student who makes mistakes but that even the experienced researcher is liable to be misled into such erroneous interpretations as may in some cases ruin his reputation as a scientific worker. Indeed, there are so many pitfalls in the correct interpretation of the material that the embryologist must always remain as watchful and alert as the worker on fossils. As an instance may first be cited the case of the *Lilium* embryo-sac whose development was repeatedly and very intensively studied by the most competent workers like Strasburger, Coulter, Mottier, Guignard and others. And yet, all of them were mistaken as the excellent work of Bambacioni (1928) showed a few years later. The embryo-sac of *Plumbagella*, long considered to be the most reduced among angiosperms, has turned out to be but a modification of the type seen in *Fritillaria* and *Lilium* (Fagerlind, 1938; Boyes, 1939). *Plumbago* has also been shown to have a development very different from that originally described for it by Dahlgren (see Haupt, 1934) and this has now been confirmed for another member of the same family, *Vogelia indica*, found in Rajputana (Mathur and Khan, 1941). In *Euphorbia heterophylla*, Sanchez (1938) recently reported a tetrasporic embryo-sac which has on reinvestigation turned out to be of the normal monosporic type (Maheshwari, 1942). Then again, the embryo-sac of *Rudbeckia*, which Palm (1934) believed to be of an entirely new type has been found to correspond with the now well-known *Fritillaria* type (Maheshwari and Srinivasan, 1944).

Another kind of error which is of frequent occurrence is the mistaking of the integument for the nucellus or *vice versa*. Even so recently as 1938 Houk fell into such an error in the case of the ovule of *Coffea* and in his confusion stated that the tissue may be regarded as an "integument-nucellus". Joshi (1938), Mendes (1941) and several other workers have shown that the nucellus and integument are both formed normally but the former soon disappears as is usual in most Sympetalæ. A similar mistake appears to have been made by Pannochia-Laj (1938) who writes that in *Lochnera rosea* the ovule is peculiar in that it is not possible here to delimit the nucellus from the integument. In another genus, *Fouquieria*, the structure which was supposed to be a "massive" nucellus is really the inner integument, the former being extremely reduced and ephemeral (see Khan, 1943). Evidently Dr. Woodcock (1943) has also been misled when he says that in *Ipomæa rubro-cærulea* the ovule "has no distinct integument" and the micropyle is formed by an "invagination at the end of the ovule next to the funiculus (see Maheshwari, 1944b).

The origin of the haustorial processes in the ovule has been another fruitful source of errors and misinterpretations. To mention only two such cases, Heinricher (1931-32), in his monograph on the genus *Lathraea*, stated that the micropylar haustoria are formed from the synergids and the chalazal from the antipodal cells. This was promptly contradicted and disproved by Glišić' (1932) who made a thorough study of *Lathraea squamaria* and found that both the haustoria are formed from the endosperm. A similar mistake made by G. O. Cooper (1942), working on *Lobelia cardinalis*, has already been commented on by me a few months ago (Maheshwari, 1944a).

Without citing further instances, I shall conclude this portion of my address merely by saying that even if the laboratory work in this subject makes greater demands upon the energy and resourcefulness of the teacher, this should not be grudged, as through this the young pupil gets such a stimulus for his mental development as is sure to be of use to him ever afterwards in his future career.

THE FUTURE

And now we proceed to the future.

It is said by some that the days of descriptive embryology are now over. This is far from true in my opinion. We need more of such investigations and will continue to do so for a long time. What needs to be emphasized, however, is that the descriptions must be full and accurate and the interpretations checked as critically as possible with preparations of the highest quality. As I have mentioned earlier, results of greater value may be expected if attention is focussed on a comparative study of only one aspect of the life-history at a time, viz., male gametophyte, ovule, embryo-sac, pollen tube, etc. Each of these requires the study of a vast amount of literature and sometimes a technique different from that used for the rest.

With respect to phylogenetic embryology there is a great scope in our country, for we have representatives of a number of families in India, Burma and Ceylon, which have either received little or no attention or which deserve more intensive study than has so far been bestowed upon them. I mention below the names of a few but the list is by no means exhaustive and is capable of amplification :—

Aristolochiaceæ	Loranthaceæ	Cyperaceæ
Balanophoraceæ	Magnoliaceæ	Dioscoreaceæ
Berberidaceæ	Myristicaceæ	Eriocaulaceæ
Burseraceæ	Myrsinaceæ	Flagellariaceæ
Callitrichaceæ	Nepenthaceæ	Hamodioraceæ
Ceratophyllaceæ	Nymphæaceæ	Juncaceæ
Cornaceæ	Pittosporaceæ	Lemnaceæ
Crypteroniaceæ	Podostemaceæ	Marantaceæ
Dilleniaceæ	Salvadoraceæ	Najadaceæ
Dipterocarpaceæ	Simarubaceæ	Palmaceæ
Droseraceæ	Sterculiaceæ	Pandanaceæ
Ebenaceæ	Symplocaceæ	Stemonaceæ
Flacourtiaceæ	Thymelæaceæ	Triuridaceæ
Fumariaceæ	Zygophyllaceæ	Xyridaceæ
Hippocrateaceæ		Zingiberaceæ

A thorough investigation of so many families requires much time and patience and the participation of a band of workers properly trained in embryological methods. Fortunately we have a number of qualified embryologists at Mysore, Bangalore, Benares, Annamalainagar, Poona, Madras, Agra, Meerut, Calcutta and other places, and I venture to hope that, with the co-operation of some of our colleagues in Europe and America, we may be able to prepare in this country a new "Comparative Embryology of Angiosperms" written more or less on the lines of Schnarf's great work (now 15 years old) in which each worker will write an exhaustive and critical account of the embryology of the particular group with which he is most familiar as the result of his *own* researches, for the literature on the subject is now too vast to be surveyed in a satisfactory manner by any one person. Students of wood structure like Record, Bailey and Wetmore, of chromosomology like Tischler, and floral anatomy like Eames and Arber are working towards the same end for their particular subjects. I expect that some of us present here will live to see the day when few families of flowering plants will need to be assigned by guess work, and in any case, whether the end comes sooner or later, we must travel hopefully towards it.

One fact to be noted in this connection is that although the embryologist cuts sections of the flower at various stages of development, he frequently confines his attention to the development and organisation of the embryo-sac and the subsequent changes which take place *inside* it or to a study of the meiotic divisions in the pollen mother-cells, while the structure of the anther and ovary wall, the placentation, the integuments, the nucellus, and the chalaza are either dealt with in a more or less cursory fashion or not described at all. This is unfortunate as all of these yield characters of great systematic value. The structure of the seed and the fruit receives even less attention probably because of the difficulty of sectioning them, but a judicious use of dilute hydrofluoric acid can sufficiently soften them in many cases without causing any appreciable harm to the tissues.

Last of all we come to the comparatively new science of experimental embryology. This is a more difficult field, but one which is full of promise in so many ways. As some one once said, the plant breeder puts pollen on the stigma and 'prays' for results in the ovary! For a scientific explanation of his successes and failures and for finding out the ways and means of increasing the former and remedying the latter, he must turn to the cytologist and the embryologist. The work that has been started in recent years on the effect of X-rays, heat, chemicals, etc., on the artificial induction of mutations is still in its infancy and it opens up vast possibilities before us. Here the breeder, the cytologist, the embryologist and the physiologist, must all join hands so that not only do we get the maximum results from what we have but we may evolve new and still better varieties of plants and thus add to the health and happiness of the world.

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