PLANT REPRODUCTIVE BIOLOGY

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PREFACE

Exponential growth in many areas of basic fundamentals made it necessary in some cases to write several chapters on the same topic which was covered in a single chapter in the earlier book. Similarly, in the present volume, separate new chapters have been written on topics which in the earlier title either did not figure at all or were each covered very briefly as a part of a chapter. In the present book, for instance in separate chapters have been written on new topics. The students of Biology at the post graduate (P.G.) under graduate (U.G.) levels needed to the recent Global changes and developments. The book is written in simple language so that the students can easily grasp the matter. Some important terms has been incorporated. So that the students may search the useful related for competitive examinations. In the recent years included in the syllabus of almost all Indian Universities in various subjects of Biology or Life Sciences as an independent evergreen subject.

Several of my students in the laboratory helped me either in writing some of the chapters or in preparing the list of references and appendix given at the end of this volume. Excellent technical help by rearranging the text and incorporating corrections in the text and figures, as and when it was necessary. I am also thankful to the publishers. I hope that the book will serve the purpose for which it is written, and that the teachers and student will find it useful for various courses in Biological Science prescribed for B. Sc and M. Sc degrees in life sciences and biotechnology. The teachers may also use this book for farming new syllabi and for revising the old ones. However, despite several rounds of reading, the book may still have some printing errors. There may also be errors and omissions of technical nature, since in a vast and fast expanding subject like Botany Biotechnology; one cannot claim to have known everything, despite his best efforts.

I am thankful to Department Head, BOS, Staff and Research Scholars (Botany) my family members, inspiration and cooperation my wife and children's (M. Hamsini) Teachers, Friends, Students and Well-Wishers (Dursheti Sai Charan, M.B.A., Certified Microsoft Office and Windows Specialist). I hope that this book will be useful to students in Life Sciences.

Dr. Mandaloju Venkateshwarlu

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CHAPTER I

MALE AND FEMALE GAMETOPHYTES

INTRODUCTION

The Microspore (pollen grain), which makes the first cell of the male gametophyte, undergoes only two divisions. With the result of first division two cells are formed a large vegetative cell and a small generative cell only. This division may take place either in the pollen grain or in the pollen tube, and gives rise to two male gametes.

The pollen grain at the time it is shed as a spherical or ovoid cell with two membranes a rough outer wall of cutin called the exine and an inner thin wall of cellulose and pectin substances called the intine**.** According to Erdtman the external surface of the pollen is characterized by various markings such as pilate, reticulate, striate, ornate, crassisexinous, tenuisexinous, subsaccate, tegillate, etc. Outside of the mature pollen in insect pollinated plants, there is an oily layer which consists of lipid carotenoids and some proteins. This oily layer is called pollenkitt. Carotenoids are responsible for the yellow or orange colour of the pollen and proteins make them sticky. As regards its function, it helps in insect attraction, and protects pollen against radiations.

Apertures.

According to Erdtman there are four types of apertures in pollen. These apertures are the weak places where exine is absent and the intine comes out as pollen tube. The apertures may be simple or compound. Long apertures are called colpi, and short ones pores. Pollen grains with simple apertures are either.

a. Colpate *(i.e.*, with colpi)

b. Porate *(i.e.*, with pores). A compound aperture consists of a central region called oral, and the outer region called colpal in colporate pollen *(i.e.*, with compound colpi), and poral in porate pollen *(i.e.*, with compound pores)

c. Sulcus, is a longitudinal furrow confined to the distal half of the pollen, crossing the polar axis at right angles

d. Ruga, is a furrow which is not confined to the meridianal region but placed irregularly on the surface of the pollen grain.

The pollens of monocots are typical monocolpate type, whereas the pollens of dicots are tricolpate.

Sculpturing

The details of the exine of the pollen constitute the sculpturing. The main types are as follows: psilate (smooth); foveolate (pitted); fossulate (grooved); scabrate (with fine projections); verrucate (warty); baculate (rod like elements); pilate (rod like projections with swollen heads); gemmate (sessile pillar like); echinate (spiny); rugulate (with elongate irregularly distributed elements); striate (with elongate parallel elements); punctate (with minute pores) and reticulate (with net like elements).

Shape

The shape of pollens varies from spherical, subprolate, prolate to perprolate. In perprolate pollen, polar axis is more than twice as long as the equatorial diameter. In peroblate pollen polar axis is less than half of the equatorial diameter. Each pollen grain has two poles at opposite ends of the polar axis. The proximal pole is at the centre of the proximal face *(i.e.*, toward the centre of the tetrad), while the distal pole is at the centre of the distal face *(i.e.*, away from the centre of the pollen tetrad). The polar axis makes always a perpendicular with the distal pole at the apex and the proximal pole at the base. The pollens are known as heteropolar when their two faces are different, and isopolar when similar. In heteropolar pollens one face possesses an aperture, whereas the other has none. The equator runs round the surface of isopolar pollen grains midway between the poles.

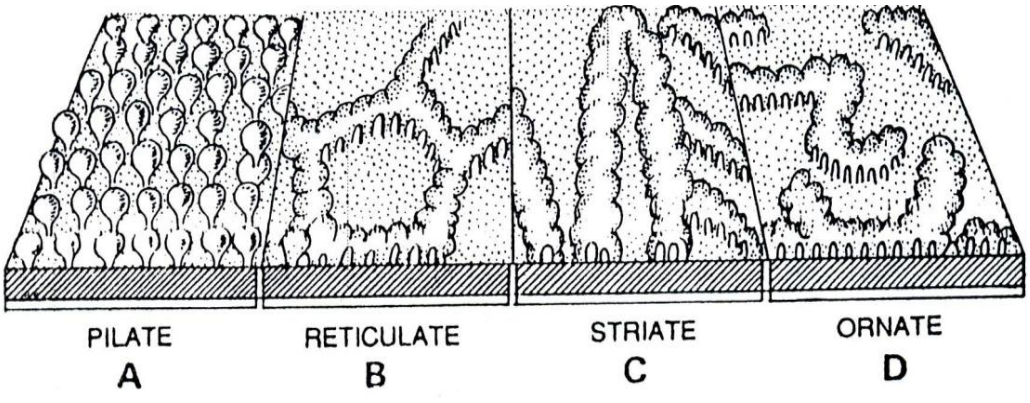


Figure 2: Sculpturing in different types of Pollen. A. pilate; B. reticulate; C. Striate; D. ornate.

Size

The size of pollens varies from nearly less than 10 µ (as in *Myosotis alpestris)* to; more than 200 p: (as in *Mirabilis jalapa* and some Cucurbitaceae).

Chemical nature of intine and exine.

The intine is pecto-cellulosic in nature, and generally destroyed during acetolysis, while the exine consists of cutin, and it is acetolysis-resistant layer. It is also resistant to biological and physical degradations.

Microspore and its germination

The microspore or the pollen grain (n) represents the beginning of the male gametophyte. The structure of microspore or pollen grain has already been given in the preceding paragraph. The newly formed microspore possesses very dense cytoplasm with a central nucleus, but as the cell increases in size, a vacuole develops and the nucleus shifts from centre to a place adjacent to the wall. The germination of microspore starts while it is still within the microsporangium or pollen sac.

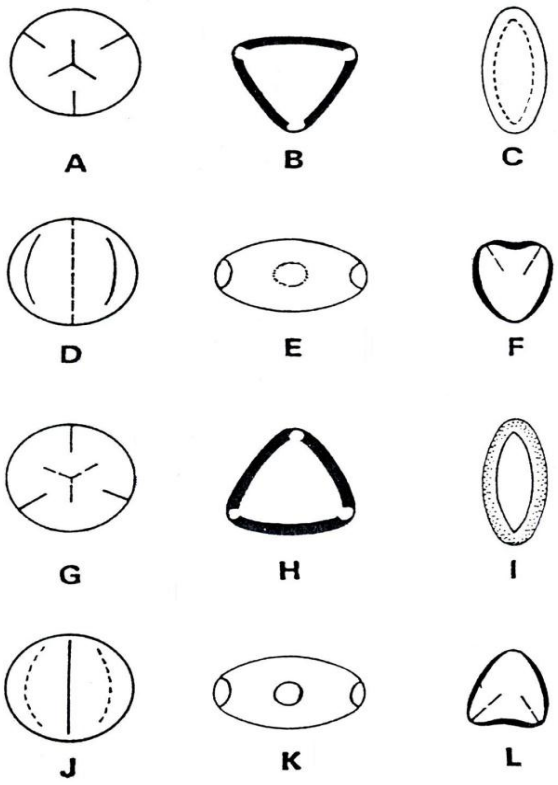


Figure 3: Different types of pores in pollen. A, tricolpate pollen with triradiate scar; D, G and J, other views of A;B, three pored pollen; E, H and K, other aspects of B;C, monocolpate pollen; F, I and L, other views of C.

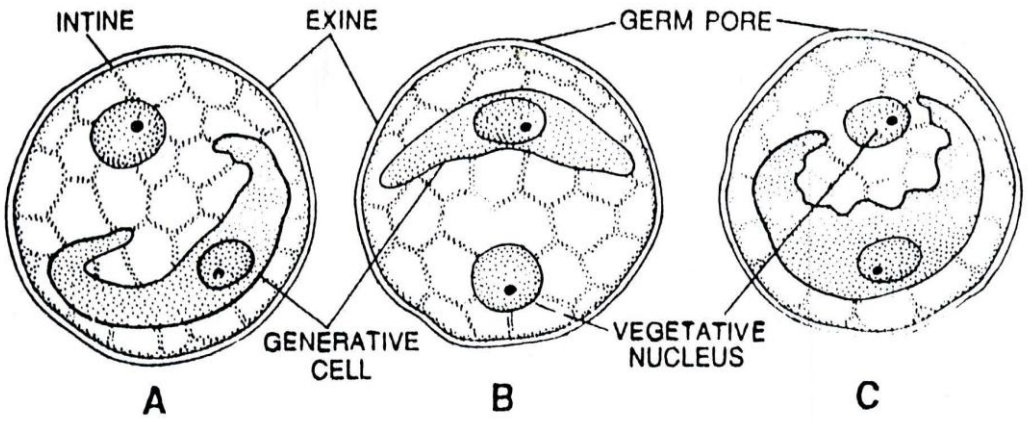


Figure 4: Male gametophyte. Pollen grains showing vegetative nucleus and generative cella. In A and B, the generative cells are much elongated; whereas in C the generative cell is of amoeboid nature.

Formation of vegetative and generative cells

The first division of the microspore gives rise to the vegetative and generative cells. The first formed walled and peripheral cell is the generative cell while the larger, naked, central cell, which fills the remainder of the spore-wall cavity, is the vegetative or tube cell. The nuclei of generative and vegetative cells differ in size, structure and in staining qualities. The nucleus of vegetative cell possesses a prominent nucleolus, while the nucleus of generative cell contains a small nucleolus. The cytoplasm of the generative cell is hyaline and is almost without RNA, whereas that of vegetative cell is rich in RNA. The DNA contents of both the nuclei are same in the beginning but later on they increase in the generative nucleus. There is considerable variation in the form of the generative cell. Usually it is elliptical, lenticular or spindle-shaped. The starch and fat are the most conspicuous substances found in the vegetative cell. Certain proteinaceous bodies have also been reported in microspores.

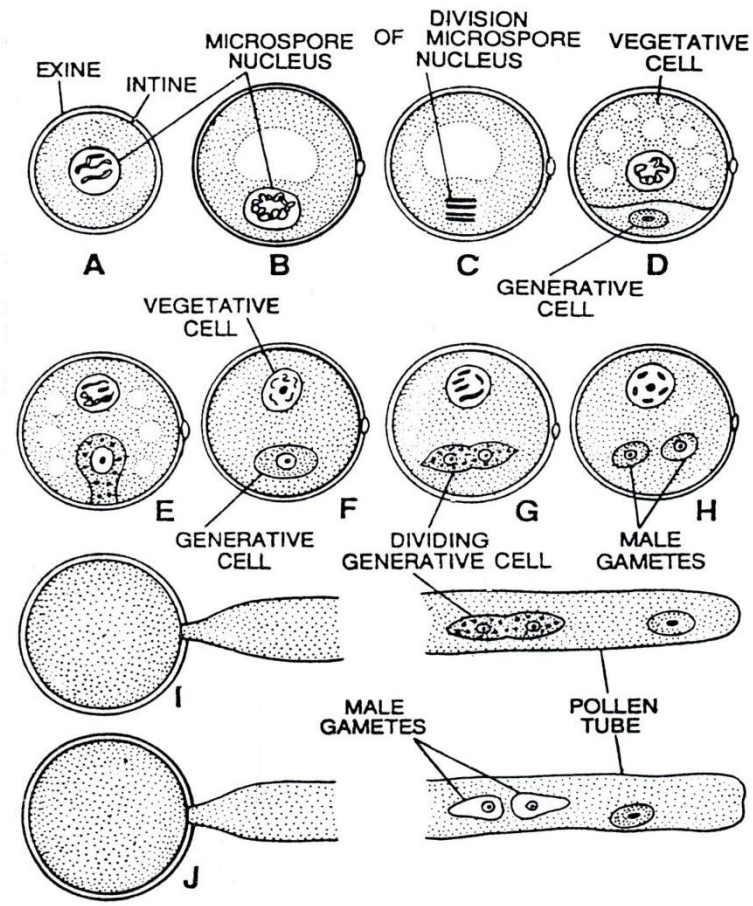


Figure 5: Male gametophyte, A-J, different stages in the development of male gametophyte.

Eventually the generative cell loses contact with the microspore wall, and is being shifted into the vegetative cell, where it may lie in any part of it. It then becomes oval or spindle-shaped. Thus the microspore becomes two-celled. Generally the microspores (pollen grains) are being shed from the microsporangium (pollen sac) in two-celled stage for pollination. It is only in rare cases that the pollen grain (microspore) becomes three-celled before its liberation from pollen sac. It seems somewhat difficult to make clear cut demaracation between two-celled pollen grains, because the generative cell may be in prophase or metaphase stage at the time the pollen grains are liberated from the pollen sac, and the process of division is continued in the pollen tube. In some cases *(e.g.,Viola, Dionaea, Nicotiana,* etc.) both two and three-celled pollen grains have been reported in the same plant. However, the division of the generative cell, whether it takes place in the pollen grain or in the pollen tube, occurs in a regular way.

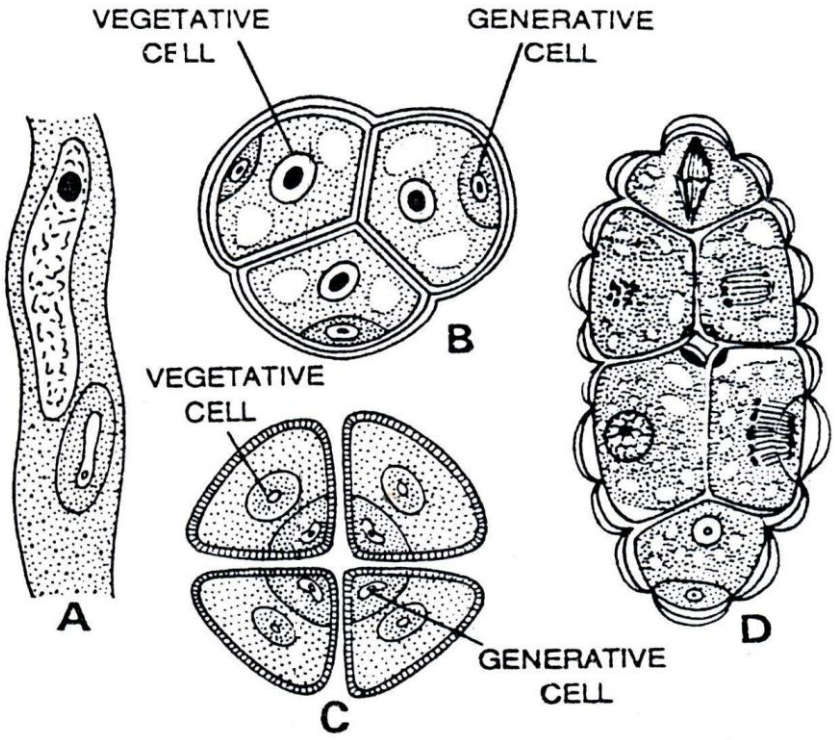


Figure 6: A, division of microspore into vegetative and generative cells; B, pollen tetrad where generative cell is cut off towards outer side of each pollen; C, pollen tetrad where cell is formed towards inner side of each pollen; D, pollinium with various stages in division of pollen.

It seems quite clear that in most of angiosperms the pollen grain has two nuclei at the time of shedding from the anther. One of these is the generative nucleus, which later on divides and thus two sperm nuclei are 'farmed. In several plants, the generative nucleus divides before the pollen is shed and thus the pollen grains are trinucleate. The early division of the generative nucleus represents one more step in the progressive compression of the gametophyte that characterizes vascular plants in general. In considering binucleate and trinucleate pollen types, it should be noted that both types produce mature male gametophytes with three nuclei; they may be differentiated at the time of the division of generative nucleus.

In any case the cells formed by the division are always unequal. However, in *Cuscuta* (Fedortschuk, 1931), where the daughter cells are sometimes of the same size, this seems to be an abnormality that leads to the formation of double microspores, each of which divides again to give rise to the vegetative and generative cells. Double pollen grains consisting of two units have also-been seen in *Podostemon subulatus.* The

separating wall between the two pollens is pitted, and only one of them produces a pollen tube, and the other may serve as a source of food material.

Unlike the simultaneous reduction divisions, in all the microspore mother cells of an anther, the microspores do not divide at the same time. In those plants where the microspores remain together in a tetrad, all four cells in a tetrad remain in the same stage of division, but not all the tetrads of an anther. A complete synchronization may occur only where the microspores are united into pollinia such as in families Mimosaceae, Asclepiadaceae and Orchidaceae. However, even in such cases exceptions are there, for example, in the pollinium of *Acacia baileyana,* where one of the microspores is in prophase, another with the tube and generative cells already formed, and still others in various intermediate stages.

According to Goebel (1933) the generative cell in the angiosperms, as a rule is cut off on the distal side, *i.e.,* ventral side of the microspore. However, Geitler (1935) reported, that the generative cell may be cut off either on the outer side, *e.g.,* in *Vaccinium* and *Acacia,* or on the inner side, *e.g.,* in *Juncus, Xyris,* and many other plants of Cyperaceae, or on radial side in *Lilium, Anthericum* and *Convallariai*. It has been established that after sometime the generative cell leaves its contact with the wall of microspore and after that, its position in the pollen grain may be changed and it may lie in any region of the pollen grain. There is considerable variation in the form of the generative cell. Usually it is elliptical, lenticular or spindle shaped. However, in *Cuscuta* (Finn, 1937) and *Ottelia* (Islam, 1950) it is long and occupies the entire width of the pollen grain.

Male cells or male nuclei

It was believed formerly that whenever the generative cell divides in the pollen grain, the sperm cells are formed, but if the division takes place in the streaming cytoplasm of the pollen tube only nuclei are formed. However, Schnarf (1941) reported, that in all cases the male gametes -are definite cells and the cytoplasmic sheath persists throughout their course in the pollen tube. In *Lilium* and other plants, the cytoplasmic sheath around the male nuclei, has been followed up to the time of their discharge in the embryo sac.

Vegetative nucleus or tube nucleus

According to earlier workers, the vegetative nucleus or tube nucleus played an important role in directing the growth of the pollen tube. It is not always found in the distal end of the pollen tube but frequently lies considerably behind the male gametes. However in *Ulmus*, *Senecio*, *Crepis* and *Secale* it degenerates even before the germination of the pollen grain, and does not enter the tube at all, and the tube continues to function normally. Poddubnaja Arnoldi (1936) regards the vegetative nucleus as a vestigeal structure without any important function in the growth of the pollen tube.

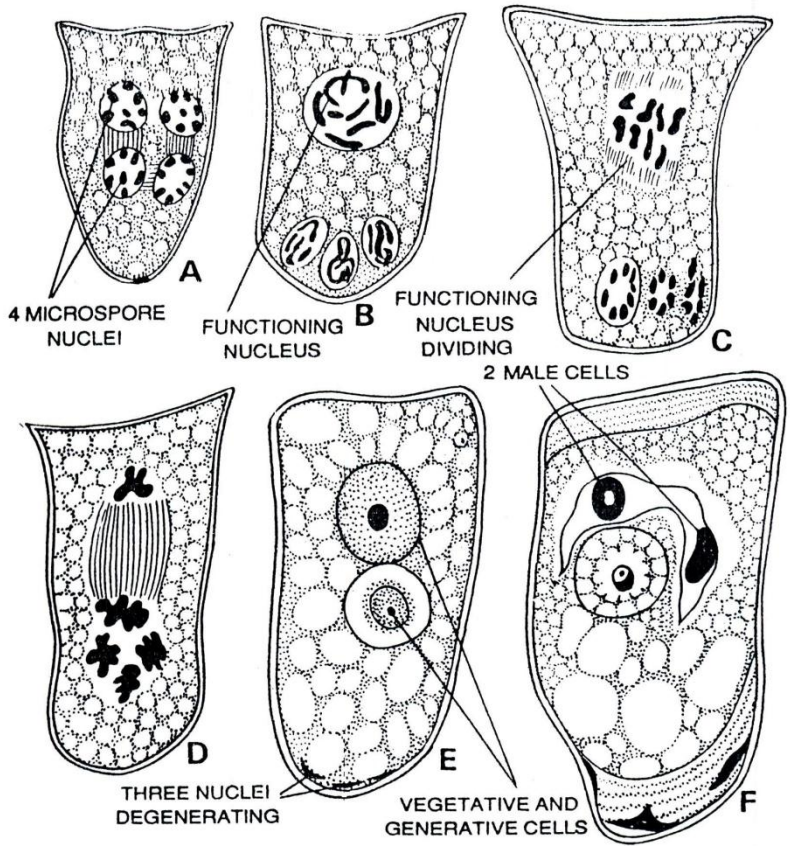


Figure 7: The different stages of development of male gametophyte in Scirpus paluster (Cyperaceae).

Pollen tube

On the stigma the pollen grain is caught in a sugary solution or even in water. The pollen grain swells up and the exine ruptures at the germ pore. The intine and contents come out in the form of a pollen tube. The pollen tube grows through the style and reaches the ovule in the ovary. The entrance of the pollen tube is either through micropyle, chalaza or side ways. It carries the tube nucleus and the two male gametes

at its tip, as it enters the ovule. After discharging its contents in the embryo sac, the pollen tube contains some enzymes such as amylase, invertase, phosphotase, pectinase, lipase, etc. The distal part of the pollen tube contains some amount of cytoplasm.

Abnormal development of pollen in Cyperaceae.

According to Juel (1900), Stout (1912) and Piech (1928), out of the four microspore nuclei produced after meiosis, only one develops further, and the remaining three are pushed towards one side of the mother cell. The centrally situated functional nucleus divides with its spindle oriented in the direction of the long axis of the cell. The cell plate is formed between the vegetative nucleus and generative nucleus and it extends around the generative nucleus to give rise to a regular plasma membrane. The generative cell soon becomes spindle-like and undergoes the division to form the two sperm cells.

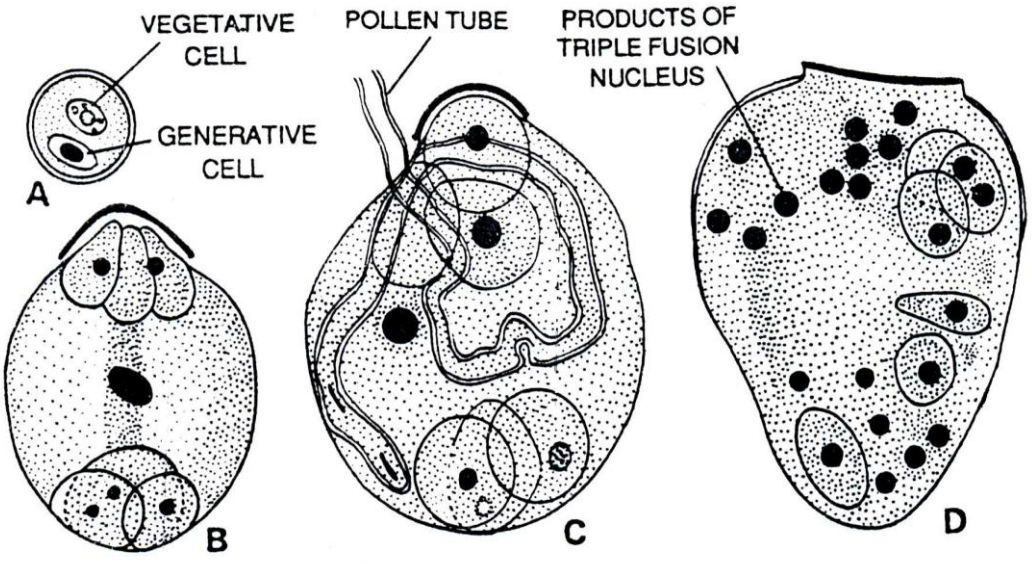


Figure 8: Different stages of fertilization of pollen embryo sacs. A, normal pollen with vegetative and generative cells; B, pollen embryo sac; C, pollen embryo sac influenced by pollen tube from pollen of another variety; D, fertilized pollen embryo sac.

Embryo-sac-like pollen grains

In 1898, Nemec noted that in the petaloid anthers of *Hyacinthus orientalis* the pollen grains sometimes form large eight-nucleate structures showing a surprising resemblance to embryo sacs. According to Nemec, they arose as the result of a

degeneration of the generative nucleus and three divisions of the vegetative nucleus. This was known as "Nemec phenomenon" after the name of its discoverer. Later on, De Mol (1923) observed this phenomenon in the anthers of other varieties of *Hyacinthus orientalis.* According to him, this abnormality was due to a duplication of the generative nuclei. Stow (1930, 1934) found similar embryo-sac-like pollen grains or "pollen-embryo-sacs" in the anthers of another variety. He believed that the primary microspore nucleus undergoes three successive divisions to form eight daughter nuclei. The 3 nuclei lie at the end where the exine is still intact, 3 at the opposite side and 2 in the centre. The six nuclei situated at the two poles change into cells while the remaining two fuse in the centre.

The cells which lie at the exine end represent the egg and synergids, the nuclei of the centre are called polar nuclei. According to Stow, it is not the divisions of the vegetative or generative nucleus which give rise to the pollen embryo sacs but those of the microspore nucleus itself. He suggested that a necrohormone is secreted by the dead pollen grains, which gives rise to the abnormal growth of remaining pollen grains. Naithani (1937) suggested that temperature treatment to the bulbs of *Hyacinthus* is the causal factor and the death of some pollen is only due to hypertrophy of others. Geitler (1941) considered the phenomenon to be under genotypic control and not reproducible through outside influences. Geitler interprets the three cells at the exine end of the pollen grain as the antipodals and the other three as egg and synergids. However, under unfavorable conditions the female potency becomes dominant over the male, forming the embryo-sac-like structures.

FEMALE GAMETOPHYTE OR MEGASPOROPHYTE

The antipodals even when present are not seen because of their being situated in the narrow chalazal end of the embryo sac, and may be seen only in median sections. In some cases, there may be reduction in the number of nuclei. For example, in *Phajus, Oncidium, Elatine, Geodorum, Bulbophyllum,* etc., the embryo sacs are six-nucleate because of a suppression of division of the two chalazal nuclei of the four-nucleate stage. In *Orchis morio* the primary chalazal nucleus of the two-nucleate stage may degenerate without any further division and so the resultant is a five-nucleate embryo sac. In *Epipactis pubescens* and *Paphiopedilum insigne,* it has been observed that two chalazal spindles of the last division fuse to form a single large spindle which

develops two diploid nuclei instead of the four haploid ones, which results in a six-nucleate embryo sac that contains a haploid micropylar quartlet, a diploid lower polar nucleus and a single antipodal cell.

The occurrence of more than eight nuclei in the embryo sac is rarely found in the members of the families Casuarinaceae, Loranthaceae, Rosaceae, Rhamnaceae, Rubiaceae, Asteraceae (Compositae), etc. This condition may arise in three ways : (1) fusion of two embryo sacs, *e.g.,* in *Elatine hydropiper* (Frisendahl, 1927), where an embryo sac contains two egg apparatuses, two pairs of polar nuclei, and two groups of three antipodal cells each; (2) migration of the nuclei of nucellar cells into the embryo sac, *e.g.*, in *Hedychium gardnerianum* (Madge, 1934) and *Pandahus* (Fagerlind, 1940) where during growth and enlargement of embryo sac, the adjacent cells of the nucellus become flattened and crushed, but their nuclei remain as such and eventually they penetrate into embryo sac; and (3) occurrence of secondary divisions of some of the first-formed eight nuclei, *e.g.*, in *Crassula schmidtii* and *Umbilicus intermedius,* it has been reported that on certain occasions there is a fourth division in the embryo sac, giving rise to sixteen nuclei, which form foursynergids, two eggs, six antipodal cells and four polar nuclei. It has been reported (Goodspeed, 1947) that in *Nicotiana,* some embryo sacs possess nine to sixteen nuclei.

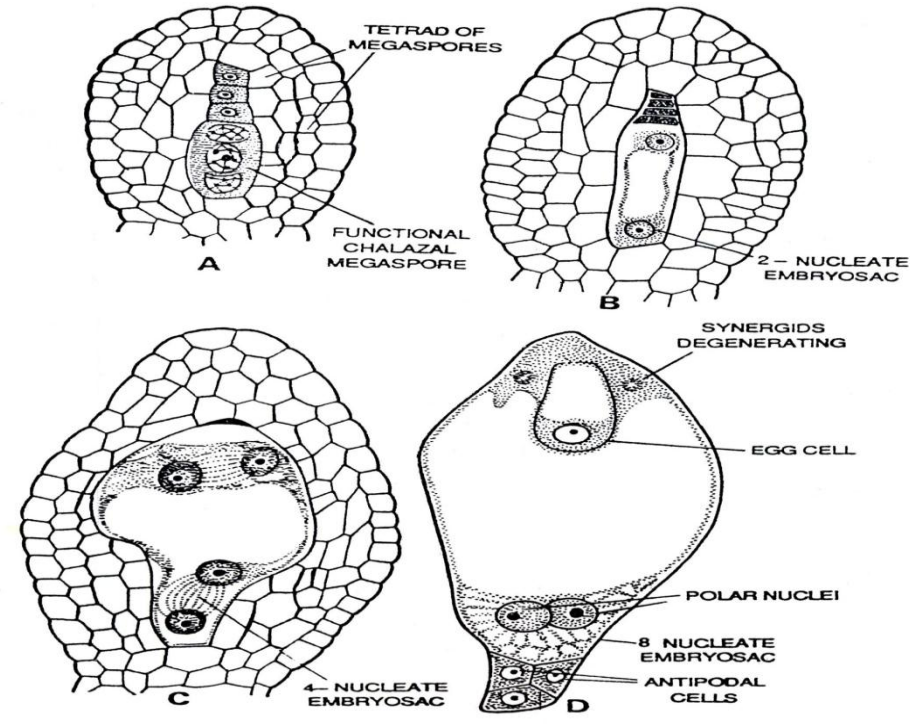


Figure 9: Development of eight nucleate embryo sac in Hydrilla (A-D).

The presence of two or three embryo sacs in the ovules of some angiosperms, due to the development of more than one megaspore of a tetrad, or the megaspores of twin tetrads has been observed in *Cassytha* (Sastri, 1962), *Echinochloa* (Narayanaswami, 1955), *Eschscholtzia* (Sachar and Mohan Ram, 1958), *Tagetes* (Venkateshwarlu, 1955) and *Utricularia* (Khan, 1954). This type of embryo-sac is the most common and generally known as the normal type. It has also been called *Polygonum* type, because for the first time in 1879, this type was reported in *Polygonum divaricatum* by Strasburger.

***Oenothera* type**

Another variation of monosporic type of embryo sac is known as the *Oenothera* type, and has been reported only in the family Onagraceae. In this type, the megaspore nucleus divides twice and thus produces four nuclei at the micropylar end. These nuclei give rise to a normal egg apparatus and a single polar nucleus. The second polar nucleus and the antipodalnuclei are absent.

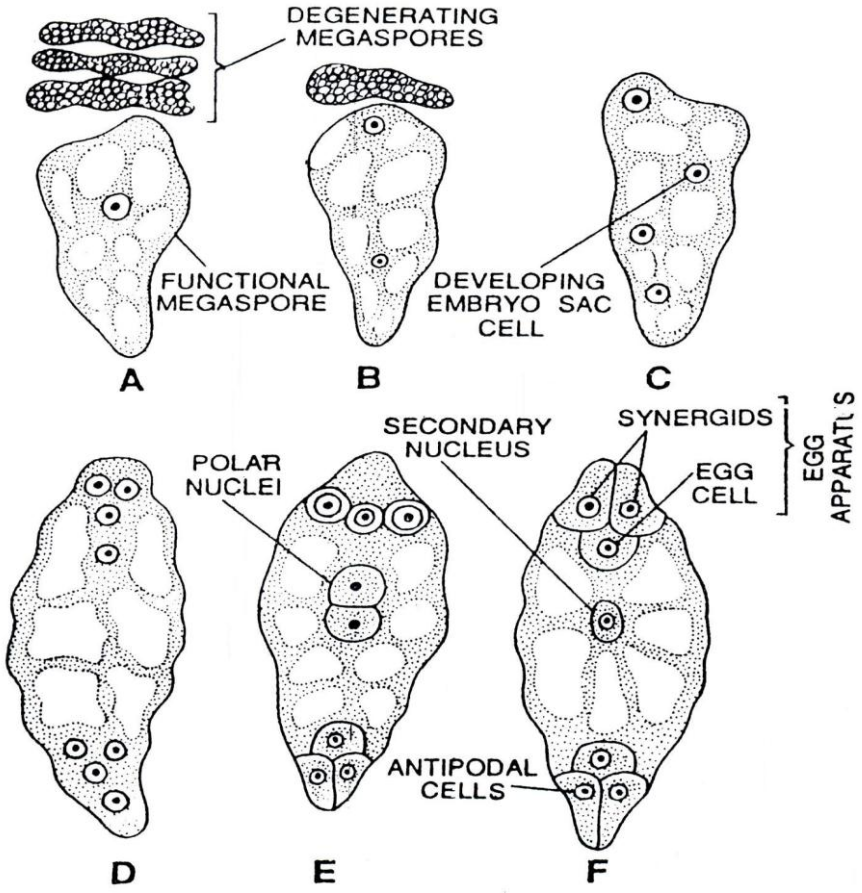


Figure 10: Female gametophyte. A-F, Development of the embryo sac (female gametophyte) of normal type (Polygonum type).

Geerts (1908) found that in *Oenothera lamarckiana* the embryo, sac is commonly formed by the micropylar megaspore of the tetrad, which undergoes only two nuclear divisions instead of the usual three that occur in the *Polygonum* type of embryo sac. Thus, four nuclei are produced which organize into the two synergids, the egg, and a single polar nucleus. The third division is absent, and therefore, all the nuclei remain situated in the micropylar part of the developing embryo sac, there is neither a lower polar nucleus nor any antipodal cells. This shows that in the development of the *Oenothera* type of embryo sac, there is growth of more than one cell of the tetrad at the same time. Eventually it is the micropylar megaspores that functions, but sometimes this may be the chalazal and at certain occasions both grow simultaneously forming 'twin' embryo sacs.

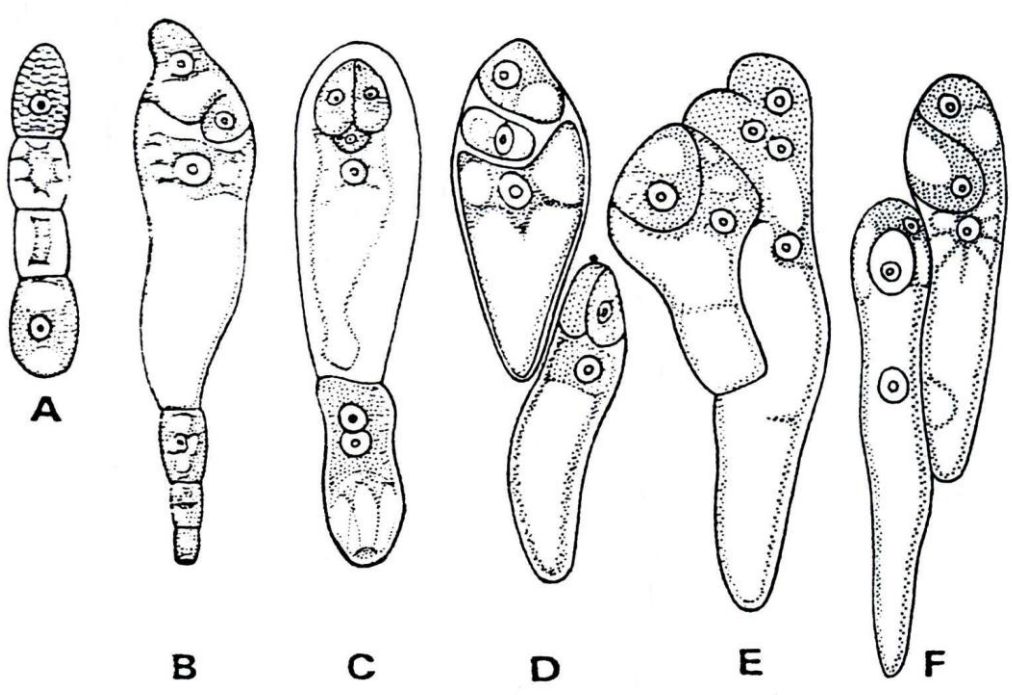


Figure 11: A-F, development of twin embryo sac in *Oenothera.*

In rare cases, more than four nuclei may be seen in an embryo sac. It is either due to (1) amitotic divisions of the polar nucleus, or (2) divisions of synergid cell, or (3) migratioh of nucellar cells into the embryo sac. On the other hand, embryo sacs with fewer than four nuclei are rare, but in Hartmannia tetraptera and jussieua repens, there are two three nucleate embryo sacs having a single synergid, an egg and a polar nucleus.

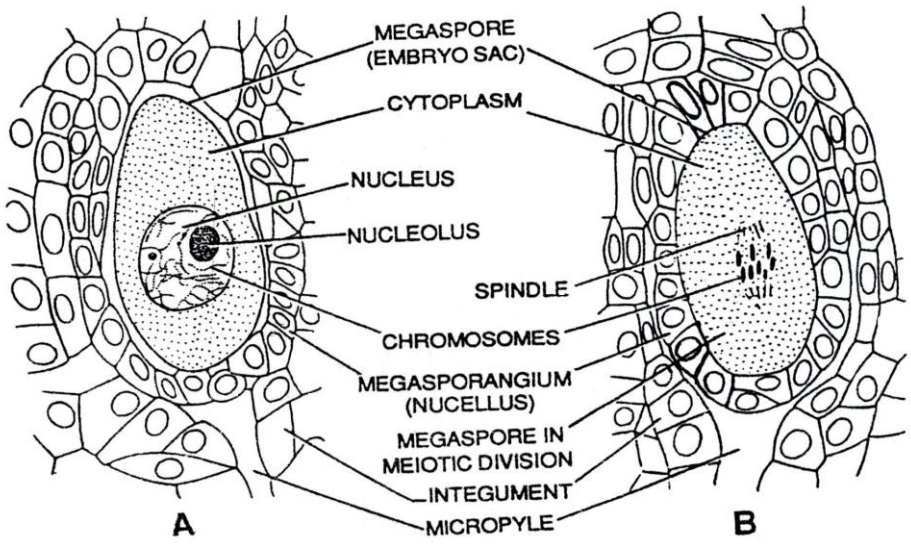


Figure 12. Embryo sac development in *Lilium*. A, uninucleate stage; B, first division.

Bisporic type.

The bisporic embryo sacs are typically 8-nucleate. They are also known as *Allium* type embryo sacs. Such embryo sacs arise from one of the two dyad cells formed after Meiosis I. Since there is no wall formation at the end of Meiosis II and both the megaspore nuclei formed in the functional dyad cell take part in the development of the embryo sac, only two further divisions of these nuclei give rise to 8-nucleate stage. Morphologically the embryo sac is formed by two spores. This type of embryo sac, *i.e.*, *Allium* type is found separately in many monocotyledonous families, such as, Liliaeae, Amaryllidaceae, Orchidaceae, Alismaceae and Butomaceae. Among Dicotyledons it is round in the genera of certain families, such as Podostemaceae, Balanopnoraceae and Loranthaceae.

Variations. In the families Alismaceae, Butomaceae, and Orchidaceae, there is a reduction in the number of nuclei at the chalazal end at the end of embryo sac. In certain genera, some species have a bisporic embryo sac, while in others it is monosporic. While working with *Cuscuta reflexa*, *Olax wightiana* and *Xyris paciflora*, respectively, John and Tiagi (1952); Shamanna (1954) and Govindappa (1954) found that in all the three species, the embryo sac is of *Allium* type, whereas in

most other species of *Cuscuta, Olax imbricata* and *Xyris indica,* the embryo sac is of *Polygonum* type, *i.e.*, monosporic eight-nucleate normal type.

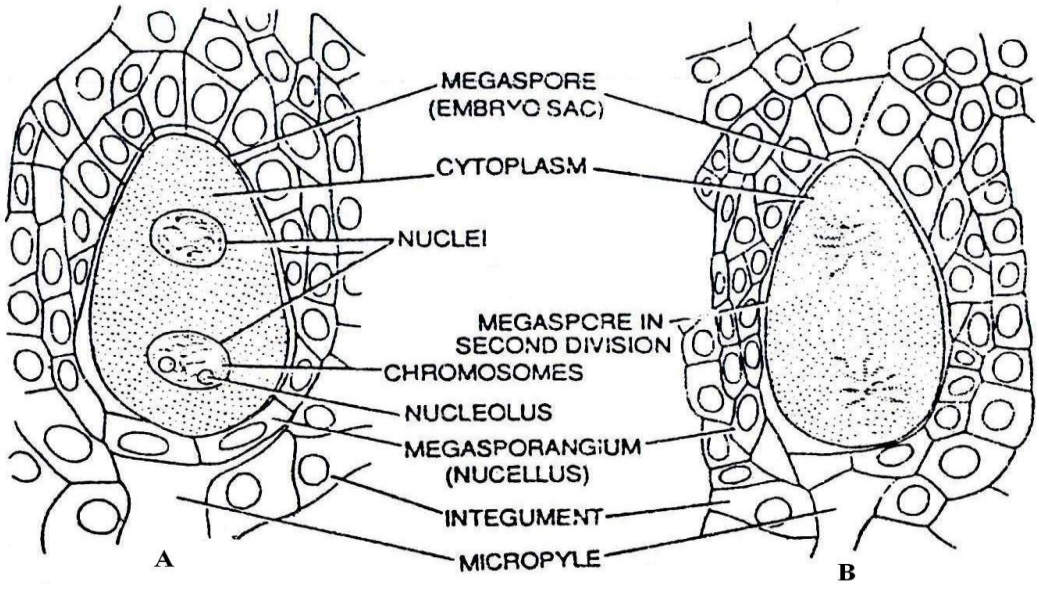


Figure 13: Embryo sac development in *Lilium*. A, first two nucleate stage; B, second division.

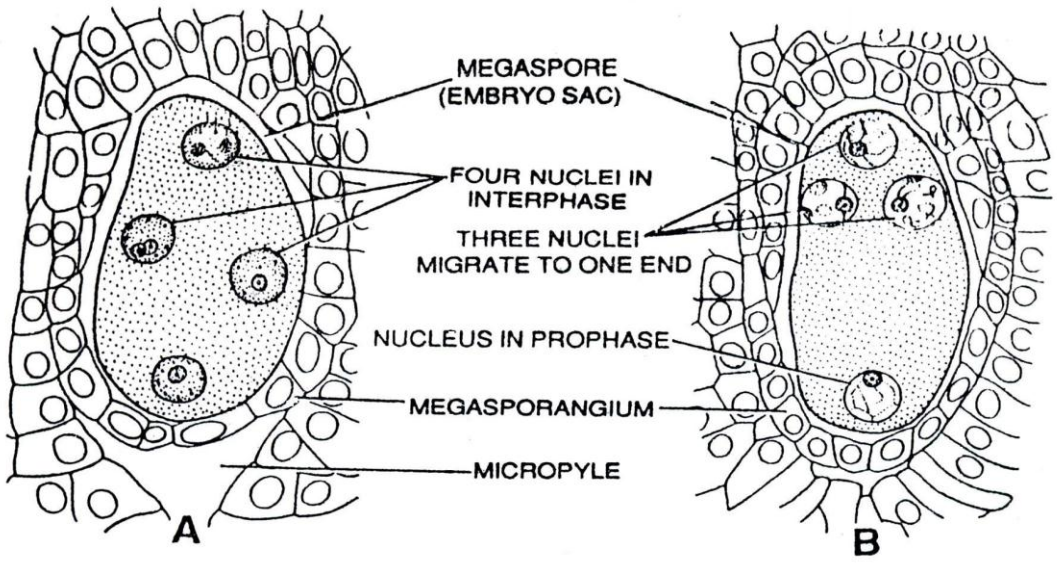


Figure 14. Embryo sac development in *Lilium*. A, first four nucleate stage; B, migration of three nuclei.

***Endymion* type.**

This type of embryo sac was reported for the first time in *Endymion hispanicus* (Battaglia, 1958). In this type, after the formation of the dyad, the lower cell may

disintegrate or its nucleus may divide once or twice producing 4 nuclei. The 8-nucleate embryo sac is formed by the nuclear division of the upper cell of the dyad.

Variations. The embryo sacs of *Indotristicha* and *Terniola* are five-nucleate (Mukkada, 1963) instead of eight-nucleate, *i.e.*, *Polygonum* or normal type. Moreover, here chalazal nucleus persists. He also reported the formation of 'pseudo embryo sac' only during the post fertilization stages.

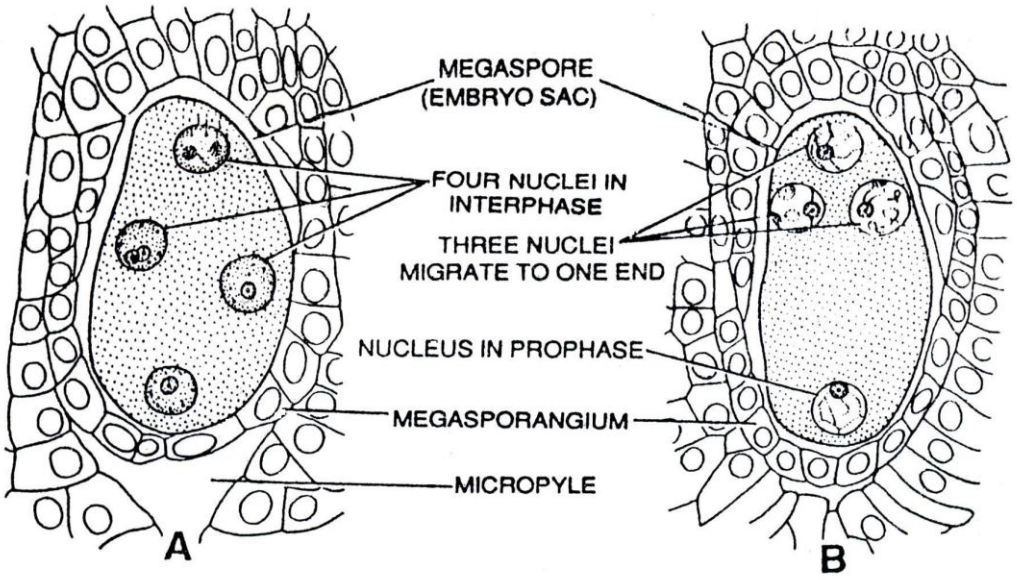


Figure 15: Embryo sac development in *Lilium.* A, third division; B, second four nucleate stage.

Tetrasporic type

In this type of embryo sac there are several variations. In several cases 16 nuclei are formed with the result of two divisions after megasporogenesis. These are further subdivided into the following types.

***Peperomia* type.**

In this sub-type each of the 4 megaspore nuclei divides twice, forming 16 nuclei which are uniformly distributed at the periphery of the embryo sac. Two nuclei at the micropylar end form an egg and a synergid; 8 are fused to form the secondary nucleus and the remaining 6 nuclei are cut off at the periphery of the embryo sac.

Variations. The chief variations concern the number of nuclei which fuse to form the secondary nucleus, and the number left over to form the antipodals. It is reported in

Peperomia pellucida that after the meiotic divisions are over, the coenomegaspore either remains spherical, or it becomes slightly pear shaped with a little tubular outgrowth at the micropylar end. In the pear-shaped embryo sacs, a three-celled egg apparatus is found, the fourth nucleus from the micropylar end and one member from each of the six peripheral pairs form the seven polars, and six nuclei are cut off to form the lateral cells. In *Peperomia hispidula,* at the eight-nucleate stage, two nuclei are seen at the micropylar end, and six at the chalazal end; at the sixteen-nucleate stage, four lie at the micropylar end and twelve at the chalazal end. Two nuclei of the micropylar end now form the egg and single synergid, as in other species, but the remaining two nuclei of the micropylar group and all the remaining twelve nuclei come near the centre and fuse to form a single large secondary nucleus. Murty (1959) reported that the egg apparatus may be two or three-celled, *e.g.*, in *Peperomia pellucida, P. comarpana* and *P. reflexa,* and four or six antipodals.

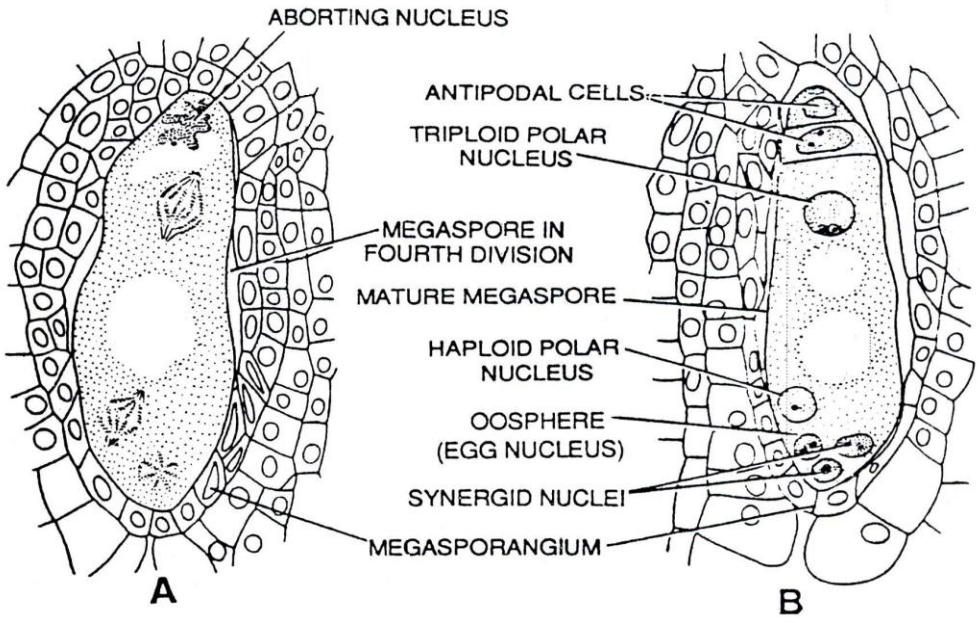


Figure 16: Embryo sac development in Lilium. A, fourth division; B, immature female gametophyte.

***Penaea* type.**

In this subtype, 16 nuclei lie in four distinct quarters which are arranged crosswise, once at each end of the embryo sac and two at the sides. Now three nuclei of each quarter become cut off as cells, while the fourth one remains free and moves towards the centre. Thus there are four triads and four polar nuclei. Here, the egg cell of the

micropylar triad alone is functional. Such embryo sacs have found in many members of Malpighiaceae.

Variations

In the embryo sac of *Acalypha indica* (Maheshwari and Johri, 1941), the development up to the sixteen- nucleate stage corresponds with that of the Penaeaceae and other species of *Acalypha,* but the organization of embryo sac shows a great variation. Here two nuclei of each quartet remain free and move to the centre of the embryo sac, while the other two organize into cells, thus forming four groups of two cells each at the periphery and eight free nuclei in the centre. In such cases, where a number of polar nuclei exist, their fusion and fertilization produce a highly polyploid primary endosperm nucleus, which seems to be associated with subsequent abortion of endosperm formation.

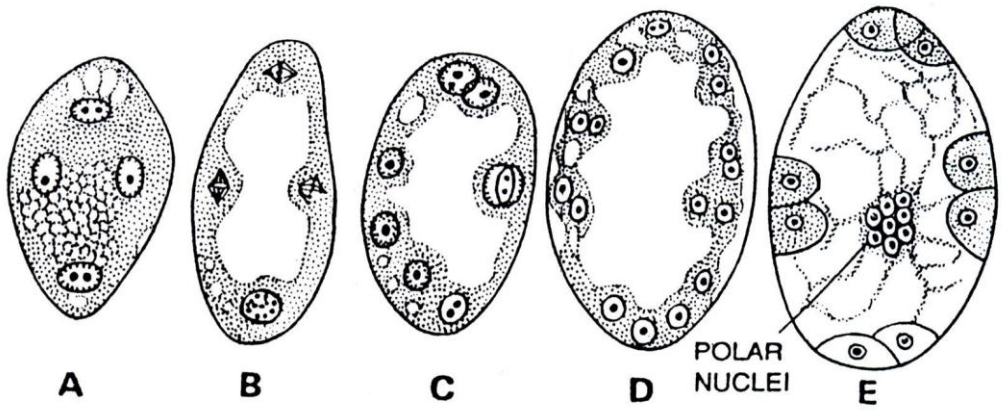


Figure 17: Development of embryo-sac in *Acalypha indica.* A, megaspore mother cell having four megaspore nuclei; B, dividing megaspore nuclei; C, eight, nucleate stage; D, sixteen-nuclete stage; E, mature embryo-sac having four peripheral pairs of cells and eight polar nuclei.

Cortini (1958) reported the following variations

(1) In such cases, the embryo sacs possess six nuclei at the micropylar end, three at chalazal end or *vice versa,* a lateral group in three nuclei and four polars. Here one lateral group is not present

(2) In such embryo sacs, the micropylar group consists of nine nuclei, a chalazal group of three nuclei or *vice versa,* and four polar or secondary nuclei. Here, both the lateral groups are absent

(3) In certain cases, the embryo sacs include the micropylar and chalazal groups, each of six nuclei. In the centre there are four polar nuclei and thus the lateral groups are absent.

***Drusa* type.**

In this type, a sixteen-nucleate embryo sac was recorded (Hakansson, 1923) in *Drusa oppositifolia* (family Umbelliferae). Here when the meiotic divisions are over, three of the megaspore nuclei pass down to the basal end of the embryo sac, and only one remains at the micropylar end. This is followed by two successive divisions forming four micropylarm nuclei and twelve antipodals. The four micropylar nuclei give rise to the egg apparatus and upper polar nucleus, and the twelve chalazal nuclei to a lower polar nucleus and eleven antipodal cells. This type has been recorded in *Mallotus, Mainthemum, Crucianella, Rubia, Ulmus* and a few other plants.

Variations. The embryo sac of *Chrysanthemum parthenium* shows a range of variation. According to Palm (1915), each of the four megaspore nuclei divides twice forming sixteen nuclei. Now these sixteen nuclei organize to form a three celled egg apparatus, two polar nuclei, and eight antipodal cells of which the basal cell is four nucleate. Later on Fagerlind (1941) studied the two specimens of the same species of *Chrysanthemum* and observed that out of four megaspore nuclei, three basal nuclei lie nearer to each other. After sometime, they separated from each other and the micropylar nucleus became larger than others. All the nuclei now divided simultaneously, forming eight nuclei of which two basal were the smallest and soon began to degenerate. After the completion of next division, there were fourteen nuclei in the embryo sac, of which three organized into an egg apparatus, two became polar nuclei and the rest formed the antipodal cells. In the other specimen, the megaspore mother cells as well as the developing embryo sacs and their nuclei were found to be of a larger size than in the first specimen. The chalazal megaspore nucleus degenerated after its formation. The remaining three nuclei divided to form six and then twelve nuclei. In the mature sac, the basal antipodal cell contains more than one nucleus, while the other antipodal cells were uninucleate.

***Chrysanthemum cinerariaefolium* type.**

Martinoli (1939) has described a peculiar mode of development in this plant. In this type the four megaspore nuclei show 1 + 2 + 1 arrangement, *i.e.*, one nucleus lies at

each pole and two in the centre. The two nuclei of the centre remain quite close to each other but do not fuse together. Now the megaspore nucleus of micropylar end divides twice forming four nuclei, but there is no regularity in the division of the nucleus of chalazal end. Thus the embryo sac may have six, nine or ten nuclei.

Sometimes the two central nuclei out of the four megaspore nuclei are fused together forming a single diploid (2n) nucleus; the next division produces six nuclei, one haploid (n) pair at micropylar end; one haploid pair at chalazal end and one diploid pair in the centre. The next division of these six nuclei produces three groups of four nuclei each. At the micropylar end the three nuclei make the egg apparatus, and one migrates in the centre forming upper haploid polar nucleus. The four haploid antipodal cells are formed at chalazal end. One of the diploid nuclei of the central quarter behaves as the lower polar nucleus and the remaining three organize them as additional antipodal cells. Thus there are twelve nuclei in the embryo sac. Sometimes less than twelve nuclei *(i.e.*, ten or seven) are developed, because of the failure of certain divisions.

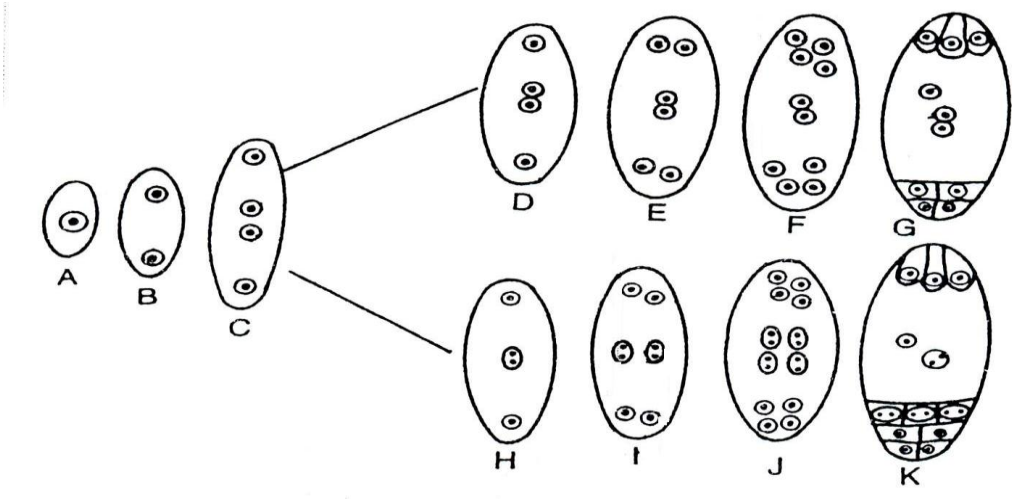


Figure 18: Two types of development of embryo sac in *Chrysanthemum cinerariafolium,*

***Fritillaria* type.**

This type of development of embryo sac has been reported in many angiosperms including *Lilium.* In *Fritillaria* and *Lilium* the behaviour of the four megaspore nuclei is peculiar. Here three of the megaspore nuclei go to the chalazal end and the fourth one goes to the micropylar end. The three chalazal nuclei come to lie very close to

each other. During the next stage the micropylar nucleus divides normally, but the three chalazal nuclei fuse together forming a triploid (3n) nucleus, which then divides mitotically forming two triploid nuclei at the chalazal end ; so that at the close of the division there are two haploid nuclei at the micropylar end and two triploid nuclei at the chalazal end; so that at the close of the division there are two haploid nuclei at the micropylar end and two triploid nuclei at the chalazal end. One more division takes place, resulting in the formation of eight nuclei, of which the four chalazal nuclei are triploid and the four micropylar are haploid. Thus a mature embryo sac consists of haploid cells of egg apparatus, three triploid antipodal cells and a tetraploid (4n) secondary nucleus formed by the fusion of two polar nuclei, one haploid and the other triploid. The endosperm nucleus, when formed after fertilization will be pentaploid (5n) in such type of embryo sac.

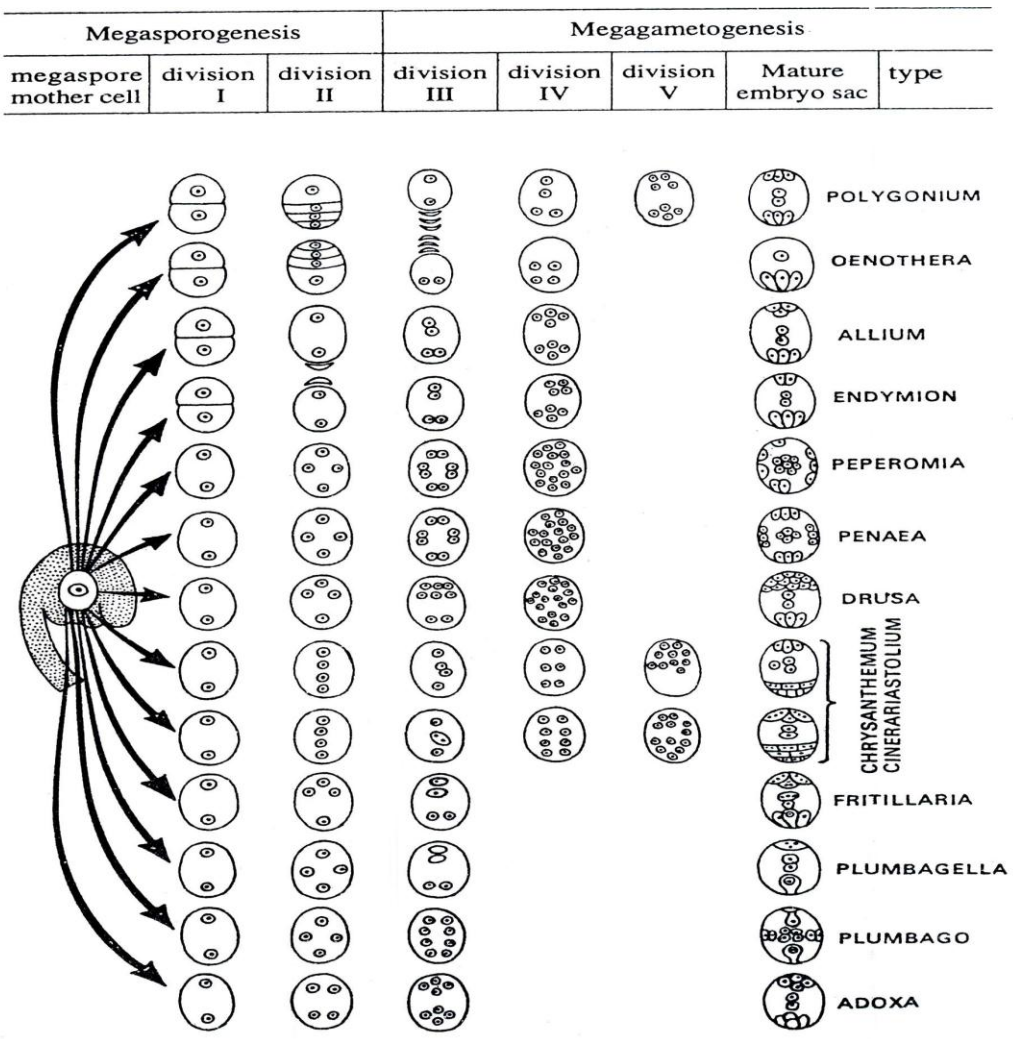


Figure 19: Female gametophyte. Development of different types of embryo sacs in angiosperms.

This peculiar type of development of embryo sac is of particular interest because it is found in a wide range angiospermic genera including *Lilium,* which formerly was known ajs *Lilium* type. The stage of the development of embryo sac in Lilium and Fritillaria was fully understood after the investigations of Bambacioni (1928) and Cooper (1935). The *Fritillaria* type has been demonstrated in a general way in Liliaceae, and several other genera of diverse families, such as *Piper, Tamarix, Armeria, Gaillardia, Euphorbia, Gagea, Erythronium, Tulipa, Clintonia,* etc. It may be noted that the fusion of the three chalazal megaspore nuclei take place when they are either in the prophase stage or in early metaphase. In the former case the secondary four- nucleate stage is preceded by a secondary two-nucleate stage, and the sequence then is as follows: megaspore mother cell, primary two-nucleate stage, primary four nucleate stage, secondary two nucleate, secondary four-nucleate and last of all the eight- nucleate stage.

In normal cases all the four megaspore nuclei are of the same size, but in some plants the micropylar nucleus becomes large and the other three nuclei remain small. In *Tulipa maximovicii.* (Romanov, 1939) the three chalazal megaspore nuclei divide abnormally in which all the telophase chromosome groups are included in a common membrane, so that, the mature embryo sac becomes five-nucleate.

***Plumbagella* type.**

This type of development of embryo sac has been reported only in *Plumbagella micrantha* (Fagerlind, 1938; Boyes, 1939). In this type, the 4 megaspore nuclei take up a 1+3 arrangement, *i.e.,* one nucleus at the micropylar end and three nuclei at the chalazal end. The three nuclei of the chalazal end fuse together forming a single triploid nucleus. Now the developing embryo sac is in binucleate stage. Both the nuclei divide once mitotically forming a second four nucleate stage, two micropylar haploid nuclei and two chalazal triploid nuclei. There are no further divisions. The nucleus situated near the micropylar end organizes into the egg; the triploid nucleus of the chalazal end forms the single antipodal cell, and the remaining two nuclei, one haploid and the other triploid, fuse to form a tetraploid secondary nucleus in the centre.

This type of development of embryo sac shows an evident relationship with the *Fritillaria* type, with the difference that in *Plambagella* type the development is checked at the secondary four-nucleate stage, and the fourth division is not there.

***Plumbago* type.**

This type of development of embryo sac has been reported in *Plumbago capensis* (Haupt, 1934). Here the four megaspore nuclei arrange crosswise and divide once to form eight nuclei in four pairs. One nucleus of the micropylar pair is now cut off to form the lenticular egg cell and one nucleus from each of the four pairs approach each other in the centre and fuse to form a tetraploid secondary nucleus. The remaining three nuclei degenerate.

The *Plumbago* type of development occurs not only in other species of the genus *Plumbago* (Dahlgren, 1937; Fagerlind, 1938) but also in two other genera of Plumbaginaceae, viz., *Ceratostigma* (D'Amato, 1940) and *Vogelia* (Mathur and Khan, 1941): This type is not known in any other family.

***Adoxa* type.**

This type of development of embryo sac was described for the first time by Jonsson (1879) in *Adaxa moschatellina* and later by Lagerberg (1909) and Fagerlind (1938). In this type, the four megaspore nuclei divide to form eight nuclei which form a normal type of 8-nucleate embryo sac, *i.e., a.* normal egg apparatus, three antipodal cells and two polar nuclei. This type of embryo sac is regular in *Adoxa* and *Sambucus.* It also occurs in some species of *Ulmus, Tulipa,* and *Erythronium.*

Variations. An interesting variation has been reported in some species of *Tulipa.* In *T. sylvestris* (Bambacioni, 1931) vacuolation frequently occurs even at the megaspore mother cell stage, and all the four megaspore nuclei gather at the micropylar end of the cell. Now they divide to give rise to a group of six cells and two free nuclei. One of the six cells represents the *egg.* Romanov (1938) reported the similar case in *T. tetraphylla.* As soon as the meiotic divisions are over, three nuclei are transferred to the micropylar end and one to the chalazal end. All the nuclei divide again, so that there are six daughter nuclei in the upper part of the embryo sac and two in the lower. This is followed by the formation of cell plates at the end of the division, resulting in

the formation of five cells at the micropylar end (one of them represents an egg) and one cell at the chalazal, leaving two free polar nuclei in the centre.

Abnormal and Unclassified Types

In addition to above mentioned distinct and well-established types of embryo sac development, the somewhat isolated types are mentioned here.

Limnanthes douglasii type.

The development of the embryo sac of *Limnanthes douglasii* is controversial. According to Stenar (1925), it is an *Adoxa* type of embryo sac and he; noticed the reduced size of the nuclei at the chalazal end. This view has been supported by Eysel (1937), but he reported an occasional reduction in the nuclei of the chalazal end of the embryo sac, because of the failure of the formation of the basal nucleus of the four nucleate stages to undergo the last division. According to him embryo sac contains nine nuclei, of which seven organize into cells *(i.e.*, four synergids, two eggs, and one of uncertain nature) and two form the polar nuclei. The third view proposed by Fagerlind (1939) differs from both of Stenar and Eysel.

He reported that megaspore mother cell contains a much vacuolated cytoplasm. The first division gives rise to two nuclei, of which the lower degenerates and shows compact homogeneous blob that lies at the bottom of the embryo sac, and the upper nucleus undergoes further nuclear division, forming two daughter nuclei of which lower one is smaller and does not divide further. Thus following meiosis, a three-nucleate stage is formed which represents a micropylar nucleus, middle nucleus and a chalazal nucleus. Only the micropylax nucleus divides further twice, forming a group of four nuclei which organize into the egg apparatus and the upper polar nucleus. The remaining two nuclei are considered as an antipodal and the other one as the lower polar nucleus.

***Balsamita vulgaris* type.**

According to Fagerlind (1939), as in other members of family Compositae the ovules of this plant are tenuinucellate. The archesporium is generally two celled, but this number may vary from one to three. After the first meiotic division two nuclei are formed of which the upper becomes larger in size. Both nuclei divide again without wall formation, and the four nuclei are formed, which take up 1+3 arrangement. Only

the micropylar nucleus functions, the remaining three degenerate. Now a lateral vesicular outgrowth develops due to the vacuolation of the cell. This tubular outgrowth gradually makes its way and travels upward into the micropyle. The functional megaspore nucleus migrates into the apex of the tube and divides twice the successive nuclear divisions forming four nuclei, which arrange in two pairs, one at each end of a large vacuole. Now these four nuclei divide again and eight nuclei are resulted, of which the upper four forms the egg apparatus and the upper polar nucleus, and the lower four give rise to three antipodal cells and the lower polar nucleus. The nuclei of the antipodal cells usually undergo some divisions but the daughter nuclei fuse once again to form a single lobed nucleus.

Organisation of Mature Embryo Sac

In majority of angiosperms, the eventual organization of embryo sac shows a uniform pattern, whereas the origin of the mature embryo sac may differ. The *Polygonum, Allium, Fritillaria* and *Adoxa* types have similar appearance at the time of fertilization *(i.e.,* three-celled egg apparatus, three antipodal cells and two polar nuclei). However, in few genera such as *Peperomia, Plumbago, Plumbagella,* etc., the basic plan of the embryo sac is different.

The egg apparatus.

Typically the egg apparatus consists of an egg and two synergids. Usually the synergids are ephemeral structures which degenerate and disappear soon after fertilization or sometimes before it. As a rule, each of the synergids is notched and possesses a prominent hook. The nucleus lies in or just below the hook and the lower part of the cell contains a large vacuole. In the egg cell, the nucleus and most of the cytoplasm lie in the lower part of the cell and the vacuole in the upper. Usually the synergids are short lived which degenerate soon after fertilization or even before that. However, in some cases they persist for some time and show signs of considerable activity.

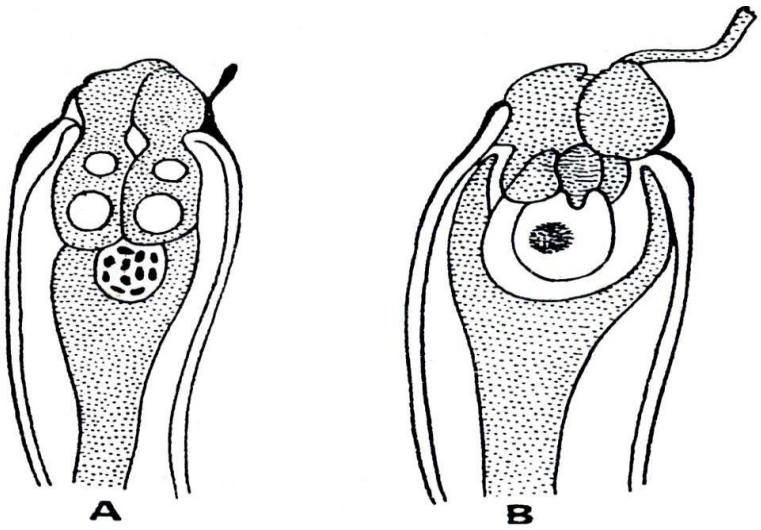


Figure 20. The hooked synergids. A, in *Santalum;* B, in *Daphne.*

According to Weber (1929), *m'Allium knifolium* and A. *rotundum,* one of the synergids begins to degenerate only after the development of embryo has started. In some members of Cucurbitaceae both the synergids become large and prominent, and it is thought that they play an important role in the nutrition of the embryo sac. In certain cases synergid haustoria develop as in *Lathraea, Lobelia* and *Angelonia.*

Antipodal cells

The antipodals are usually short-lived. However, they frequently show a considerable increase in size or number. An increase in the number of antipodal cells and the number of nuclei per antipodal cell is frequently found in the Compositae. The antipodal cells of some members of Ranunculaceae become greatly enlarged and glandular in appearance. The antipodal cells are not present in four-nucleate embryo sacs of the *Oenothera* type. In some members of Gentianaceae, the three antipodal cells divide to form about ten to twelve cells, and in the family Gramineae a still larger number of cells are produced.

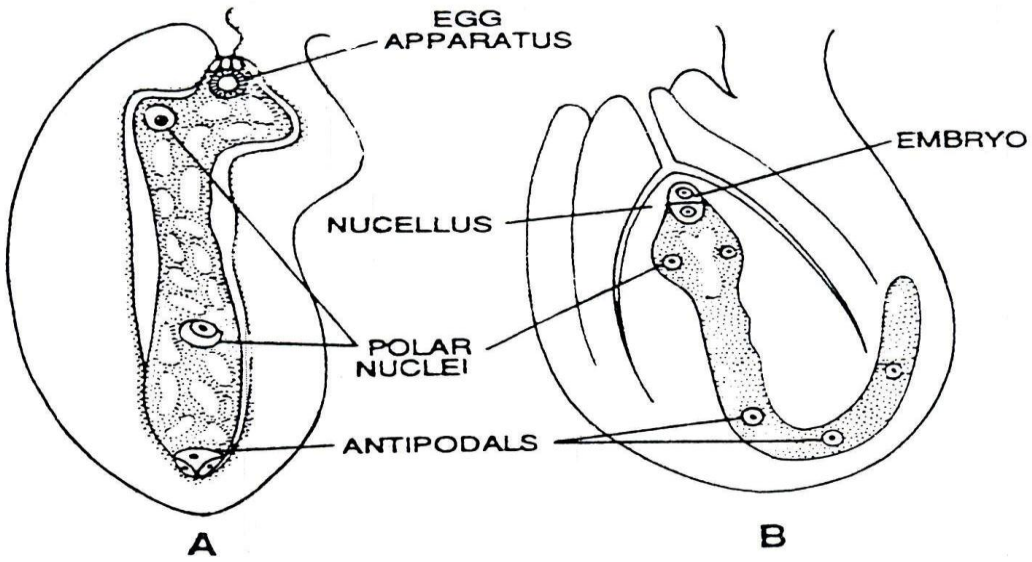


Figure 21. Embryo sac haustoria. A, micropylar end of the embryo sac protrudes out of the ovule, breaking down completely the nucellus, above it; B, the chalazal end of the embryo sac enlarges in size and digests its way through the nucellus and integument.

In many genera of the family Rubiaceae *(e.g.,* in *Putoria),* the basal antipodal cell elongates in size and acts as hausorium. Less than three antipodal cells have been reported in Plumbaginaceae, Scrophulariaceae and Compositae. Multicellular antipodal complexes are found in the families, such as Ranunculaceae, Papajveraceae, Compositae, Gentianaceae and Gramineae. The enlargement of the antipodal cells, without increasing in number beyond three has been reported in *Crocus, Iris* and *Gladiolus* of family Iridaceae, and in a few other monocotyledonous plants, such as *Narsissus, Ornithogallum* and *Commelina,*. In these examples, the enlargement is downward and may be associated with invasion of the chalaza. In *Aconitum napellus* (of Ranunculaceae), the enlargement takes place upward into the embryo sac itself. However, the arrangement of the three antipodal cells is very, much variable. Generally, the two cells remain at the base of the embryo sac, and the remaining third one above them. On the contrary, linear groupings both longitudinal and transverse also occur. Antipodal cells are synthetic and they remain engaged in absorption and conduction.

Polar nuclei or central cell

The central portion of the embryo sac contains polar nuclei, and eventually gives rise to the endosperm and therefore, known as endosperm mother cell usually the two

nuclei coming from two different poles are similar in appearance, but sometimes the micropylar polar nucleus is bigger one. The fusion of the polar nuclei may occur either before, or during, or sometimes after entry of the pollen tube inside the embryo sac. The central cell is the largest cell of embryo sac. The nuclei of central cell called polar nuclei are large, and each of them possesses a well defined nucleolus. The cytoplasm of central cell contains plastids, mitochondria, dictyosomes, ribosomes, etc. Generally the micropylar nucleus of the pair of polar nuclei is larger than the chalazal nucleus. But sometimes contrary to it the chalazal nucleus is larger than the micropylar nucleus, as in the embryo sac of *Fritillaria* type. The resultant of the fusion of the polar nuclei is called, the secondary nucleus, the central nucleus or the primary endosperm nucleus, respectively.

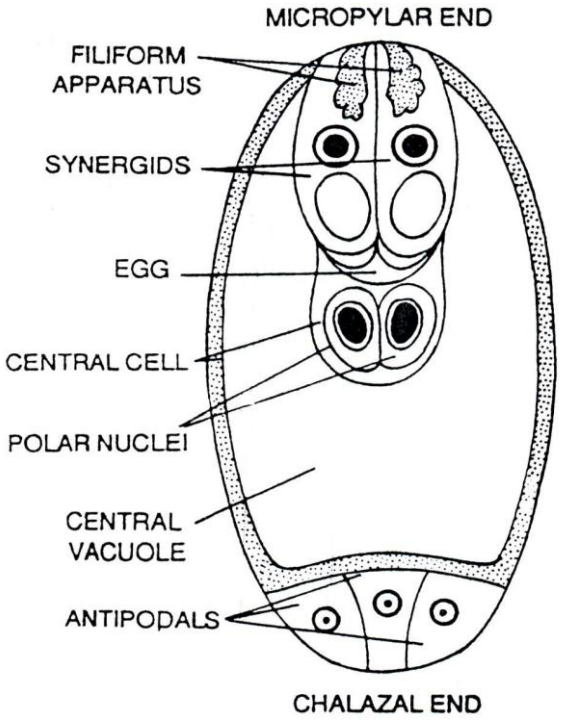


Figure 22. Structure of embryo sac.

The number of polar nuclei varies in different species. For example, in four-nucleate embryo sacs of *Oenothera* type only one polar nucleus is found. In sixteen-nucleate embryo sacs, as in *Plumbago* type the number of nuclei combining to form the primary endosperm nucleus varies from three to fourteen with a high degree of polyploidy in the endosperm nucleus. In *Fritillaria* the lower polar nucleus, like the antipodals is triploid and the primary endosperm nucleus before fertilization is thus tetraploid. The secondary nucleus formed after fusion usually moves towards the egg

and remains separated from the antipodal cells by a large vacuole. When the secondary nucleus is situated in the centre it shows connection with the egg apparatus by a thick strand of cytoplasm. According to List and Steward (1965), the vacuole of central cell acts as reservoir of amino acids, sugars and inorganic salts.

Food reserves in the embryo sac

Generally it is assumed that the angiosperm embryo sac is devoid of any appreciable food reserves. However, there are now several records of the occurrence of starch in embryo sacs, and in the families Aizoaceae, Cactaceae, Portulacaceae, Bruniaceae, Tiliaceae, Crassulaceae and Asclepiadaceae this is a common phenomenon. A few cases have been recorded in which the starch occurs not merely in the cavity of embryo sac but also in the cells of egg apparatus and rarely even in the antipodal cells. The higher concentration of starch grains stored during the development of the embryo sac is utilized at the time of development of endosperm and embryo. After fertilization endosperm provides a considerable amount of food material (Maheshwari, 1950; Wardlaw, 1955).

The various types of haustoria developed in the embryo sac are the tools for the absorption of the food materials from ovarian, placental and stylar tissue. Arnoldi and Zinger (1961) reported the presence of fat in the living ovules. It has been suggested, that fat is also brought into the embryo sac by the pollen tube and it accumulates near the egg apparatus and polar fusion nucleus. Thus, the physiological and biochemical changes in the female gametophyte are of significance since they serve in subsequent development leading to the production of fertile seeds.

Embryo sac haustoria

In normal cases the general surface of the embryo sac is absorptive in function but in certain cases the ends of the embryo sac show more active growth and convert into haustoria; which absorb the food not only from the nucellus and integuments but also from the placental tissue to which they directly contact. As shown in Fig. 21 A, in certain genera of Loranthaceae the embryo sac shows sufficient elongation of the micropylar end where it enters into the tissue of the style and grows into it. In other examples as shown in the Fig. 21 B, the embryo sac grows downward. Here the chalazal end of the embryo sac acts as haustorium and digests its way through the nucellus.

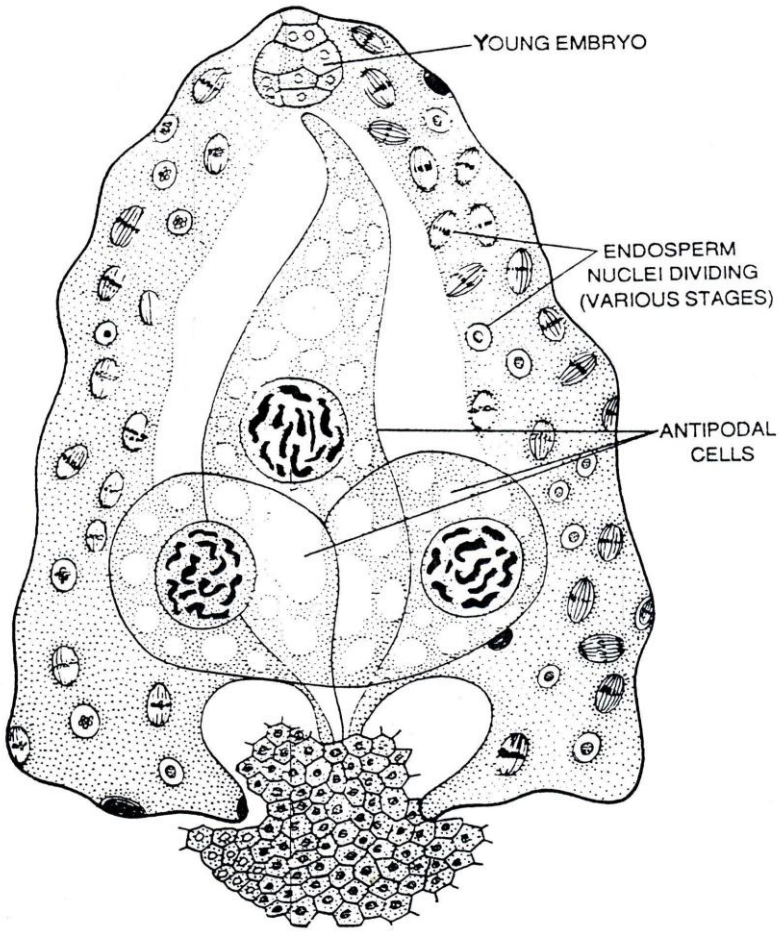


Figure 23. Structure of embryo sac in Aconitum napellus containing three large antipodal cells.

Morphology of Female Gametophyte

According to Schnarf (1936) the monosporic eight-nucleate embryo sac is the most primitive, and all the other types have been descended from it. This idea has been followed by several other plant embryologists. The chief argument in the support of this view is that this type is the most widely distributed in angiosperms, and on the other hand the female gametophytes of the pteridophytes and gymnosperms are also monosporic. Another argument in the favor of this view is that all the other types can be easily derived from it, while the reverse is not possible. In the *Oenothera* type, only two divisions intervene between the functioning megaspore stage and the differentiation of the egg apparatus, and all the four nuclei remain restricted to the micropylar part of the embryo sac.

In the *Allium* type, wall formation does not take place after meiosis II, and each dyad cell contains two megaspore nuclei; now two further divisions are required to give

rise to the eight- Nucleate stage. In the tetrasporic types no wall formation takes place after any of the meiotic divisions, with the result all the four megaspore nuclei lie in a common cavity, and may be organized in various ways, one pair of nuclei lying at the micropylar end, and the other at the chalazal, or one nucleus at the micropylar end and three at the chalazal end, or one nucleus at micropylar end, one at chalazal end, and the remaining two at the sides. The megaspore nuclei may divide twice or only once. The 2+2 position of nuclei represents the *Peperomia* and *Adoxa* types; the 1+3 position represents the *Drusa, Plumbagella* and *Fritillaria* types.

Considering the monosporic eight-nucleate embryo sac as the fundamental type there are three main theories as to its homologies. They are as follows.

1. Genetalean theory.

According to this theory the embryo sac of angiosperms is derived from a form like *Gnetum* in which all the nuclei of the embryo sac possess the same morphological value, and any of them can function as an egg and may give rise to an embryo. This view was put forward for the first time by Hofmeister and Strasburger, and was given the name 'the Gentalean theory'. In *Gnetum* (a gymnosperm), the sac contains many free nuclei all of which have potential for the formation of an egg. According to this theory the angiospermic embryo sac has been derived from an embryo sac of Gnetalian type by reduction, in number of nuclei. The sac of *Gnetum* is also a highly modified gametophyte, specialized by the multiplication, rather than the reduction of potential eggs. In angiosperms the synergids are the 'new features' which are not present in gymnosperms, and thus double fertilization is also a 'new feature'. If the embryo sac of *Gnetum* represents a transitional stage in between that of most gymnosperms and that of angiosperms, it should show some evidence in transitional form rather than an entirely different type of gametophyte. The Gnetalean theory has been supported by many plant embryologists.

2. Porsch's theory.

According to this view the embryo sac of angiosperms is derived by reduction from the female gametophyte of some gymnosperm and consists of only two archegonia without any prothallial tissue. Here the embryo sac of angiosperms can be compared with two archegonia, where one archegonium is represented by the egg apparatus and micropylar polar nucleus and the other by the antipodals and chalazal polar nucleus.

The archegonium found at the micropylar end is functional and consists of two neck cells (synergids), oosphere (egg cell) and a venter canal nucleus (micropylar polar nucleus). The archegonium of the chalazal end is without any type of function. This view was first put up by Porsch (1907), and has been supported by Nilson (1941) and Schnarf (1942). However, this theory has been criticized by P. Maheshwari (1950). The objections are as follows.

a) In the archegonia of pteridophytes and gymnosperms, the venter canal nucleus is situated just above the egg. On the other hand in angiosperms, the upper nucleus, which should have formed the venter canal cell, is supposed to develop the egg, whereas the lower cell which should have organized into the egg represents the venter canal nucleus.
b) In some species of *Peperomia* and in *Acalypha indica* the egg is associated with a single synergid. It is then supposed that there is an archegonium in which one neck cell has disappeared, leaving the other to perform the function of both.
c) The other objection is that in the angiosperms there are several known instances of embryos developing from synergids, either as the result of fertilization or even without fertilization. There is no known record where the neck cell of a true archegonium acts in a similar way.
d) Another objection is that the components of the chalazal quartet, *i.e.*, the antipodal cells and lower polar nucleus, show a great variation in their behavior which is not known in archegonia.

3. Schurhoffs hypothesis.

This view was first propounded by Schurhoff (1919) and later supported by him in 1928. According to this hypothesis, the egg apparatus and micropylar polar nucleus represent the remnants of two archegonia and the chalazal group of four cells (three antipodal cells and one chalazal polar nucleus) represents the prothallial tissue. According to this view one synergid and egg constitute the first archegonium, the synergid is supposed to be equivalent to the ventral canal cell, and the other synergid and the upper polar nucleus make the second archegonium. Neck cells are not found, and fertilization takes place in both the archegonia, one gives rise to the embryo and the other to endosperm.

The main objection to this view is that one synergid is sister cell to the egg and the second to the upper polar nucleus, and that these two pairs of nuclei make two separate archegonia. Langlet (1927), however, produced evidence to show that the synergids are developed from one pair of sister nuclei and the egg and upper polar nuclei s from another pair. According to above mentioned views, the embryo sac seems to be the result of extreme reduction and it is constituted of 'archegonia and their contents' or of 'vestiges of archegonia', containing a few or no prothallial cells. Thus the angiospermic gametophyte is so much reduced that apparently no interpretation of its nature can be well supported even if the embryo sac is considered without double fertilization, which is shown only after the maturity of the embryo sac.

CHAPTER II

POLLINATION MECHANISMS IN ANGIOSPERMS

In angiosperms, the pollen grain is being transferred from the anther to the stigma, and is termed pollination. This phenomenon was first discovered by Camerarius (1694) in the end of seventeenth century. According to him pollination is essential for the production of the seed. Several agencies are indulged in bringing about this transfer of pollen from the anthers to the stigma. The pollination may be of two types self pollination and cross-pollination. The transfer of the pollen from the anther of a flower to the stigma of the same flower is known as self pollination, whereas cross-pollination is the transference of the pollen from one flower to another flower. The cross pollination is of three types.

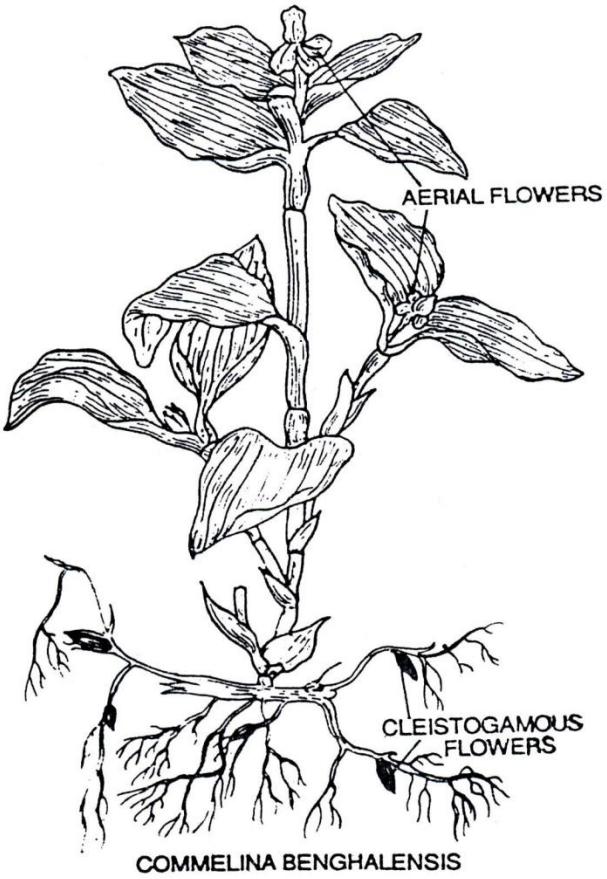


Figure 24. Homogamy-Cleistogamy. The cleistogamous flowers are born on underground branches of *Commelina benghalensis.*

1. Xenogamy. In this type the pollination takes place between flowers borne on two different plants of the same species.

2. Geitonogamy. This type of pollination takes place between the flowers developed on the same plant.

3. Hydridism. Such pollination takes place between two flowers of two different plants of the allied species or sometimes even allied genera. In the condition in which the pollen is discharged from the anther, they show considerable resistance to environmental changes. Sometimes they remain viable for several weeks.

In certain cases even in hermaphrodite flowers self-pollination does not take place. This happens because of heterostyly, *e.g.*, in *Primula vulgaris;* dichogamy, where the maturity of male and female sex organs of the flowers is attained at different times, *e.g.*, in *Impatiens* ; herkogamy, in which the structure of male and female sex organs in the flowers acts as barrier for self- pollination, and selfsterility, as found in *Petunia axillaris.* As mentioned in preceding paragraph, this phenomenon involves the transference of pollen grains from the anther of a flower to the stigma of the same flower or of another flower of the same or sometimes allied species. On the bursting of the anthers, the pollen grains are released, and scattered. Some of the pollen grains are carried over to the stigma by means of various agencies such as wind, water, insects, animals, etc. Pollination is of two kinds.

1. Self-pollination or autogamy *(auto* = self ; *gamos* = marriage)
2. Cross- poliintion or allogamy *(alios* = different).

1. Self-pollination

This kind of pollination is this transference of pollen grains from the anther of a flower to the stigma of the same flower or from a flower (male or bisexual) to a flower (female or bisexual), both found on the same individual plant. Here only one parent plant is concerned to give rise to the offspring. The self-pollination is, however, prevented in unisexual flowers borne by two separate plaints, and also in many bisexual flowers. In the normally self-pollinated plants, occasional cross-pollination in some generation is a biological necessity to maintain the vigor of the offspring. The under mentioned adaptations are commonly found in flowers to achieve self- pollination.

Homogamy *(homos*=same).

In this condition, the anthers and the stigmas of a bisexual flower mature at the same time.

- Here some of the pollen grains may reach the stigma of the same flower through the agency of wind or insects, thus effecting self-pollination.
- The filaments of the anthers may recoil, and bring the mature anther close to the stigma *(e.g.,* in *Mirabilis jalapa).* The anthers then burst and discharge their pollen right on the surface of the stigma. In some cases; the stigmas move back and touch the anthers to achieve self-pollination when cross-pollination fails *(e.g.,* in Asteraceae [Compositae] and Malvaceae families).
- In some drooping flowers the style is longer than the filaments, whereas in certain erect flowers the reverse may be the case.
- Sessile or sub-sessile anthers may lie at the mouth of the narrow corolla tube and the stigma, while pushing out through the tube brushes against anthers *(e.g.,* in *Ixora*, *Gardenia* and *Vinca).*

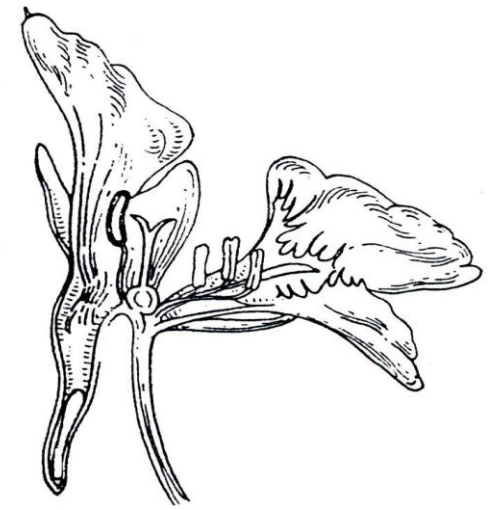


Figure 25. Nectary. V.S. flower of garden nasturtium showing the nectar concealed in the spur.

Cleistogamy *(Kleistos* = closed)

Some of the bisexual flowers do not open and are known as cleistogamous or closed flowers. In such flowers, the pollen grains are distributed on the stigma of the same flower. Such cleistogamous flowers are very small and inconspicuous. They are not colored, and do not secrete any nectar. These flowers are not even scented. This type of pollination is found in the underground flowers of *Commelina bengalensis, Viola, Drosera, Oxalis,* etc.

Cross-pollination: **Modes of cross pollination**

The cross-pollination is induced by external agents who carry the pollen grains of one flower and deposit them on the stigma of another flower, the two being borne by two separate plants of the same or closely allied species. These agencies may be insects *(e.g.*, bees, flies, moths, etc.), animals *(e.g.*, birds, snails, etc.), wind and water. The allogamy (cross-pollination) is the rule in unisexual flowers borne by two separate plants, while in bisexual flowers it also occurs generally. Nature favors cross-pollination and there are so many adaptations in flowers to achieve this type of pollination.

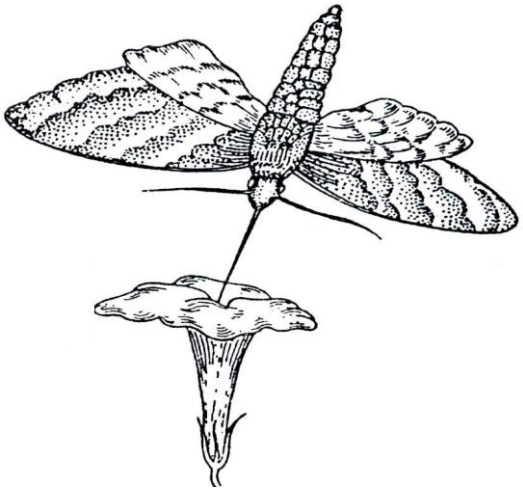


Figure 26. Entomophily. Flower of Petunia being visited by a moth.

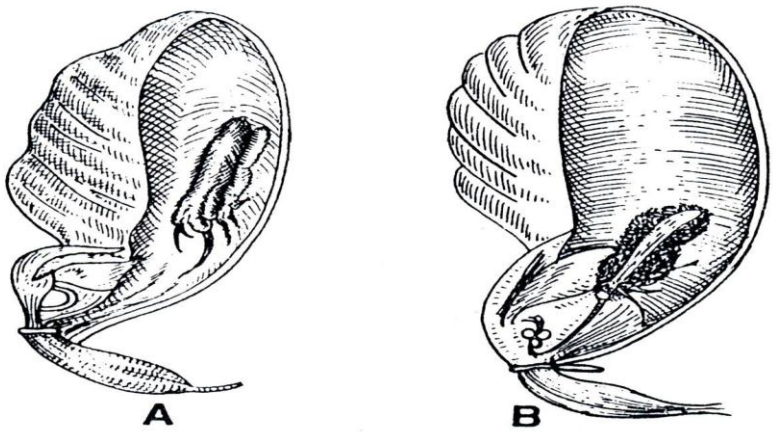


Figure 27. Entomophily. A-B, V, S. Flowers of *Cyprepedium* (an orchid) showing pollination by bee.

Entomophily *(entomon* = insect, *phileo,* to love)

This type of pollination takes place through the agency of insects. It is of general occurrence among plants. The insect-loving flower possesses various adaptations by which they attract insects and use them as carriers of pollen grains for the purpose of cross-pollination. The main such adaptations are color, nectar and scent. Besides these, there are certain special adaptations in some flowers.

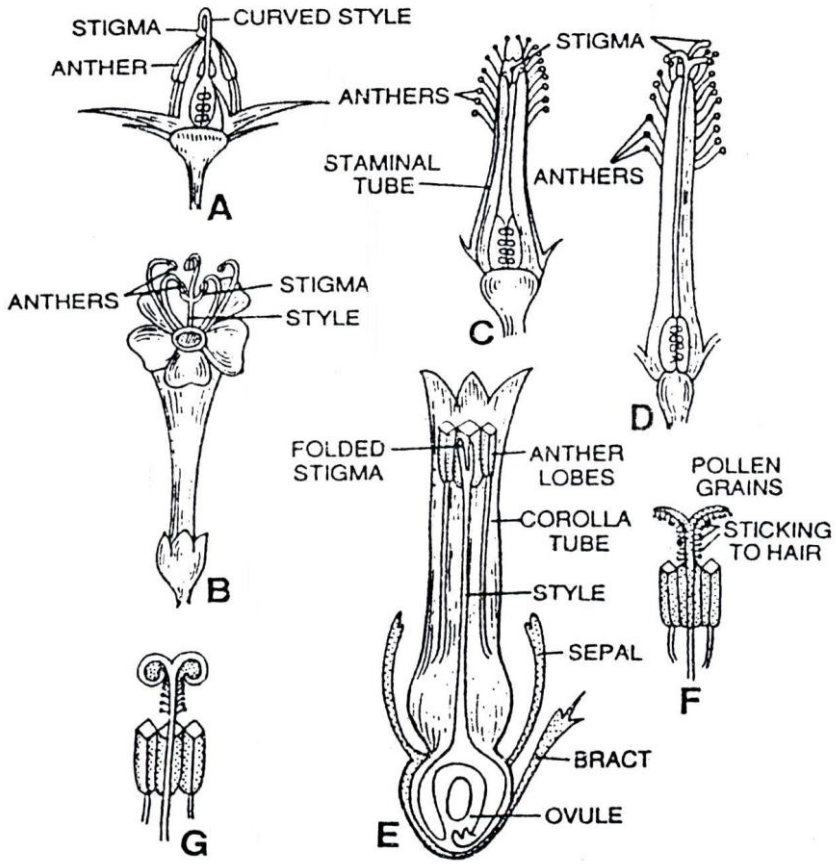


Figure 28. Modes of cross-pollination. A, in *Solanum tuberosum* the stigma bent over the stamens; B, in flpwer of *Mirabilis jalapa* the anthers bent over the stigma; C-D, in the flowers of *Hibiscus rosa-sinensis* the stigma brushes against the anther lobes to receive pollen; E-G, in flower of *Helianthus annuus* the stigmatic lobes curl back ward to receive the pollen sticking to hair on the outer stigmatic surface and the style.

Color

The colour of the petals (corolla) and tepals (perianth) is one of the most important adaptations. The bright colors and irregular shapes of the flowers are responsible to a great extent to attract the insects. In some cases, where the flowers are inconspicuous,

other parts may become colored and showy to attract insects. For example, in *Mussaenda*, one of the sepals becomes modified into a large white or colored leafy structure and serves as an advertising flag [to attract insects. In *Bougainvillaea* and *Euphorbia pulcherrima*, the bracts become highly coloured and attractive. In other cases *(e.g.,* in Musaceae and Araceae) the spathes become brightly colored to attract the insects.

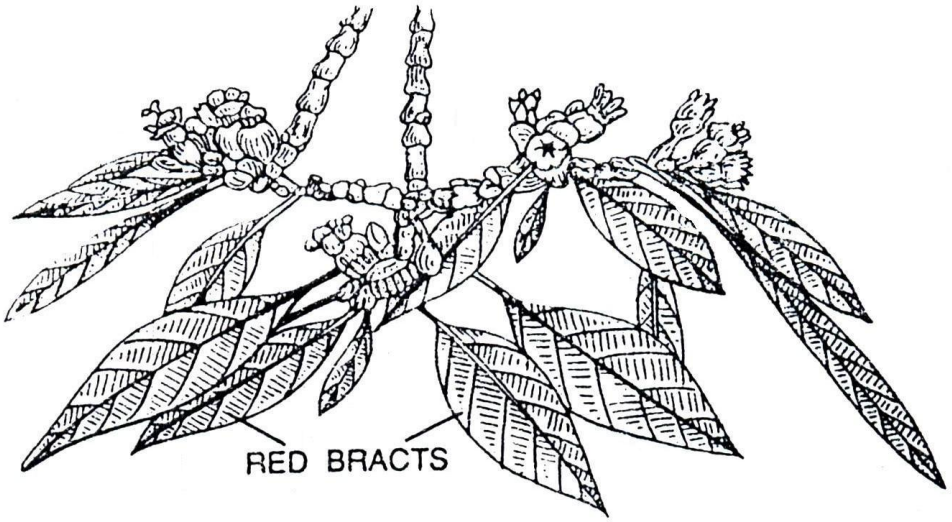


Figure 29. Modes fof cross pollination colour. In *Euphorbia pulcherrima* colored leaf like bracts that surrounded the cyathia, attract the insects for the purpose.

Nectar

The nectar is also an important adaptation to attract the insects. Most of the flowers with gamopetalous corolla secrete nectar which makes a positive attraction to the insects like bees. The nectar is generally found in a special gland known as nectary. Generally the nectary occurs at the base of one of the floral whorls, and as the bees come to the flower to collect the nectar from the nectary, they bring about pollination. Usually in course of a day a single bee can easily pollinate over one hundred flowers. It is a common sight to see a swarm of bees hovering over a shrub or tree in full bloom.

Scent

The other important adaptation is the scent. Most of the flowers which open in the night are insect-loving and they emit at night a sweet scent which attracts insects from a distance. At night when color is not seen, the scent becomes quite useful directing

the insects to the flowers. Thus flowers opening in night are generally sweet-smelling *(e.g., Nyctanthes, Cestrum nocturnum*, jasmines, *Quisqualis ituiica* and several others). In some cases where the smell is offensive the nauseating to human beings, is immensely liked by small insects. For example, the appendix of the mature inflorescence of *Amorphophallus* emits a stinking smell, and always attracts a swarm of carrier flies, and pollination is effected through them.

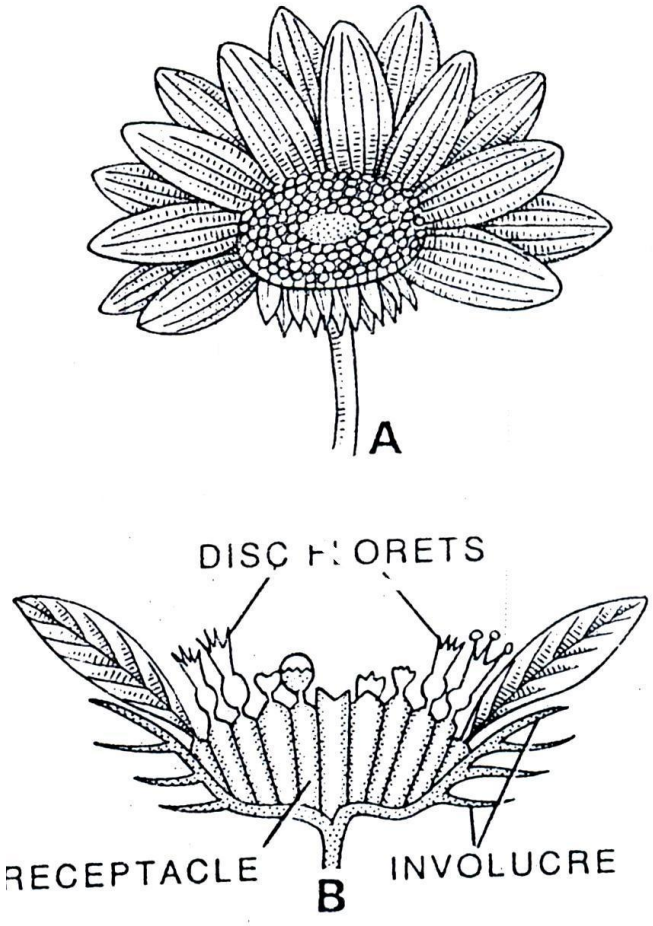


Figure 30. In capitulum inflorescence of *Helianthus* (A-B), the closely arranged flowers have every chance of pollination.

Generally, the pollen grains of entomophilous flowers are sticky. The stigma is also sticky. Pollen grains and nectar are very often used as food materials by the insects. Flowers generally attract insects by their color, nectar or scent, or they visit the flowers in search of food, or shelter from sun and rain. Thus, as the insects visit the flowers their body gets dusted with pollen grains, and when they fly and visit other flowers, they brush against the stigma which being sticky at once receives the pollen grains from their body. Thus cross-pollination is achieved.

Special adaptations.

In sunflower and other members of Asteraceae (Compositae), *Anthocephalus, Acacia,* etc., where the individual flowers are small and inconspicuous, they are massed together in a dense inflorescence which becomes much more showy and attractive. This type of inflorescence with dense masses of flowers has another advantage; flowers being close together have every chance of pollination. In several species of *Ficus* the insects enter the chamber of the inflorescence (hypanthodium) through the apical pore, and as they move over the unisexual flowers inside the chamber the pollination is achieved.

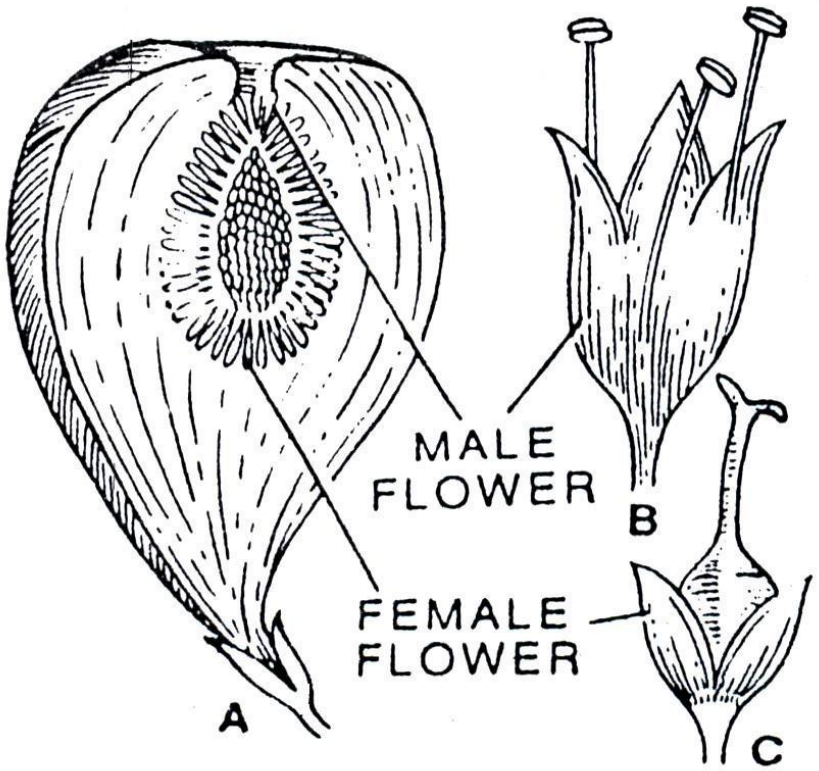


Figure 31. The insects anter throukh the apical pore of hypanthodium in *Ficus*, to ensure pollination (A-C).

Female flowers lie at the base of the cavity and open earlier, whereas male flowers lie near the apical opening and open later so that pollen grains have to be brought over from another inflorescence. Flowers are generally adapted for pollination by some specific insects. For example, in snapdragon and other such flowers with saccate corolla, only the insects of particular size and weight can open the mouth of the

corolla. On the other hand, long- tongued insects can pollinate the flowers with long corolla tubes.

Pollination in *Calotropis*

This is member of Asclepiadaceae and is pollinated by bees. In this flower, the Filaments of stamens form a tube around gynoecium. The anthers are fused with the stigma to form a 5-angled disc called gynostegium. The staminal tube gives out distinct lobes called the corona. It is fused with the petals. The anther lobes that are fused with the stigmatic disc have straight and parallel sides and are separated only by long narrow clefts. Within these clefts there are interstaminal chambers. The pollen grains in the anthers are grouped in the form of mass called pollinium. The pollinium develops a rider mechanism or the translator. It consists of two armslcalled the caudicles. The caudicles carry the two pollinia on one side and are fused to form a black and sticky dot on the other side. This dot-like structure is called the corpusculum. There are five corpuscula at the angles of gynostegium from two adjacent anthers.

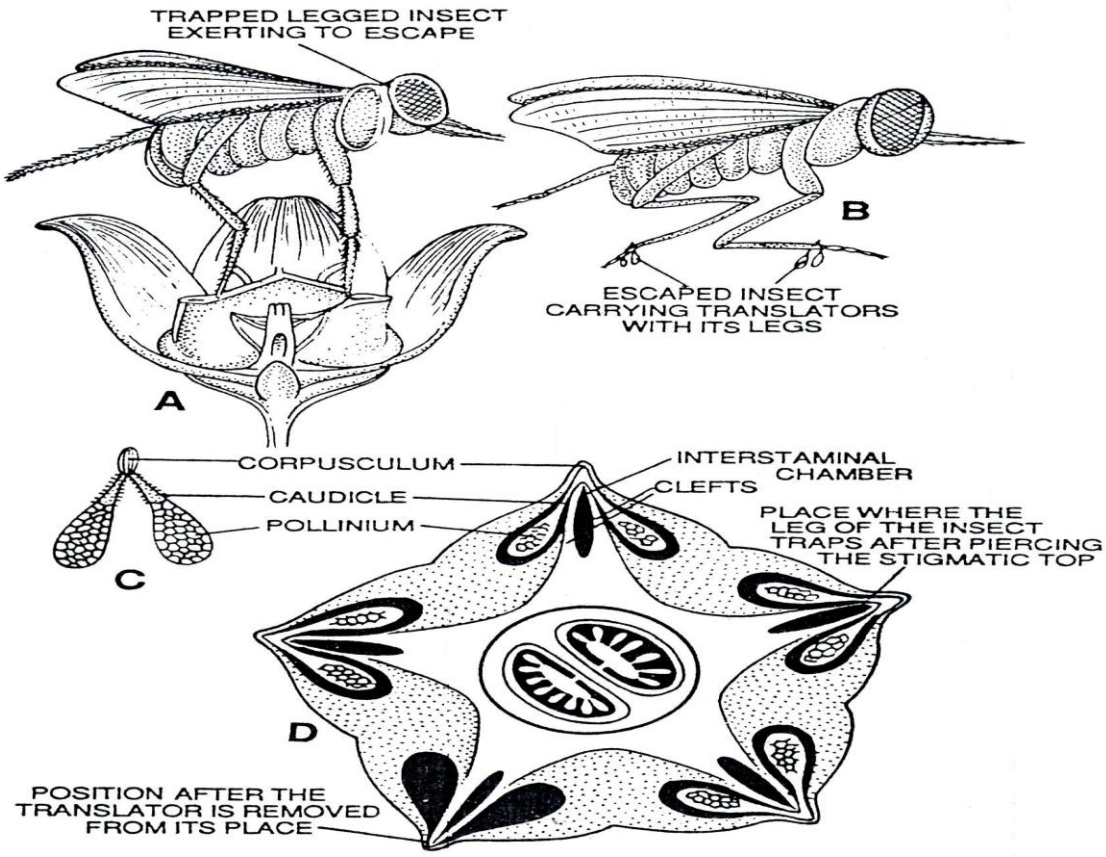


Figure 32. Pollination in *Calotropis.*

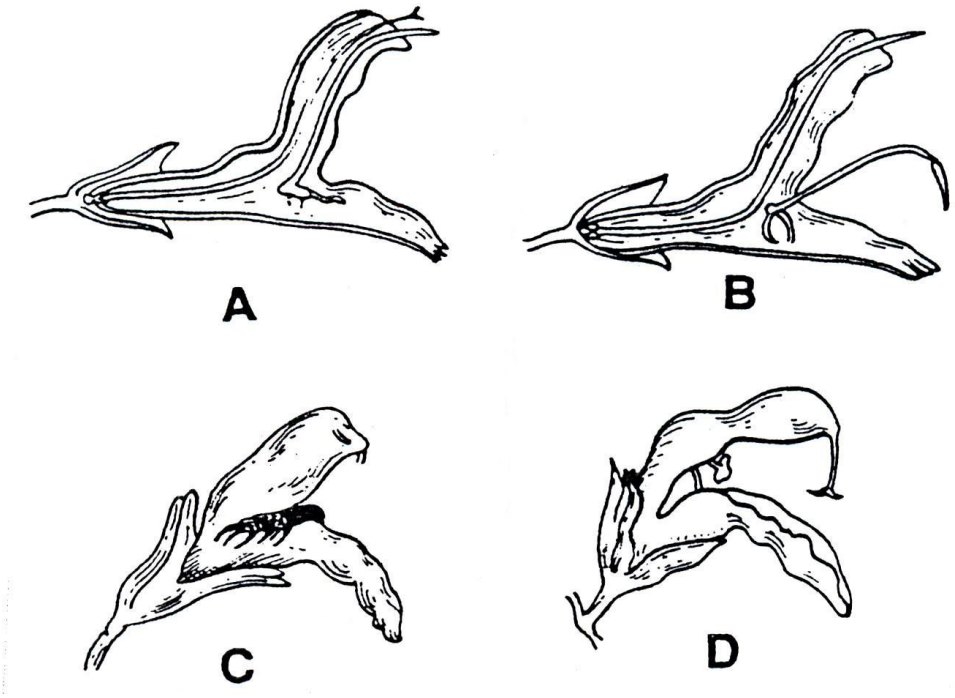


Figure 33. Entomophily. An interesting type of cross pollination in *Salvia* by insects.

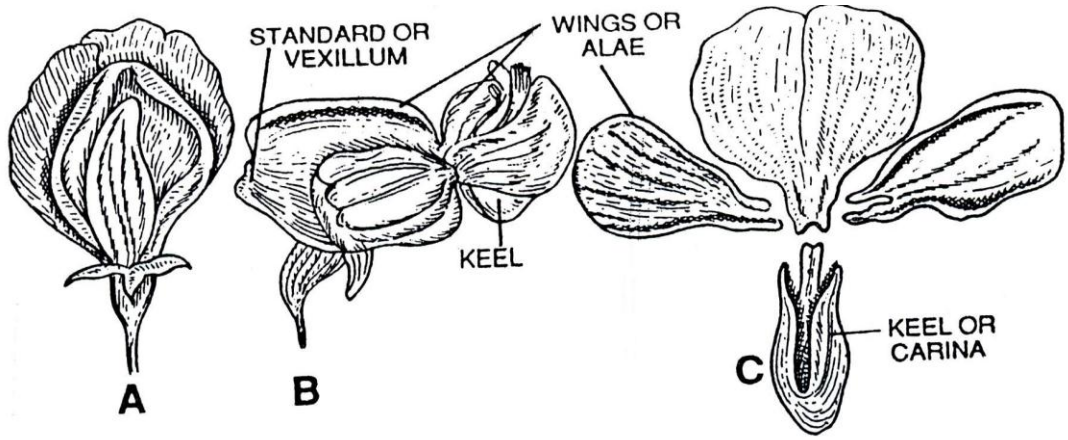


Figure 34. Entomophily. The diagram (A-C), shows the structure of pea flower that is pollinated by bees.

An insect crawling about over the flowers is sooner or later trapped, through one of its legs becoming caught in one of the clefts between adjacent anthers. The insect can release itself only by drawing the leg upwards through the clefts and this it does, but as the leg becomes free at the top of the cleft, it catches in the notch of the corpusculum so that further movements pull this together with its attached pollinia, away from its anchorage on the gynostegium. The released insect in due course visits another flower and again becomes caught by the leg in the same way. While drawing

the leg, this time, through the anther cleft the pollinia brought from the previous flower are torn away from corpusculum and are deposited in the inter staminal chamber. Th4 new translators are carried away and there is repetition of the whole process.

An interesting type of cross-pollination takes place in *Salvia* by insects. In this flower there are two stamens. The two anther lobes of each stamen are widely separated by the elongated curved connective which plays freely on the filament. The upper lobe is fertile and the lower one sterile. In the natural position, the connective remains upright. When the insect, enters the tube of the corolla it pushes the lower sterile anther lobe of each stamen; the connective swing round with the result that the upper fertile lobe comes down and strikes the back of the insect with force and dusts it with pollen grains. The flower is protandrous, and on the maturity of the stigma it bends down and touches the back of the insect and receives the pollen grains from it. Thus, pollination is effected.

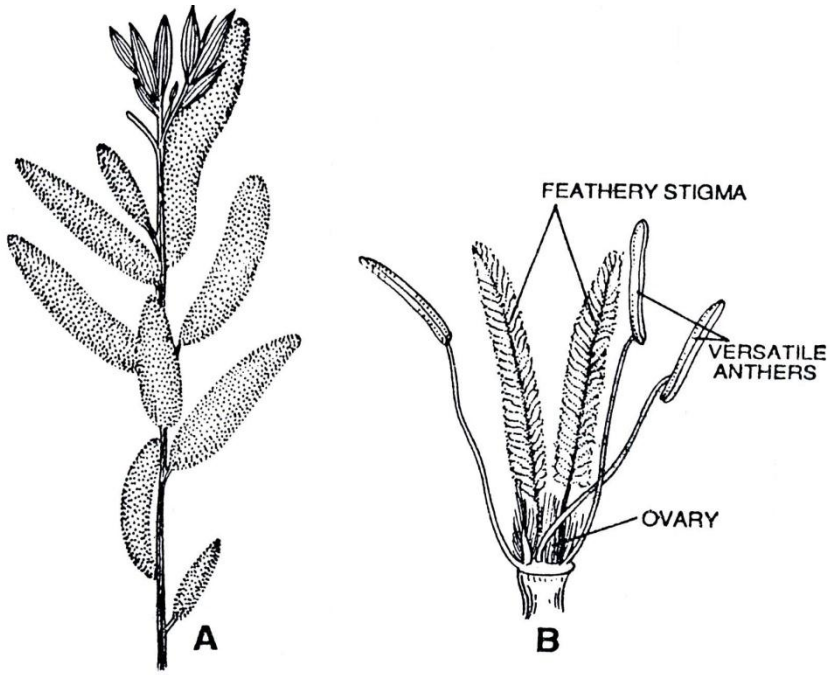


Figure 35. Anemophily. A, the male catkins of *Salix;* B. the flower of *Avena sativa* with feathery stigmas.

Anemophily *(anemos* = wind)

In many cases pollination is achieved by wind. The wind pollinated flowers are small and inconspicuous. They are neither colored nor showy. They do not have any smell

and they do not secrete any nectar. The anthers produce an immense quantity of pollen grains. A large quantity of pollen grains is being wasted during transit from one flower to another. The pollen grains are quite light and dry, and sometimes are provided with wings *(e.g.,* in *Pinus)* for facility of distribution by wind. In the wind-loving flowers the stigmas are comparatively large and protruding, sometimes branched and often feathery *(e.g.,* grasses, bamboos, cereals, millets, sugarcane, etc.). The maize plant makes a good example of this type. The plant bears a large number of male flowers in a terminal panicle, and in the lower part of the plant one or two female spadices, each in the axil of a leaf, surrounded by spathes. A cluster of fine, silky and long styles is seen. On the maturity, the anthers burst and a cloud of dust-like pollen grains is seen floating in the air near the plant. Some of these pollen grains are entangled by the protruding stigmas and thus pollination is effected.

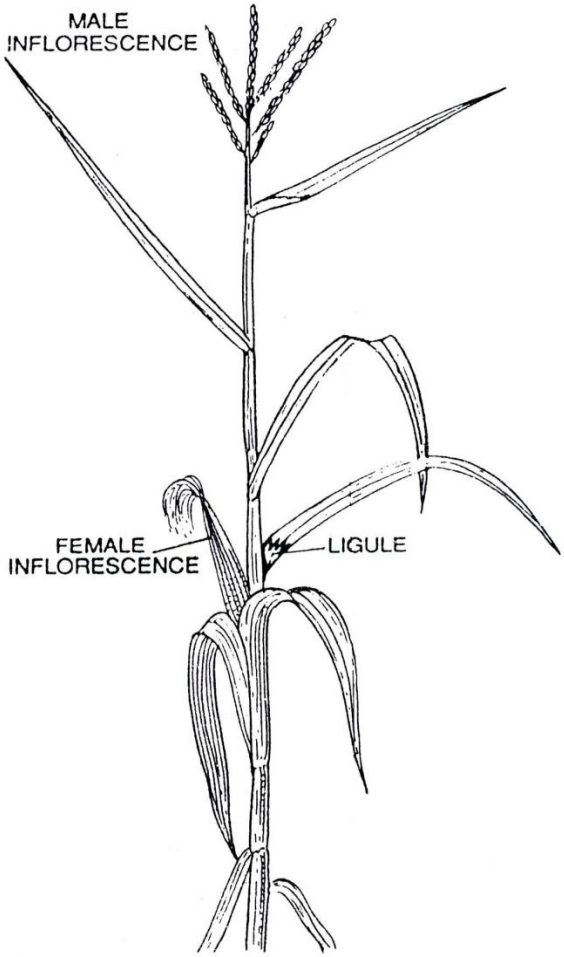


Figure 36. Anemophily. Plant of Zea mays having male flowers in tassels above the foliage and female flowers in spathes with silky long styles.

Hydrophily *(hydor* = water)

In some aquatic plants, the pollination is brought about through the medium of water, *e.g.*, in *Vallisneria, Hydrilla,* etc. In these, aquatic plants where the flowers remain above the water the pollination is achieved through insects or wind. The mode of pollination in *Vallisneria* (submerged aquatic plant) is as follows. The plant is dioecious. The male plant bears a large number of minute male flowers in a small spadix surrounded by a spathe and borne on a short stalk, whereas the female plant bears solitary female flowers each on a long slender pedicel.

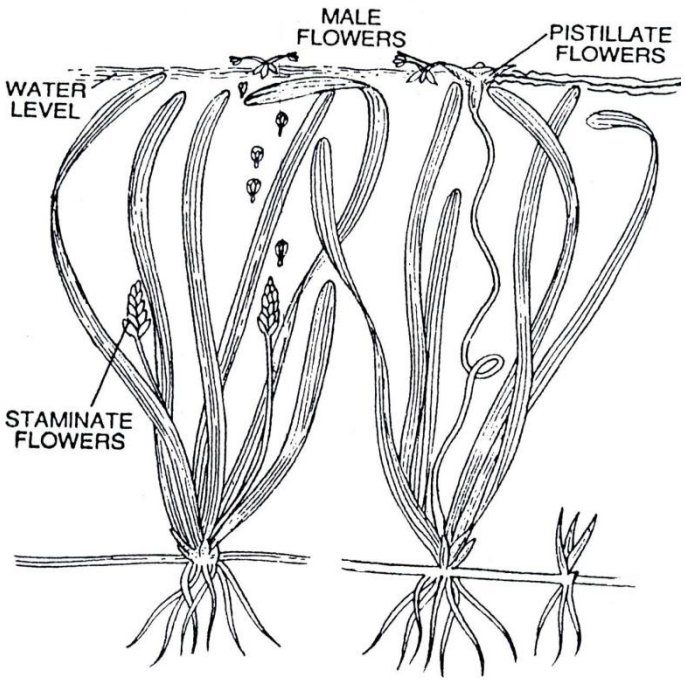


Figure 37. Hydrophily. Pollination by water in *Vallisnaria.*

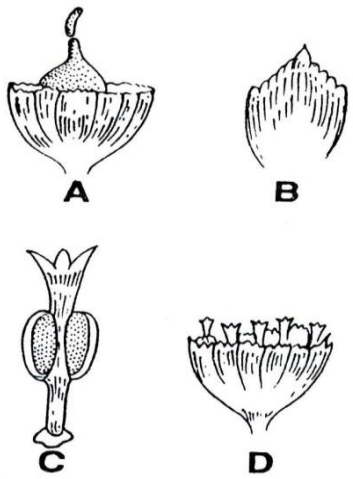


Figure 38. Hydrophily. The structure of male and female flowers of *Ceratophyllum,* which are pollinated by water.

This stalk of the flower elongates and takes the female flower to the surface of the water. The spathe bursts releasing the male flowers from the spadix, while still closed, and float on the surface of the water. The floating male flowers come in contact with the female flowers. The anthers burst and the sticky pollen grains adhere to the surface of trifid stigmas which thereafter close up. As the pollination is over the stalk of the female flower becomes spirally coiled and pulls the female flower down into the water. The fruit develops and matures under water a little above the bottom.

Zoophily *(zoo* = animals)

There are so many animals such as birds, squirrels, bats, snails, etc., which aire involved in cross-pollination. For example, in *Bombax* and *Erythrina* the birds and squirrels are held responsible to bring about pollination. Bats bring about pollination in *Anthocephalus*, while snails in aroids (of Araceae). In aroids, the inflorescence is a spadix; the female flowers remain situated at the base of the spadix and the mate: flowers towards top. The Stigmas mature first and the pollen grains are brought from another spadix.

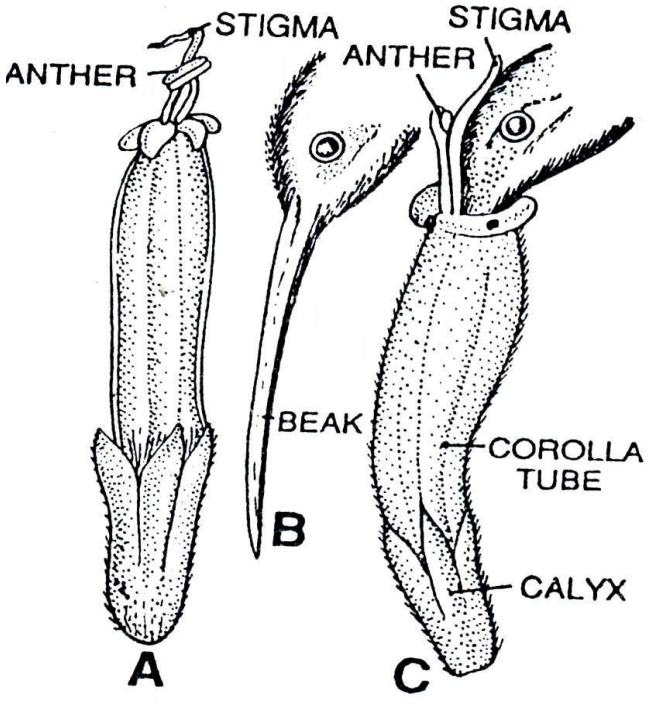


Figure 39. Zoophily. Many flowers are pollinated by birds (Ornithogamy).

CONTRIVANCES FOR CROSS-POLLINATION

Certain structural devices in the flowers favor cross pollination. These are as follows.

1. Unisexuality

The stamens and carpels lie in separate flowers-male and female, either borne by the same plant or by two separate plants. There are two kinds of unisexuality: *(i)* where the male and female flowers lie on the same plant and the plant is said to be monoecious *(e.g.,* members of Cucurbitaceae, castor, maize, etc.) *(ii)* Where the male and the female flowers are borne by one plant and the female flowers lie on another plant, it is known as dioecious *(e.g.,* palmyra palm, *Carica papaya, Moms alba,* etc.). In monoecious plants, there may be self-or cross-pollination, while in dioecious plants, cross-pollination is a basic necessity.

2. Self-sterility

In certain flowers the pollen grains are unable to germinte on its own stigma. It is noted in some orchids that the pollen has an injurious effect on the stigma of the same flower. In this case on the application of pollen to stigma, the stigma dries up and falls off, *Abutilon, Passiflora, Malva, Primus* and *Pyrus* are self-sterile. To effect the successful cross-pollination in these cases the pollen must be from two such parents which differ genetically. Cross-pollination is the only method in such cases for the setting of seeds.

3. Dichogamy *(dicha* - in two)

In many bisexual flowers, the anthers and stigmas often mature at different times. This condition is known as dichogamy. As the anther and the stigma mature at different times, dichogamy often checks the self-pollination. There are two types of dichogamy.

protogyny *(protos* - first ; *gyne* - female) where the gynoecium matures earlier than the anthers of the same flower, and in such cases, the stigma receives the pollen grains brought from another flower and .thus cross-pollination becomes indispensable *(e.g., Ficus, Mirabilis, Magnolia, Annona,* etc).

protandry *(protos* = first; *andros* = male) where the anthers mature earlier than the stigma of the same flower and hence the pollen grains, are carried over to the stigma of another flower *(e.g., Clerodendron, Hibiscus rosa-sinensis, Abelmoschus esculentus, Helianthus annuus, Coriandrum sativum,* etc.)

Heterostyly *(heteros* = different)

Some plants bear flowers of two different forms. One form possesses long stamens and a short style, while the other form possesses skort stamens and a long style. This kind of bearing of stamens and styles is known as dimorphic heterostyly. In such cases, the chances of self- pollination decrease whereas chances of cross-pollination increase. In the flowers of this type, cross-pollination readily takes place between stamens and styles of the same length borne by different flowers. Dimorphic heterostyly is seen in *Oxalis, Linum, Polygonum fagopyrum, Woodfordia,* etc.

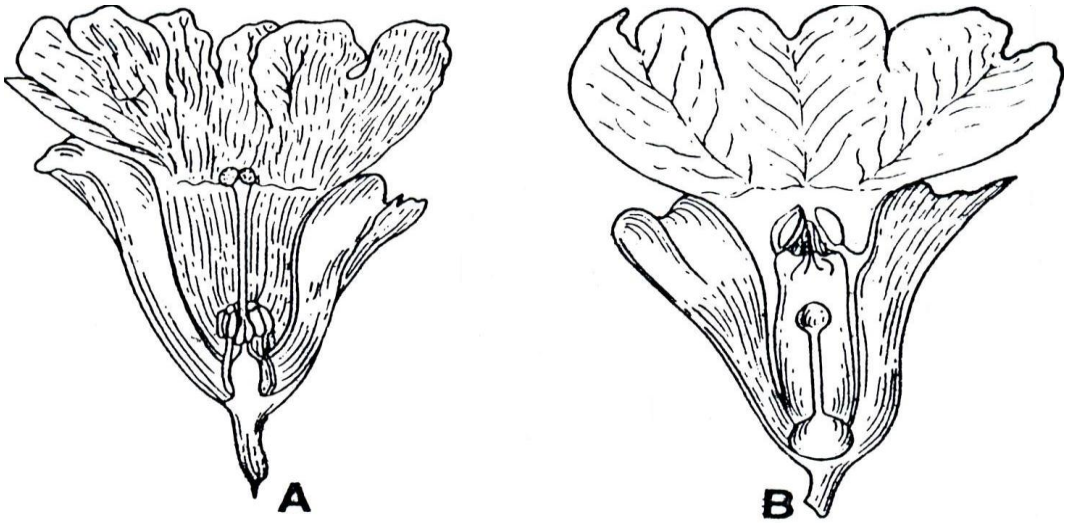


Figure 40. Heterostyly in the flowers of *Primula.*

Herkogamy *(hercos* = barrier)

In some homogamous flowers, there are certain structural peculiarities of the floral parts which act as a barrier to self-pollination and thus favor cross- pollination by insects. Here are cited some important examples. For example in *Calotropis* and orchids, the pollinia are located at places where they can never come in contact with the stigma by themselves and can only be carried away by insects. The lever mechanism in *Salvia* also promotes cross-pollination and avoids self-pollination. In *Viola tricolor* the stigma is protected by a flap or a lid that prevents contact between the stigma and anther. This flap is pushed aside by the insect and thus cross-pollination is effected.

ADVANTAGES AND DISADVANTAGES OF SELF AND CROSS-POLLINATION

Advantages of Self-pollination. This type of pollination is almost certain in a bisexual flower, if the stamens and carpels of the flower mature at the same time.

Disadvantages of Self-pollination. Continued self-pollination generation after generation definitely results in weaker progeny.

Advantages of cross-pollination.

- It always gives rise to healthier offspring in subsequent generations which are better adapted in the struggle for existence.
- More abundant and viable seeds are produced.
- New varieties can be developed by this method.
- The adaptability of the plants to their environment is definitely better by this method.

Disadvantages of cross-pollination

- The plants have to depend upon external agencies for pollination) (such as wind, water, insects, and animals)
- Various devices are needed to fulfill the needs of outer agencies.
- There is always a considerable waste of pollen where wind is the pollinating agent in cross-pollination.

CHAPTER III

FACTORS INFLUENCING POLLINATION

Abiotic Factors

Wind pollination (anemophily) is the most prevalent mode of the abiotic transfer of pollen. About 98 percent of all known examples of the abiotic type conform to the anemophily. Although primary anemophily is considered to occur in diclinous angiosperms (Meeuse 1972), early angiosperms have been insect-pollinated (Hesse 1980c; Hoekstra 1983). Anemophily is frequent in monocotyledonous families such as Cyperacpae, Juncaceae and Poaceae. Gymnosperms and generally wind-pollinated, and *Ginkgo* and conifers are suggested to have primary anemophily. Incidence of insect-pollination in however common in some Cycadales such as *Encephalartos* species (Wester 1910), *Macrozamia tridentata* (Schuster 1932), and *Zamia integrifolia* (Wester 1910). Cycad pollen is typically buoyant and wind-borne, but the transfer on the surface of megasporophylls is dependent on insects (Baird 1938).

Wind-pollination is prevalent in Gnetales but insects are known to collect sticky pollen in the sweet scented nocturnal staminate flowers and the female possesses a sweetish pollination droplet, strongly suggestive of insect-pollination syndrome (Hendryck 1953). *Ephedra aphylla* and *E. campylopodia* are partly and wholly entomophilic, respectively, and may represent on initial phase of the evolution of entomophilous syndrome from an originally anemophilous condition (Bino and Dafni 1983). In most angiosperms, anemophily may have been secondarily derived from a primary entomophily, as discernible in *Ambrosia*, *Fraxinus* and *Thalictrum* (Faegri and Van Der Pijl 1979). Occurrence of nectaries in anemophiles such as *Cannabis* and *Urtica* may be interpreted as relict and as evidence of a secondarily-derived anemophilous condition (Faegri and Van Der Pijl 1979; Stager 1902).

Anemophilous plants produce great quantities of pollen. The abundance of pollen attracts anthophilous insects such as the collector bees seeking nutritivepollen. Principle pollen sources of this kind to the bees are gymnosperms and members of Amentiferae, Cyperaceae, Plantaginaceae and Poaceae (Stelleman 1983). While anemophily and entomophily concur in certain species of Plantaginaceae (namely *Plantago lanceolate* and *P. media* : Stelleman 1983; Clifford 1962), Cyperaceae and Poaceae (Stelleman 1983), numerous features of a typical wind-pollination syndrome tend to discourage biotic transfer of pollen. These

features include a highly reduced perianth (Stanley and Linskens 1974; Poroctor and Yeo 1975). Since attractants such as color and fragrance of the blossom do not have any selective value, bracts and perianth are generally green or dark brown to reddish in anemophiles. A showy and colorful perianth or distinct fragrance in wind-pollinated blossoms is relict (Faegri and Van Der Pijl 1979).

Anemophilous pollen grain is small (20-60 microns in dia), though smallest (below 20-micron dia) and largest (above 60-micron dia) are found in entomophiles. Exceptionally large anemophilous pollen has a very low density as in *Pinus* (Pohl 1937b). Exine is modified to enhance buoyancy or floatability in air (Kozar and Aaron 1976). In many anemophiles, air-filled cavea increase the pollen buoyancy (Harrington and Metzger 1963). Wind-transferred pollen grains are smooth and round (Kozar and Aaron 1976). They have little effective pollen-kitt, so that they are dry, do not adhere and are dispersed individually (Hesse 1978, 1979a, 1979b, 1979c, 1980a, 1980b, 1980c). Starchy pollen grains are typical of anemophiles, in addition to species that are self-pollinated, pollinated by lepidoptera or by birds (Baker and Baker 1983). Gymnosperms as a group are also reported to lack pollen-kitt (Hesse 1980c). Number of anthers/flower in anemophiles is typically low and pollen grains/ovule ratio is very high (Pohl 1937a). Anthers are versatile and dangle in an open anemophilic flower (Baker and Baker 1983).

Staminal filaments are frequently long to place the anthers sufficiently high for effective dispersal by wind, as in Cyperaceae, Plantaginaceae and Poaceae. In grasses, the release of pollen is almost explosive (Ponomarev 1966), and in Urticaceae, the filaments are under strong tension released simultaneously with the opening of flower and anther (Mosebach 1932). Anther of anemophiles does not open unless weather is favorable and peak emission of pollen typically occurs in the mid-morning about a few hours after the sunrise (Ogen, Hayes and Raynor 1969). Blossoms frequently have certain mechanism to arrest pollen until wind is sufficiently strong to blow. Number of ovules per flower is low. Stigmatic surface is greatly enlarged to collect drifting pollen. Stigma may be feathery as in grasses, or brush-like as in *Typha*. Air-borne pollen is generally capable of traversing great distances and the record distance is as high as 1300 km as in conifers. However the grain will in all probability be dead upon its arrival on stigma after travelling such a distance. The average maximum transport distance is thus in the vicinity of about a dozen kilometers or at least 50 km (Faegri and Van Der Pijl 1979).

Hydrophily or pollination brought about by water is a derived condition and is of two types. Ephydrophily is the pollination occurring on the surface of water while hyphydrophily is the under-water pollination (Delpino 1868-75). In ephydrophily, the pollen grains are dispersed in two-dimensions increasing the efficiency of pollen considerably. As in anemophiles, perianth in typical hydrophiles is highly reduced. In ephydrophiles, the number of pollen grains per flower is highly reduced but this is adequately compensated for by highly efficient pollen grains. In *Aschynofnene* and *Neptunia* species, pollen grains collect and form foam on water surface. Pistillate flowers emerge into this foam, get pollinated and are then withdrawn (Mahabale 1968). Ephydrophilous pollen grains are water-repellent and floatable (Faegri and-Van Der Pijl 1979). In hyphydrophiles, the exine is highly reduced. (Fritsche 1837; Schwanitz 1967a, 1967b; Pettitt and Jeromy 1975; Ducker and Knox 1976). In *Amphibolic antarctica* and *Thalassodendron ciliatum,* poor exine development is preceded by the absence of callose in sporocyte wall (Ducker, Pettitt and Knox 1978).

Pollen grains of submerged aquatic sea nymph *(Amphibolis* species) possess mucoid content, unlike those of floating plants. This content is related to the remarkable adhesive property of pollen in sea water. Pollen grains of most sea grasses showing hyphydrophily are reported to be filiform or thread-like (Schwanitz 1967b; Pettitt and Jermy 1975; Ducker, Pettit and Knox 1978; Clavaud 1878; Hofmeister 1852; Bornet 1864; Pettitt 1976; Yamashita. 1976). Grains of *Amphibolis antarctica* are a record 3000-5000 microns long (Ducker, Pettitt and Knox 1978). Cotton-woollike shape of grains might be an adaptation to submerged environment (Clavaud 1878; Hofmeister 1852). The confervoid shape of pollen grains is unique among the flowering plants and observed only in Cymodoceaceae, Posidoniaceae and Zosteraceae (Ducker, Pettitt and Knox 1978).

As in anemophiles pollen production per flower is very high in hyphydrophiles. *Zostera* produces so many pollen grains that a cloud of pollen filled the surrounding water at anthesis (Ducker, Pettitt and Knox 1978). In *Posidonia australis,* grains have neutral buoyancy and drift with water (McConchie, Knox and Ducker 1988). Hyphydrophilous stigma is sticky due to proteinaceous secretion for trapping pollen (Ducker and Knox 1976). Still water appears necessary for hyphydrophily, as in *Zosten marina* (Clavaud 1878). Although rain-pollination *(Ombrophily)* has been described in a number of species (Hagerup

1950) the observation that rain seriously damages pollen of certain plants originally described as ombrophiles casts doubts on the occurrence of ombrophily (Daumann-1970).

Biotic Factors

Biotic pollination is of two types depending upon the construction of blossom. In the *topocentric* pollination, the construction is such that the mobile pollinator unwittingly contacts pollen and stigma bringing about the transfer. In the *ethodynamic* type, which is very rare, the pollinator actively approaches mature anthers, loads pollen and transfers to the pistil of another blossom and deposits actively (Galil 1973). Blossoms possess a wide variety of attractants for drawing the pollinators to bring about pollen transfer. In fact, a blossom should be essentially viewed upon as a pollinator's environment. It is a place of trade where the visitor is promised a suitable reward for the pollinating services. The rewards include nectar, pollen, brood-places, nutritious tissue, resin and sugary mucilage (Renner and Feil 1993). Most dioecious species have "specialized flowers adapted to specific pollinators" (Renner and Feil 1993).

With regard to the character of visits during the biotic pollination, blossom can be *polyphilic* (visited by many different taxa), *oligophilic* (visitors of related taxa) or *euphilic* (visited by one single or closely related species of pollinators). Likewise, an allophilic blossom is the one that shows no adaptation for guiding visitors and is available for pollination by any visitor; while hemiphilic blossom is partially adapted to receive pollinator, and *euphilic* one is strongly adapted and restricted to pollination by highly specialized and sometimes unique visitors (Faegri and Van DerPijl 1979). Regarding their adaptation for the blossom visit, the pollinators can be categorized as *dystropic,* when they are unadapted or counter-adapted; *allotropic,* when they are poorly adapted and the food obtained for any-one blossom constitutes only a part of mixed diet; hemitropic or hemilectic, showing intermediate specialization; and eutropic or eulectic, when the pollinators are fully adapted. With respect to the character of visit activity, the pollinator can be *polytropic* (visiting many different taxa), *oligotropicloligolectic* (visiting related taxa), or *monotropicl monolectic* when visiting one single or closely related plant species only (Faegri and Van Der Pijl 1979).

Not all visitors to the blossom can be categorized as pollinators and not all pollinators are quantitatively important (Bohart, Nye, Hawthorn 1970). In *Ceiba,* bats are the major pollinators while moths, humming birds and bees form minor pollen vectors (Baker.

Cruden and Baker 1971; Baker and Baker 1975a). Absolute one blossom-one pollinator relationship (euphily-monolecty) is observed rarely, as in *Angraecwn sesquipedale. Yucca* and *Ophtys* species (Faegri and Van Der Pijl 1979). Usually a blossom: is visited by many different and often unrelated pollinator species. Adaptation to a combined pollination system or a non-specialized blossom has an innate insurance for pollination by virtue of its appeal to a diversity of pollinators (Baker 1961).

Appearance of blooms generally coincides with the emergence of pollinator populations (Thien, White and Yatsu 1983). Over the ages, the blossoms: have co-evolved with the pollinators, perfecting a symbiosis. Blossom-pollinator co-adaptation is a dynamic process in which changing interrelationships establish and reproductive barriers in breeding systems are overcome (Young, Schaller and Strand 1984). Convergent evolution due to similar pollination mechanism in unrelated taxa through pollinator-sharing has resulted in floral mimicry and synchronous anthesis of sympatric species (Mactor 1971). *Cochleanthes lipscokbide* (Orchidaceae) is a floral mimic of sympatric legume (host) *Clitoriajavacensis* and flowers of both species are presented at the same level. Naive englossine bees while collected nectar from the legume blossom also pays exploratory visits to the blossom of *C. lipscombiae,* effecting pollination in the latter (Ackerman 1983).

Mutual interdependence between the blossom and the pollinator has often resulted in the evolution of highly specialized flowers than can be pollinated by a single species or a closely related group of pollen vectors. Such a narrow specialization has on one hand removed the uncertainties about the mutual needs of the blossom, and the pollinator, whereas on the other, made the plant vulnerable to failure of reproduction in case the pollinator fails to emerge. Understandably, most successful colonizers of weeds have very unspecialized pollination systems (Baker and Hurd 1968).

Flowers that are small tend to collaborate by producing clusters or inflorescences multiplying attraction for the pollinators. Capitulum or the head of Composites is one such typical cluster of inconspicuous flowers uniting to form a disc-shaped platform on which the insect pollinator conveniently lands and simultaneously pollinates scores of flowers in a single go. Apiaceous umbels produce flowers in large numbers that are small and closely spaced so that each insect visitor is a potential and probable pollinator (Bell 1971). Inflorescence size is in fact considered as an indicator of plant fitness (Schemske 1980). Gregarious flowering also aids biotic pollination as the effect of attractant is accentuated on

account of it being produced simultaneously in great quantities, like in *Coffea, Passiflora* and orchids (Faegri and Van Der Pijl 1979). In *Platanthera blepharoglottis,* each inflorescence blooms over a period of 2 weeks and an individual flower is receptive for upto 10 days. Both tactics prolong availability to the pollinator and enable consistently successful reproduction regardless of microhabitat (Cole and Firmage 1984).

A variable reward structure may be less preferred by pollinators (risk-aversive foraging: Feinsinger 1978; Real 1981; Waddington, Allen and Heinrich 1981). Food appears to be among the earliest and commonest reward that the blossom promises to the pollinator. Blossoms produce nutritively rich nectar (with 25-75% sugar content: Waddington, Allen and Heinrich 1981; Percival 1961; Gottsberger, Schrauwen and Linskens 1973). Besides offering surplus pollen to the foraging pollinator. In addition to sugars, nectar also contains amino acids and lipids (Baker and Baker 1975a, 1975b). For the butterflies, nectar nitrogen is potentially the most important (Faegri and Van Der Pijl 1979). A plant's nectar propeities are an integral part of its strategy for pollination success (Pleasants 1983). Nectar production rate (or NPR) is as important part of plant's pollination syndrome as the flower large amount of nectar of fulfill the requirements of energy demanding (bees) and large bodied (birds) pollinators. Nectar dominated by sucrose is significantly related to big bee-pollination (Baker and Baker 1990).

Presence of nectar in flowers before they open has been demonstrated in a number of plants (Feinsinger 1978), including *Ipomopsis aggregata* of Polemoniaceae (Pleasants 1983). Overnight production is common in ornithophiles requiring larger amounts of nectar. Resorption of nectar by the blossom is reported to occur at night in alfalfa (Pedersen and Fevre 1958). UV-fluorescent nectar occurs in many bee-pollinated blossoms and function and direct visual clue by which bees evaluate the quantities of nectar '(Thorp, Briggs, Estes and Erickson 1975). In *Hamamelis mollis, Ranunculus acris, Rhododendron fortunei* and *R. Schlippenhachii,* nectar vomited by the bee reinforces the attachment of pollen to the body of the vector to facilitate pollination (Hesse 1980d).

In red raspberries *(Rubus idaeus),* synchronous flowering minimizes the incidence of floral predation and makes even low nectar blossoms sufficiently attractive by reducing flight time and energy expenditure of the pollinator. High reward flower with more dilute nectar is attractive to the long-tongued bumble bees (Whitney 1984). In vipers bugloss *(Echium vulgare),* low volumes of concentrated nectar allows small-bodied andrenids to operate

profitably (Corbet 1978). Aggregation of flowers forming large inflorescences compensates for the low nectar content per flower in sugar maple *(Acer saccharum)* and yellow birch *(Betula alleghaniensis;* Whitnev 1984).

Presence of nectar also attracts the non-pollinating or illegitimate visitors, particularly the nectar thieves, to the blossom. The proboscis of butterfly is so thin that it may thieve nectar from the bee blossom without contacting anther/stigma. Queens of *Bombus qffinis* perforate *Aquilegia* spurs and steal nectar. They however collect pollen entering the blossom ordinarily (Macior 1966). Some insects appear to be habitual nectar thieves. For instance, *Bombus mastrucatus* bites hole in corolla into which it might have easily crept and reached nectar ordinarily (Faegri and Van Der Pijl 1979). Nectar thieves need not always do harm to the cause of pollination. In fact, the reduced level of nectar may make the pollinator search more frantically in order to reach the residue, thus enhancing its body contact with the sex- organs of the flower and improving the chances of pollination. The nectar of certain blossoms may be poisonous to the illegitimate visitors.

Toxin content of the nectar of ornithophilous blossom may be sufficient to kill illegitimate insect visitor without harming the large-bodied legitimate pollinator. The toxin *of Rhododendron* nectar is reported to be acetylandromedol (Leach 1972). Pollen is another rich source of food: offered by the blossom to the prospective pollinator. Whenever pollen is attractant, it is produced copiously in the blossom, like in *Rosa* and *Papaver*. Pollen grain consists of 16-30% protein, 1-1:0% fats, 1-7% starch and many vitamins (Barth 1985). Pollen rich in lipids and dissolved carotenoids are sought by bees and flies. Bees reportedly cannot digest starch (Baker and Baker 1983). Starch less grains are typical of bee pollination particularly if there is no other reward, whereas starchy grains are typical of species pollinated by Lepidoptera and birds (Baker and Baker 1983). Lepidoptera and birds, however, do not appear to use pollen nutritively but visit flowers for the nectar they contain. These correlations are however not absolute.

If a pollen grain is less than 25 microns in diameter, it will be starch less whatever the pollination mechanism. Pollen grains of Composites are exceptionally starch less and rich in lipids which agree for majority of species that are bee-pollinated. Yet there are also wind-pollinated species of Composites *(Artemisia* and *Ambrosia)* showing same features of pollen grains (Baker and Baker 1983). Pollen also plays a vitamin role (Stanley 1971) and contains substances that stimulate secretion from the hypopharyngeal gland of nurse

bees (Doull 1973). In *Lagestroemia indica* (Harris 1914) and *Verbascum thapsus* (Faegri and Van Der Pijl 1979), the blossoms contain two types of anthers: (i) feeding anthers, producing pollen for consumption by the visitor, and (ii) fertilization anthers, producing normal pollen. In *Exacum.* feeding anthers gather in one part of the flower, inducing zygomorphy. In *Tripogandm grandijlora* and *Cassia* species, the pollen produced by feeding anthers is degenerate (Lex 1961).

Pollinators collect both nectar and pollen from blossoms. Usually there is a strong binding for the pollen source (oligo- or monolecty) combined with a wider variety of nectar sources (Faegri and Van Der Pijl 1979). Commonly, bees collect most nectar and little pollen early in the morning. During the day, pollen collection increases and nectar collection drops progressively (Free 1962). Pollen alone may not deliver enough energy to the pollinator. *Bombus luconun* is reported to interrupt its pollen flights by visits to nectariferous blossoms for refuelling (Brain 1957).

Several tropical members of Iridaceae, Krameriaceae, Malpighiaceae, Orchidaceae and Scrophulariaceae have oil-secreting organs or elaiophores as a part of their pollination syndrome and offer oil as nutritive reward to the solitary bee pollinators of the family Anthophorideae (Vogel 1969; Simpson, Neff and Siegler 1977). At least two unrelated genera *Dalechampia* (Euphorbiaceae) and *Clusia* (Guttiferae) attract pollinators by secreting resin. Bee pollinators collect resin for use in nest construction. Floral resins are slow in hardening and this facilitates collection and storage by bees. Resin secretion is considered originally as a defence mechanism against herbivores and secondarily assumed the role of pollinator reward (Armbruster 1984). Trap flowers of certain members of Araceae produce food bodies as primary attractant to the pollinator. Blossom of *Victoria amazonica* attracts, traps and feeds food bodies to its pollinator during confinement. The trap forces the pollinator to stay longer and ensure pollination (Valle and Cirino 1972).

Relationship between floral colors, including UV-reflectance features of the blossom, and the pollinator behavior have been recognized for about a century (Knuth and Locw 1895-1905). Acting as a secondary attractant, colorful perianth is essentially a signpost or display board advertising the presence of nectar and pollen in the blossom to this foraging pollinator. Colorful patterns on the petals visually guide the insect to the nectar sites M the flower (McCrea and Levy 1983). Due to a certain positional shift of the visibility spectrum into the ultraviolet range, insects can visualize UV-radiations unlike the humans. In the

trichromatic vision of bees, near UV (of the human visibility spectrum or HVS) appears as blue to insects, blue (of HVS) as green, and green (of HVS) as red. Nectar guides in many flowers are in the form of UV-reflectance patterns that disappear following pollination (McCrea and Levy 1983; Kevan 1972, 1978, 1979). Insects are red-blind due to shifted visibility spectrum. Red flowers are thus as good as black ones for insects. It may be a reason why red blooms appear in summers when the background is bright. Pure red blossoms are rare in temperate regions with poor sunshine (Barth 1985). Petaloid stamens (in *Canna*) or pistil (as in *Iris)* may supplement perianth in the long distance advertisement drive of the blossom (Faegri and Van Der Pijl 1979).

Blossom of *Mentzelia tricuspis* provides a mating place to the pollinating insect *Megandrena mentzeliae* (Zawortnik 1972). Similar relationship is rejported between certain Annonaceae and beetles (Gottsberger 1970), Owing to strong physiological activity such as the production of odour (Meeuse 1966, 1975), the temperature of the blossoms in several members of Araceae may be several degrees higher than the surroundings (Budel 1959). Warmer interiors of these blossoms may be attractive to the pollinator as shelter sites (Faegri and Van Der Pijl 1979). In the arctic, mosquitoes bask in open flowers at a temperature upto 6°C higher than the surroundings. The insects are dark-colored and take position near the focal point of heliotropic flowers and may result in pollen transfer (Kevan 1975).

Like the color, fragrance is ordinarily a secondary attractant and plays an important role in the advertisement drive of the blossom in the overall pollination strategy. It works as an odorous guide to the foraging sites in the blossom. Odor apparently is an older secondary attractant than the color (Faegri and Van Der Pijl 1979). Since at night the visual display by colorful petals will not work, nocturnal blossoms often use odour as an attractant. The nocturnal pollinators are commonly the "'non-appreciating" type such as moths, beetles, mosquitoes, cockroaches and even bats.

Certain orchids (about 10% of total species). *Gloxinia speaosa* and some members of Araceae *(Anthuhum* and *Spathiophyllum)* and Gesnenaceae not only attract male solitary bees (of the genera *Euglossa, Eulaema* and *Euphisia)* with the characteristic odors of their blossoms but also allow the perfume to be taken away as the reward. Fragrance in such instances acts as a primary attractant. In addition, the narcotic or intoxicating .action of the perfume ensures pollination as the male bees tend to enjoy the sensation and return

repeatedly to the blossom over a long period of time before tiring and living away (Dodson and Frymire 1961: Williams and Dodson 1972).

In orchids, the odorous substances are secreted generally by the glandular apices of the petals, referred to as osmophores (Vogel 1962. 1966, 1990). Osmophores in myophilous *Restrepia* secrete foul-smelling substances simulating the natural breeding substrate of flies (Pridgeon and Stern 1983). Production of stinking substances by the blossoms that releases the pollinator instinct for oviposition and causing sapronryophily is known in members of Annonaceae, Araceae. Aristolochiaceae, Asclepiadaceae, Burmanniapeae. Hydnoraceac. Orchidaceae, Rafflesiaeeae. Sterculiaceae and Taccaceae (all highly evolved families: Faegri and Van Der Pijl 1979) The imitative syndrome of *Arum canophattoides* consists of a peculiar blossom odor simulating the small of mammal skin, attracting small blood-sucking midges to bring about pollination (Knoll 1923, 1956). Stench from about 2-mctcr-high beetle pollinated blossom of *Amorphopliallus titamun* is so suffocating that curious onlookers drawing too close to the blossom arc known to faint (Barth 1985). Aminoid smell emanating from the blossoms of *Stopelio giganiea* and X *nobilis* and the odor characteristic of an over-ripe orange or pineapple from the blossoms of *Caralluma schweinfurthii* stimulates the ovipositor) instincts of flies, resulting m sapronryophily (Agnew 1976).

Deception-based pollination is common m orchids. Orchid blossom may mimic food source of the pollinator as in *Arethusa bulbosa,* (*alopogon tuberosus* (Thicn and Marcks 1972), *Oncidiuin* species (Nirenberg 1972). *Orchis iwaclitica* (Dafni and Ivri 1981). and *Pagonia ophioglossoides* (Thien and Marcks 1972). In *Epipactis consimilis,* the blossom mimics aphid shape and attracts aphidophagotis hoverflies especially *Shhaerophoria ruepelli* and. V. *scripta,* resulting in pollination (Ivri and Dafni 1977). Female scolicid wasps *(Campsofueris)* mistake the labellum of *Brassia* and *Calochilus* for the insects they prey upon, sling and cause pollination (Van Der Pijl and Dodson 1969). Orchid blossom may even mimic territorial intruders of the pollinating insects, so that the aggressive advances of the pollinator brings about pollen transfer (Van Der Pijl and Dodson 1969).

Orchid blossoms arc in fact extremely specialized in exploiting insect visitors to produce pollination. Flowers of many orchids (such as *Oplvys* and *Cataselum)* initiate the female of the pollinator insect to the extent that the male visitor desirous of sex grasps the flower with the us copulatory organs extended and the abdomen resting on the largest petal or labellum.

As its back jerks the flower, as if in search of something, pollinarium with its sticky iscidium is loaded onto its head. When the insect in lured into the) next flower under its sexual urge, the pollinarium collected from the previous visit is inadvertently transferred onto the stigma (Barth 1985: Van Der Pijl and Dodson 1969: Stoutamirc 1975), Furriiicss of the Iabcllum provides tactile stimulus during the act of pseudocopulation (Van Der Pijl and Dodson 1969). Due to a close and specific relation between the insect pollinator and the orchid blossom acting as pseudo female, each orchid species is specialized for a particular insect pollinator species. Thus the fly orchid or *Ophrys insectifera* is adapted to burrowing wasps *(Gorytes* sp.), *0. speculum* to the scobid wasp *(Campsocolia* sp.). *0. araneifera* with its spider-like form to the andrenid bees; and bee-fly orchid *0. bombylifera,* to the bees belonging to the genus *Eucera* (Barth 1985). *Leporella fimbriate!* presents a bizarre instance of pseudocopulation with a male ant (Bates 1979).

As if to reinforce the act of pseudocopulation., the orchid blossoms not only imitate shape of the female insect, but also produce odorous attractant for the male pollinator (Kullenberg 1956; Dodson 1970). Gamma-Cadinene appears to be the key odorous substance in triggering sexual reactions in susceptible insect pollinator (Kullenberg 1973; Priesner 1973). In *Catasetum* species, the males of wild solitary bees (mostly *Eufhesta auriceps)* may collect and use these odorous substances as pheromones to mark their territory (Kull inberg and Bcrgstrom 1975).

All orchid flowers that are pollinated by sexual deception do not bear a close resemblance to a specific female insect (Bino, Dafni and Meeuse 19S2). Two sister species of *Disa,* the largest South African orchid genus, arc pollinated exclusively by male wasps. *D. atricapilla* being pollinated by *Podalonia canescens* (Sphecidae), while *D. hivalvata* is being pollinated by *Hemipepsis hilaris* (Pompilidae). The two orchids have overlapping distributional ranges, occupy similar habitats, flower at the same time, and often occur sympatrically. The absence of a floral reward, the exclusive presence of male wasps as primary pollinators, the lack of female visitors and the mate-seeking behavior of wasps when approaching and visiting flowers implicates pollination through sexual deception (Steiner, Whitehead and Johnson 1994). Unlike most orchids' pollinated by sexual deception, the flowers of *D. atricapilla* and *D. hivalvata* do not resemble a female wasp, instead the entire inflorescence acts as an attractive unit.

The buds and old closed flowers of *D. atricapilla* provide the greatest optical stimulus to males seeking mates especially the black shimmy lateral sepals that are prominent in bud (Sterner. White/head and Johnson 1994). The optical and textural components of the flower, even in pseudocopulatory orchids like *Ophrys,* are only secondary in importance to the odour for attracting pollinators (Kullenberg 1961, 1973a, b; Bergstrom 1978, Borg-Karlson 1990). Even in the presence of the appropriate odour, male bees do not require an exact image of the female in order to be sexually deceived. Rather, only certain key optical features such as the shine, color and/or markings, on the lip and overall size of the flower contribute to the effectiveness of pollinator attraction (Paulus and Gack 1990). It is believed that sexual deception has evolved from ancestors whose flowers were non-rewarding generalized food mimics (Steiner, Whitehead and Johnson 1994, Dafni 1987).

Like the orchids, Asclepiads arc extremely specialized entomophiles. As if to ensure cross-pollination, the adaptive design of their floral display includes: i) blooming of inflorescence for a long duration and ii) prolonged receptivity of the flower (Willson and Rathoke 1974). Flowers also show a very low level of self-compatibility (Wyatt 1976). Pollinial Asclepiads are apparently melitophiles (Shukla 1974. 1980; Macior 1965). In *Pergularip daemia,* as the pollinator bee *(Apis dorsata)* attempts to land on the blossom, its legs slide on the slippery staminal corona into the stigmatic grooves harboring pollmaria with associated non-sticky translators. When the bee withdraws from the blossom, the bristles on the insect leg hook on the characteristically cup-shaped corpusculum of the translator, so that entire pollinarium is pulled out by the departing bee. When the bee visits next flower in search of nectar, the pollinia collected from the previous visit are inadvertently thrust into the receptive stigmatic chamber (Shukla 1974; Vijayaraghavan and Shukla 1980).

CHAPTER IV

POLLEN - PISTIL INTERACTIONS

Fertilization in flowering plants is a unique phenomenon and involves two fusion processes syngamy between one of the male gametes and egg, and triple fusion between the other male gamete and fusion nucleus. Successful fertilization leads to fruit and seed development. The processes leading to fertilization are initiated on the stigma. Soon after pollination the crucial decision of recognition followed by subsequent acceptance or rejection of the male partner is made by the pistil. A chain of sequential, integrated processes in the pistil following pollen recognition regulate the post-pollination behavior of the pollen. If the pollination is compatible, the pollen grain germinates on the stigma, and the pollen tube grows through the style and eventually reaches the embryo sac where it discharges the male gametes. If the pollination is incompatible, the pistil will effectively prevent fertilization, by inhibiting either pollen germination, entry of the pollen tube into the stigma, or growth of pollen tube in the style. Pollen-stigma interaction, therefore, is of paramount importance in the biology of sexual reproduction, because the vital functions of selection of the male gametes in flowering plants is performed not by the egg, but by the pistillate tissue (see Shivanna 1979).

For a rational understanding of the pollen-pistil interaction and fertilization it is important to understand the structure of the pollen grain and the pistil in relation to their function of recognition and rejection. Although the basic events of pollen-pistil interaction leading to fertilization became known by the end of the nineteenth century, studies remained basically descriptive until 1950. Following the realization of the significance pf plant hybridization programme in crop improvement, limitations of these traditional approaches to studies on fertilization became apparent. This realization, coupled with the general trend of plant sciences to become experimental, resulted in the interaction of histochemical, ultra structural, and biochemical techniques to unravel the mysteries of fertilization. Significant progress has been made on these lines during the last two decades. This chapter deals largely with these latter developments, on the processes from pollination to fertilization. Sexual incompatibility (both intra and interspecific), and methods of overcoming incompatibility are also discussed in greater detail.

The Pollen Grain

Pollen grains are surrounded by a two-layered wall, the exine composed of sporo pollenin and the pectocellulosic intine. The exine is a more elaborate structure differentiated into an outer sculptured layer (the sexine), and an inner non-sculptured layer (the nexine). The sculptured layer is made up of radially directed rods, the baculae, which may be enclosed above to form a tectum (tectate grain), or stand free or joint together to form various patterns non-tectate grain). In tectate grain the tectum is invariably perforated by micropores.

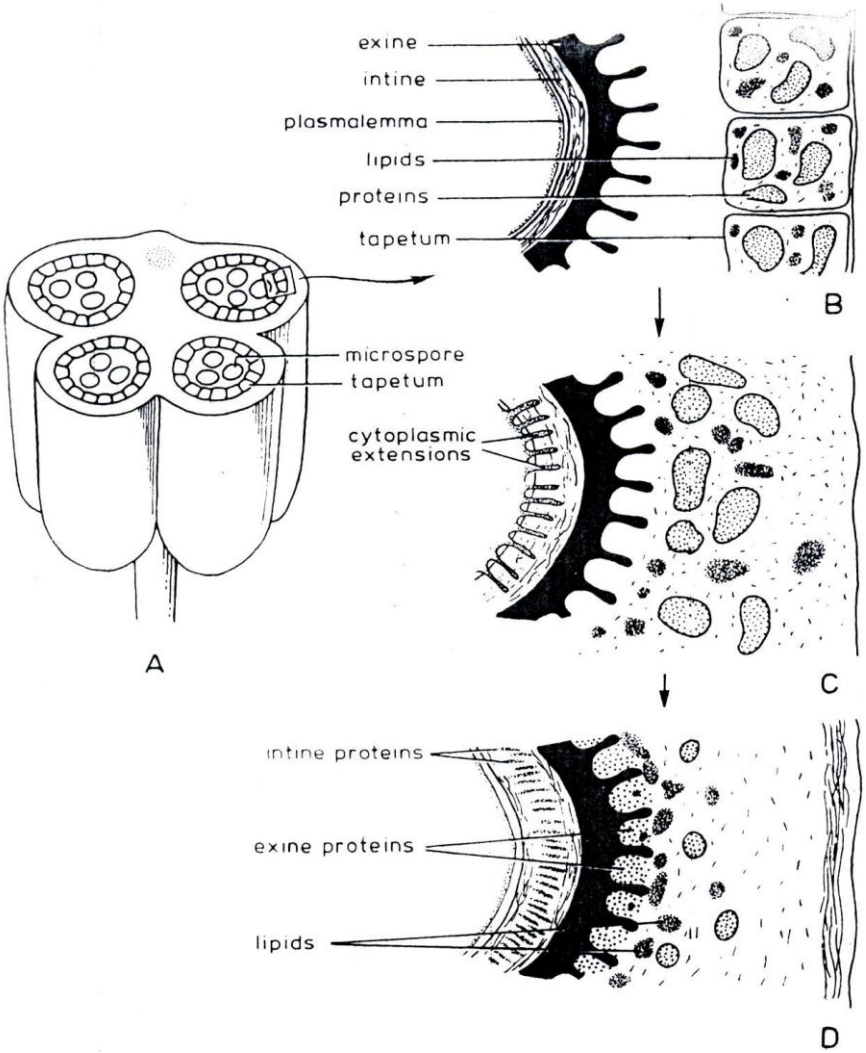


Figure 41. Origin of the intine and exine proteins. A Anther cut transversely to show young microspores surrounded by a layer of tapetum. Only a part of the microspore wall and ta-petum are shown in B-D. Note the origin of intine proteins in the pollen cytoplasm, and of exine proteins in the tapetum. (After Shivanna 1977).

Most of the classic studies were made with acetolyzed pollen and concerned with the morphology of exine as a means of identification and phylogeny. Recent progress in our understanding on the structure of pollen grain comes from the studies of the unacetolyzed pollen wall. Both wall layers of the pollen grain, the exine and intine, have now been demonstrated to contain a large amount of mobile proteins (Knox and Heslop-Harrison 1969, 1970, 1971 a; Heslop-Harrison et al. 1973, 1975 a). These include many hydrolytic enzymes; and proteins responsible for pollen allergy (Knox et al. 1970). The intine proteins are present in the form of tubules or leaflets, and are generally concentrated near the germpores. In the exine the proteins are located in the sculptured region of the exine in the chambers between the baculae (tectate grains) and surface depressions (non-tectate grains). These wall proteins are readily released into the medium on moistening.

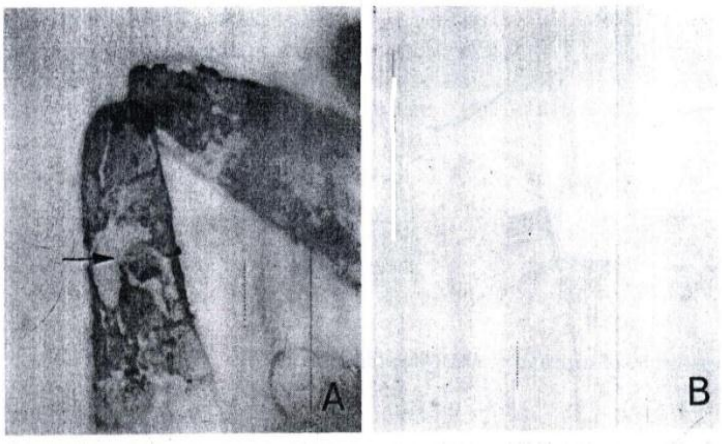
The origin of these pollen wall proteins is diagrammatically represented in Fig. 41. The intine proteins are produced in the pollen cytoplasm and incorporated into the intine during its development (see Heslop-Harrison 1975 a). As the deposition of intine progresses soon after the release of microspores from the tetrad, the plasmalemma of pollen cytoplasm sends out radially oriented tubules into the developing intine. Eventually these tubules, with their protein inclusions, are cut off from the plasmalemma and sealed off from the cell surface by the deposition of a layer of intine free from tubules. In aperturate pollen the intine-proteins are principally concentrated in the region of germpore (Heslop-Harrison et al. 1973); in non-aperturate monocotyledonous pollen they are distributed throughout the intine (Knox 1971; Heslop-Harrison 1975 a). Similar tubules in the intine, apparently derived from the plasmalemma, have been described in ultrastructural studies of many other species (see Heslop-Harrison 1975 a).

In *Cosmos bipinnatus* the incorporation of intine proteins is rather unique. Instead of tubular evaginations, leaflets of plasmalemma with their protein inclusions get separated from the cytoplasm and become incorporated in the intine in the form of a series of tangentially oriented lamellae (Knox and Heslop-Harrison 1970; Heslop-Harrison 1975 a). The exine proteins, on the other hand, originate in the cells of the surrounding tapetum, a sporophytic tissue (Heslop-Harrison et al. 1974). During meiosis of microspore mother cells, proteins, and lipids accumulate in the tapetal

cells. When the tapetal wall breaks down towards the end of pollen development, proteins and lipids are released into the thecal cavity and, eventually, become deposited in the surface depressions of exine. In tectate grains the protein fraction passes through the micropores of the tectum and accumulates in the spaces between the baculae; the lipids remain on the surface of tectum (Heslop-Harrison et al. 1973). Thus, the intine proteins are the products of pollen cytoplasm, the male gametophyte; and the exine proteins the products of tapetum, the sporophytic tissue. The significance of this differential origin of the exine and intine proteins in controlling the breeding behaviour of the species is discussed later.

The Pistil

Traditionally, two basic types of stigma have been recognized: the wet type in which the receptive surface becomes covered (to various degrees) with the exudate; and the dry type in which the receptive surface is free from any apparent exudate. Recently, the variations in these two major categories of stigma have been divided into further groups (J. Heslop-Harrison 1976; J. Heslop-Harrison et al. 1975 b). Y. Heslop-Harrison and Shivanna (1977) studied and classified the stigmas of about 1,000 species covering 900 genera belonging to over 250 families (Table 1). The details of the origin and composition of the exudate have been studied only in a few systems. In *Petunia* the exudates is secreted by the cells of stigmatic tissue and accumulates on the stigma surface by rupture of the cuticle (Konar and Lin-skens 1966 a). In *L,ilium,* on the other hand, the cells of the stigma are non-secretory; the exudate is presumably secreted by the canal cells of the hollow style and extruded on to the stigmatic surface through the stylar canal (Dashek et al. 1971; Labarca and Loewus 1973).



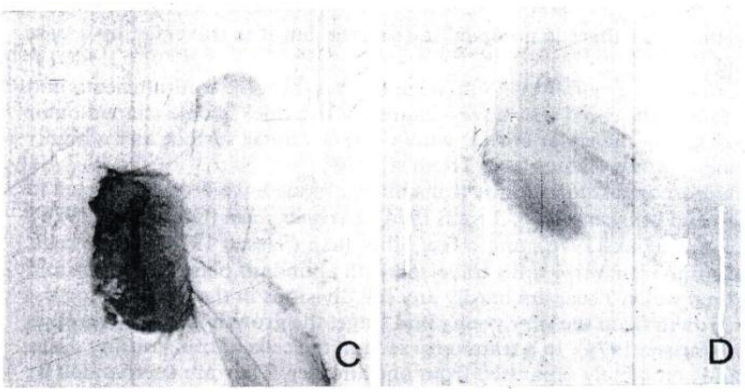


Figure 42. A-D. Histochemical localization of stigma-surface esterases using a-napthylacetate as a substrate in a coupling reaction with fast blue B salt. A, B Stigmatic papillae of *Acidan-thera bicolor* (dry stigma, group IIB). A with substrate. The pellicle is seen as a dark sheath investing the cuticle, but is torn at places *arrow* and reveals the underlaying cuticle. B Control for A (without the substrate). C, D Stigrr.a of *Vigna unguiculata* (wet stigma, group III). Esterase activity is clearly seen on the exudat; in C. D Control for C (without the substrate).

The composition of the exudate is highly variable, and contains varying proportions of lipids, carbohydrates, phenolic compounds, and proteins (Martin 1969; Konar and Linskens; 1966 b; Labarca *et al*. 1970, Dumas 1974; Rosen 1971; Heslop-Harrison 1975 b; Shivanna and Sastri 1976). In *Petunia* the exudate is largely lipoidal, and has no nutritive role for the germinating pollen. In *Lilium* the exudate is aqueous consisting largely of carbohydrates and a small amount of proteins (Kroh *et al*. 1970; Rosenfield and Loewus 1975). Arabinose is one of the major carbohydrates of the exudate and forms about 28% of the dry weight. When the exudate is added to the pollen culture medium, it stimulates pollen germination and tube growth, and is taken up by the pollen tubes.

The lipoidal substances of the exudate help in trapping the pollen grains and to protect the stigma from desiccation and wetting. The phenolic compounds are presumed to be helpful in protecting the stigma from microbes and pests. Recently, there have been evidences to implicate phenolic compounds in pollen nutrition, and in selective promotion, or inhibition, of pollen grains on the stigma (Martin 1970, 1972; Martin and Ruberte 1972; Tara and Namboodiri 1976; Sedgley 1975).

Table 1: General classification of angiosperm stigma types based on the morphology of the receptive surface, and the amount of secretion present during receptive period. (After J. Heslop-Harrison 1976; Y. Heslop-Harrison and Shivanna 1977). Some examples for each group are given in parenthesis.

Dry stigmas (without apparent fluid secretions)
Group I Plumose, with receptive cells dispersed on multiseriate branches (Gramineae).
Group II Receptive cells concentrated in distinct ridges, zones or heads.
A - Surface non-papillate (Acanthaceae)
B - Surface distinctly papillate
i. Papillae unicellular (Cruciferae., Compositae)
ii. Papillae multicellular
a. Papillae uniseriate (Amaranthaceae),
b. Papillae multiseriate (Bromeliaceae, Oxalidaceae)
Wet stigmas (surface secretions present during receptive period)
Group III Receptive surface with low to medium papillae; secretion fluid flooding interstices (some Rosaceae, some Liliaceae)
Group IV Receptive surface non-papillate; cells often necrotic at maturity; usually with more surface fluid than Group III (Umbelliferae)

The dry stigmas are free from any apparent secretory products, and are generally covered by an unruptured cuticle of varying thickness. Recently, the presence of a hydrated proteinaceous covering (together with lipoidal fraction), termed pellicle, has been shown to be invariably present on the surface of cuticle (Mattsson *et al*. 1974; Y. Heslop-Harrison and Shivanna 1977). The pellicle can be easily localized histochemically by its intense non-specific esterase activity (Fig. 42A-D), and sensitivity to pronase digestion. Although the details of the origin of pellicle are yet to be investigated, the cuticle has been shown to have discontinuities through which the pellicle is presumed t3 be extruded on to the surface of papillae (Fig. 43). The "dry" stigma is, therefore, not really dries as it was thought earlier, but covered with the pellicle which is physiologically comparable to the exudate of the wet stigma. The surface proteins, either as a component of the exudate or the pellicle, have an important role during pollen-stigma interaction (discussed later).

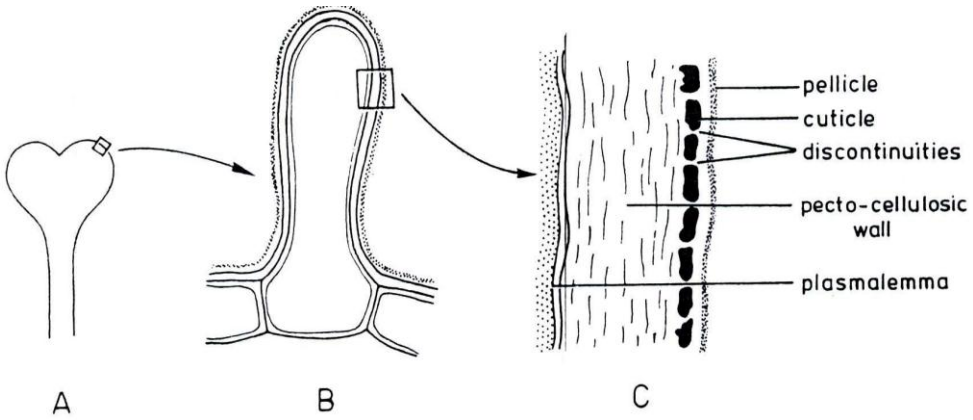


Figure 43. The structure of stigmatic papilla.

The styles are of two main types - hollow (open) and solid (closed). In the former the style is traversed by a stylar canal and lined with a layer of glandular cells, canal cells; in the latter there is no canal in the style but it is traversed by a core of transmitting tissue. The canal cells are generally glandular, and often become multinucleate and polyploid. In *Lilium* the canal cells have a characteristic thick, dome-shaped outer tangential wall (facing the stylar cavity) with a smooth outer surface and a highly convoluted inner surface (Rosen and Thomas 1970). Electron microscopic studies of the transmitting tissue have been conducted in *Petunia* (Pluijm and Linskens 1966; Sassen 1974), *Lycopersicum* (Cresti *et al*. 1976), *Nicotiana* (Bell and Hicks 1976), and a few other taxa (Sassen 1974). These cells, in general, have thin transverse walls traversed with abundant plasmodesmata and thick longitudinal walls. There are hardly any cell divisions in the transmitting tissue during its growth from the very young bud stage; the growth is largely through cell elongation (Sassen 1974).

In a transverse section the cells of transmitting tissue are circular, and completely separated from one another. They are surrounded by an intercellular substance of different electron density than the cell wall. The intercellular substance, a secretion product of the transmitting tissue, is not comparable to the middle lamella. It is more complex than the middle lamella, and comparable to the secretion fluid found in the stylar canal (Sassen 1974). Recently, the intercellular substance in *Lycopersiciim peruvianum* has been shown, histochemically, to contain proteins (Cresti *et al*. 1976). According to Sassen (1974), there are no basic differences between the structure of solid and hollow styles. The cells of the transmitting tissue of cotton, however, are not surrounded by any intercellular substance. The lateral walls are 7-10 um thick,' and

consist of four distinct layers, including the outermost pectinaceous middle lamella (Jensen and Fischer 1969).

Pollen-Pistil Interaction

The first visible change in the pollen, soon after it lands on the stigma, is hydration. Simultaneously with hydration, pollen wall proteins, first the exine proteins and then the intine proteins, are released on the stigmatic surface (Knox and J. Heslop-Harrison 1971 a; Knox 1973; J. Heslop-Harrison *et al*. 1975 b). The outflow of the pollen wall proteins can be readily detected by immunofluorescence, as well as by histochemical techniques. In dry stigma the pellicle is the receptor site for the pollen-wall proteins. Initially, the wall proteins are not bound to the stigma and hence may be easily removed by saline leaching. But soon the wall proteins bind to the pellicle establishing a close interaction, and the pellicle looses its identity as a discrete layer at this point. Recognition of the pollen appears to take place during this interaction, and results in the activation of the male gametophyte and papilla (Heslop-Harrison 1975 a).

If the pollen grain is compatible, the pollen tube soon emerges and comes in contact with the cuticle of the stigmatic papillae. The cuticle is eroded at the point of contact; the pollen tube enters the inner pecto cellulosic wall and grows down the papillae. The events from pollination to pollen tube entry into the papillae are rapid and completed in less than 30 min in many taxa (J. Heslop-Harrison et al. 1975 b). In cotton, which is also characterised by dry stigma with a covering of cuticle, the pollen tubes have been reported to grow down the papilla on the surface of the cuticle without entering the cuticle (Jensen and Fischer 1969). However, recent studies have clearly shown that in cotton as well as in many other related taxa, pollen tubes invariably enter the cuticle, and grow through the wall of papillae (Shivanna and Y. Heslon-Harrison, unpublished).

Pollen Tube Growth

In taxa characterised by wet stigma the cuticle is generally ruptured during the de position of exudate, and the pollen tubes enter into stigmatic tissue through pecto cellulosic layer. In taxa characterized by a hollow style the stigmatic surface is in direct contact with stylar canal. In *Lilium* the cuticle of stylar canal is ruptured during the secretion of exudate and, hence, the pollen tubes grow on the surface of canal cells

bathed in exudate. In *Crocus,* in which cuticle remains intact on the papillae, as well as on the cells of stylar canal, pollen tubes bore through the cuticle of stigma and grow down the stylar canal between cuticle and canal cells (Y. Heslop-Harrison 1975 a). In *Petunia* (Pluijm and Linskens 1966), and other taxa characterized by solid styles, pollen tubes grow through intercellular substance. In cotton it grows through wall layer three and not through the middle lamella (Jensen and Fischer 1969).

The pollen tube utilizes nutrients from the pistil for its growth. In many hollow-styled members such as *Aegle marmelos, Fritillaria,* and *Lilium* stylar tissue of the unpollinated pistils contain abundant starch. Following pollination and pollen tube growth the starch is broken down, apparently taken up by the growing pollen tubes (see Vasil 1974). By studying the incorporation of labelled glucose and myoinositol in the detached pistils of *Lilium,* Loewus and his associates (Kroh et al. 1970; Labarca et al. 1970; Labarca and Loewus 1973) have demonstrated that both glucose and myoinositol are taken up by the growing pollen tubes from the pistil and utilized for the synthesis of pollen wall materials. Evidences for the utilization of stylar carbohydrates by the growing pollen tubes have also been obtained in *Nicotiana* (Tupy 1961), *Oenothera* (Kumar and Hecht 1970), and *Petunia* (Krdh and Helsper 1974) from labeled studies.

In cotton there is no evidence to indicate the utilization of stylar carbohydrates (Jensen and Fischer 1969), as no depletion of starch or lipid takes place in the tissues of style following the growth of tube. On the other hand, the growth of pollen tube through the transmitting tissue results in the deposition of callose in the pit fields sealing off the cells adjacent to the pollen tubes. Pollination initiates many physiological changes in the pistil. It increases respiratory activity, changes patterns of RNA and protein synthesis (Linskens 1975), and initiates marked increase in the activity of several enzymes (Roggen 1967). Some of these physiological changes are initiated even in those regions of pistil in which the pollen tubes have not yet entered. For example, in *Petunia* a wave of enzyme activity precedes the growing pollen tubes (Roggen 1967).

Also, within a few minutes after pollination an electrical signal characteristic of compatible or incompatible pollination can be measured at the base of style (Linskens and Spanjers 1973). In cotton (Jensen and Fischer 1968), and many other taxa, one of

the synergids begins to degenerate even before the pollen tube enters the ovule. Thus, some stimulus precedes the growing pollen tube in the pistil, and initiates responses suitable for the normal growth of pollen tube. Generally, a larger number of pollen tunes enter the ovary than the number of ovules, although only one pollen tube enters each embryo sac. There are a few reports of more than one pollen tube entering the embryo sac. However, in *Triticum durum* (Rudramuniyappa and Panchaksharappa 1974) and *Persea americana* (Sed-gley 1976) - both uniovulate systems - only one pollen tube enters the ovary. In *P. americana* though, on an average, over 66 pollen grains germinate on the stigma, most of the tubes cease growth after traversing up to various distances in the style, and only one tube reaches the ovary. In twin embryo sacs generally two pollen tubes reach the ovary. Based on these studies, Sedgley (1976) suggested that the embryo sac has a control over the growth of pollen tubes in the style.

Chemotropism

One of the controversial aspects of progamic phase is the nature of chemotropic factors which guide the pollen tubes from the stigma to the embryo sac. Many in-vestigators have demonstrated; using in vitro experiments, positive chemotropic effect of various parts of the pistil to pollen tubes (Iwanami 1959; Welk et al. 1965; Rosen 1961, 1971). In cultured pollen grains of *Antirrhinum majus* Mascarenhas and Machlis (1962, 1964) demonstrated calcium to be the chemotropic factor. They also found a gradation in the distribution of total calcium from the stigma to ovules in the pistil of *A. majus.* Based on these evidences they suggested that calcium may be the universal chemotropic factor in pistils of angiosperms. However, calcium is chemotropically inactive for pollen tubes of *Lilium* (Rosen 1971) and *Oenothera* (Glenk *et al*. 1970). Subsequently, Mascarenhas (1966) studied, cytochemically, the distribution of soluble calcium; in the pistils of *Antirrhinum majus* and found it to be almost constant throughout the length of style.

The micropyle and the embryo sac did not show higher concentrations of calcium. Placenta and ovary wall, on the other hand, showed the highest concentration of calcium. This is contrary to expectations if calcium is to be a chemotropic factor guiding the pollen tube to the embryo sac. Following these studies, Mascarenhas (1966) suggested that, in addition to calcium, some other factors are involved in

directing pollen tubes. Glenk et a1. (1970) also reported lack of calcium gradient from the stigma to embryo sac in the pistils of *Oenothera.* Kwack (1969) observed that calcium did not play any role in chemotropism in *Clivia* and *Crinum*, although it promoted germination in both taxa. According to Jensen and Fischer (1969), cells of the transmitting tissue in cotton, with their file-like arrangement, provide a path of least mechanical resistance for the growth of pollen tubes and, thus, there is no need for a chemotropic gradient in the style.

Lack of chemotropic gradient in the style was also demonstrated by Iwanami (1959), by. a series of elegant experiments in *Lilium.* He demonstrated by stylar grafts that pollen tubes grow down the grafted style irrespective of its orientation. Also, when pollen grains were put in the stylar cavity by making a window, the pollen tubes grew in both directions - towards the pistil as well as the ovary. Ascher (1977) has shown that in *Lilium*, even when the ovarian end of the excised style was pollinated, pollen tubes grew normally towards the stigma.

Recently, Mascarenhas (1975) proposed a hypothesis according to which any growth factor may act as a chemotropic factor by causing a shift in the angle made by the centre of growing tip of the pollen tube with respect to the rest of the tube. Once this change in the direction of the growth takes place, the tube would continue to grow straight without the presence of a concentration gradient until another shift in the direction occurs. According to this hypothesis, a chemotropic factor is necessary in the pistil only for changing the direction of the pollen tube growth, from the placenta towards the micropyle.

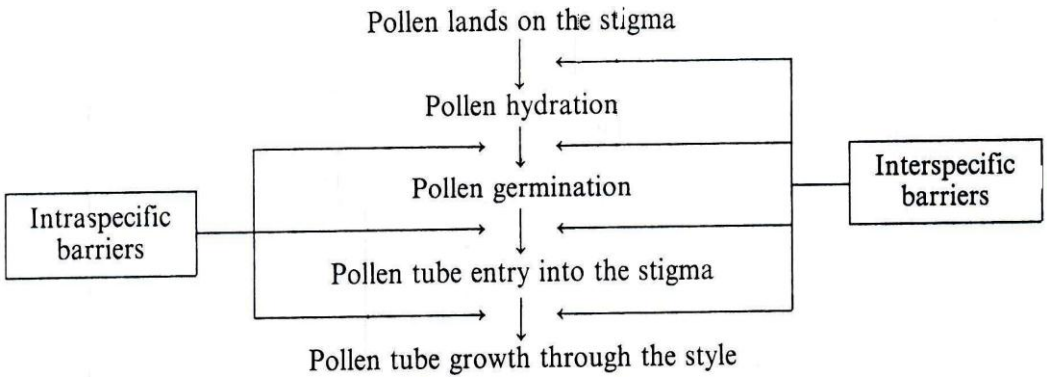


Figure 44. Major post pollination events that occur during pollen pistil interaction. Intra specific barriers operate at the last three stages, and inter specific barriers operate all stages.

Many suggestions have been put forward to explain the nature of the chemotropic factors originating in the ovule itself. Some investigators consider synergids, particularly the filiform apparatus, to be the source of chemotropic factor (Coe 1954). According to Chao (1971) the chemotropic substance in *Paspalum arbiculare* is produced by the dissolution of integumentary cells at the region of micropyle (see also Chao 1977).

Fertilization

The male gametes are released in the synergid through a pore formed at the tip,,of the pollen tube, or by rupture of the tube tip. Many hypotheses have been proposed to explain the causative factor for the rupture of pollen tube in the embryo sac. The only explanation which has some experimental basis is the one proposed by Stanley and Linskens (1967). According to this hypothesis, the rupture of the pollen tube is caused by low oxygen tension in the embryo sac. Experimental evidence for this concept comes from the studies of Linskens and Schrauwen (1966), and Stanley and Linskens (1967). Linskens and Schrauwen (1966) measured oxygen tension in the stylar canal in *Hippeastrum hybridum.* Unpollinated pistils showed high oxygen tension in the stigma and style, and a sharp drop in the lowermost 5 mm of style and ovary. In the pollinated pistil there was a drop in oxygen tension which progressed along the growth of pollen tubes. These results have been interpreted as evidences to indicate that pollen tubes grow aerobically up to the base of style, and switch over to anaerobic state soon after entering the ovary.

Stanley and Linskens (1967) could induce rupturing of the pollen tube tips of *Lilium* in vitro by decreasing oxygen tension in the medium. This led to the suggestion that, probably, lower oxygen tension in the embryo sac sets up cell wall stress in the pollen tube tip causing it to rupture. However, reducing oxygen tension in the medium did not induce the rupture of pollen tube in cultured pollen grains of *Petunia.* Thus, oxygen tension as the cause for the rupture of pollen tube is not tenable in solid-styled systems. Apart from the classical studies on the process of double fertilization by Geras-simova (1933) and others, not much progress has been made in recent years on the process of fertilization per se. The only progress that has been made on these lines is the fine structural details of fertilization in a few taxa (see Kapil and Bhatnagar 1975 for details). One of the important features of fertilization that has been observed

in many systems is the presence of gaps in the cell wall between the synergid and egg, and between the synergid and central cell. The male gamete enters into the cytoplasm of egg and central cell through these gaps. Eventually, the nuclear membranes of the male gamete and the egg fuse, resulting in nuclear fusion (for details see Jensen 1973).

CHAPTER V

INCOMPATIBILITY

(INTRASPECIFIC, INTERSPECIFIC, HETEROMORPHIC, HOMOMORPHIC AND OVERCOMING INCOMPATIBILITY)

The details of the progamic phase discussed so far are those following compatible pollination in which pollen germination and pollen tube growth proceed normally, and result in double fertilization. However, there are many instances in which pollinations are incompatible, i.e. they do not lead to fertilization. This is due to the arrest of post-pollination events at different levels (Fig. 44). Incompatibility occurs between species (interspecific incompatibility), as well as within the species (intra-specific incompatibility or self-incompatibility). Whereas interspecific incompatibility prevents fertilization between gametes of distantly related species, intraspecific incompatibility prevents fertilization between gametes of the same or other individuals of the same species.

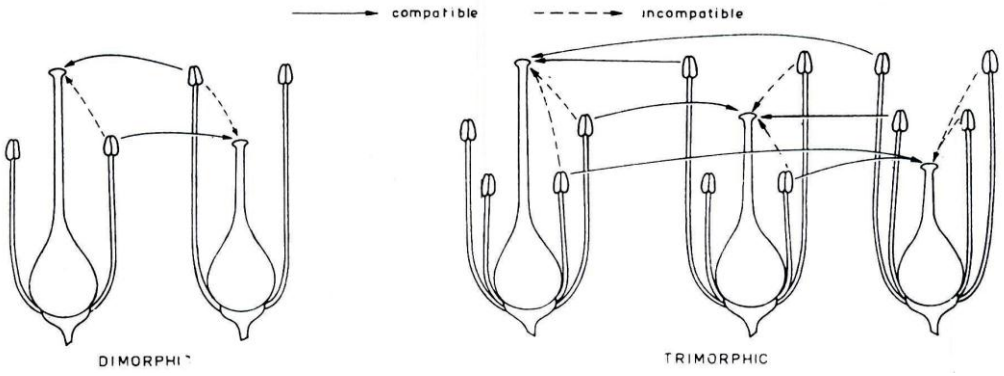


Figure 45. Heteromorphic intraspecific incompatibility.

There are two types of iniraspecific incompatibility-heteromorphic and homomorphic. In heteromorphic incompatibility different individuals of the species produce either two or three types of flowers differing in the length of stamens and style. Each plant produces only one type of flower. Pollen grains either from the same plant, or any other plant bearing the same type of flower, will be nonfunctional; only the pollen grains from other plant bearing any other type of flower are functional. These are diagrammatically represented in Fig. 45. In homomorphic type of incompatibility, on the other hand, all individuals of the species produce only one type of flower. Homomorphic incompatibility is governed by multiple alleles termed S alleles. Pollen tube having a particular S allele is inhibited in the style carrying the same S allele. In

the majority of taxa incompatibility is determined by multiple alleles at one locus; in grasses incompatibility in controlled by multiple alleles at two independent loci, S and Z. In some members of Ranunculaceae and Chenopodiaceae (Lundquist 1975; Larsen 1977), and Cruciferae (Lewis 1977) incompatibility is controlled by multiple alleles at three or four loci.

Incompatibility reaction in pollen may be controlled by the S allele present in the pollen itself (gametophytic), or by both the S alleles of the parent sporophyte (sporophytic). Determination of the genetics of self-incompatibility is a laborious process involving extensive breeding programme for many generations; hence, the detailed genetical analysis has been carried out only on a few systems. Gametophytic incompatibility is found in members of Solanaceae, Leguminoseae, and Gramineae, and sporophytic incompatibility in members of Compositae, Cruciferae, and Convolvulaceae. In heteromorphic systems also, incompatibility in pollen is determined by the genotype of the parent, and hence is of sporophytic type. The cytology of the pollen (two- or three-celled at the time of shedding) shows an interesting correlation with the zone of inhibition and the genetics of self-incompatibility. The taxa characterized by two-celled pollen show gametophytic type of incompatibility and the pollen tubes are inhibited in the style.

The taxa characterized by the three-celled pollen, on the other hand, show sporophytic type of incompatibility and the pollen tubes are inhibited on the stigma (Brewbaker 1957; Lewis 1956). Although this correlation holds good in a large number of taxa, there are many exceptions. For example, members of Gramineae are characterized by three-celled pollen with gametophytic type of incompatibility. In species *of Oenothera* the incompatible pollen tubes are inhibited in the stigma, but pollen grains are two-celled and incompatibility is of the gametophytic type. In many heterostylic taxa also, this correlation does not hold good. J.Helsop Harrison et al. (1975b) and Y. Heslop-Harrison and Shivanna (1977) analyzed the morphology of pistil and observed that sporophytic type of incompatibility is invariably associated with taxa characterized by the dry type of stigma whereas the gametophytic type may be associated either with wet or dry type. Intraspecific incompatibility is invariably a prefertilization barrier and is due to active inhibition of pollen tubes.

Studies on cytological details of post-pollination events following interspecific pollination are limited. The arrest of post-pollination events may occur at any level (Fig. 44) depending on the extent of reproductive isolation of the two parents. For example, the stigma of *Gladiolus* (Knox et al. 1976) allows hydration and germination of the pollen of *Crocosmia* (belonging to the same family, Iridaceae), but not the entry of pollen tubes into the papillae. When the stigma of *Gladiolus* was pollinated with pollen of *Gloriosa* (belonging to a different family, Liliaceae) pollen hydration itself is inhibited. From the above discussions it is apparent that the pistil has mechanisms to recognize compatible pollen from incompatible pollen, and to reject effectively incompatible pollen. In recent years significant progress has been made in understanding the details of recognition and rejection.

Factors Involved in Recognition

Pollen-Wall Proteins

As described earlier, pollen-wall proteins have dual origin; the intine proteins are gametophytic and exine proteins sporophytic. This demonstration led to the suggestion that the exine proteins (sporophytic) are involved in sporophytic type of incompatibility and the intine proteins are involved in the gametophytic type of incompatibility (J. Heslop-Harrison et al. 1973). Subsequent experimental investigations have produced strong evidences to support this concept. Upon pollination, whether compatible or incompatible, pollen-wall proteins are released on to the stigma and come in contact with the stigma-surface proteins.

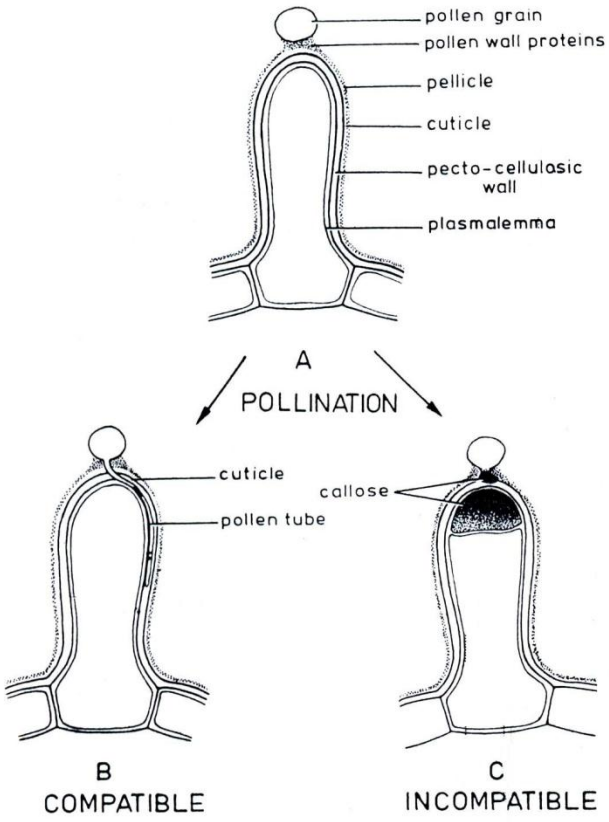


Figure 46. 6A-C. Pollen-stigma interaction in Cruciferae, and Composi-tae. Pollen-wall proteins are released on to the pellicle A where recognition takes place. The compatible pollen tube penetrates the cuticle and grows down the papilla. The incompatible pollen tube, although penetrates the cuticle, is inhibited from further growth. Note the deposition of callose in the stigmatic papilla below the incompatible pollen.

If compatible, pollen grains germinate and the tubes enter the stigma, and grow through the style. If incompatible, the pistil will initiate rejection reaction. The details of the rejection reaction vary from species to species. In *Raphanus* (Dickinson and Lewis 1973 a, b), *Iberis* (J. Heslop-Harrison et al. 1974), and *Cosmos* (Howlett et al. 1975) - all characterized by sporophytic type of incompatibility. The incompatible pollen either fails to germinate or produces a short tube which will, at the most, erode the cuticle of the papilla but fails to make any growth in the papilla. Generally, pollen tube tip gets plugged witty callose. Thus, the cuticle is not the effective barrier in preventing pollen tube entry, as many of the incompatible tubes do penetrate the cuticle. Interestingly, the stigmatic papilla also shows characteristic rejection reaction. Just at the region of contact of pollen grain, the papilla shows deposition of callose between the plasmalemma and pectocellulosic layer (Fig. 46). This callose deposition

is specific to incompatible pollen, and develops within 3-6 h after pollination (Fig. 47 A, B). In *Cosmos bipinnatus* the callose plug can be seen even 15 min after pollination (Howlett et al. 1975).

The characteristic rejection reaction of the stigma has been used as a bioassay for identifying the components of pollen involved in incompatibility. In species of *Iberis*, J.Heslop-Harrison et al; (1974) successfully induced rejection reaction on the stigma by deposition of an agarose film into which exine proteins were allowed to diffuse. More importantly, even the isolated fragments of the tapetum (before releasing their protein loads into the thecal cavity) have been shown to be effective in inducing characteristic rejection reaction on the stigma. The efficacy of exine -borne material in inducing rejection reaction has also been demonstrated in *Raphanus* (Dickinson and Lewis 1973 b). These elegant experiments provide direct evidence for involving exine proteins in recognition and rejection reactions. As the exine proteins are the products of sporophytic tissue, the tapetum, the genetic basis of sporophytic incompatibility is satisfactorily explained (see J.Heslop-Harrison 1975b).

In a majority of taxa having gametophytic incompatibility, the pollen tubes are not inhibited on the stigma, but in the style. It is, therefore, difficult to carry out comparable experiments on gametophytic systems showing pollen tube inhibition on the stigma. However, grasses are the well-known exception with gametophytic system showing pollen tube inhibition in the stigma. This makes them suitable for conducting experiments to test the role of pollen-wall proteins in incompatibility. Investigations have been carried out on species of *Gaudinia*, *Saccharum*, and a few other graminaceous taxa (J. Heslop-Harrison 1975 c; Shivanna et al. 1976; see also Shivanna 1977, 1979). In grasses there is no callose deposition on the stigmatic papillae subsequent to incompatible pollination. However, the callose deposition is very conspicuous in pollen tube (Fig. 47 C-F).

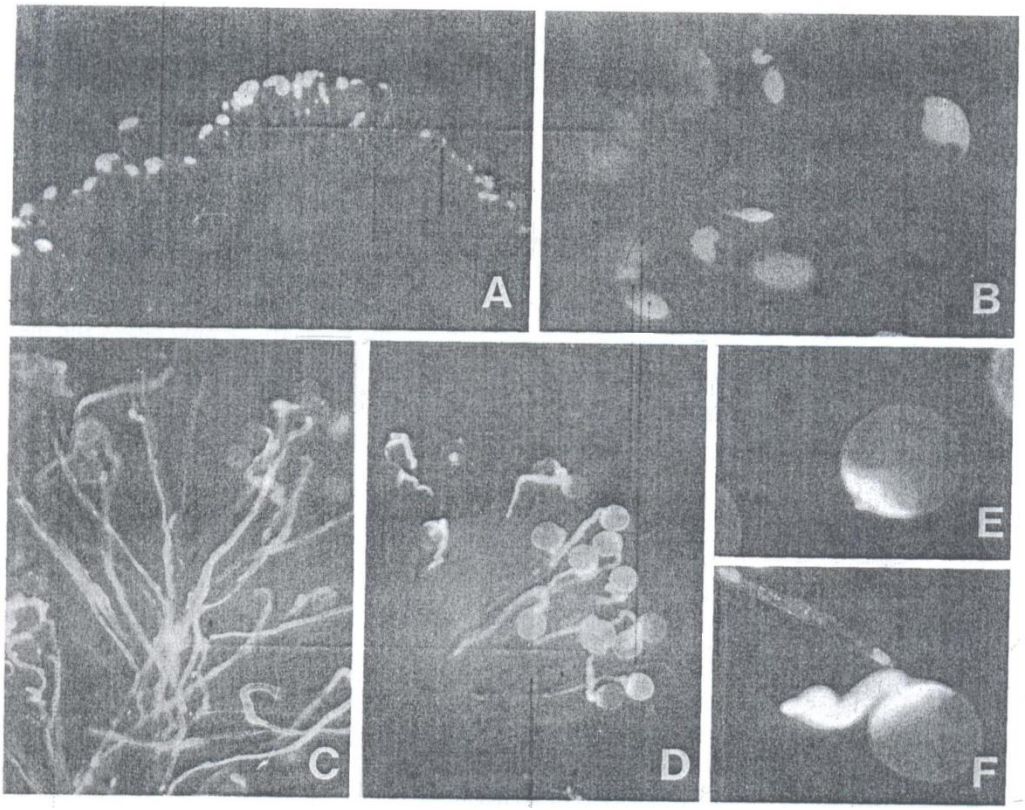


Figure 47. A-F. Fluorescence micrographs of stigma 6 h iafter pollination following staining with decolourised aniline blue. A, B *Brassica campestris* portions of stigma 6 h after self-pollination. Observe characteristic rejection reaction at the tips of papillae. A few papillae are shown at higher magnification in B. C-F *Saccharum hengalensis.* C Part of the compatibly pollinated stigma; observe profuse growth of pollen tubes. D Part of the incompatibly pollinated stigma. Pollen tubes are inhibited after growing a short distance in the stigma. E, F Incompatible pollen grains. E Germ pore itself is blocked with callose inhibiting germination. F Pollen tube is arrested before entering the papilla.

In extreme cases the pollen germination itself is inhibited by deposition of callose in the germpore. In all the grasses investigated, release of only the exine components on to the stigma does not initiate rejection reaction. However, release of intine proteins triggers off rejection reaction immediately. The recognition reaction in grasses is completed within a few minutes after pollination, and subsequent acceptance or rejection become distinct in less than 10 min. Thus, in grasses there are strong evidences to implicate intine proteins in incompatibility. No data are available on any other taxon having gametophytic incompatibility.

Stigma-Surface Proteins

Most of the investigations implicating ^stigma-surface proteins are confined to the taxa with dry type of stigma. In members of Caryophyllaceae (J. Heslop-Harrison and Y. Heslop-Harrison 1975) and *Gladiolus* (Knox et al. 1976) digestion of stigma-surface proteins with pronase does not affect pollen germination but inhibits the entry of pollen tubes into the papillae. Some factors in the pellicle, therefore, are required for effective operation of the cutinase system in the pollen. In *Ra-phanus sativus* also, enzymatic digestion of the pellicle reduces pollen germination and inhibits the entry of even; the compatible tubes into the papilla (Shivanna et al. 1978).

Studies of Knox et al. (1976) on *Gladiolus* provide more direct evidences for the involvement of stigma-surface proteins in pollen-pistil interaction. They showed that concanavalin A (con A) binds specifically to the pellicle. Stigmas of very young buds free from pellicle did not bind to con A. Following con A binding stig-matic surface did not inhibit pollen germination, but prevented the entry of pollen tubes. Therefore, components involved in con A binding were necessary for pollen tube entry, and not for pollen: germination. Washing of the stigma with sodium deoxycholate removed the ability of the stigma to support pollen germination as well as its ability to bind to con A. Knox et al. (1976) suggested that stigma-surface receptors are composed of many components; some of them are involved in pollen germination and others in entry of tubes.

Studies on intraspecific incompatibility also have implicated stigma-surface proteins in pollen recognition and rejection. S allele-specific proteins have been shown to diffuse from intact stigma (Nasrallah and Wallace 1967). Also, water-soluble substances released from the undamaged stigmas selectively inhibit in vitro germination of self-pollen, but not of cross-pollen (Ferrari and Wallace 1976). Obviously, these proteins emanate from the pellicle.

Sequence of Recognition and Rejection

Intraspecific Incompatibility

As no data is available for heteromorphic systems, the discussion is confined to homomorphic systems only. A probable sequence of recognition and rejection that occurs during pollen-pistil interactions are presented in Fig. 8. This is based largely on the site of recognition and rejection, and the factors involved in recognition. In

taxa characterized by sporophytic type of incompatibility, recognition of incompatible pollen as well as rejection are completed on the stigma. As pointed out earlier, exine proteins and pellicle proteins seem to be involved in these processes. In taxa characterized by the inhibition of pollen tubes in the style, the sites of recognition and the factors involved in recognition are not apparent. In *Petunia hybrida* distinct differences have been observed between self- and cross-pollinated styles in the synthesis of RNA, DNA, and the size of free nucleotide pool within 3 h after pollination (van der Donk 1974, 1975).

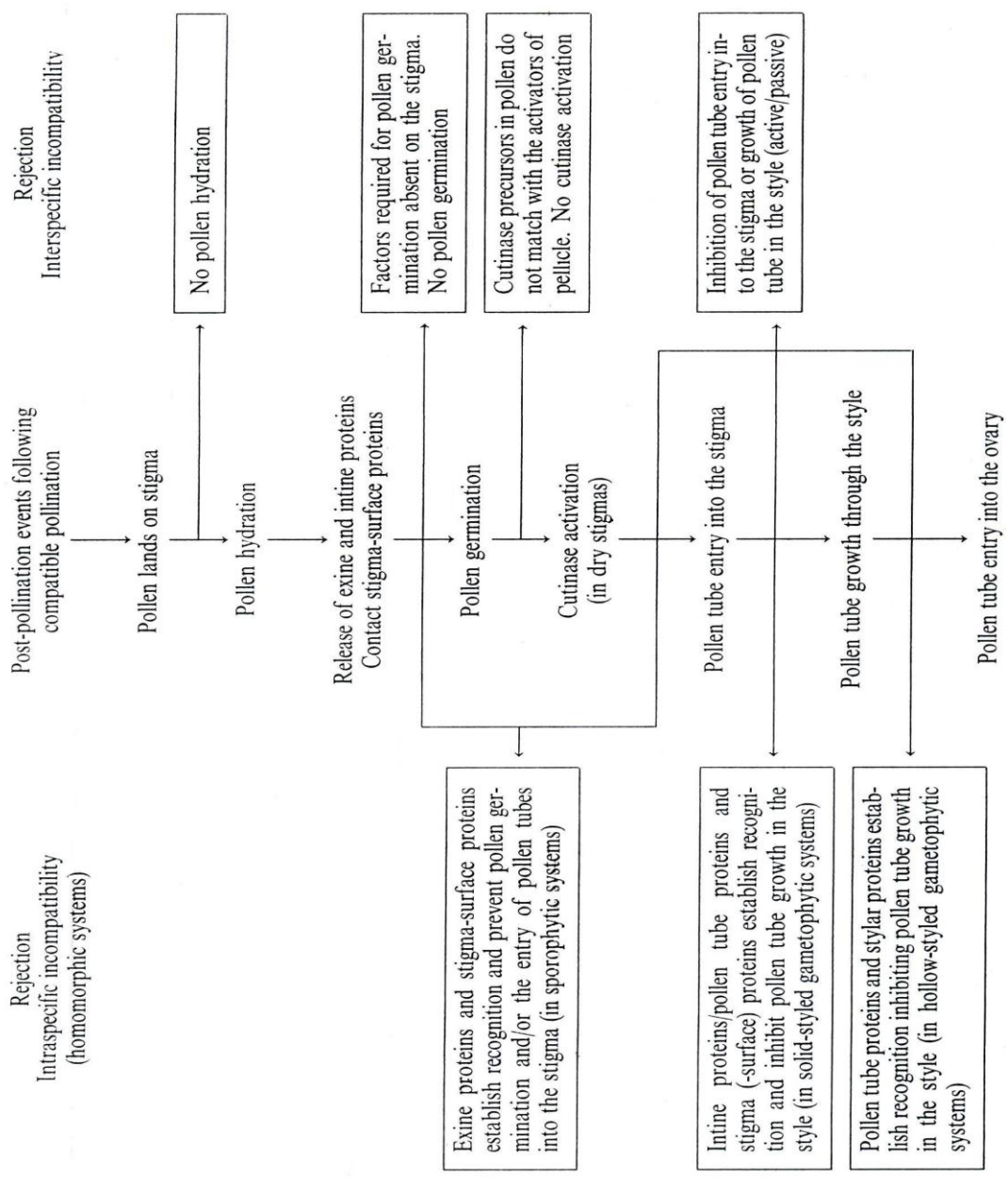


Figure 48. Probable sequence of recognition and rejection during pollen pistil interaction.

It was suggested that these differences were the result of recognition of pollen taking place much earlier, on the stigma itself, soon after pollination. In *P'unus avium* (Raff and Knox 1977) the presence of stigma is necessary for pollen tube inhibition (Fig. 49 A). When pollen grains were deposited on an artificial stigma made on the cut stump of the style (after removing the stigma), both compatible and incompatible tubes grew through the style and reached the ovary. Thus, in *Petunia, Prunus,* and probably other genera with solid style recognition and rejection are separated in time and space, recognition takes place soon after pollination in the stigma, and rejection is completed in the style about 24 h later. Intine proteins and stigma/stigma-surface proteins are likely to be involved in recognition.

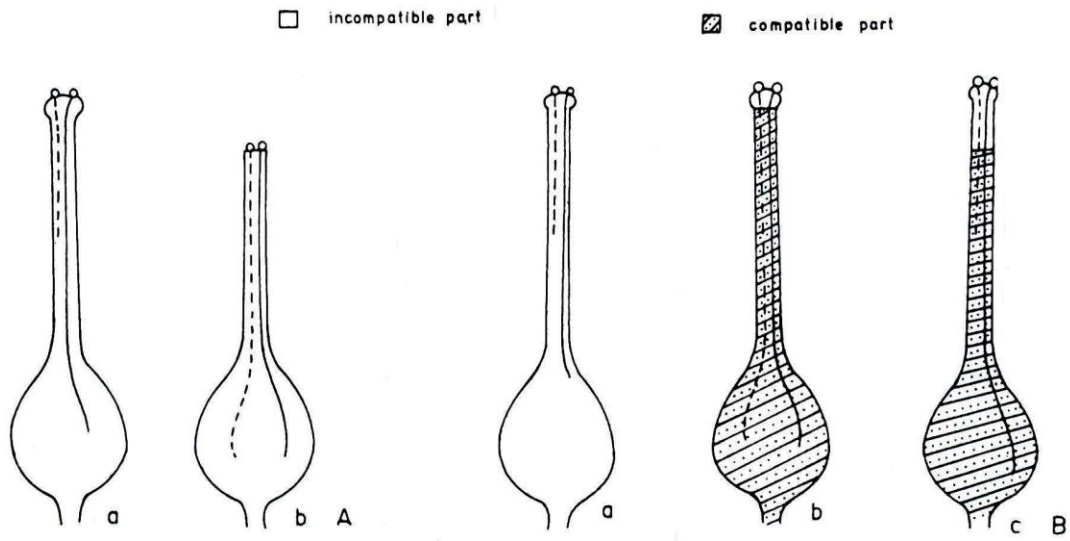


Figure 49. A, B. Responses of self- *broken line* and cross- *solid line* pollination to various treatments in *Prunus avium* A, and *Lilium henryi* B. In both taxa incompatible pollen tubes are inhibited in the style. In *P. avium* removal of stigma was effective in overcoming pollen tube inhibition indicating that incompatible message is received in the stigma. In *L. henryi* grafting of incompatible stigma on compatible style was not effective in inhibiting incompatible pollen. However, when incompatible stigma together with upper quarter of the style was grafted on to compatible style, incompatible pollen tubes were promptly inhibited.

In *Lilium,* which has a hollow style, the stigma does not seem to play any role in pollen recognition. Details of grafting experiments conducted in *L. henryi* (Law-son and Dickinson 1975) have shown that the incompatible message is not received in the stigma, but is received only after the pollen tube has grown through a quarter of the

style (Fig. 49 B). Similar results have been obtained in *L. longiflorum* also (Gladding and Paxton 1975). Further, hot water treatment to the pistil of *L. longiflorum* is known to inactivate incompatibility. Application of hot water treatment only to stigma was not effective in overcoming incompatibility, but hot water treatment only to the lower part of the style was effective in overcoming incompatibility (Fett et al. 1976). These studies clearly demonstrate that in *Lilium* both recognition and rejection events are confined to the style, and stigma has no role in these processes. In *L. longiflorum* the removal of loosely bound wall materials (proteins and carbohydrates) does not affect pollen germination and pollen tube growth as well as self-incompatibility reaction (Fett et al. 1976). Thus, it appears that in *Lilium*, and probably other hollow-styled systems, pollen wall proteins and stigma proteins are not involved in recognition. Both these processes occur in the style, and proteins synthesized in the pollen tubes and those present/synthesized in the style are involved in recognition and rejection function.

Interspecific Incompatibility

The data on interspecific incompatibility are fragmentary. It is apparent that the operation of interspecific incompatibility is more diverse and much less understood as compared with intraspecific incompatibility (Fig. 48). Pollen-wall proteins and stigma-surface proteins are probably involved in inhibition of pollen hydration, pollen germination, and cutinase activation. Besides stigma-surface proteins, non-proteinaceous factors such as phenolic compounds and carbohydrates may also play a role in pollen hydration and pollen germination. Phenolic compounds have now been shown to promote or inhibit selectively pollen germination (Martin 1970, 1972; Martin and Ruberte 1972). In *Impatiens balsamina* (Tara and Namboodiri 1976) failure of the stigma of a mutant to support pollen germination has been correlated to the absence of some of the phenolic compounds (present in other varieties, and which support pollen germination). Thus, lack of pollen germination following interspecific pollinations may be due to the absence of some substance(s) on the stigma (needed for germination). Such mechanisms represent passive rejection, and there is no need for pollen recognition.

Another method of passive rejection would be the inability of pollen tubes to penetrate the cuticle of the stigma, as has been shown in the cross involving *Gladiolus*

and *Crocosmia* (Knox et al. 1976; see also J. Heslop-Harrison 1975b). These passive rejections are more like a "lock and key" mechanism: absence of a suitable key with one of the partners for the lock present with the other partner results in incompatibility. Closely related species would have the right key at the stigma level, but may not be able to grow through the style. No information is available on the factors involved in pollen tube inhibition in the style. Inability of the pollen tubes to utilise stylar nutrients, which may be due to the lack of positive recognition, may often be the reason. In very closely related species it may be due to active inhibition. The sequence of recognition events, presented in Fig. 48, is a tentative one and indicate prominently the lacunae in our understanding of the details of pollen-pistil interaction. As more data become available the scheme is bound to be appended and/or modified.

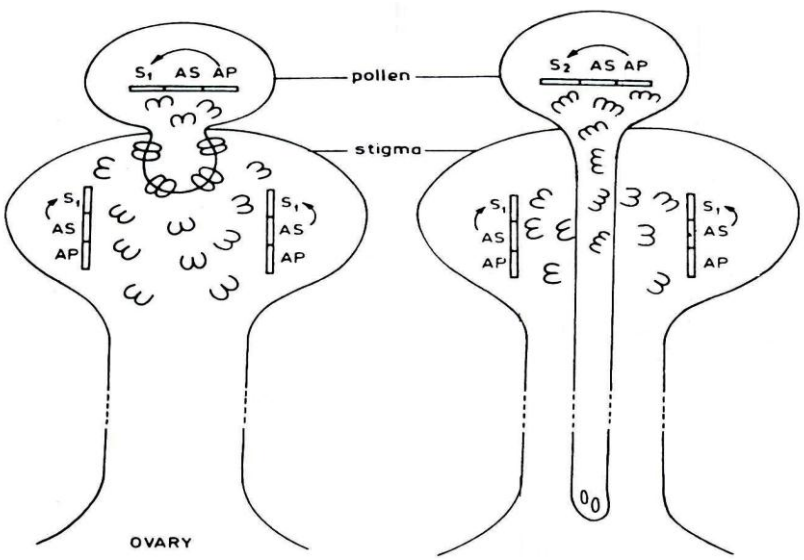


Figure 50. A generalised model to explain the function of S allele. This is based on the tripartite nature of the S locus (Lewis 1960) having a specificity part *S* common to both pollen and style, and two activity parts controlling the reaction in pollen *AP* and style *AS*.

Mechanism of Inhibition

Intraspecific Incompatibility

Many models have been proposed to explain the mechanism of inhibition (Lin-skens 1965; Lewis 1965; Ascher 1966; see Nettancourt 1977). Basically, these models envisage the production of S allele-specific polypeptide identical in pollen and style. When the polypeptide of the pollen comes in contact with the identical polypeptide in

the style, it dimerises on the surface of the pollen tube to form a repressor which inhibits the growth of the pollen; tube (Fig. 50). Production of S allele-specific proteins has been demonstrated in *Oenothera* (Lewis 1952; Lewis et al. 1967), *Petunia* (Linskens 1960), and *Brassica* (Nasrallah and Wallace 1967). Similarity of S allele-specific proteins in pollen and pistil has been shown only in *Petunia;* in *Oenothera* the investigations are confined only to pollen, and in *Brassica* S allele-specific proteins have been found only in the pistil and not in pollen.

Detailed studies on *Brassica oleracea* by Nasrallah and his associates (Nasrallah and Wallace 1967; Nasrallah et al. 1970, 1972; Nasrallah 1974) have shown that each S allele-specific protein had a different electrophoretic mobility and, hence, each of them could be localized to a specific band on the gel. These proteins were heritable as shown by the presence, in the heterozygous plants, of both S allele-specific bands of the parents (Nasrallah et al. 1970). Studies: of F_1 and F_2 progenies, involving crosses of different homozygous self-incompatible genotypes showed that S allele-specific proteins present in the progenies were exactly correlated with the segregation of S alleles as determined by genetical analysis (Nasrallah et al. 1972). Recent studies of Nishio and Hinata (1977) on isoelectric focusing of stigma proteins of *B. oleracea* several fractions of proteins rather than a single protein, and S allele specificity is expressed by a combination of the protein fractions.

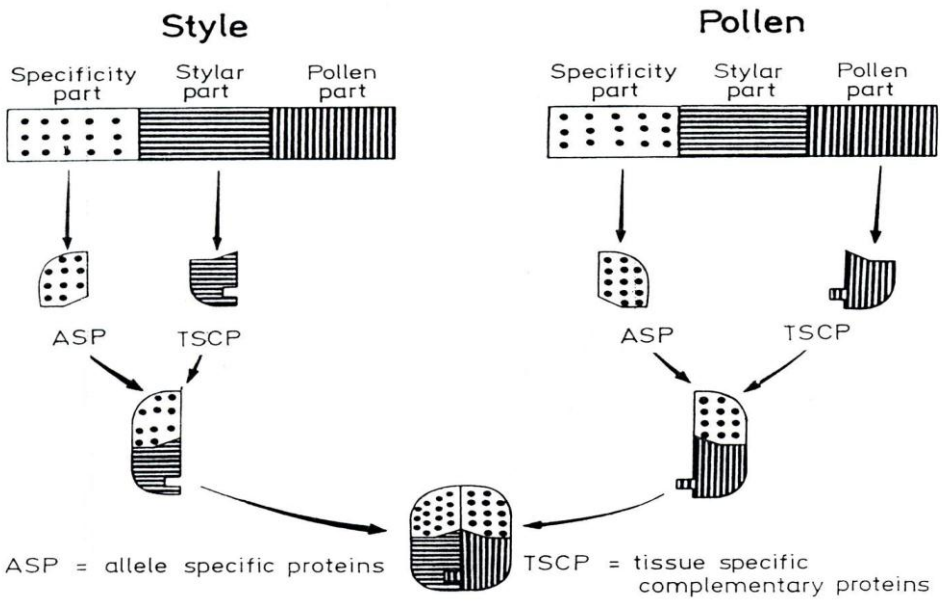


Figure 51. The hypothesis concerning S gene products. Identical specificity proteins in the pollen and style, form a complex with tissue specific adaptive

proteins. This model explains the formation of mutually reactive S allele specific proteins in the pollen and style.

One of the limitations of these models, which envisage identical S allele-specific proteins in pollen and style, is the lack of explanation for the reactivity of identical proteins (of the pollen and style), and the mechanism that prevents the formation of repressors by polypeptides of the pollen or the style themselves. To overcome these difficulties, Pandey (1975) modified the concept of Lewis (1960) concerning S gene determination based on the tripartite nature of S gene. According to this hypothesis the S allele proteins in the pollen and style have an identical specificity protein and a tissue specific adaptive protein (Fig. 51). This model explains the failure of the stylar or the pollen proteins to react amongst themselves, but allow for the interaction between them to produce a repressor of the genes involved in pollen tube growth.

The mechanism by which the repressor Inhibits the growth of the pollen tube is not clear (see Nettancourt 1977; Shivanna 1979). The concept that self-incompatibility inhibition is mediated through the inhibition of protein synthesis in pollen tubes is in agreement with presen knowledge on pollen tube inhibition (Nettancourt 1977; Nettancourt et al. 1974, 1975; Cresti et al. 1977). Although earlier investigators considered the mechanism of inhibition in sporophytic systems different from that in gametophytic systems (Christ 1959; Kroh 1966), recent evidences have indicated that the basic mechanism in both systems may be similar (Ferrari and Wallace 1977; Shivanna 1979) model, based on the inhibition of protein. Ferrrari and Wallace (1977) put forward a synthesis, to explain self-incompatibility inhibition in both sporophytic and gametophytic systems.

Interspecific Incompatibility

Our knowledge on the details of inhibition following interspecific pollination are meagre. As pointed out earlier, in a large number of instances it may be a passive inhibition due to the lack of some factor(s) in the pistil, required for pollen germination or pollen tube growth. In crosses involving closely related species, particularly in instances of unilateral incompatibility, the inhibition may be active. According to some investigators the S allele has a dual function and is involved in both intra- and interspecific incompatibility (Lewis and Crowe 1958; Pandey 1968). However, many recent genetical studies suggest that interspecific incompatibility is

controlled by genes different from S genes (Abdalla 1974; Takahashi 1974). Hogenboom (1975) includes all interpopulational incompatibility, not controlled by S alleles, under incongruity. Incongruity is due to the lack of genetic information in one partner about some relevant character of the other. It is a by-product of evolutionary divergence and, hence, Varies from system to system depending on the extent of evolutionary divergence. It may also be concerned with the post-fertilization barrier. Thus, incongruity represents a passive rejection, whereas incompatibility involves active rejection as a result of S gene action.

The hypotheses to explain unilateral and interspecific incompatibility are based largely on genetical studies. Very little work has been done on the physiological and biochemical aspects. A few ultrastructural and physiological data available are not unequivocal. Nettancourt et al. (1973 a, b, 1974, 1975) studied ultrastructural details of pollen tubes inhibited in self-incompatible and unilateral incompatible reactions. They observed that, in both reactions, the pollen tubes showed accumulation of a large number of bipartite particles at the tip, and the formation of characteristic concentric endoplasmic reticulum. The only difference which is consistent between the tubes inhibited in self-incompatibility and those inhibited in unilateral incompatibility, was the nature of the outer wall of the pollen tube; it was very thick in the former but rather thin in the latter. Based on these and other genetical studies, Nettancourt et al. (1975) concluded that interspecific unilateral incompatibility results from the interaction of S elements in the pollen grains with one or several non-identified stylar genes.

Roggen and Linskens (1967) studied pollen tube growth and respiration pattern following intergeneric crosses between *Petunia hybrida* and *Salpiglossis sinuata.* The morphological abnormalities of the incompatible pollen tubes (branching and swelling of tube tip, increased callose deposition, etc.), and the respiration pattern following intergeneric crosses were similar to those following selfing. They suggested that the mechanism of inhibition of pollen tubes is similar in both self- and intergenericibility. However, in *Lilium,* unlike self-incompatibility which can be overcome by giving hot water treatment to the pistil, interspecific incompatibility could not be overcome by hot water treatment (Ascher and Peloquin 1968), indicating that the mechanism of action in both types of incompatibility is different. Further physiological and bio-

chemical studies on interspecific incompatibility are necessary for a better understanding of the mechanism of inhibition.

Methods of Overcoming Incompatibility

There are many effective methods to overcome intraspecific incompatibility. Success in overcoming interspecific incompatibility is limited only to a few systems. Also, interspecific incompatibility in many of the closely related crosses operates after fertilization, and the techniques of embryo culture have been effectively used to overcome post-fertilization barriers (see Raghavan 1976). Table 2 lists most of the well-established techniques used to overcome barriers to fertilization, with some examples. The details of many of these techniques have been adequately covered by Nettancourt (1977). A few of the recent techniques are dealt here in greater detail.

Recognition Pollen/Mentor Pollen

As discussed earlier, pollen wall proteins have been implied in intraspecific incompatibility. Knox and his associates indicated that they are involved in controlling interspecific incompatibility also. Knox et al. (1972 a, b) attempted to cross *Populus deltoides* and *P. alba* by mixing killed compatible pollen (either by irradiation, or by treatment with organic solvents) with live incompatible pollen. By this method they could obtain a significant number of hybrids. Even the proteinaceous diffusates obtained from the wall of compatible pollen were effective in overcoming interspecific incompatibility. They presumed that the proteinaceous recognition factors released from the wall of killed compatible pollen mask the rejection reaction on the recipient stigma, thus making it ineffective in inhibiting incompatible pollen tubes. Sastri and Shivanna (1976 b) attempted to overcome interspecific incompatibility between *Sesamum indicum* ar d *S. mulayanum* by this technique of recognition pollen.

Table 2. Effective techniques to overcome incompatibility.

Methods	Species
Intraspecific incompatibility	
Induced mutations	*Oenothera, Prunus, Petunia, Trifolium, Nicotiana*
Induction of autotetraploaidy	*Prunus, Pyrus, Petunia, Tradescantia, Lolium*
Irradiation of Pistils (X-rays/UV rays)	*Petunia, Lilium, Rubus, Ribes, Licopersicum, Nicotiana*
Bud pollination	*Brassica, Raphanus, Petunia, Nicotiana*
Delayed pollination	*Brassica, Lilium*
Hot water/high temperature treatment	*Malus, Pyrus, Prunus, Oenothera, Trifolium, Brassica, Raphanus, Lycopersicum, Lilium, Secale, Nemesia*
Treatment of stigma with organic solvents (hexane)	*Brassica*
Increased atmospheric humidity	*Brassica*
Mutilation of the stigma	*Brassica*
Application of growth substances	*Lilium, Petunia, Tagetes, Trifolium, Brassica, Lycopersicum*
Electric aided pollination	*Brassica*
End season pollination	*Nicotiana, Petunia, Abutilan*
Use of recognition pollen (mentor pollen)	*Theobroma, Cosmos, Brassica, Malus, Petunia, Nicotiana*
Placental pollination	*Petunia*
Interspecific incompatibility	
Application of growth substances	*Lilium*
Stump pollination	*Solanum, Nicotiana*
Use of recognition/mentor pollen	*Populus deltoids* x *P.alba*
	Nicotiana forgetiana x *N. langsdorffii*
Intra ovarian pollination	*Argemone Americana* x *A.ochroleuca*
Placental pollination	*Melandrum album* x *M. rubrum*
	M. album x *Silene schafta*
Treatment of stigma/pollen with organic solvents	*Populus*

Although recognition pollen was effective in overcoming incompatibility on the stigma, it failed to overcome incompatibility barrier in the style. These experiments provide rational explanation for the success of distant hybridisation by the use of mixed pollen and mentor pollen by many investigators, particularly in Russia. As pollen-wall proteins are involved in controlling intraspecific incompatibility, at least in sporophytic systems, it should be possible to overcome intraspecific

incompatibility by using the technique of recognition pollen. This has been achieved in many taxa. In *Cosmos bipinnatis* (Howlett et al. 1975), a strictly self-incompatible species with a sporophytic incompatibility, the application of exine diffusate of compatible pollen on the stigma before self-pollination, resulted in significant increase in seed set (as much as 22% of the control).

The application of crude ether extract of bee collected pollen of *Brassuica napus*, largely containing pollen coat material, on to the stigma of Brussels sprouts (*B. oleracea* L. var *gemmifera*) greatly increased selfed seed set, to as much as was obtained with bud polli lination (Roggen 1975). *(B.napus* is compatible with *B.oleracea).* The application of recognition-pollen along with self-pollen has also been effective in increasing the selfed seed-set in *Petunia hybrida* (Sastri and Shivanna 1976 a) and many varieties of apple (Dayton 1974). Recently, Pandey (1977) also reported success in overcoming both intra and interspecific incompatibility by using mentor pollen in some species of *Nicotiana.* Detailed analysis of his results suggested that the role of mentor-pollen in overcoming incompatibility may be that of providing extra .free pollen growth promoting substance rather than providing recognition, factors.

These treatments are basically aimed at manipulating the pollen proteins involved in incompatibility. Comparable manipulations can also be carried out with the stigma-surface proteins, the other partners involved in incompatibility. In interspecific hybridisation of *Populus,* Willing and Pryor (1976) showed that the treatment of stigma with many organic solvents, such as anhydrous hexane and ethyl acetate, before pollination was remarkably effective in overcoming interspecific incompatibility. In some crosses the seed-set following incompatible pollination was almost as good as compatible pollination.

Intra-Ovarian Pollination and Test-Tube Fertilization

All the techniques, described so far, are aimed at overcoming incompatibility re-taining the zone of inhibition in the pistil (i.e. the stigma and style). Theoretically, any attempt which aims at eliminating the stigma and style altogether, thus bringing the pollen in direct contact with the ovules and achieving fertilization and seed development, is the most effective technique. Significant success has been achieved on these lines at the Department of Botany, University of Delhi (see Rangaswamy

1977). Initial attempts were made on members of Papavaraceae, as they are most suitable for such studies. The procedure of intraovarian pollination was the first technique standardised, and involves injecting pollen grain (suspended in a suitable medium) directly into the ovary, achieving pollen germination, pollen tube entry into ovule, and fertilization. Viable seeds following intra-ovarian pollination have been obtained in *Papaver somniferum* (Fig. 52 A, B) *P. rhoeas, Argemone mexicana,* and *A.ochroleuca.* This technique has also been applied to achieve interspecific hybridisation between *A. mexicana* and *A. ochroleuca* (Fig. 52 C) (Kanta and Maheshwari 1963; Maheshwari and Kanta 1961).

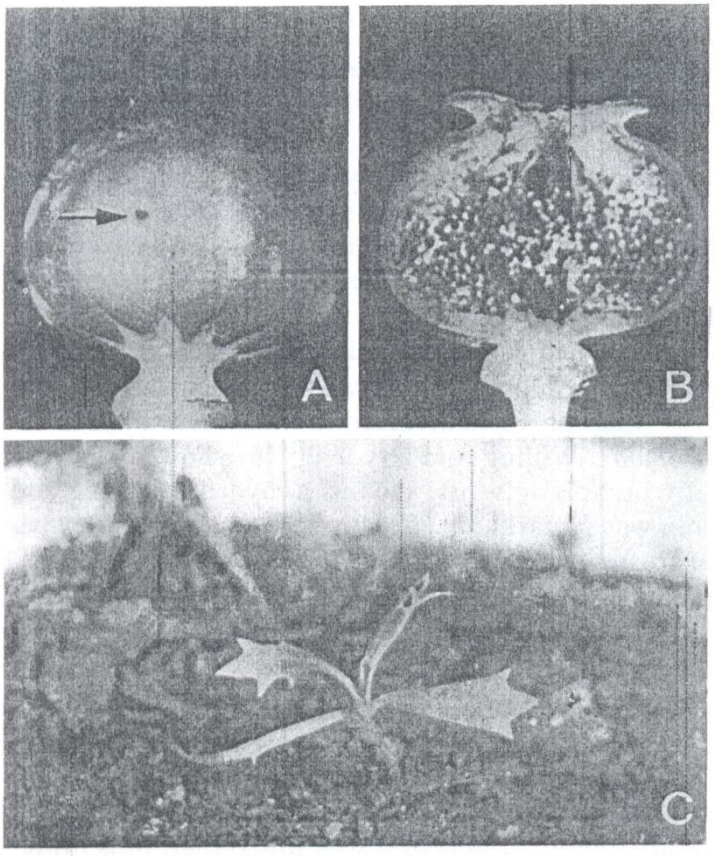


Figure 52. Intra-ovarian pollination. A, B 27-day-old fruits of *Papaver somniferum* developed as a result of intra-ovarian pollination. *Arrow* in A shows point of injection of pollen suspension. In B vertical half of the fruit is shown; note the seeds. C 6-week-old hybrid seedling *(Arge-mone mexicana* x *A. ochro-leuca)* raised from seed obtained through intra-ovarian pollination.

There are many limitations in extending the technique of intra-ovarian pollination to other taxa. It is not suitable to the taxa in which there is not enough space in the ovary

to inject pollen suspension. Also, in taxa in which sugar is required for pollen germination, injection of pollen suspension containing sugar makes the ovary prone for bacterial and fungal infection. These limitations have been overcome in techniques involving aseptic culture of whole pistils or ovules to achieve test-tube fertilization. Cultured pistils of *Nicotiana* (Dulieu 1963, 1966; Rao 1965), *Petunia* (Shivanna 1965), and *Antirrhinum* (Usha 1965), pollinated in vitro, have been successfully grown to obtain mature, viable seeds. As expected, the culture of whole pistils and pollination on the stigma was not effective in overcoming self-incompatibility (Shivanna 1965). This is because the inhibitory zone (the stigma and style) remains intact in the pistil. However, the culture of isolated ovules (bringing them in direct contact with pollen grains) has been very successful.

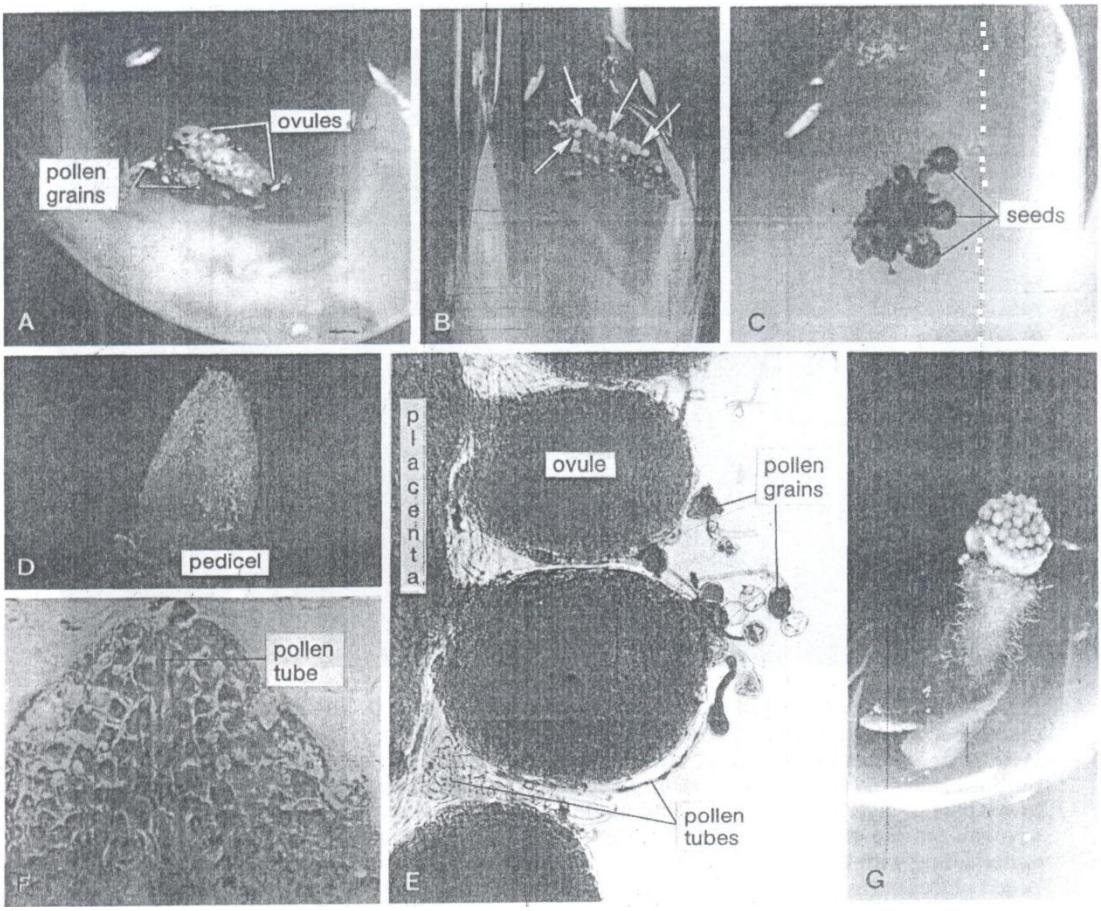


Figure 53. A-G. Test tube fertilization. A, B *Papaver somniferum.* C *Argemone mexicana.* D-G *Petunia axillaris.* A Portion of ovules and placenta cultured on nutrient medium and pol linated with pollen. B 7-day-old culture showing many developing seeds *white bodies.* C About 4-week-old seeds developed on nutrient medium following ovule pollination. D Both placentae of an ovary with its entire

mass of ovules dusted with pollen ready for culture. E Free-hand transection through self-pollinated placentae 24 h after culture; note mar ginal portion of one placenta, three ovules and many pollen grains. Also note pollen germination and profuse growth of pollen tubss. F Longisection of micropylar part of ovule, 2 days after selfing, to show entry of pollen tube. G 24 days after placental self-pollination; notemature seeds.

Kanta et al. (1962) cultured excised unpollinated ovules of *Papaver somniferum* on nutrient medium and dusted pollen grains on and around the ovules (Fig. 53 A). Following ovule pollination, pollen grains germinated normally, pollen tubes entered the ovules, and affected double fertilization. Interestingly, fertilized ovules developed into mature seeds on the same medium (Fig. 53B, C). Subsequently, the technique of test-tube fertilization was extended to *Argemone mexicana, Eschscholzia californica, Nicotiaria tabacum* (Kanta and Maheshwari 1963; Maheshwari and Kanta 1964), *Dianthus caryophyllus* (Zenkteler 1965), and *Dicranostigma franchetianum* (Rangaswamy and Shivanna 1969). All these taxa are self-compatible, and fertilization achieved in vitro was through compatible pollen.

Rangswamy and Shivanna (1967) attempted to overcome self-incompatibility in *Petunia axillaris* by using the technique of test-tube fertilization. The culture of isolated ovules or groups of ovules together with the pollen grains did not result in fertilization or seed development, even with compatible pollen, although pollen germination was abundant. They modified the technique: instead of pollination of isolated ovules, the entire ovule mass of the ovary intact on the placentae together with a short length of pedicel was cultured on the medium and the ovules dusted with pollen (Fig. 53 D). This refined technique, termed placental pollination, helped to bring the pollen in direct contact with the ovules without disturbing their original arrangement and, thus, preventing any injury to them. Following placental pollination, pollen grains germinated readily on the ovules and placentae (Fig. 53E), pollen tubes entered the ovules (Fig. 53 F), and effected fertilization. The fertilized ovules showed normal development of the embryo and endosperm, and mature viable seeds were obtained in 3 weeks after fertilization (Fig. 53 G). The same period is required for the seeds to mature in vivo. Unlike stigmatic pollination in which self-pollination is

invariably a failure, in placental pollination both self-and cross-pollinations are equally effective in inducing seed-set.

The technique of placental pollination (in *Petunia)* was further modified to treat the ovules on the two placentae, of the same ovary, differently (Shivanna 1971; Rangaswamy and Shivanna 1971a). The two placentae were separated, mechanically, by introducing a piece of cellophane between them (Fig. 54 A). When one of the placentae was maintained as control, and the other pollinated with self-or cross-pollen grains, the ovules on the Control invariably shrivelled, whereas many of the ovules on the pollinated placenta developed into seeds, irrespective of self- or cross-pollination. When one of the placentae was selfed and the other crossed, seeds developed on both the placentae equally well (Fig. 54 B), thus demonstrating conclusively the equal efficacy of both self- and cross-pollination.

These experiments demonstrated that once the stigma and style are eliminated, the ovules do not show any preferential receptivity to crossed pollen, and they led to further modification of the technique to study the interaction between stigmatic pollination and placental pollination. The technique of two-site pollination was devised in *P. axillaris* (Rangaswamy and Shivanna 1971 b). The ovary wall was carefully peeled to expose one of the placentae, retaining the ovary wall on the other placenta, and the style and stigma intact (Fig. 54 C); Thus, the pollination could be carried out both on the stigma and on the exposed placenta in the same pistil. A series of experiments using two-site pollination demonstrated that the stigmatic self-pollination does not affect the response of placental self-pollination, and vice-versa (Fig. 54D, E).

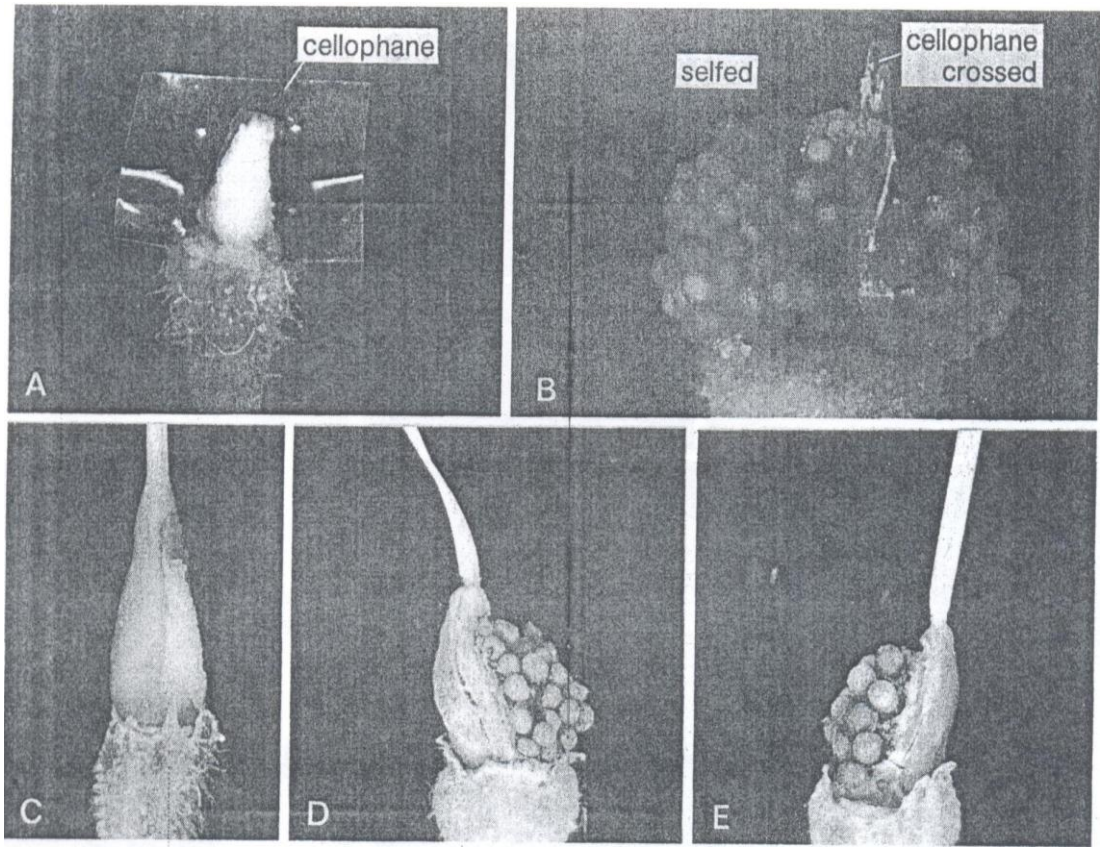


Figure 54. A-E. Placental pollination in *Petunia axillaris* A and B. Differential pollination ofplacentae. A Explant after insertion of a cellophane partition between the placentae, face view of one placenta. B 21 days after differential pollination; note the formation of seeds on both selfed *left* and crossed *right* placentae. C-E Two-site pollination; only ovary and lower part of style are shown. C Pistil made ready for two-site pollination by removing ovary wall on one of the two palcentae *right.* Ovary wall on the other placentae *left* was retained along with the style, and stigma (not shown). D 24-day-old culture in which both exposed placenta *right* and stigma were self-pollinated. E 21-day-old culture in which exposed placenta *left* was cross-pollinated, and the stigma self-pollinated. Only exposed placentae showed seed-set in both.

The success of test-tube fertilization attracted the attention of many investigators, and attempts have been made to use the technique in many fundamental and applied aspects. It has also been successfully used to overcome self-incompatibility in *Petunia hybrida* (Niimi 1970, 1976; Wagner and Hess 1973). Balatkova and Tupy (1968) could achieve in vitro fertilization and seed formation in *Nicotiana tabacum*, by using already germinated pollen grains. They also showed that the pollen lubes in which

gamete formation had occurred, when deposited on the unpollinated ovules, could effect successful fertilization. These studies demonstrate the feasibility of treating either the male gametes, or female gametes, without affecting each other. The technique of two-site pollination has been used (Wagner and Hess 1973) in *Petunia hybrida* to test the relative fertilization competence of pollen grains deposited on the stigma and or the ovule surface. The pollen deposited on the stigma was found to have a better chance for effecting fertilization, despite the longer way the pollen tube has to traverse, than the pollen deposited on the ovule.

Pollination with killed pollen, irradiated pollen, and pollen of alien species occasionally stimulate unfertilized egg to develop parthenogenetically. Logically, such pollinations carried out directly on the ovules would be more effective in inducing parthenogenesis, than those carried out on the stigma, as the factors which stimulate the egg are brought much closer to the embryo sac. The feasibility of this has been demonstrated by the report of Hess and Wagner (1974), which successfully induced parthenogenesis by using the technique of test-tube pollination. Their attempts to obtain androgenic haploids by anther culture technique in *Mimulus luteus* were unsuccessful. However, when its ovule mass was pollinated in vitro with the pollen of *Torenia fournieri*, 1 % of the ovules developed parthenogenetically and gave rise to haploid plantlets.

Zenkteler (1967, 1970), and his associates (Zenkteler et al. 1975), successfully applied the technique of test-tube fertilization to raise interspecific and even intergeneric hybrids (Fig. 55 A-F). They could successfully obtain hybrid progeny following the cross *Melandrium album* (M) x *Melandrium rubrum* (F), as well as *M. album* (M) x *Silene schafta* (F). They also attempted to obtain hybrids between *M. album* (M) and many other taxa belonging to Caryophyllaceae, Cruciferae, Solanaceae, and Campanulaceae (Fig. 55). Following such crosses, they reported many pre-fertilization as well as post-fertilization abnormalities. In many of them there was normal fertilization and initiation of embryo and endosperm but, later, the embryo degenerated. Isolation of young embryos, and culturing them on a suitable nutrient medium, would probably enable the production of hybrids in these crosses also. Thus, the technique of test-tube fertilization offers immense possibilities not

only to overcome intra- and interspecific incompatibility, but also in many fundamental studies concerning fertilization.

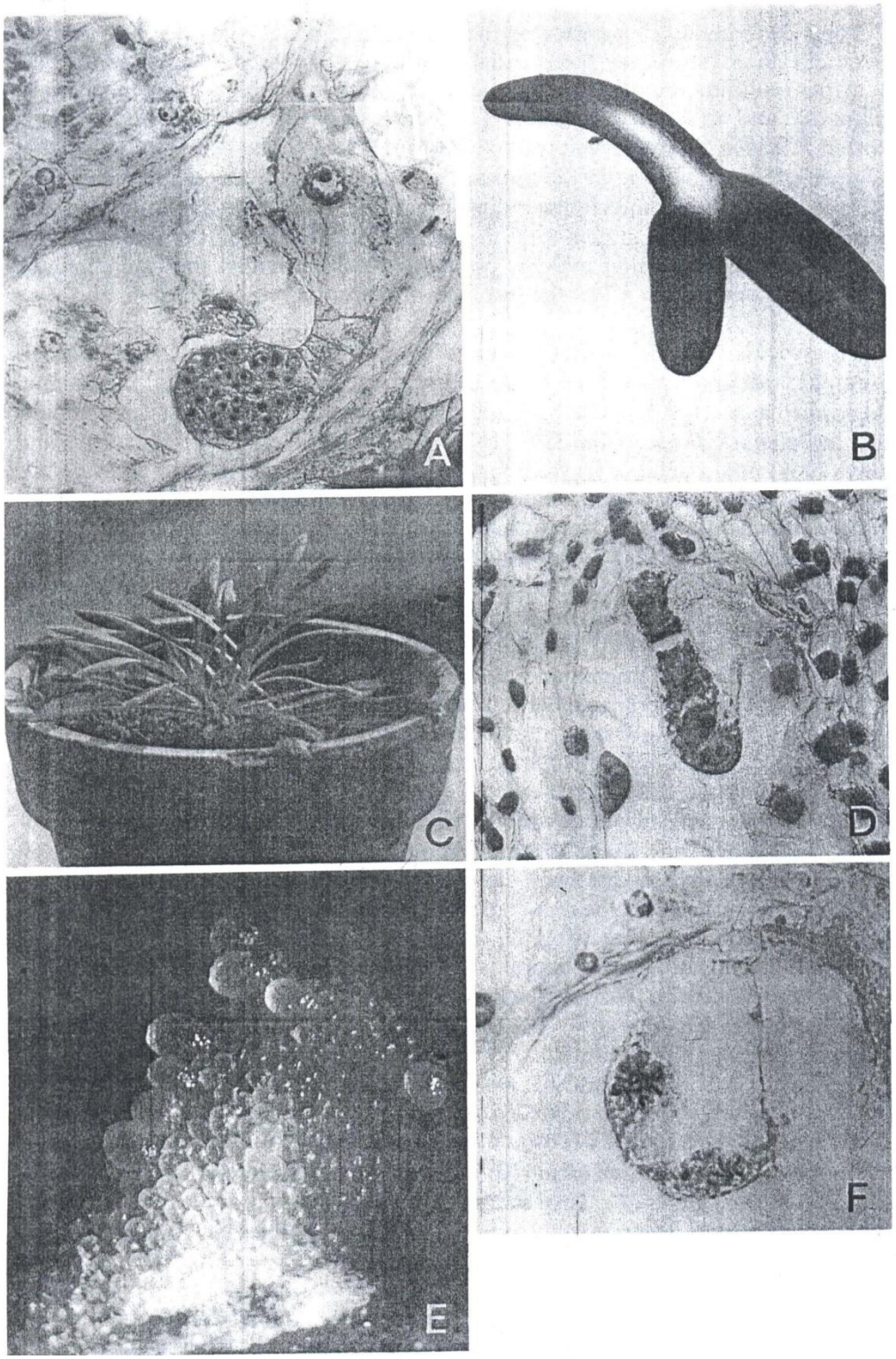


Figure 55. A-F. Interspecific hybridization through test tube fertilization. A *Melandrium album* x *Viscaria vulgaris,* hybrid embryo and endosperm, 5 days after test tube pollination. B *M.album* x *M.rubrum,* wholemount of embryo 12

days after pollination. C *M.album* x *V. vulgaris,* hybrid plants raised from seeds obtained in test tubes. D *Nicotiana tabacum* x *Petunia hybrida,* hybrid, linear embryo 4 days after pollination. E *N. tabacum* x *Hyoscyamus niger,* development of seeds 4 days after pollination. F *M. album x P. hybrida,* male gamete has entered the egg; male gamete and egg nucleus are undergoing mitotic division.

Concluding Remarks

From the foregoing discussion it is obvious that the progress in our understanding of the biology of pollen-pistil interaction and fertilization, although impressive, is far from complete. Detailed investigations, whether structural, physiological, or biochemical, have so far been confined only to a few selected taxa. In addition to the need for intensification of studies on the established taxa, it is important to extend them to other systems.

Much more attention must be paid to studying pollen and stigma, as their interaction during initial stages of pollination determines the subsequent events in the pistil. Localization of pollen-wall proteins and stigma-surface proteins, and their implication in pollen recognition, have been important achievements in recent years. Although the details of the origin of pollen-wall proteins, and their incorporation, have been followed in a few systems, the details of the stigma-surface proteins, particularly in dry types, are yet to be investigated. Attempts must also be made to characterize the heterogenous components of stigmatic surface and pollen wall, and in identifying the role of individual components in pollen physiology, stigma receptivity, pollen recognition, and eventual acceptance or rejection of the pollen tube. The investigations of Knox and his associates on *Gladiolus* are significant in this direction.

Extensive physiology and biochemical studies have been carried out on the growth of pollen tube in the pistil. Yet, |our knowledge of many of the important aspects of pollen tube growth is totally inadequate. Some of these aspects are: the nature and origin of chemotropic substance, the nature of the stimulus which precedes the growing pollen tubes, and the mechanism of the release of male gametes and of fertilization. Our knowledge of details of interspecific incompatibility is very inadequate, and extensive studies need to be conducted.

Recent investigations on various aspects of self-incompatibility have been rewarding. Most of the studies are confined only to homomorphic systems, largely to members of Cruciferae, Compositae, Liliaceae, and Solanaceae. The evidences in implicating exine proteins in controlling sporophytic incompatibility are more direct and conclusive, but implication of intine proteins in gametophytic incompatibility needs conclusive evidences. There |s an urgent need to extend these studies to other taxa, including heteromorphic systems. The ultimate aim of these investigations would be to isolate and characterize the proteins involved in pollen recognition and rejection.

There has not been much progress in understanding the mechanism of incompatibility. Many hypotheses have been put forward to explain the gametophytic incompatibility, on the basis of interaction of identical pollen and pistil proteins. However, the similarity of S-specific proteins from the pollen and pistil has been immunologically shown only in *Petunia*. Further immunological and electrophoretic investigations, similar to those carried out on *Brassica* by Nasrallah and his associates, should be conducted on other systems.

The efficacy of a large number of techniques has been demonstrated to overcome intraspecific incompatibility. Often, more than one technique works in a given system. It is, therefore, necessary to select the best technique suitable for the particular requirement. Attempts to devise and/or standardize simpler and more effective techniques should be continued. The techniques of recognition-pollen and in vitro fertilization have great potential not only in overcoming self-incompatibility, but also in achieving interspecific and intergeneric crosses. Besides its practical application, the technique of in vitro pollination and fertilization offers great advantages in basic studies concerned with pollen-pistil interaction and fertilization. This is because of its suitability in controlling the environmental factors, and in carrying out experimental treatments.

Studies on the receptive surface of stigma and the path of pollen tubes in the pistil have been extended to many other systems. Apart from esterases, acid phosphatases are also present on both dry (Ghosh and Shivanna 1980 a) and wet stigma (Herrero and Dickinson 1979). Electron microscopic evidence indicates that the exudate is secreted by ER (Dumas at al. 1978); golgi vesicles do not seem to have any role (Kristen et al. 1979). Even in taxa characterized by wet stigma, the younger stigmas

are comparable to the dry stigma, with a cuticle-pellicle layer (Shivanna and Sastri 1981). In solid-styled systems such as *Petunia* and *Nicotiana*, the pellicle is disrupted during the secretior of exudate. In hollow-styled systems such as *Amaryllis* and *Crinum*, the exudate is secreted by the tissues of stylar canal, and the pellicle-cuticle layer is not disrupted (Shivanna and Sastri 1981). The stigma of marine angiosperms *(Enhalus, Halophila* and *Thalassia)* is dry with a pellicle-cuticle layer, and is comparable to tha: in terrestrial plants (Pettitt 1980). In watermelon (Sedgley and Scholefield 1980) and *Acacia* (Kenrick and Knox 1981) pollination stimulates a second phase of stigmatic secretion which results in accumulation of copious exudate on the stigma.

The structure of transmitting tissue has been investigated in *Persea* (Sedgley and Buttrose 1978), *Vitis* (Considine and Knox 1979), *Petunia* (Herrero and Dickinson 1979), *Actinidia* (Hopping and Jerram 1979), members of Gramineae (J. Heslop-Harrison 1979 a, J. Heslop-Harrison and Y. Heslop-Harrson 1980), and *Primula* (Y. Heslop-Harrison et al. 1981). Besides the presence of extracellular proteins on the surface of stigma, proteins are also present in intercellular spaces of the transmitting tissue (Herrero and Dickinson1979, J. Heslop-Harrison and Y. Heslop Harrison 1980, Y. Heslop-Harrison et al. 1981). Considerable significance is attached to the presence of extracellular proteins on the stigma, and in the path of the pollen tube, because of the possibility of its involvement in pollen recognition and incompatibility responses.

Investigations on pollen-pistil interaction highlight the importance of pollen adhesion and pollen hydration (J. Heslop-Harrison 1979 b, Clarke et al. 1979, Stead et al. 1980, Woittiez and Willemse 1979). Adhesion of pollen is largely determined by the extent of wetness of the stigma, and on the sculpture of pollen wall (Woittiez and Willemse 1979). Wet stigma supports adhesion of both powdery and sticky pollen. This is largely mechanical, and does not seem to involve any specificity. The adhesion of pollen on dry stigma is more critical, and depends on the extent and composition of the pellicle and amount of surface-coat substances on pollen. Often the adhesion may involve morphological complementation between the pollen and stigma and, in many species, acts as a barrier for incompatible pollen. A theoretical consideration of pollen hydration on the stigma is provided by J. Heslop-Harrison (1979 b). The rapidity of pollen hydration depends upon the nature of stigma. If the stigma is of dry type,

hydration is gradual, and controlled by the water potential of stigma. When the stigma is covered with an aqueous exudate, the hydration is more rapid.

There are evidences to indicate that, in desiccated pollen, the plasmalemma is in a dissociated condition, but its integrity is restored during hydration (J. Heslop-Harrison 1979b; Shivanna and J. Heslop-Harrison 1981). The role of stigma-exudate in pollen germination varies from species to species (Shivanna and Sastri 1980). In species characterized by solid style, the exudate does not seem to contain factors involved in pollen germination. In species with hollow style, however, the exudate seems to contain pollen germination factors.

Observations on pollen-pistil interaction in a seagrass; *Amphibolis* (where pollination occurs under submerged condition), has revealed many interesting features (Pettitt et al. 1980). Pollen grains, soon after coming in contact with the stigma, are held tenaciously by a meniscus of adhesive material formed from the surface-coatings of both pollen and stigma. The adhesive binding, in contrast to terrestrial plants, is water-proof. In *Amphibolis* the pollen grains are filiform, and lack a preformed aperture. The aperture is formed by the focal autolysis through which the tube emerges. Anderson (1980) reported an unusual phenomenon in some members of Malphighiaceae with produce both chasmogamous and cleistogamous flowers. In cleistogamous flowers pollen grains germinate inside indehiscent anthers, pollen tubes grow through the filament of anther into the receptacle and, eventually, reach the carpel and the ovules. These observations need further study.

There has been significant progress in our knowledge on heteromorphic incompatibility. The presence of extracellular proteins, comparable to homomorphic systems, has been demonstrated in *Linum* (Ghosh and Shivanna 1980 a), and *Primula* (Y. Heslop-Harrison et al. 1981). *In Primula* there is no basic difference in the receptive surface of stigma of the two morphs (Y. Heslop-Harrison et al. 1981); in *Linum* the stigma of long-styled form is dry, that of short-styled form wet (Ghosh and Shivanna 1980 a). Also, the surface proteins of the two morphs of *Linum* show qualitative and quantitative differences which may have a role in incompatibility responses.

Unlike the earlier presumption which implied that incompatibility in heteromorphic systems was controlled by the absence of morphological complementation between

the stigma and pollen, and differences in the osmotic pressure of pollen grains and styles of the two morphs (see Nettancourt 1977) investigations on *Linum* (Ghosh and Shivanna 1980 b) hs.ve demonstrated the operation of physiological mechanisms - similar to those in homomorphic systems - in controlling incompatibility. Reports on *Primula vulgaris* (Shivanna et al. 1981) indicate that inhibition of incompatible pollen takes place at many levels - pollen hydration, germination, pollen tube entry into stigma and its growth through the stigma and style. The macromolecular components in the intercellular spaces of transmitting tissue of pistil appear to be involved in inhibiting the growth of incompatible tubes (Shivanna et al. 1981).

The cytology of pollen-pistil interaction (following compatible and incompatible pollinations) has been followed in several homomorphic taxa: members of Gramineae (Sastri and Shivanna 1979, J. Heslop-Harrison 1979 a, J. Heslop-Har-rison and Y. Heslop-Harrison 1980), *Petunia* (Herrero and Dickinson 1979, 1980 a, b, 1981, Cresti et al. 1979), and members of Commelinaceae (Herd and Beadle 1980, Owens 1981). S-allele specific proteins have been identified in the pistil of *Nicotiana alata,* through isoelectric focusing (Bredemeijer and Blass 1981). Ferrari et al. (1981) isolated and purified S-allele specific glycoprotein in *Brassica oleracea* var. *capitata.* It eluted as a single peak from Sephadex column, appeared as a single band (which stained with coomassie blue and periodic acid Schiff reagent) after polyacrylamide-gel electrophoresis and had protein to carbohydrate ratio of 1:3. In vitro pretreatment of pollen with the glycoprotein obtained from self-stigma prevented pollen germinating even on compatible stigma. Pie-treatment of pollen from the glycoprotein obtained from compatible stigma did not affect germination.

In homomorphic incompatibility also, contrary to the earlier concept which considered self-incompatibility as a one-step reaction, it has been convincingly proved that incompatibility barriers operate at many levels within a system,-par-ticularly in sporophytic systems. In *Brassica* Stead et al. (1979, 1980) and Roberts et al. (1980) reported significant differences in the adhesion and hydration of pollen, and the mobility of pollen wall components following compatible and incompatible pollinations. Earlier data, although[1] not emphasized, had brought out the differences in the proportion of pollen grains germinating on the stigma, and pollen tubes entering the stigma in selfed and crossed pistils. Incompatibility barrier, at least in sporophytic

systems, therefore, operate at all levels of pollen-pistil interaction- pollen adhesion, pollen hydration, pollen germination, pollen tube entry into the stigma, and pollen tube growth through the style. Thus the details shown in Figs 44 and 48 require modifications. The numbers of pollen grains completing successive post-pollination stages become progressively reduced at each level. Such a realization raises serious doubts about the validity of the hypotheses on the operation of incompatibility based on a single mechanism such as the inhibition of protein synthesis in the pollen/pollen tube.

It would be appropriate to consider self-incompatibility as a system of many superimposed mechanisms controlled by different genes. Such a basic system has undergone modifications in a number of taxa due to inactivation of one or more of the mechanisms and, thus, altered the phenotypic manifestations of self-incompatibility. Recent genetic studies also indicate that self-incompatibility controlled by many alleles is more common (than was thought earlier), and covers both gametophytic and sporophytic systems (see Lewis 1979). Our understanding of the operation of intraspecific incompatibility is likely to change radically in the coming years.

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CHAPTER VI

POST POLLINATION EVENTS

Fertilization involves the fusion of a male gamete with a female gamete. In angiosperms the female gametophyte is seated deep in the ovarian cavity, quite away from the stigma. The pollen (male gametophyte) is, normally, held at the stigma, and there is no device for them to reach the egg inside the female gametophyte. To effect fertilization in this group of plants the pollen grains germinate on the stigma by putting forth tubes (pollen tubes) which grow through the style and find their way into the ovules (Fig. 56), where they discharge the sperms in the vicinity of the egg.

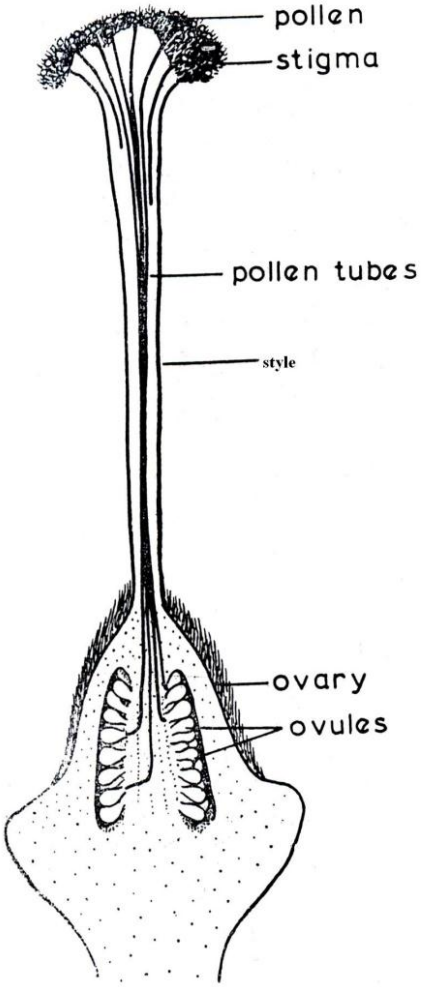


Figure 56. Longitudinal section of a pollinated pistil of Okra. The pollen tubes have grown to various lengths in the style; some have reached the ovules.

One of the sperms fuses with the egg (forming zygote) while the other fuses with the polars or the secondary nucleus (forming primary endosperm nucleus). The distance a pollen tube has to travel in order to reach the egg depends on the length of the style

which is quite variable in different species. For example, in sugar-beet it' has to grow only a few millimetre whereas in corn it grows as much as 450.mm.

POLLEN GERMINATION AND POLLEN TUBE GROWTH

Normally only one tube develops from a pollen grain. In polysiphon-ous grains, however, more than, one tube may emerge from a grain; up to 10 pollen tubes have been observed in *Althaea rosea*, and 14 *in Malva neglecta.* As a rule, only one of them makes further growth. In plants where pollen grains are united into tetrads (orchids) or pollinia (Asclepiadaceae) several pollen tubes are produced at the same time and all of these may grow (Fig. 57). Branching of pollen tubes is quite common in the members of some Amentiferae.

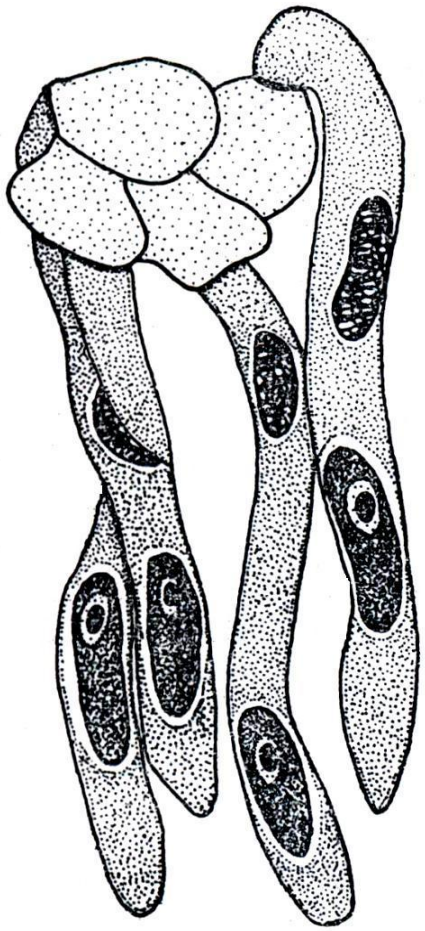


Figure 57. A germinated pollen tetrad of *Cymbidium bicolor.*

Growth and Structure of Pollen Tube

The pollen tubes, as a rule, emerge at the germ pores on the pollen grains. Almost the entire contents of the grain move into the tube. Rapid growth of the tube is restricted

to the tip region. In a growing tube most of the cytoplasm is confined to the apical region, anther large vacuole fills the grain and the older region of the tube (Fig 58 A, B). To restrict the cytoplasm to the apical region of the growing tube, a series of callose plugs are formed at a regular distance behind the tip. As a result a fully grown pollen tube is subdivided into many compartments due to these plugs.

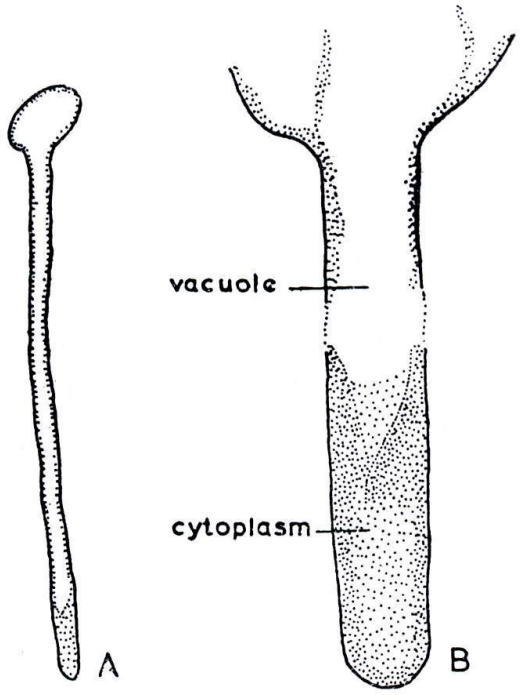


Figure 58. A. A germinated pollen grain to show that in a growing pollen tube the cytoplasm is confined to the apical portion. B. Enlarged apical portion of the pollen tube shown in A.

The plugs originate as a ring on the inner side of the wall. They gradually grow toward the centre reducing the lumen and, finally, sealing the tube (Fig. 59 A-C). The small amount of cytoplasm that is left behind the plug on the side of the grain gradually degenerates. Under a high power light microscope the extreme tip region of the tube appears hemispherical and transparent (Fig. 59A). The zone behind it looks granular. The transparent apical zone is called "cap block". It exists only as long as the tube is growing and disappears when the growth ceases (Fig. 60). The cytoplasm behind the cap block is rich in the usual cell organelles, namely, mitochondria, golgi bodies, rough and smooth endoplasmic reticulum, vesicles, amyloplasts, and lipid bodies. In the cap block region the aforementioned structures are absent, with the exception of vesicles which are present in abundance. These vesicles are rich in

polysaccharides or RNA, and are associated with wall formation. Un germinated pollen grains contain free ribosomes. Polysome assembly begins immediately after water uptake. Protein synthesis starts as soon as polysomes appear. Some enzymes needed for pollen germination and pollen tube growth are produced at this time. Once the pollen tube is initiated polysomes break-down.

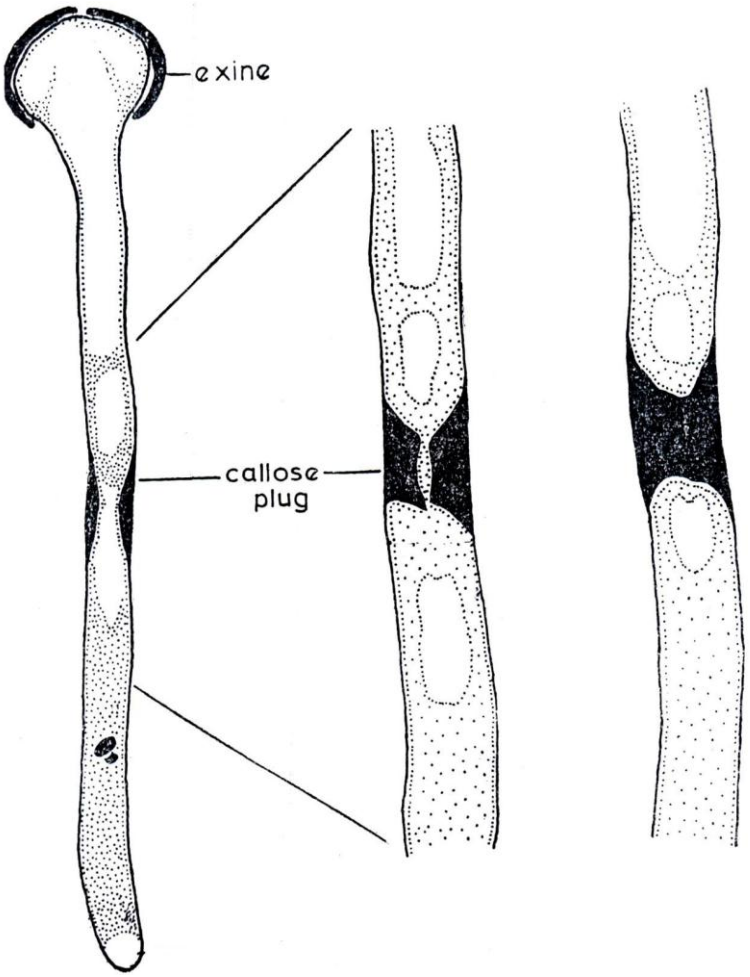


Figure 59. A-C. Stages in callose plug formation in a growing pollen tube. A. Initiation. B. Intermediate stage. C. Lumen of the tube is completely sealed.

Pollen tube wall

It is made up cellulose and pectin. Wall at the cap block region is very rich in pectic content which gradually decreases toward the pollen grain. The cellulose microfibrils are arranged at random at the tip whereas in the older regions the microfibrils are oriented in two directions, both at angles of approximately 45 degrees to the main axis of the tube. The pollen tube also synthesizes callose which is deposited on the wall behind the growing region. Only when the tube stops growing does the callose get

deposited at the tip. A large part of information on the factors influencing pollen germination and pollen tube growth has been collected through the culture of pollen grains in nutrient medium. In cultures it is possible to handle the pollen under controlled conditions and check their response to various substances. We shall, therefore, deal first with-pollen germination and pollen tube growth in nutrient medium *(in vitro)* and then in the stigma, style and ovary *(in vivo).*

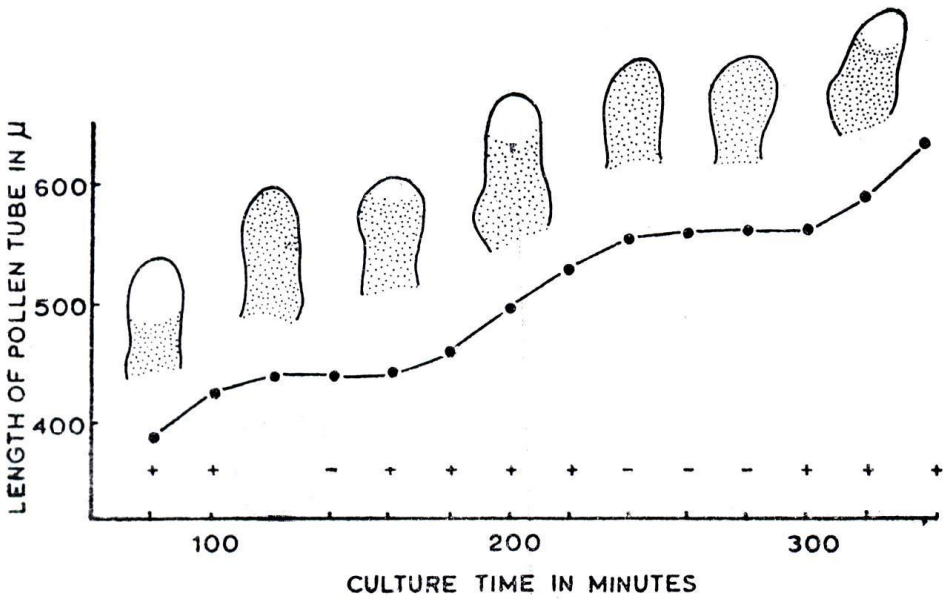


Figure 60. The relationship between pollen tube elongation and the appearance of "cap block" in *Lilium longiflorum.* When the cap block disappears (indicated by—) the tube ceases to grow, and when the cap block re-appears (indicated by +) the tube starts growing,

In vitro

Pollen grains are resting plant organs. Uptake of water leads to swelling of the grains and their activation. Therefore, high relative humidity (RH) is the first essential requirement for pollen germination, whether *in vitro* or *in vivo.* Pollen of some plants germinates readily in saturated atmosphere. Other factors which have been found important for pollen germination and pollen tube growth are as follows.

1. Carbohydrates

For germinating pollen grains in nutrient medium a sugar is always necessary. Two roles have been ascribed to sugars: *(a)* control of osmotic pressure, and *(b)* to serve as the respiratory substrate. Pollen of many species would burst when placed in water.

Addition of a definite amount of sugar limits the diffusion rate of water into the pollen and thus, prevents pollen tube bursting. Of the many sugars tested for their ability to promote pollen germination and growth. Sucrose (the sugar present in most pollen) is most effective. According to Iwanami (1959) the time taken between imbibition and the beginning of tube initiation depends on the kind of sugar present in the grain at the time of shedding. The pollen grains of *Impatiens balsami* contain mainly glucose and require only 2-3 minutes for germination whereas *Li Hum* pollen have largely sucrose and need 30-40 minutes for germination.

2. Boron

Among inorganic substances, boron, in the form of boric acid or borate, has most dramatic effect on pollen germination and pollen tube growth. Pollen of most species is deficient in boron content. In nature this deficiency is made up by comparatively high levels of boron in the stigma and style. When such pollen grains are grown *in vitro*, high amounts of boron (10-200 ppm) are supplied exogenously. Boron reduces bursting of pollen tubes as well as enhances percentage germination and pollen tube growth. Some of the roles attributed to boron are.

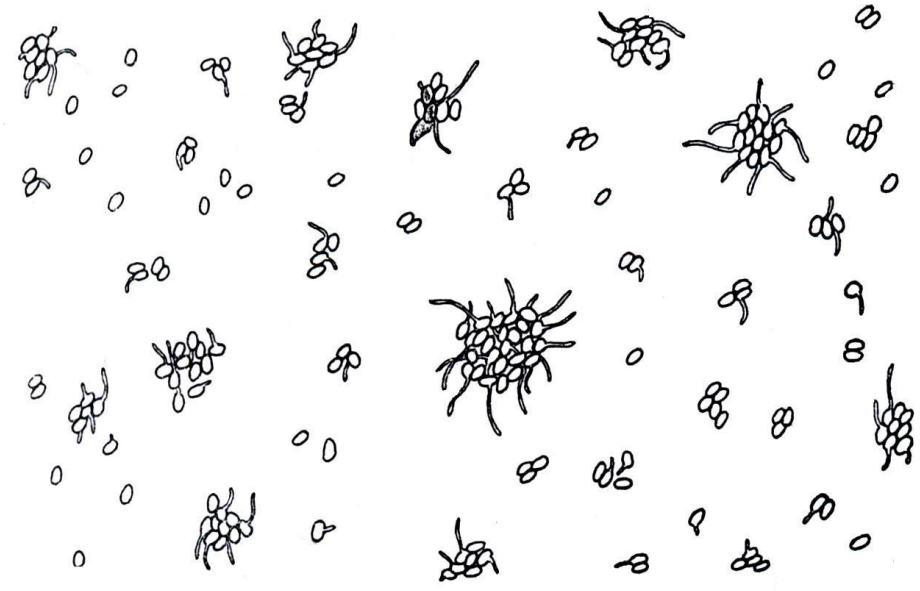


Figure 61. Percentage pollen germination and the growth rate of pollen tube is better when a large number of pollen grains are grouped together as compared to when they are planted singly on the nutrient medium.

- Effect on water relationship and, thus, preventing pollen tube bursting.
- Translocation of sugars.

- Direct or indirect influence on enzymatic steps in the biosynthesis of carbohydrates.

Table 3. Composition of Nutrient Medium for Germinating Pollen Grains.

Constituents	Amount/litre
Sucrose	100 g
H_3BO_3	100 mg
Ca $(NO_3)_2$. H_2O	300 mg
$MgSO_4$. $7H_2O$	200 mg
KNO_3	100 mg

3. Calcium

The percentage of pollen germination and pollen tube growth is far better when a large population of grains is grown as compared to when they are placed separately on the medium (Fig. 61).mThis observation led to the recognition of what has been called "population effect", "mutual effect", or "crowding effect". The population effect is now known to be brought about by Ca^{++} ions. On calcium-supplemented medium following features are noticed.

- The growth of pollen tube is more vigorous.
- Pollen tubes are more straight and rigid.
- Pollen as well as pollen tubes are less sensitive to minor changes in the medium.
- Permeability is controlled. Omitting calcium ions from the germination medium leads to an increase in the permeability of pollen tube membranes and causes the loss of internal metabolites.

Calcium also antagonises the inhibitory effects of certain heavy metals. Pollen grains contain very small amount of calcium. In aqueous medium calcium diffuses out rapidly leaving a low amount of it in the pollen grains which is insufficient for pollen germination. When pollen grains are present in large groups on the surface of semi-solid medium the diffused out Ca^{++}may be trapped in between the pollen grains and, thus, bring about the population effect. The effect of calcium is dependent on the presence of a suitable osmotic milieu, oxygen, and "borate. It is enhanced by a methyl donor, such as methionine and other inorganic cations, especially Mg^{++}, KC, Na^{+}, and H^{+}.

4. Enzymes

Cellulase, pectinase and callase are present in pollen grains. Cellulase and pectinase are released immediately after the pollen is placed in the germination medium. When supplied exogenously, these enzymes increase the rate of tube elongation. Cellulase and pectinase are assumed to affect tube elongation through an increase in the plasticity of the tip region.

5. Plant hormones

Promotion of pollen tube growth by auxins and gibberellins has been recorded but the effect is not appreciable.

6. Physical factors

Among the physical factors affecting the pollen tube growth, temperature is the most important. The growth rate is appreciably enhanced with an increase in temperature. An optimum range of temperature is 20-30°C. However, in some incompatible self-pollinations high temperatures have been reported to retard pollen tube growth.

A simple method to demonstrate pollen germination is to grow them in a hanging drop. It requires a cavity-slide and a coverglass. Take a clean and dry coverglass and in the middle of it place a small drop of nutrient medium (for composition *see* Table 3). Sprinkle pollen grains on the medium directly by shaking the dehisced anthers or transfer them with the help of a camel, hair brush. On the slide apply vaseline around the rim of the cavity. Lift the coverglass with a pair of forceps and turn it upside down. Place it on he slide in a manner that the culture drop hangs right above the middle of the cavity. Care must be taken that the drop does not touch the cavity wall.

When the objective is to study and compare the effects of some substances on percentage germination and pollen tube growth the method described above is not satisfactory. This is because the distribution of pollen grains in the medium is not uniform. Consequently, the results are also not uniform (for explanation *see* population effect on page 107). In such a situation the .method described by Iwanami (1959) may be used: In this method semi-solid medium is employed (prepared by adding about 1 % agar to the liquid medium) instead of liquid medium. The medium containing agar is heated to dissolve the latter and poured on a slide or in a petriplate. On cooling a thin agar-nutrient-plate will be formed. Now spread pollen on a

coverglass as evenly as possible. Scrape with the edge of another coverglass the pollen spread out and touch it on the surface of the medium. The pollen grains thus placed on the medium are in a perfect straight row. Pollen sown in this manner will show comparatively more uniform results with regard to percentage pollen germination and pollen tube growth.

In vivo

Stigma possesses a specialized surface for catching and holding the pollen. The main function of the stigma is to provide the pollen with water necessary for their germination. In many plants it also supplies the necessary medium for pollen germination in the form of exudates. The chief components of the exudates are of lipid and phenolic nature. In addition, small amounts of free sugars, amino acids, proteins, and peptides are also present. The composition of the exudates may vary from species to species. The stigmas which secrete exudates are called wet stigmas *(Petunia)* and those which do not are called dry stigmas (cotton).

In certain plants, such as crucifers *(Diplotaxis tenuifolia)* the stigma is covered with cuticle. In such cases pollen grains degrade the cutin enzyma-tically in order to obtain water necessary for germination. Pollen grains contain an elaborate set of enzymes. Some of these are present in the wall and are available as soon as the pollen grain makes contact with the stigma.

Path of pollen tube

After pollen germination, the pollen tubes grow on the surface of the stigmatic papillae *(Gossypium)* or through the cellulose-pectic layer of their walls *(Lilium).* Upon reaching the base of the papillae the tube grow in the intercellular spaces of the stigmatic tissue. The subsequent course of the tubes depends on the nature of the style. Styles are chiefly of two types, viz. solid and hollow. *Gossypium hirsutum* and *Petunia hybrida* the style is solid. Here the pollen tubes grow by making a pathway through the pectin-rich wall layer of the special, cells constituting the "conducting tissue" or "transmitting tissue". The cell walls in this tissue are exceptionally thick (Fig. 62).

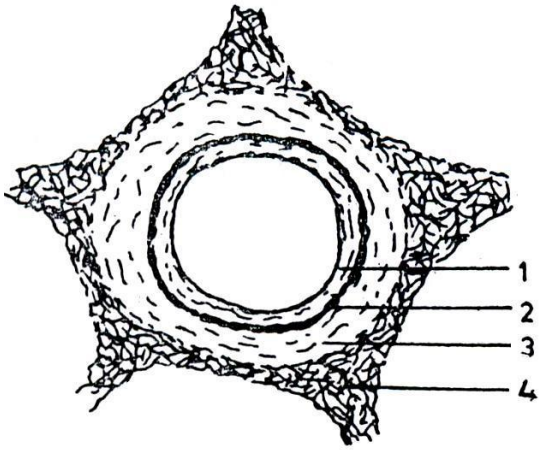


Figure 62. The composition of the cell wall layers of the transmitting tissue in the solid style of cotton. The pollen tube grows through layer 3 which is very rich in pectic material.

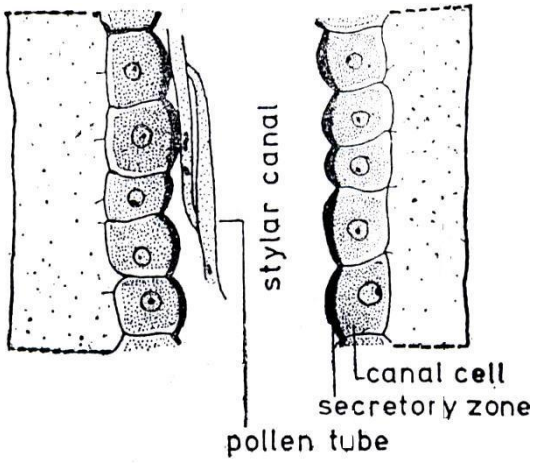


Figure 63. A portion of hollow style in longisection. Note the large cells lining the stylar canal.

In *Lilium*, on the other hand, the style is hollow and the pollen tubes creep on the surface of Special cells lining the stylar canal (Fig. 63). These cells are called "canal cells". The canal cells are secretory in nature. The most striking structural feature of the canal cells is the presence of an 8-14 µm thick, domed 'secre tory zone" on the side facing the canal (Fig. 63). The secretory zone consists of three regions (Fig. 64).

1. An outer 1 u.m thick wall layer (L_1). It chiefly consists of cellulose fibrils.
2. 7-13 *µm* thick granular-fibrillar wall layer (L_2) which extends as irregular projections into the adjacent cytoplasm. The cellulose microfibrils in this layer are randomly dispersed and are continuous with the cellulose fibrils in the L_1. The granules in this region are pectic in nature, probably complexed with proteins.

3. An irregular interphase between L_2 and the adjacent cytoplasm. It contains aggregates of tubules and vesicles. The aggregations have been termed Paramular bodies.

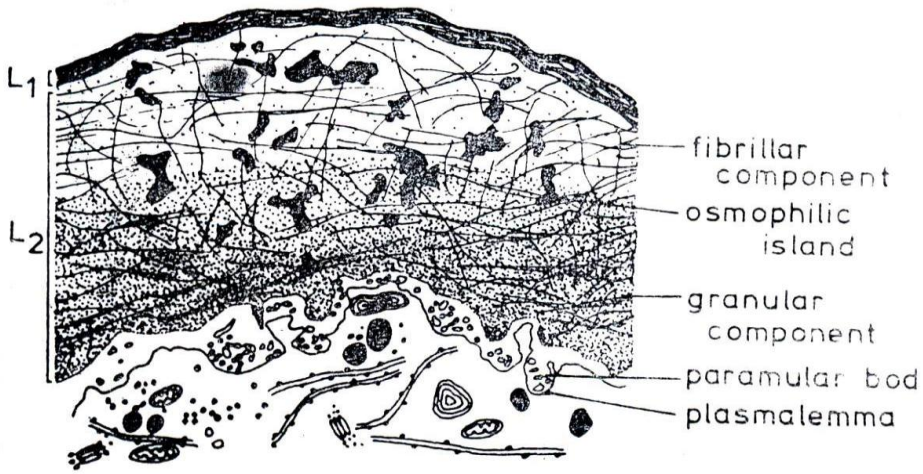


Figure 64. Diagrammatic representation of the secretory zone and adjacent cytoplasm of a typical canal cell of *Lilium*.

It has been suggested that in *Lilium* the parenchyma cells Undernet canal cells synthesize some mucilaginous substances and transport then the latter (canal cells) which, in turn, secrete them into the stylar. After pollination the canal is filled with the secretion which serves as nutrition for the growing pollen tubes. Rosen and Thomas (1970) have show in lily the stigmatic cells and canal cells are functionally alike (secretory) but a secretory zone is not found in the stigmatic papillae.

Figure 65. Surface test to demonstrate the chemotropic attraction for pollen tubes by pistil tissue; all pollen tubes are growing toward the pistil tissue.

With regard to the fact that the pollen tubes always grow in the direction of the ovary, Strasburger (1887) stated that the path of pollen tube in the pistil is guided by a secretion of the ovule. Subsequently, it was demonstrated that the ovules, placenta, the inner epidermis of the ovary and the stigma attract pollen tubes (Fig. 65). Moreover, if a slice of the pistil is placed on the medium and later' removed the tubes grow toward the spot where it was lying. Schildknecht and Benoni (1963) suggested that amino acid and amino mixture coupled with sugars is responsible for the chemotropic attraction of pollen tubes in *Oenothera* and *Narcissus.* Rosen (1964) and Mascarenhas and Machlis (1962). However, did not observe such a response with Lilium and *Antirrhinum,* respectively. In 1962, Masearanhas and Michalius suggested that Ca^{++} ions are the naturally occurring chemotropic agent in the pistils of *Antirrhinum majus.*

They also suggested that calcium-controlled unidirectional growth of pollen tubes in the pistil may be of universal occurrence. However, the distribution of Ca^{++} ions in the pistil of *A. majus,* as measured cytochemically, docs not correlate well with the, distribution of chemotropic activity (Mascarenhas, 1966). The concentration of Ca^{++} ions throughout the length of the style is very low and almost constant. It is slightly higher in the stigma and the ovary. In the ovary the concentration of Ca^{++} is extremely high in the placenta and the ovary wall but the ovules have comparatively, low concentration and there is no increase in it in the micropyle or the embryo sac. A similar distribution of calcium ions occurs in the pistil of *Oenothera* (Glenk *et al.* 1967). This anatomical distribution of calcium ions in the pistil would not be expected if increasing Ca^{++} gradient was necessary for directing pollen tube growth from stigma to ovules (Mascarenhas, 1975).

Moreover, Ca^{++} is chemotropically inactive with the pollen tubes of lily (Rosen, 1964); corn (Cook and Walden. 1967), *Clivia* and *Crinuin* (Kwack, 1969). These two observations argue against the suggestion that Ca^{++} alone controls the unidirectional growth of the pollen tubes in the pistil. Mascarenhas (1975) has proposed an altogether new hypothesis for the mechanism controlling unidirectional growth of pollen, tubes in the style. According to this theory for straight growth of pollen tube a gradient of chemotropic substance/s is not necessary. It would only require the tropic factor to be present along the path of pollen tube at a; concentration above a certain threshold value. A steep gradient of the: tropic factor is necessary only in localized

regions of the pistil where pollen tube has to take a sharp turn, e.g., at the placenta for the pollen tube to turn towards the ovule.

This hypothesis of Mascarenhas is supported by early work of Iwanami (1959), which clearly demonstrates the lack of a gradient of chemotropic factor in the pistils of lily; Iwanami took different segments of the style and stigma and studied the direction of pollen tube growth. If the pistil was placed horizontally and pollen grains germinated on the inner surface of the style by making a hole in it about 55 per cent pollen tubes grew towards the ovary while the remaining 45 per cent tubes grew towards the stigma. Similarly, if the part of the style was cut off and placed upside down and pollen germinated at the top almost all pollen tubes grew downwards. These observations clearly suggest the lack of a gradient of chemotropic factor within the style.

Entry of pollen tube into the ovule

After arriving in the ovary pollen tube finds its way into an ovule. Depending on the place of pollen tube entry into the ovule, fertilization is of three types (Fig. 66).

1. ***Porogamy:*** In this type, which is most common, the pollen tube enters through the micropyle.
2. ***Chalazogamy:*** This refers to a situation where pollen tube enters the ovule at the chalazal end. This type is found in Causurina.
3. ***Mesogamy:*** In this the entry of pollen tube into the ovule is through the funiculus *(Pistacia)* or through the integuments *(Cucurbitd).*

With respect to porogamy it has been suggested by some workers that the entry of pollen tube into the ovule and its subsequent growth toward the embryo sac is regulated by a chemotropic substance secreted by the filiform apparatus into the micropyle. The following points have been raised against the suggestion of filiform apparatus as the source of the stimulus.

- Pollen tubes may even enter such ovules which abort before the formation of the embryo sac.
- Pollen tubes may enter such ovules in which embryo sac has been fertilized.
- Pollen tubes may also enter such ovules in which embryo sac lacks a synergid *(Plumbago).* With regard to this point it may be mentioned that in *Plumbago* a

filiform apparatus-like structure is present in the egg.

The idea of a chemotropic guidance of pollen tube growth toward the embryo sac is also supported by Rosen (1965). However, he holds the view that the substance is secreted by the micropyle itself rather than the synergid. Chao (1972) has carried out a detailed cytological investigation on *Paspalum orbiculare* and demonstrated that the distal part of the integuments, by dissolution of its cells *in situ*, secretes the mucilaginous substance into the micropyle which provides a way of least resistance for the pollen tube and guides it toward its ultimate destination. The mucilaginous secretion is largely water soluble carbohydrate and it aids the pollen tube growth both mechanically and chemotropically.

A special structure which facilitates the entry of pollen tube into the ovule is the obturator. It forms a sort of bridge for the pollen tube to reach the ovule. After fertilization the obturator shrinks and disappears. Unfortunately, there is no explanation available for the mechanism of pollen tube entry into the ovules showing chalazogamy or misogamy. In Loranthaceae there is no structure like an ovule. Here the embryo sacs undergo remarkable elongation and meet the pollen tubes at some point in the stylarcanal.

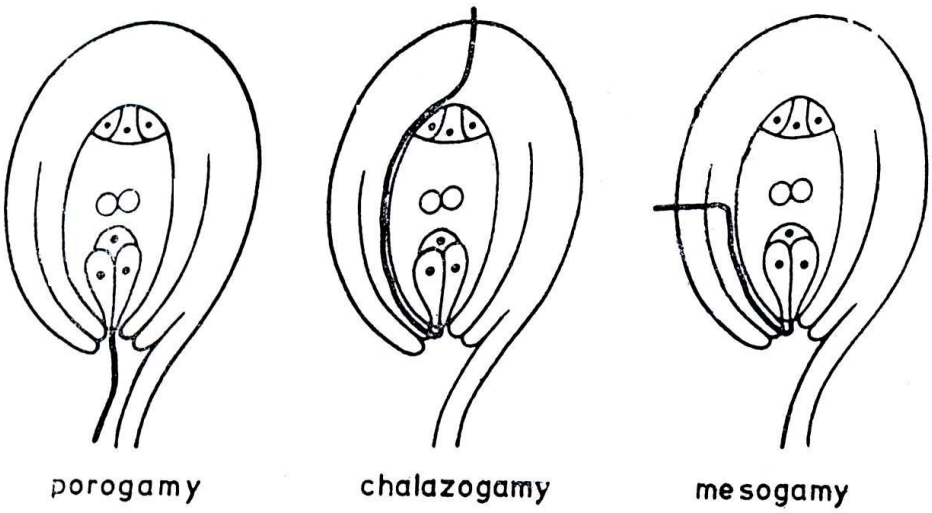


Figure 66. Modes of pollen tube entry into the ovule.

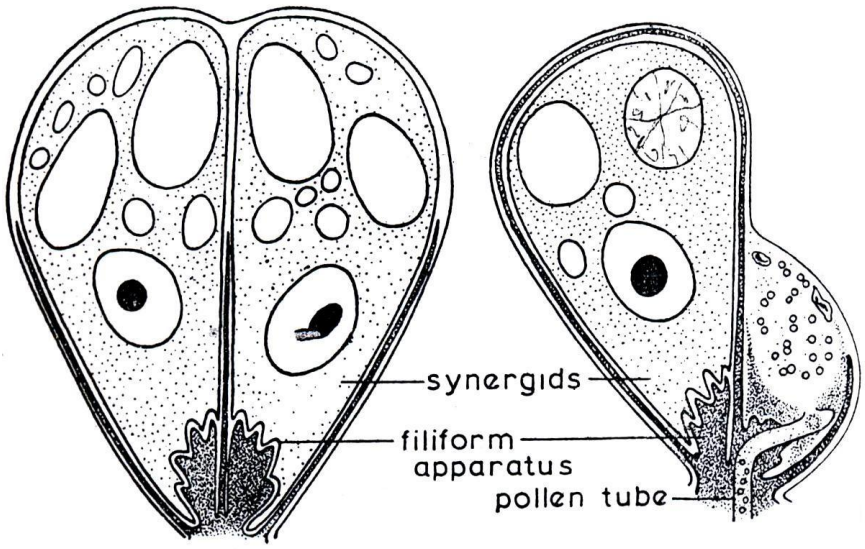


Figure 67. Diagrammatic summary of the changes in the synergids after pollen tube discharge. *A.* Synergids in a pollinated flower. *B.* Synergids after pollen tube discharge.

Entry of the pollen tube into the embryo sac

Irrespective of the place of entry of pollen tube into the ovule, it invariably enters the embryo sac at the micropylar end (Fig. 66). Three modes of pollen tube entry into the embryo sac have been described in the light microscopic studies:

- Between the egg and one of the synergids,
- Between the wall of the embryo sac and one of the synergids, or
- Directly into one of the synergids.

In the recent electron microscopic studies only one uniform picture has emerged. The tube enters at the apex of the filiform apparatus and after growing through it arrives in the cytoplasm of the synergid (Fig. 67). Which one of the two synergids a pollen tube would enter seems to be predetermined. Mostly the penetrated synergid starts degenerating before the arrival of the pollen tube, but after pollination. However, in *Petunia* the synergid visited by pollen tube does not show any visible change until the arrival of the tube. The contents of pollen tube are discharged in the synergid, and the tube does not grow beyond it in the embryo sac. The process of discharge takes place in seconds. In cotton the contents of the tube are discharged through a subterminal pore which is invariably on the Side facing the chalaza (Fig. 67-69). In *Epidendrum, however,* the pore described as terminal.

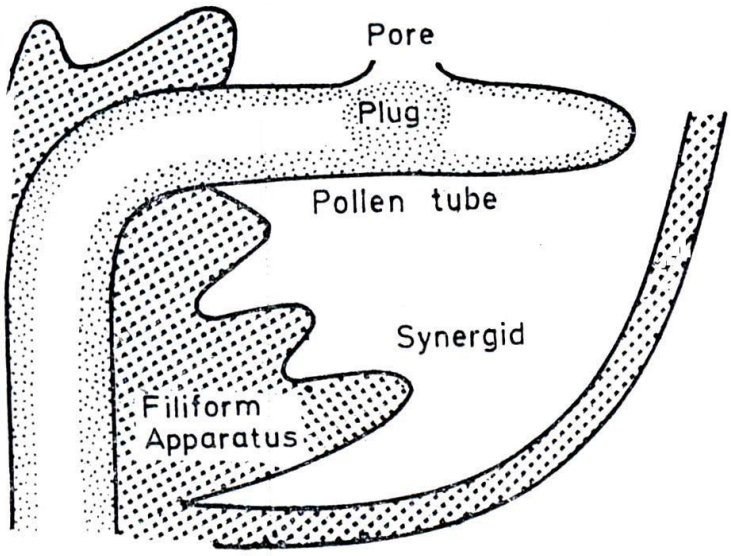


Figure 68. A ortion of the penetrated synergid in figure 67 enlarged to show the subterminal pore on the pollen tube.

The pollen tube discharge

It includes two sperms, the vegetative nucleus, and a fair amount of cytoplasm. A portion of the cytoplasm is retained in the pollen tube where it rapidly degenerates. The cytoplasms released by the pollen tube and that of the synergid hardly show any mixing. They remain as two separate entities. The synergid cytoplasm is confined to the micropylar end and that of pollen tube is restricted to the chalazal end of the cell. The latter can be easily recognised by the presence of thousands of tiny polysaccharide spheres (0.5-1.0 µm).

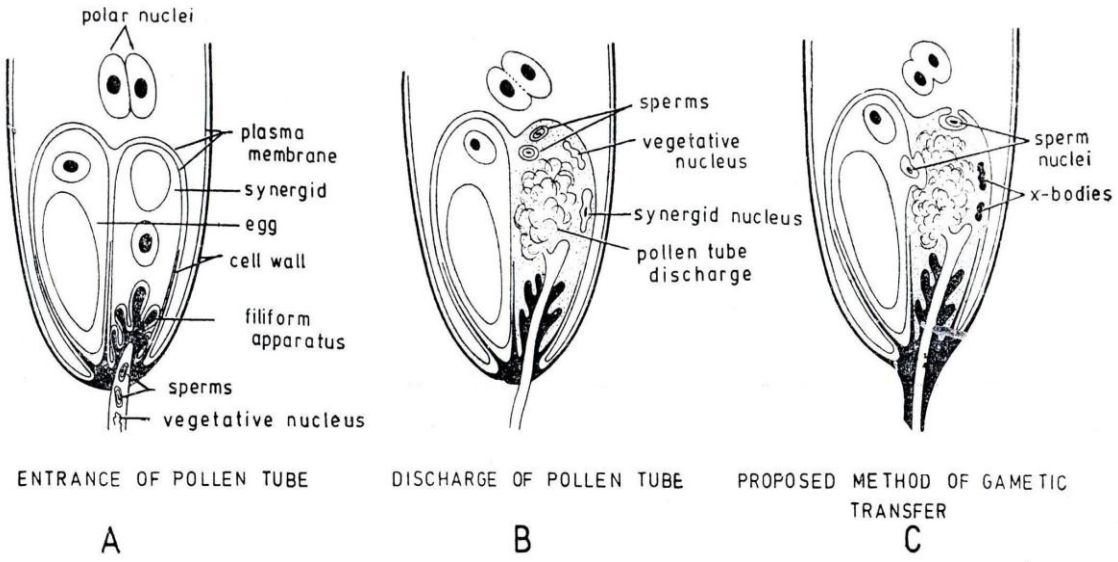


Figure 69. Summary diagram to show sperm transfer in the embryo sac.

Sperms

The two sperms in a pollen tube often change their shapes. They are true cells, each bound by a plasma membrane. In the tube the two sperms are usually placed close to each other. Living sperms discharged from the ruptured pollen or pollen tube of barley has been shown to remain in contact for considerable period. However, no explanation has been offered for this sperm adherence. The sperm cytoplasm contains the usual cell organelles. Microtubules have been suggested to be associated with the motility of the sperms. The nucleus contains a distinct nucleolus.

Post-pollination changes in the embryo sac

As mentioned earlier, the synergid which is visited by the pollen tube degenerates faster than the other. The former is called degenerating synergid and the latter is known as the persistent synergid. Some of the characteristic features of the de generated synergid are.

- The large chalazal vacuole disappears.
- The nucleus and the nucleolus are somewhat flattened, and the nuclear membrane disappears.
- The organelles get disorganised.
- Crystals appear in the cytoplasm.

X-bodies

For long the embryologists have observed two darkly staining oval bodies in the synergid which has received the pollen tube discharge (Fig. 69 C). Since the nature of these bodies was not clear they were denoted as X-bodies. From time to time these X-bodies have been variously interpreted as remains of vegetative nucleus, remains of synergid nucleus, cytoplasm of the sperms, adjacent nucellar cells, remain of degenerated megaspores, etc. Based on the shape and their distribution in the synergid, and the fact that they contain DNA, Jeffsen (1972) has interpreted one of them as the remains of synergid nucleus and the other as the remains of vegetative nucleus (Fig. 69 B, C).

DOUBLE FERTILIZATION

S.G. Nawaschin (1898) was the first to show that both the sperms released 6y a pollen tube are involved in fertilization. They fertilize two different elements of the embryo

sac. The phenomenon is unique to angiosperms and is called double fertilization. The nucleus of one of the sperms fuses with the egg nucleus (syngamy) and that of the other migrates to the central cell where it fuses with the polars or their fusion product, the secondary nucleus. Most of the plants have two polar nuclei. Consequently, the second fertilization involves the fusion of three nuclei. This phenomenon is called, triple fusion. However, in some plants there is just one polar nucleus, and there are some others which have more than two polar nuclei.

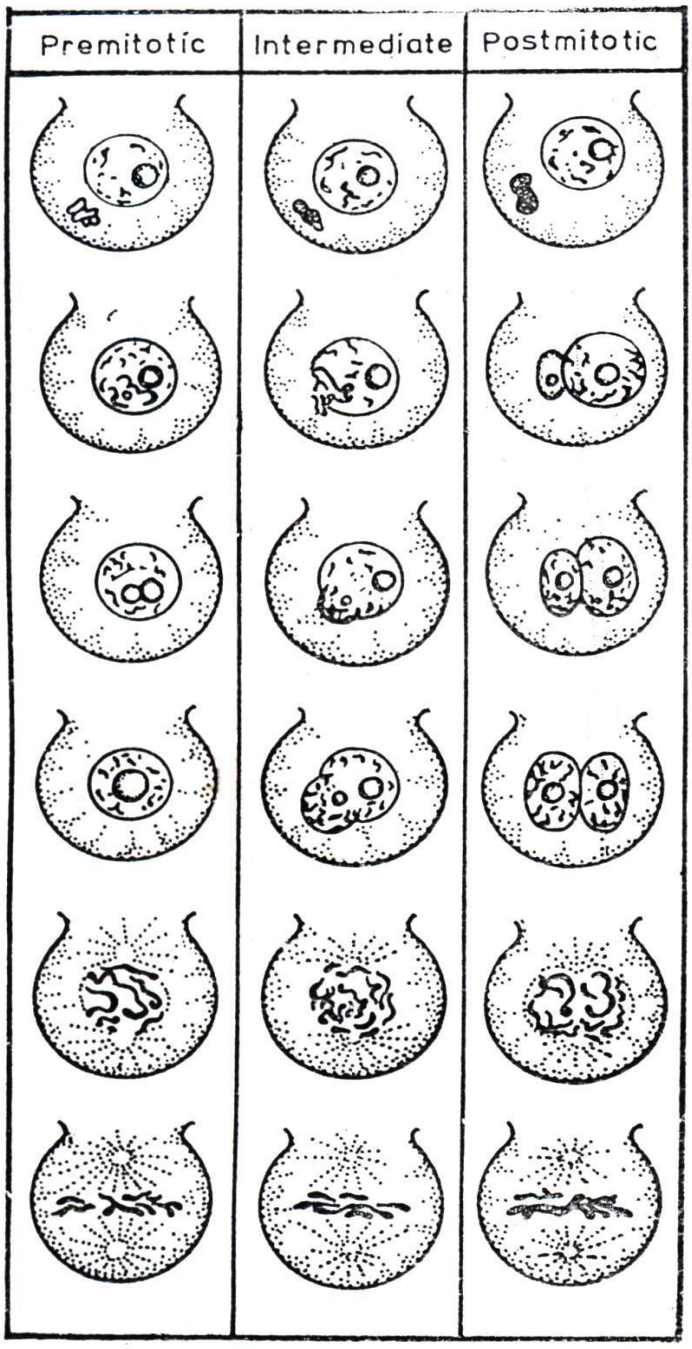


Figure 70. Types of Syngamy.

Passage of the sperms

The sperms are released in the synergid as intact cells but only their nuclei migrate out of it. The mode of sperm transfer is a matter of speculation at present. The latest view held by Jensen (1973) states that one of the sperms comes in contact with the plasma membrane of the egg cell while the other contacts the plasma membrane of the central cell. The membranes at the points of contact dissolve and the sperm nuclei are released, one in the egg, and the other in the central cell (Fig. 69 A-C). After entering their destined cells the male nuclei are passively carried along the cytoplasmic stream to the egg nucleus or to the polars as the case may be. The male nucleus reaches the egg nucleus before the other male nucleus reaches the polars. However, the fusion of the egg nucleus and the sperm nucleus takes much longer than the fusion of the male nucleus and the polars. The reason for this is the active state of central cell as compar-ed to that of egg which is inert (Jensen and Fisher, 1967).

Syngamy

Fusion of the egg nucleus with sperm nucleus is called syngamy. Gerassimova – Navashin (1960) has described three types of syngamy (Fig. 70). According to her at the time when the two nuclei come in contact with each-other the egg nucleus is in a state of deep mitotic rest whereas the male nucleus is at the telophase of the previous mitosis.

Type 1-*Premitotic:* The sperm nucleus fuses immediately on coming in contact with the egg nucleus, and the zygote nucleus divides subsequently; *e.g.* Gramineae, Compositae.

Type *2-Postmitotic:* The sperm nucleus and the egg nucleus remain in contact for a while and fuse only after both the nuclei have entered into divisions (zygotic mitosis); *e.g., Lillian, Fritillaria.*

Type 3. *Intermediate:* The sperm nucleus fuses with the egg nucleus after completing its previous mitosis. Even after the fusion of the nuclear membranes the contents of the two nuclei show incomplete mixing. At the prophase of zygotic mitosis often the two sets of chromosomes can be seen separate; *e.g., Impatiens.*

UNUSUAL FEATURES

Polyspermy

This refers to a situation where more than two sperms ate released in an embryo sac. This may result because of the formation of more than two sperms in a pollen tube or due to the penetration of an embryo sac by more than one pollen tube. Polyspermy occurs only as an abnormality. Normally, an embryo sac receives only two sperms. Polyspermy may bring about fertilization of egg by more than one male nucleus or the supernumerary sperms may fertilize other components of the embryo sac, such as synergids or antipodals. In the embryo sac which receives two or more pollen tubes the sperm nucleus fusing with the egg nucleus may be derived from one pollen tube and the one fusing with the polars may be derived from another (hetero-fertilization).

Persistent and branched pollen tubes: In angiosperms the pollen tube is normally an unbranched structure which collapses soon after fertilization Some reports, however, claim that rarely the pollen tube may persist for as long as three weeks. In *Cucurbita* and some member's of the Onagraceae branching of the terminal portion of the pollen tube has been observed Ramanna and Mutsaerts (1971) reported that in spinach 10-12 percent of the pollen tubes branch in the stylar region and near the micropyle, and the tubes grow like fungal mycelium producing haustoria-like structure. A reinvestigation of pollen tube growth in spinach by Wils (1974) has revealed that branching of the pollen tube occurs mainly in the micropyle and they grow between and around the inner and outer integuments.

According to Wils branching of pollen tubes is probably a post-fertilization phenomenon. Initially, many pollen tubes may enter the nucellus but once a pollen tube has entered the embryo sac further entry of pollen tubes into the nucellus is checked at the micropyle where they branch, Ramanna and Mutsaerts described that the pollen tube branches penetrate into the integuments, nucellus, and other ovular tissues and ascribed them a haustorial function. Wils does not agree with this. According to him the branches of pollen tube do not penetrate into the integument or other tissues. The pollen tube branches simply ramify between the nucellus and the surrounding tissue. He could not detect any haustorial structure. Consequently, the haustorial function of the pollen tubes is doubtful.

CHAPTER VII

FLOWER INDUCTION AND DEVELOPMENT

VEGETATIVE DEVELOPMENT in higher plants depends very much on optimum conditions of light and temperature; morphological development may vary considerably depending on whether plants receive relatively short or long periods of daylight with alternating periods of darkness. Thus vegetative development of the shoot is very much subject to the conditions of the external environment. Even more profound and dramatic are the effects of temperature and photoperiod which convert the plant from vegetative growth the production of stem and leave to reproductive growth with the formation of flowers.

It is an old idea that plants pass through a series of distinct phases or stages in development. The seed germinates, the seedling develops, and then a phase of vegetative development ensues. When a particular stage of vegetative growth is reached, the plant, subject to the conditions of the environment, can become reproductive. Dramatic changes occur in the vegetative apical meristem, cell divisions become rapid and localized, and the meristem elongates or enlarges, forming floral primordia and finally flowers. The initiation of flowering is a profound morphological change, and in many plants it is controlled in a specific and selective way by the environment, particularly temperature and day length.

According to the German physiologist Klebs, the plant must reach a certain stage of vegetative development before it can respond to the environment. Klebs described a stage in vegetative development, which he called "ripeness-to-flower," brought about by low-temperature exposure. Once the "ripeness to-flower" condition has been achieved, which in itself has no morphological manifestation, and then flower initiation and development would ensue.

The Effects of Low Temperature

One of the earliest known and still most striking cases of a low-temperature effect on flowering, first clearly explained by Gassner around 1918, is to be seen in the spring and winter varieties of cereals, such as oats, rye, or wheat. Spring varieties are sown in the spring of the year, bloom in the summer, and are harvested in the autumn. Winter varieties, which differ genetically, are sown in late fall and begin seedling development, overwintering in the soil as young plants. They show rapid vegetative

development in the spring, flower in early summer, and are harvested in late summer. Winter varieties require a period of low temperature in order to flower. If they fail to get low-temperature exposure, they do not flower, or flower only after a prolonged period. One can give winter cereals, which have particularly desirable food qualities, an artificial low-temperature treatment and then handle them as spring varieties. This process is called "vernalization"—that is, inducing spring-like behavior.

Table 4. Vernalization and Devernalization in Spring Rye and the Winter Variety of Petkus Rye.

	Duration of Cold Treatment (5^0C) Followed by Long Days	**Number of Leaves Forms Before Flowering**	**Comments**
Spring rye	None	7	No cold requirement
Winter rye	None	27	
(Petkus)	4 Days	26	
"	4 Weeks	14	
"	14 Weeks	7	Completelyvernalized
"	14 Weeks, followed by 2 days at 40^0C	25	Devernalized

One of the most extensive studies; of temperature control of floral initiation was that carried out by the English workers Gregory and Purvis on the cereal Petkus rye. In order for the winter variety of Petkus rye to flower it must first receive a low-temperature treatment at the seedling stage. Without the cold treatment, Petkus rye will grow and form many leaves but will not flower for a prolonged period of time. The comparison can be made to a related spring rye which grows vegetatively, produces approximately -.seven leaves, and then flowers without any previous cold treatment. Gregory and Purvis found that winter rye would flower progressively sooner in proportion to the length of the period of cold treatment. Experimentally, they were able to establish a scale for effective vernalization (Table 4).

After 14 weeks at 5°C, the fully vernalized seed was still at the early germinating seedling stage, held by the low temperature and reduced water supply. It could be handled as seed and planted in the field. Note that flowering in vernalized winter rye depends, not only on low-temperature treatment, but also on proper day length—that is, flowering required low-temperature treatment followed by long days. Over the period (1934—1955) Gregory and Purvis studied Petkus rye, trying to work out the

mechanisms of the low-temperature effect on subsequent flowering behavior. They found that the whole seed could be vernalized without any externally supplied nutrients. The seeds required water—that is, they had to be partially imbibed and also required a supply of oxygen.

Note in Table 4 that the effect of temperature begins immediately. Continued cold treatment up to 14 weeks caused a progressive increase in response (and decrease in number of leaves formed) until a minimum leaf number (7) before flowering was reached. After 14 weeks of cold treatment, the winter rye was indistinguishable from spring rye in flowering behavior. Successful vernalization could be given to isolated embryos of winter rye after excision from the seed, cultured on a nutrient medium containing a carbohydrate source such as sucrose. Purvis was even able to show that one could excise and culture the isolated shoot apex in a nutrient medium, give it low-temperature treatment, and grow from it a whole plant which was effectively vernalized. Thus the low-temperature effect is perceived in the apical meristem, the site of the cell divisions.

Purvis and Gregory further showed that seed which had been effectively vernalized by cold-temperature treatment could be successfully "devernalized" by exposing the seed for short periods, not in excess of two days, to relatively high temperatures around 40°C (see Table 4). Spring rye or unvernalized winter rye were unaffected by such heat treatment. The effectiveness of the heat treatment depended upon the length of the previous cold treatment, or the "intensity" of vernalization achieved. Usually incomplete devernalization was achieved by heat treatment. After devernalizing, the seed could be treated again by cold and be effectively vernalized. Thus the vernalization was heat-reversible.

All of the temperature treatments discussed above was treatments applied to mature seed which has been soaked and begun germination. In winter rye and other winter cereals, it was possible to show that embryos still developing in the immature seed attached to plants could be effectively vernalized. In the field Gregory and Purvis placed the flowers of intact plants in vacuum bottles with crushed ice for periods up to 24 days, starting at different times after pollination. Later they did similar experiments with refrigerated whole plants. They were able to show that only 5 days after fertilization the young embryo was sensitive to the low-temperature treatment, which

was reflected weeks or months later in the initiation of flowers in the mature plant. Apparently the early stages of active embryo development were especially sensitive to cold treatment.

This observation is a remarkable one. Somehow the cells of the apical meristem of the embryo perceive the low-temperature effect, are in some way changed by it, yet proceed through normal vegetative development and produce normal leaves. Then, induction having been caused by the low-temperature treatment given to the embryo, flower formation occurs from mature vegetative plants. The mechanism of this self-perpetuating change is not understood. It has profound importance for any experimentation on flowering, because the past history of the seed, beginning at its inception in the parent plant, must be controlled. This fact also has its practical importance. Vernalizing temperatures affecting the seed at the time of formation can determine the subsequent flowering behavior; temperature must therefore be taken into account in selecting geographical locations for seed production areas and also presents problems in raising seeds in one latitude to be sown in another, especially if flowering is not desired.

Petkus rye, studied so extensively by Gregory and Purvis, is not the only plant subject to vernalizing temperatures. Many plants require low-temperature exposure in order to flower. The so-called "biennials" develop over two years, usually because of the low-temperature requirement for flower induction. A particular strain of the henbane *Hyoscyamus niger* which has been much studied is a biennial which requires cold treatment before it will flower; otherwise it remains vegetative, even for years. If given cold treatment as a vegetative plant, which it usually would get over winter in northern latitudes, it must then have long days to cause photoinduction. Thus it has two absolute requirements for flowering: (1) low temperature, (2) followed by long days. Flowering behavior in *Hyoscyamus* was studied extensively in Germany by Lang and Melchers. In such biennials, apparently a rosette stage of vegetative development must be reached before the plants are susceptible to low-temperature treatment to induce flowering. Here, as in Petkus rye, the site of the cold response is the apex itself. It was found that one could graft a plant which had been vernalized to a nonvernalized plant and induce the latter to power. Thus it appeared that a substance was produced in vernalised plants which would pass a graft union. The substance was

called "vernalin" and was considered a flowering hormone, or perhaps' a precursor to a flowering hormone.

Lang and Melchars, and later Gregory and Purvis, proposed a scheme which takes into account many of the facts which have been summarized. It outlines possible processes, probably chemical reactions, leading in cold requiring plants (Figure 71). At low temperature goes to B and B can accumulate. At normal temperatures, Process III (destruction of *B)* proceeds much faster than I, and *B* constantly disappears 4nd never accumulates. Thus only at low temperature can *B,* required for flowering, reach a critical amount. High temperature (40°C) either causes *B* to go to *D* or back to *A.* However, *B* can be converted to *C* upon vernalization and, once formed, is not destroyed by high temperature (that is, the plant cannot be devernalized). This product in turn, under long days in the case of Petkus rye and *Hyoscyamus,* is the flowering stimulus or is somehow needed for its production. Vernalin would be compound C in the above scheme. The chemical nature of "vernalin" is not known.

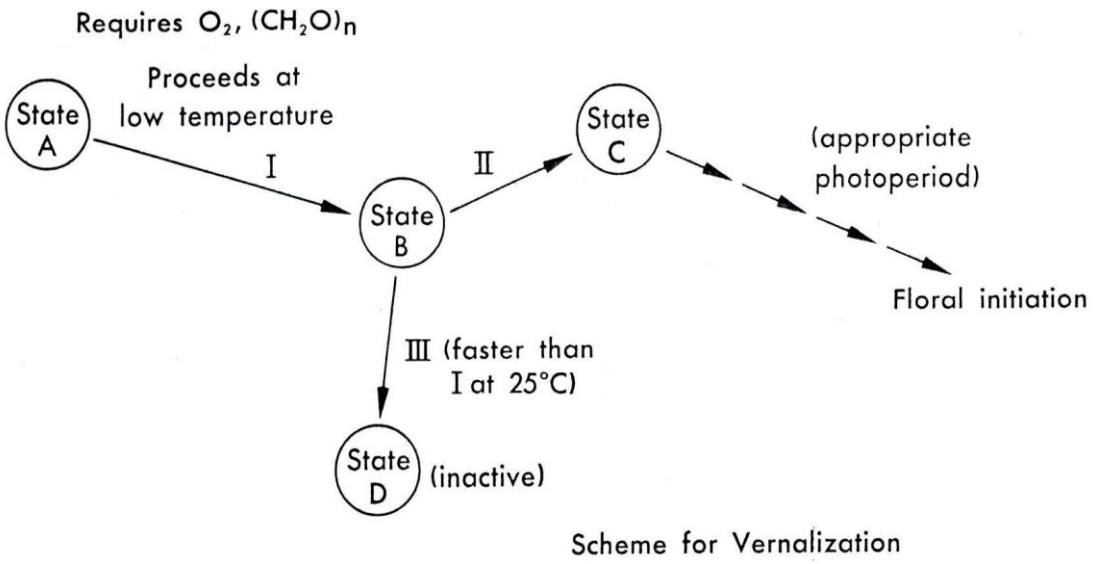


Figure 71. Schematic representation of possible relationships among processes involved in vernalization of flowering plants.

Harada and Nitsch in France isolated a substance from extracts of cold-requiring long-day plants of hollyhocks taken at the time of "bolting" (flower stalk formation). The extracts applied to hollyhocks caused stem elongation without flowering; however, when applied to the long-day plant *Rudbeckia,* the extracts caused flowering in non-inductive conditions. Again the chemical nature of this extract is unknown, although

in its biological properties and the known chemical properties, it seems very similar to a gibberellin.

Most interesting perhaps is the work with gibberellins in these cold-requiring plants. A number of plants which require cold treatment in order to flower, such as the biennials or rosette plants can be made to flower without cold treatment by application of gibberellin. In one species, application of .1 µ g per day for 20 days was fully effective in replacing the cold treatment. In some such plants, a long-day period may still be required for flowering and the gibberellin only replaces the cold-temperature effect leading to stem elongation. In other species, gibberellin replaces both cold treatment and the long-day requirement. Unfortunately, gibberellin has little or no effect when applied to Petkus rye.

The Russian plant physiologist Chailakh van showed that extracts of gibberellins can be made from. Cold-requiring plants that have been vernalized, and that this extracts show higher gibberellin levels than non induced plants. The suggestion follows that perhaps the vernalin postulated by Lang and Melchers is in fact one of the different gibberellins and could be fitted into the scheme as compound *C.* There is some evidence that gibberellin is not itself the flowering hormone vernalin, but in some way, yet to be worked out, it leads to the formation of flowering hormone in cold-requiring long-day plants. It does seem fairly certain that gibberellin is closely involved in the biochemistry of floral initiation in cold-requiring plants.

The Effects of Day Length

In all of our discussions about the effects of low temperature on floral initiation, closely related light effects have been almost inevitably involved, especially the duration of the alternating periods of light and dark. It was not until 1920 that there was any realization of the important role of the length of day and night in floral initiation. Then Garner and Allard at the U.S. Department of Agriculture did the first experiments to make clear this relationship. They had observed that in certain species no matter when seeds were planted in the greenhouse, beginning in late winter or early spring or even into June, plants of different ages all came into flower at the same time. These plants appeared to respond to lengthening days up into early summer. Other plants flowered in the fall in relation to shortening days. They made a careful

study of the requirements shown by different plants for alternating periods of light and dark for flower formation.

They found that many plants require day lengths shorter than a critical length before they would flower. If kept in long days, they would remain vegetative. Other plants had just the opposite requirement, needing light periods greater than a certain minimum before they would flower. Thus, for example,

Short-day plants flower if given light periods alternating with dark periods in which the light period is shorter than a critical length.

Thus,
Xanthium (cocklebur) is a short-day plant. It is induced to flower by 14 1/2 hours or less of light per 24 hours. It must have a continuous dark period of at least 8 1/2 hours. Other short-day plants are *Chrysanthemum, Cosmos,* and *Poinsettia.*
Long-day plants will flower on light periods greater than a critical day length.

Thus,
Hyoscyamus is a long-day plant. It will flower if the light periods are longer than 10 hours per 24-hour period. Examples of other long-day plants are *Spinacia* (spinach), *Lactuca* (lettuce), and *Raphanus* (radish).

Short-day plants and Long-day plants are not separated on the basis of their requirements of different absolute length of illumination but rather on whether these light exposures are longer or shorter than a critical period.

Day-neutral or indeterminate plants flower more or less without respect to day length. These plants include, for example, *Lycopersicon* (tomato), *Capsicum* (pepper), most cultivated varieties of *Gossypium* (cotton).

In addition to these rather clear-cut types of responses, there are plants which show still different behavior to photoperiods. Thus, clear evidence exists for plants which require exposure to short days then to long days or, conversely, plants requiring exposure to long days followed by short days. These differences in behavior do not seem to follow broad taxonomic lines. The behavior may not be the same even within a given species, but rather may occur differently among different varieties or strains.

ANALYSIS OF EFFECTS OF DAY LENGTH

Flowering in response to photoperiod. Much of the analysis of the photoperiodic requirement in plants has centered on particularly sensitive species. For this, the cockle-bur *Xanthium* is especially suited. It is a short-day plant and can be induced to flower if transferred from long-day conditions to short-day conditions for one day. Thus, one long night and then return to long days is all that is needed to induce flowering in cocklebur. Most other plants require several cycles of the proper day length, or even weeks, in order to be effectively induced to flower. Long-day plants differ from short-day plants in showing no requirement for a dark period. They will bloom in continuous light. In fact, darkness tends to inhibit flower initiation in long-day plants.

Although the effect of cold temperature on floral initiation is perceived in the shoot apex, the light receptor system is mainly in the leaves, particularly the young, half- or fully-expanded leaves. Thus, the buds arc probably not directly affected and one is led to conclude that a hormone, synthesized in the leaves in the dark (in short-day plants), later becomes stable to light and moves, probably via the phloem, to the apex where floral initiation ensues. Grafting experiments support the general idea of the transmission of a hormone. A leaf from an induced plant can be grafted to a non-induced plant of the same species and cause flowering. In fact, a leaf or shoot from an induced long-day plant grafted to a noninduced short-day plant will cause flowering. Thus, the stimulus is not species-specific and apparently the same stimulus is effective in both long- and short-day plants.

The stimulus may be quite stable. In *Perilla,* a leaf from an induced plant, grafted successively into several noninduced plants, induced flowering in each. Thus far, no one has been able to induce flowering routinely in short-day plants with external applications of a substance which would act as a flowering hormone. In the case of long-day plants, however, Lang found that many plants held under noninductive day lengths could be made to flower by treating with spray applications of gibberellin. In the long-day plant *Samolus,* grown in short days, 10 µg of gibberellin daily for 3 weeks was fully effective in floral induction. Here, gibberellin replaced the required long-day inductive treatment. Since it is generally believed from evidence from grafting experiments that the effective flowering stimulus in short-day plants and

long-day plants is the same, Lang concluded that gibberellin was not the flowering stimulus itself, but that it may be instead directly involved in the synthesis of the flowering hormone.

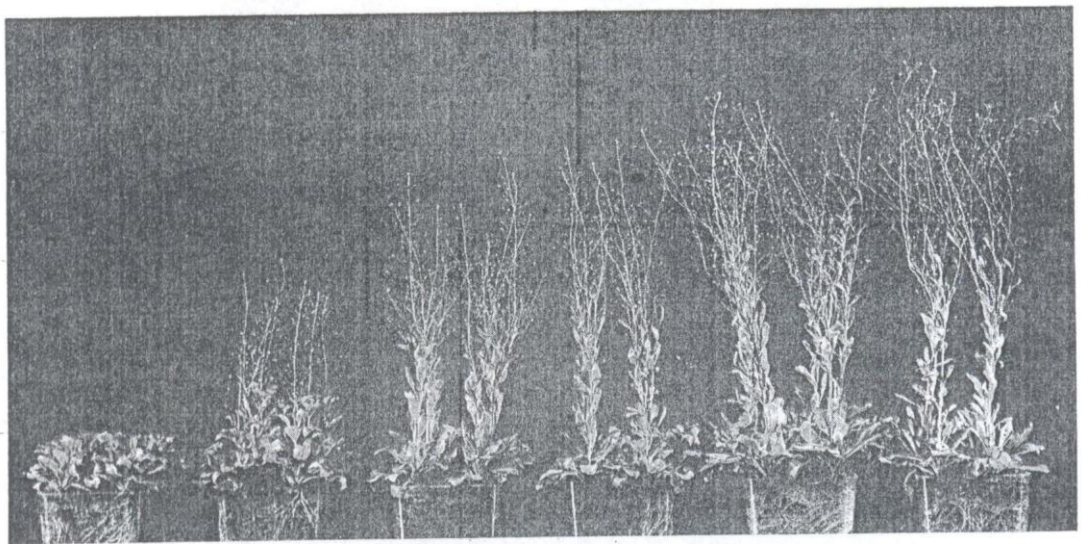


Figure 72. Response to treatment with gibberellic acid shown by *Samolus*, grown under noninductive photoperiods. Left to right: Untreated control plants, then plants treated with 1, 2, 5, 10, and 20 µg, respectively, of gibberellin daily. Notice that plants have been induced to elongate ("bolt") and to flower.

Samolus in its vegetative state is a % rosette plant and upon floral induction shows the typical "bolting" phenomenon with marked increase in cell divisions in the stem axis and rapid accompanying cell elongation (Figure 72). This stem elongation response precedes but usually seems to be associated with the flower induction, whether it occurs in response to day length or to gibberellin treatment. Thus, the flowering response is a complex sequential reaction not easily or simply interpreted.

There is an interesting quantitative effect of increasing numbers of inductive cycles on floral development in those plants which require several to many days of appropriate day length in order to flower. Thus, in the short-day plant *Chrysanthemum* a few cycles of short days are effective in inducing the conversion of the vegetative apex to a flower apex with its raised receptacle. If after 8 short days, the plant is transferred to noninductive long days, flower development is arrested at the "crown bud" stage. Further appropriate short-day cycles are required for normal flower development to proceed to floret formation and normal flowering.

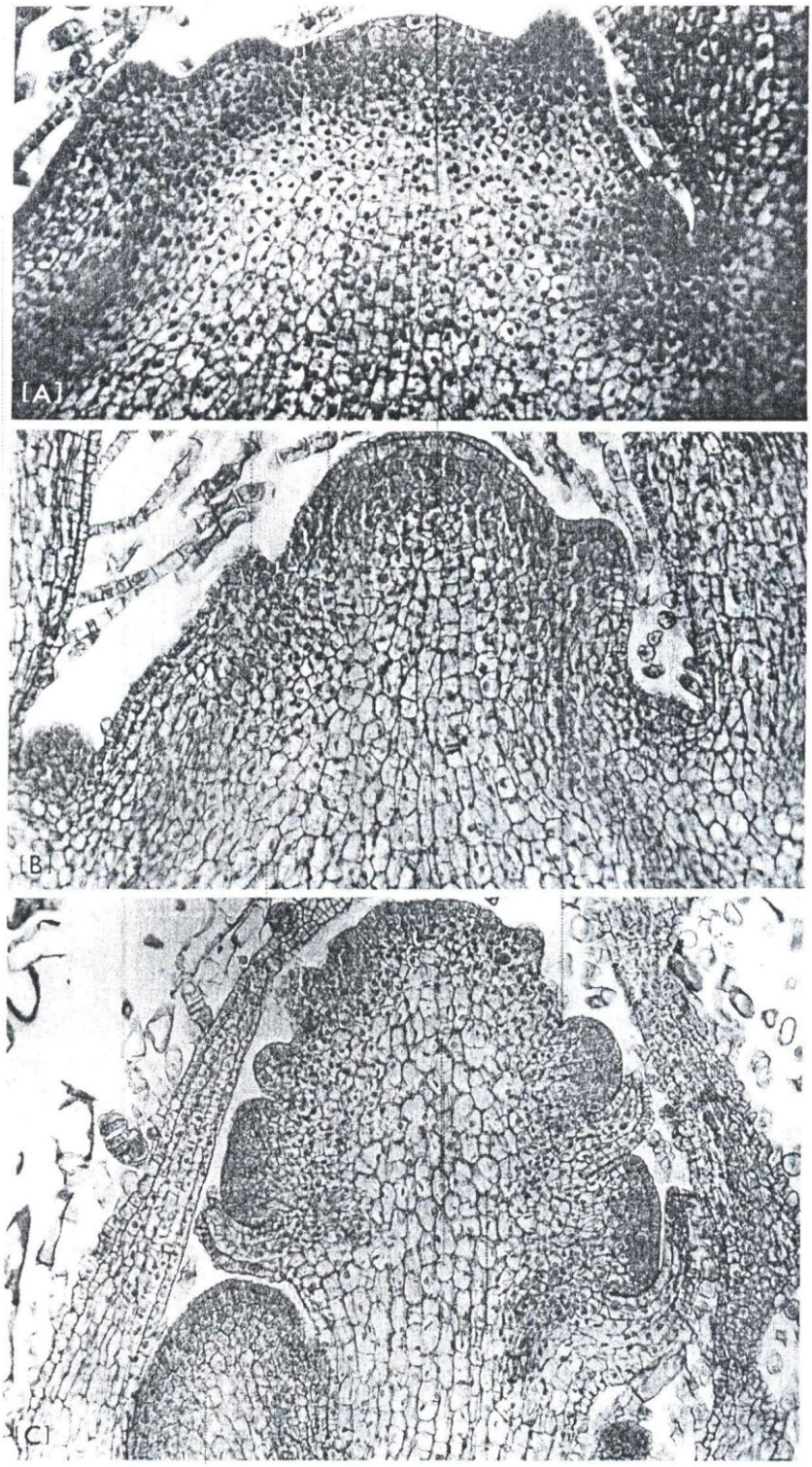


Figure 73. Median longitudinal sections of shoot apices of *Xanthium pensylyanicum*, grown on different day lengths. A: Vegetative apex. Plant grown under long-day conditions. B: Early floral apex. Plant exposed to two short days, then fixed after two additional days. C: Well-developed floral apex. Plant exposed to two short days and fixed eight days later. Note flower buds in the axils of bracts.

In this case, the day length is influencing not just the initiation process, but also the subsequent stages of floral development. The same quantitative effects of increasing numbers of inductive cycles are seen in *Xanthium* (Figure 73). Associated with early floral induction in short-day plants studied, one can demonstrate histochemically sharp increases in RNA and increased protein in the cells of the apical meristem, suggesting the synthesis of new proteins necessary for the development of new structures. At the same time, a decrease in basic proteins, notably the histories, occurs in the cells of the apex and it has been suggested that floral induction in some way involves the derepression of gene action. Clear evidence to support such speculation remains to be obtained.

The Role of Phytochrome in Flowering

In the study of the light requirements and spectral sensitivity of short-and long-day plants, it has been found most convenient to grow plants under noninductive day lengths, then transfer them for the minimum number of days to inductive conditions. Short-day plants require a period of uninterrupted darkness for floral induction. As we have seen, *Xanthium* is an especially sensitive short-day plant. If maintained on $14^{I}/_{2}$ hours or more light out of every 24 hours, it will remain vegetative. However, one cycle in which the night (or dark) period is longer than $8^{1}/2$ hours is all that is required to induce the plant to flower. Even if subsequently returned to long days (greater than $14/_{2}$ hours of light per 24-hour day), it will flower in about 2 weeks. Thus, in order to flower, *Xanthium* requires one long uninterrupted dark period of greater than $8^{l}/_{2}$ hours.

It was established in the 1940 s that a brief exposure of light at a critical point during the dark period would completely remove the effectiveness of an inductive long night. In Biloxi soybean, another quite sensitive short-day plant, a 30-second "flash" of light of 15-foot candles in the middle of inductive dark periods completely prevents flower initiation. This light response is perceived in the leaves and involves a remarkably sensitive system. Apparently, whatever happens during the long dark period is reversed by a light interruption. Short-day plants might better be called "long-night plants." It is generally conceded that the dark period allows the generation of some stimulus leading to flowering and that a light interruption destroys the stimulus. In order for a long inductive nig] it to be effective in promoting flowering in short-day

plants, there must be a preceding light period with high intensity light, presumably allowing photosynthesis, since CO_2 must be available during the high intensity light period.

In long-day plants, a brief treatment given during the dark period of noninductive conditions, acts in just the opposite way—that is, the dark interruption actually stimulates flowering. Thus short- and long-day plants show similar sensitivity to light regimes, but their responses is opposite to each other.' Experimentally, the effectiveness of the dark interruption by low-intensity short-duration light treatment allows one to study the nature of the receptor system by a determination of the action spectrum for floral inhibition (in short-day plants) or flower stimulation (in long-day plants). Such studies have been carried out over the last 20 years, especially by Borthwick and Hendricks (U.S. Department of Agriculture).

In the short-day plant *Xanthium,* red light was found to be maximally effective, with a peak of effectiveness at 660 mμ. It was further found that far-red irradiation at 730 mμ behaved essentially as darkness—that is, it had little or no effect alone. If, however, brief far-red irradiation followed red irradiation, the effect of red light was negated and the plants would flower. Successive red and far-red irradiation behaved as in the case of seed germination discussed earlier that is, the last irradiation was the effective one. Here, then, in short-day plants the same reversible photoreceptor pigment system seems to be involved.

One can test long-day plants in essentially the same way—that is, grow them under short days and long nights (noninductive conditions) and then give them a long-night interruption with red or far-red light. In this case, red light promotes flowering and far-red light alone acts essentially as darkness. Here again, red and far-red reversibility was found. Thus, it seems clear that the low-intensity light effect, interrupting the dark reactions in both long- and short-day plants, involves the pigment phytochrome. In short-day plants, when P_{FR} is present, flowering is inhibited; when P_R is present, flowering proceeds.

Here arises a difficult question: does darkness result in the removal of an inhibitor or the synthesis of a stimulator? At the present time, evidence seems to favor the idea, that, in short-day plants at least, the pigment P_{FR} in some way actively inhibits the'

flower initiation process and conversion back to P_R removes the inhibition, leaving an inactive form which allows flowering to proceed. A very important further observation has been made concerning the effectiveness of the long-night interruption. If one gives red light for a brief period in the middle of the inductive long night in *Xanthium*, the plant will be inhibited from flowering. If the red light is followed immediately by a brief irradiation with far-red light, the plant will flower since the red light effect is negated. However, if, after an inhibitory red light treatment is given, the; far-red irradiation is delayed for a period of time—about half an hour or more—then the far-red is no longer effective in reversing the effect of the red light; that is, the system has escaped from the reversibility by light.

This suggests that when the pigment is in the P_{FR} state, it is coupled to chemical processes which proceed immediately. Thus, P_{FR} seems to be the physiologically-active form of the pigment in photoperiodic responses. At 25 °C, 30 minutes is long enough for these steps to have reached a point where pigment conversion to P_R no longer can stop the process. This time period between irradiations for effective reversal can be extended by lowering the temperature to 5°C, further suggesting chemical events tied to the P_{FR} form of the pigment. The nature of the chemical events tied to P_{FR} is one of the major subjects being pursued in current research.

The photoperiodic behavior of plants with respect to flower initiation appears to depend on diurnal fluctuations in light and dark, centering on a periodicity of 24 hours. To a remarkable extent, plants show periodic activities, some of which seem to be timed by the rhythm of the changing external environmental conditions. Many such periodicities, however, can be shown to persist in cycles even when the environment is made constant. These endogenous rhythms are of considerable interest to biologists who have been puzzled about mechanisms whereby the organisms could keep track of time.

In this connection, one should point out the possibilities in this reversible pigment system for a time-measuring mechanism—that is, a biological clock. There is some evidence to suggest that, in addition to a light-catalyzed destruction of phytochrome, there is also a spontaneous dark conversion of P_{FR} to the P_R form. From physiological experiments on flower response, tie half-life for reversion of P_{FR} to P_R is thought to be about 2 hours. It is postulated that, after a period of light, dark conversion of P_{FR}

wil1 proceed during darkness at a rate such that about 3 per cent of its initial activity as P_{FR} is still left after 10 hours of darkness. This time period comes close to the range of critical night lengths for floral induction in many plants. Variations in this timing could well represent a system for measuring the length of the dark period. When the critical dark period is reached, flower initiation may proceed. This may be an accumulative phenomenon in which many successive critical night lengths are required for induction or, as in *Xanthium*, a single long night is all that is required for induction.

At the present time, postulated timing mechanisms involving phytochrome interconversions are purely speculations. Much more needs to be known about the chemical nature of phytochrome and its biochemical and biophysical properties as well as its distribution in plants and the nature of its physiologically active form. Other timing mechanisms could be involved, such as the utilization of photosynthetic products or endogenous rhythms which are built-in, usually on 24-hour cycles, but which can be reset by external environmental changes such as light.

Flower Development

The foliar nature of certain floral parts is quite evident. The sepals and frequently the floral petals are flattened, leaf like structures, which may even show the typical green pigmentation of leaves. Even brightly colored floral petals are clearly modified leaves, differing largely from vegetative foliage in their attachment to the axis, elaboration of surface area and pattern, or in pigmentation. Less obvious is the foliar nature of the stamens and carpels in many flowering plants. Yet the foliar nature of the stamen and carpel is especially evident in certain groups of the angiosperms which on a number of grounds, both morphological and anatomical, are believed to be evolutionarily primitive groups. These flowering plants belong to the family Winteraceae in the large order of the Ranales.

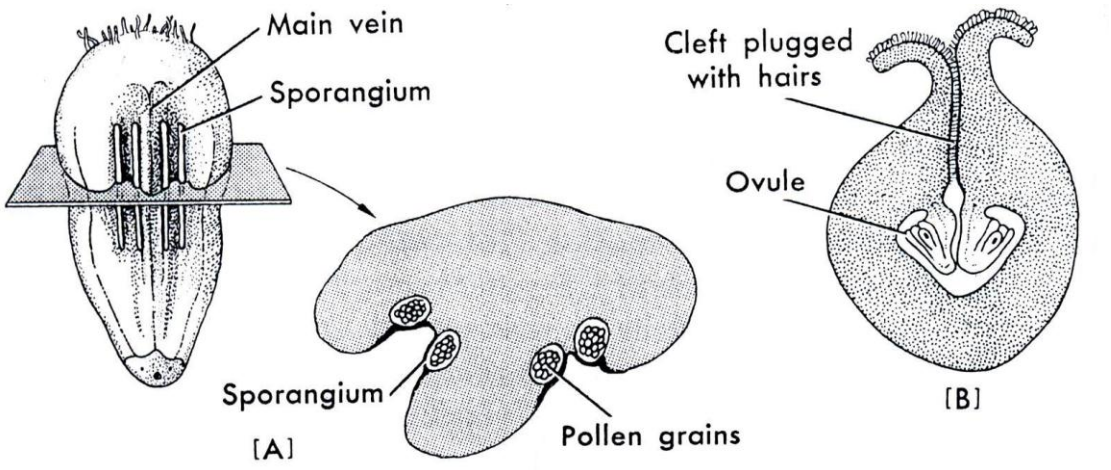


Figure 74. Diagrams of foliage-like stamen and carpel of primitive Ranalian flowering plants. A: Stamen of *Degeneria* as seen in abaxial view and in transection. B: Transection of the conduplicate carpel, of *Degeneria,* showing the position of the ovules on an infolded leaflike structure.

Among other primitive characteristics, members of this family lack true vessels in the xylem. The foliage-like structure of the reproductive parts has been pointed out by Bailey in his studies of this group. In the genus *Drimys*, the carpel, or so-called "conduplicate carpel," is a green leafy structure constructed as if from a leaf folded lengthwise upon it. On the inner folded surfaces are borne the ovules (Figure 74 B). The foliage-like structure of the carpel is seen also in such a fruit as the pea pod which encloses the maturing ovules or seeds Foliar stamens are seen in other members of the Winteraceae—for example, in the genus *Degeneria* in which the pollen sacs are embedded in the flattened, undersurface of a foliage-like appendage (Figure 74A).

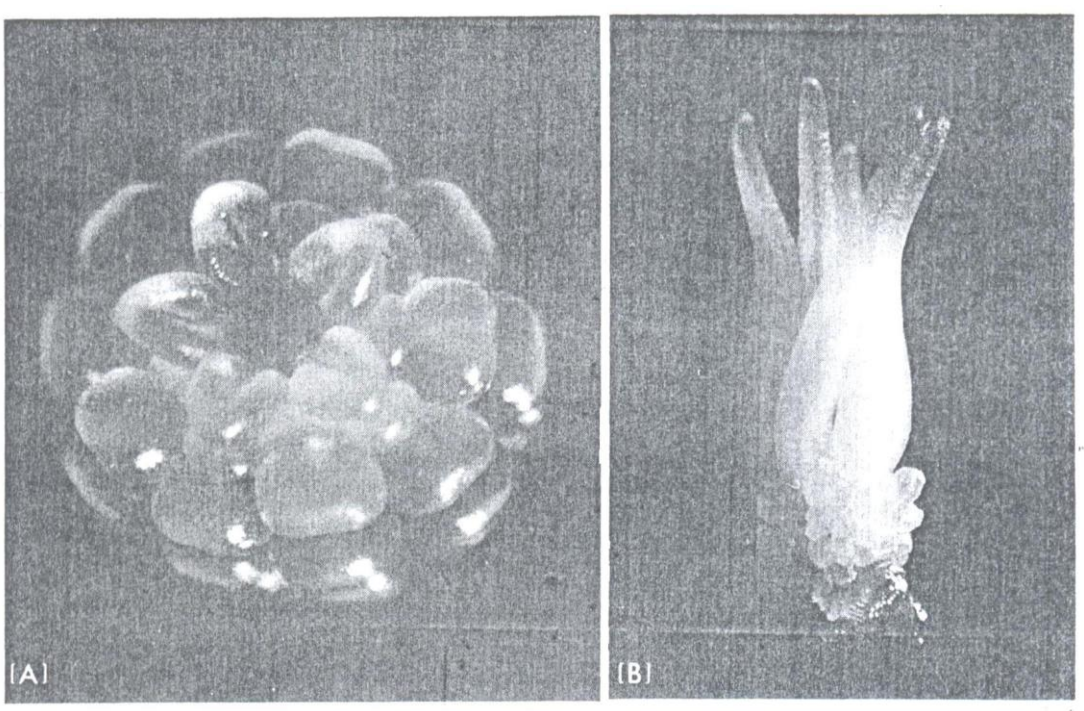
From a developmental point of view, floral appendages are specialized lateral appendages produced at the apex. Instead of flattened green foliar lamina, extensively modified structures are formed. Thus, in ontogeny, the vegetative apex converts after induction to new activities which lead to modified appendages. The future generation of the plant lies within the carpel. Following pollination and fertilization, the embryo within the ovule develops from the zygote and the outer layers of the ovule become the investing seed coats. The tissues surrounding the ovule—in particular, the ovary wall of the carpel and the basal point of attachment of the flower, the receptacle—form the major supporting tissues of the ovule and become the fruit.

From the onset of floral initiation through the progressive development of the floral parts, pollination, and formation of the fruit, present evidence clearly shows that there exists in the reproductive axis a complex of interactions involving hot only nutritional components—that is, the rapid accumulation of food materials within the developing fruit—but also, and probably crucial to normal development, the action of different hormonal systems, including those we have already "discussed in vegetative development—namely, the gibberellins, the cytokinins, and the auxins. Before turning to the details of fruit development, it is interesting to consider briefly the much less studied and rather poorly known aspects of the actual formation and early development of the floral appendages. From our earlier discussions of the photoperiodic control of 'floral initiation, it was clear that, under the proper inductive photo-period,' there was a continuous input from the environment which drove the floral differentiation along its course.

If the appropriate inductive conditions were changed, floral development could be arrested and not reach final floral formation (for example, there was the induction of so-called crown buds in *Chrysanthemum).* Such a quantitative effect of inductive days might be interpreted in terms of the generation of a hormonal stimulus (or the removal of an inhibition) which required a period of time. Thus a continuous exposure to the new conditions is essential. In order to attempt to test the importance of hormones in floral development, recent studies have been undertaken using isolated immature floral apices grown in sterile culture.-In *Aquilegia,* a Ranalian flower with many floral parts formed in whorls of five, Tepfer and his associates had some success in culturing isolated floral buds taken as early as the presepal stage when sepal primordia had not yet developed. They were able to culture such buds in a complex medium containing sugar, minerals, mixed vitamins, and coconut milk. When IAA, GA, and kinetin were also provided, the buds initiated all primordia and, depending on the balance (that is, relative concentration) of these hormones, one or another of the specific floral organs developed best.

Interestingly enough, once formed, the sepals (the lowermost set of appendages) inhibited the development of all the other primordia. These appendages had to be removed surgically in order to study the development of petals, anthers, and carpels. In nature, the petals of *Aquilegia* form long spurs at the base of a cup-shaped lamina.

This complete structure was not attained in vitro, although some success toward spur format on was achieved by manipulation of the hormone balance. Stamens begin development, form anther and filament, and even pollen sacs, but then suddenly alport in vitro, suggesting that they require a specific and different hormone or nutritional environment in order to continue. Carpel development has been achieved from initiation in the apex through to structures of 2 cm in length, which is the size of mature carpels in the adult flower (Figure 75).



Fiure 75. Isolated floral apices of the columbine *Aquilegia,* grown in sterile culture. A: Floral apex shortly after initial excision at the grooved-carpel stage, showing staminodia and stamen primordia. Approximately X60. B: Same floral apex as in A, photographed ati approximately half the magnification after 19 days in culture on a complex medium. Note especially the development of the carpels. Stamens and staminodia have aborted.

The medium clearly favors both initiation and development. Even so, mature ovules have not been formed and there is much to learn. Clearly the hormonal supply seems crucial and it may well be that changing hormonal levels operate to control primordia formation, perhaps acting more or less directly through gene control. Similar studies have been made on the control of sex expression in flowering plants—that is, whether a flower will be only male or female or whether it will be hermaphroditic (a complete

flower with both stamens and carpels). One of the most carefully studied cases of sex expression is that of the cucumber *(Cucumis)*. Here genetic strains have been developed for commercial use which produces almost exclusively female flowers, each in turn leading to a marketable fruit.

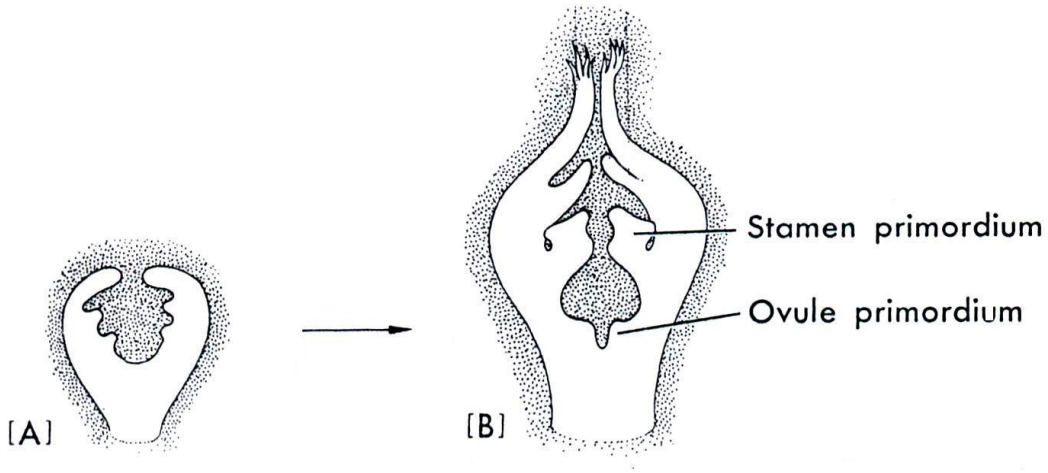


Figure 76. Diagrammatic representation of stages in the development of the floral primordia in cucumber *(Cucumis)*, seen in median longitudinal sections. A: Floral apex, 0.3 mm wide, with primordia of sepals, petals and stamens apparent. B: Later floral apex, 0.7 mm wide, showing well-developed stamen primordia. The ovaries containing the ovule primordia have begun to develop.

In the case of cucumber, the flower initiated in the primordium is bisexual up to a given stage (Figure 76). Then the expression of maleness or femaleness in the flower depends first on the genetic constitution and thereafter on the environment, where both temperature and photoperiod can affect the expression. Some years ago it was found that, if sprayed with auxin, plants which were potentially male or produced bisexual flowers would produce mostly female flowers. Thus it appeared that auxin stimulated or favored development of the carpels and suppression or abortion of the stamens. More recently, this general picture has been confirmed by Galun and Lang from cultures of isolated floral primordia of cucumber. In primordia which were genetically determined to be male, auxin provided in the medium resulted in promotion of ovary development. The stimulation to ovary development could be reversed by gibberellic acid (GA_3), but GA_3 alone in the medium had no effect. Once the ovary induction had occurred, stamen development was inhibited. When applied to whole plants as a spray, GA_3 favored stamen development in cucumber.

In hemp *(Cannabis)*, where the sexes are borne on separate individuals—that is, the species is dioecious, Heslop-Harrison has reported that in male plants, treatment with auxin in early-flowering stages actually converted stamen development into carpel development. Thus in this case there was a conversion of structures from one developmental path to another under hormonal control.

CHAPTER VIII

MOLECULAR BIOCHEMICAL CHANGES DURING FRUIT DEVELOPMENT

Floral Induction is the beginning of a complex sequence of events leading to fruit development, seed formation, and the completion of the life cycle. In herbaceous annuals, this process usually ends in the death of the plant; in perennials, the process is repeated annually, involving new floral apices each year. The development of the fruit really begins at the time of floral initiation with the conversion of the vegetative apex to a floral apex. The classical interpretation of the flower is that it is a system composed of an axis and determinate lateral members; it is a short shoot bearing floral leaves, comparable in organization to a vegetative shoot. Unlike the vegetative shoot, the floral shoot has determinate growth which usually terminates in the development of male and female parts, the stamens which bear the pollen, and the carpels which bear the ovules. The fruit may be defined as those tissues which support the developing seeds of the plant.

As we have seen, fruit development begins with the induction of flowering when the vegetative apex is converted to the formation of floral structures. It is at this time that the changes required for the development of the receptacle and the carpels begin to occur. For practical purposes, however, pollination is the event which sets in motion the physiological processes ultimately leading to the formation of the mature fruit. These processes are complex and, as new information becomes available on the interwoven hormonal activities, the subtlety of a programmed control affected by internal and external circumstances becomes clearer.

Effective pollination results in what is called "fruit-set." When pollen is deposited on the receptive stigmatic surface of a flower, a course of events is set off which results in two major physiological changes. In the first place, pollination usually prevents the abscission of the floral apex. In the absence of pollination, an abscission layer typically develops at the base of the receptacle of the flower and the undeveloped carpel abscises. In some way, pollination also triggers the development of the tissues of the fruit itself including the ovary walls and receptacle of the flower which begin t6 enlarge.

Pollen is a rich source of auxin. As early as 1909 fitting showed that orchid pollinia produced a water-soluble substance which, when extracted from pollen and applied to the stigma of the flower, would cause the initiation of swelling of the ovary. In the early 1930's, it was demonstrated that pollen contained auxin. In 1936 Gustafson found that by cutting off the stigma of tomato flowers and applying synthetic auxin in paste to the cut surface, he could effect fruit-set in the absence of pollination. He was able to produce mature tomato fruits which were, however, parthenocarpic—that is, they lacked seeds since no fertilization had occurred. Since that time, parthenocarpic fruits have been produced experimentally by auxin treatment of unpollinated flowers in a number of plants, including cucumber, squash, beans, strawberries, peppers, and others. Some practical use has been made of this knowledge. For instance, it has been found that tomatoes grown in greenhouses during winter months may not receive sufficient sunlight for normal pollen development. Under these conditions, natural fruit-set will be very low. If the blossoms, which are otherwise normal, are sprayed with an auxin solution, fruit-set may be increased markedly and healthy mature fruit produced.

Fruit-set cannot be achieved artificially with auxin in all plants. Notable exceptions in early experiments were the so-called “stone fruits." Such flowering trees as peaches, cherries, and plums, in which a large single seed or "stone" is formed, showed no response to auxin treatment. In these plants it was discovered that fruit-set can be achieved in the absence of pollination by using spray treatments' of gibberellins. Similarly, other fruits, such as grapes, apples, and figs, respond to gibberellic acid treatments in the absence of pollination, producing parthenocarpic fruits. In at least some of these species, the pollen normally produces gibberellins.

According to some views, both auxins and gibberellins are involved in the early stages of fruit development, including the process of fruit-set. Depending upon the species of plant studied, auxin or gibberellin may play the primary role in the control of early fruit development. The effect of externally applied auxin or gibberellin may then be interpreted as interacting in some way with the endogenous levels of hormone to produce the effect observed. Thus, auxin or gibberellin application may cause parthenocarpic fruit development or increased fruit size in normally pollinated species. The effect of auxin applied early in fruit development at the flower stage is

sometimes to accelerate fruit abscission, sometimes referred to as "June drop," a response opposite to what one might expect. This effect is usually interpreted as resulting from an interaction of the applied auxin with the normal endogenous gibberellin levels, in which the auxin antagonizes the action of gibberellin on effective fruit-set. The mechanism of such an interaction is unknown.

The second major phase of fruit development following pollination is fertilization. In flowering plants, double fertilization is involved, one male gamete fusing with the *egg* cell to produce the zygote from which the future embryo develops, the other sperm fusing with polar nuclei in the embryo sac to form the endosperm. The latter nucleus multiplies first to form the endosperm tissue in which the embryo later develops. Both of these structures, which are held within the ovule, are sites of intense hormone synthesis first in the endosperm and later in the embryo. At this stage in fruit development, cell division is intense in the developing seed; and may also be occurring at a rapid rate in the surrounding ovary wall. The evidence seems fairly clear that in many fruits, the developing endosperm may be rich in all three types of hormones—auxins, cytokinins, and gibberellins. Liquid endosperm has been a prime source for chemical studies of growth-promoting materials.

Coconut milk, the milk stage of maize endosperm, and other young fruit tissues have been the sources of natural auxins, gibberellins, and cytokinins. In fact, the various tissues of young fruits are so rich in hormones that it has not been easy to unravel the respective roles of these hormones in fruit development. Certainly there is no single simple scheme that would be applicable to all fruits. Early fruit development, when mitotic rates are high, is especially -associated with relatively high concentrations of cytokinins. Thus, the extraction of immature green apples in bulk has given evidence of a naturally-occurring cytokinin at this early stage, associated apparently with the rapidly dividing tissues of the ovary wall. The high level of cytokinin seems thus to be correlated with the endosperm stage of fruit development which is primarily a stage of rapid cell division.

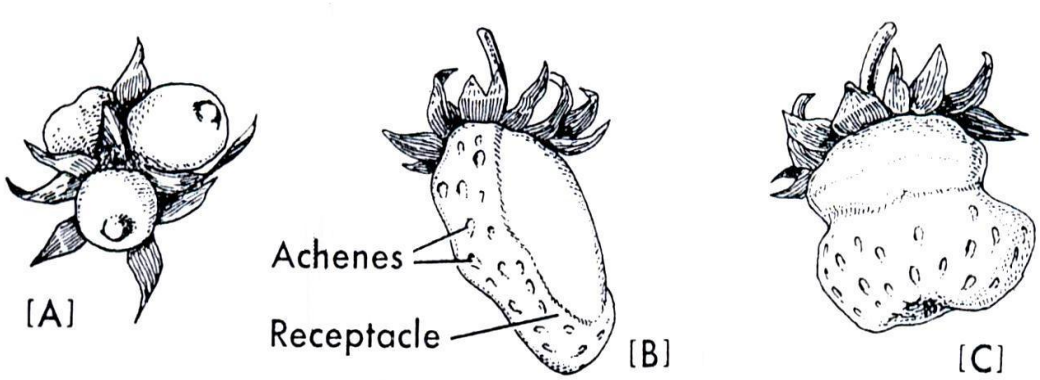


Figure 77. Malformed strawberry fruits produced by removal in different patterns of many of the achenes present at the beginning of fruit development. Only, those tissues of the receptacle associated with the auxin supply from the remaining achenes show cell enlargement and development.

When cell divisions are largely completed, the tiny fruit continues its development by rapid and remarkable cell enlargement. That auxin may play a decisive role in fruit enlargement was clearly demonstrated by Nitsch who showed that removal of the achenes (the seedlike structure on the fruit surface) from the receptacle of a young strawberry *(Fragaria)* fruit stopped its enlargement. Replacement of the achenes by small dabs of auxin-containing paste allowed resumption of growth to almost normal fruit size (Figure 77). In this case, the ovule is the source of auxin, and the receptacular tissue or the tissues of the ovary respond to the auxin by cell enlargement. Nitsch showed that while the receptacle itself produced no auxin, the achenes were active sources of auxin, showing increased auxin production after fertilization until the twelth day, and then the auxin level decreased to a constant level. The enlargement of the receptacle could be closely correlated with cell enlargement which was auxin dependent. Misshapen fruits in many species frequently result from the uneven distribution of seeds within the developing fruit.

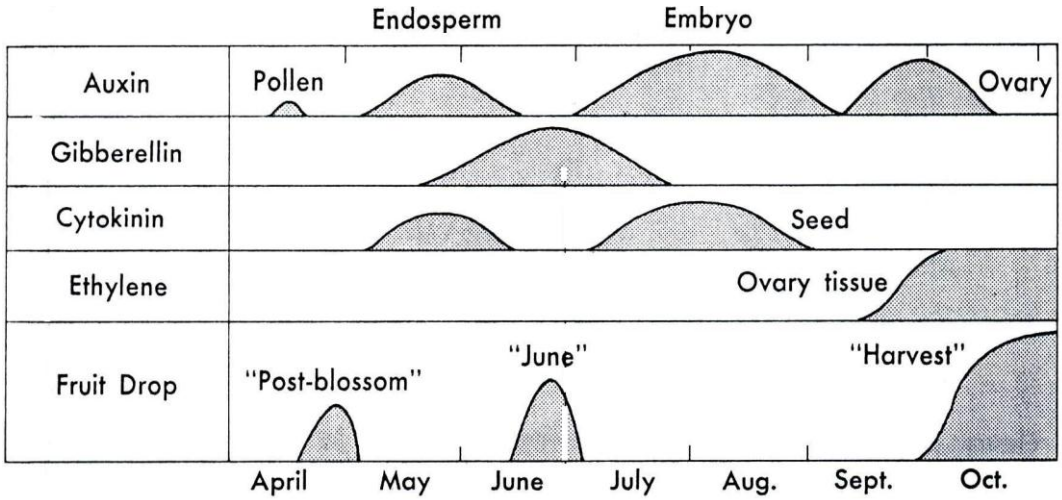


Figure 78. A schematic representation of the timing of hormone production and fruit abscission for a typical pomaceous fruit. For each hormone, the graphic representation of increasing concentration is given in a generalized form, especially relating to any specific tissue. The relative incidence of abscission is indicated in the bottom graphs.

This may result either from failure of certain ovules to be fertilized, or from destruction of ovules by insects or other pests. In some plants, the cell-enlargement phase of fruit development appears to be largely under control of gibberellins rather than auxin. This situation is particularly evident in some varieties of seedless fruits such as grape. Here, auxin seems not to be limiting, and the cell-enlargement phase of the fruit development is associated primarily with high levels of endogenous gibberellin; in such fruits, external application of gibberellins may increase fruit size still further. In still other species, both auxins and gibberellins apparently act together to produce the cell-enlargement phase in the fruit.

During the final stages of fruit enlargement, sugar content of the cells increases rapidly. It is believed that in many cases, such as in grape, the growth rate of the fruit can be most closely correlated with increase in sugar levels. At this stage there is claimed to be an osmotic effect leading to rapid cell enlargement, limited not by hormone level but by the level of osmotically active sugar in the fruit. Figure 78 shows a scheme summarizing some of the physiological events involved in the development of a hypothetical fruit, illustrating the possible sequential changes in hormones and their association with events observable in the developing fruit.

The mature fruit shows many other physiological and chemical changes associated with ripening. In fact, the ripening of fruit is a field of study all in itself, centering as it does on the processes which make the mature fruit palatable, nutritious, and attractive to man and animals as a food source. Early in the maturation and ripening of the fully developed fruit, the hormone-like substance ethylene is produced by the fruit tissues. This volatile material appears to be associated with the processes of ripening—that is, conversion of starch to sugars, softening of the fruit flesh due to pectin hydrolysis, and change of pigmentation from green (due to chlorophyll) to the bright anthocyanin pigmentation typical of many ripe fruits. Other complex changes occur as well in a coordinated and programmed fashion, leading ultimately to development of a separation zone at the base of the fruit and finally abscission of the fully ripe fruit.

One thus finds a complicated sequence in fruit development in which phases of mitosis, cell enlargement, and cell differentiation and maturation follow one another in sequence under the control of progressively changing hormonal and metabolic states. Many variations in the detailed hormonal controls can be found among the wide varieties of fruits studied. Clearly, much work remains to be done before we will be in a position to outline with any precision the sequence of events and the control mechanisms actually at work in fruit development.

CHAPTER IX

POLLEN STORAGE AND VIABILITY

The main objective of plant breeders is to produce new and improved plants for not only better yield and quality but also which are resistant to pests and diseases. Hybridization is one of the most commonly used techniques for plant improvement. However, many of these attempts fail since selected parents flower at different times or at distant places. The barriers imposed by time and space can be overcome by collecting and storing pollen, may be for a short duration and utilize it at appropriate time for hybridization purposes. Storage of pollen also eliminates the necessity to continuously grow lines frequently used in crosses. Plant breeders are increasingly using stored pollen as a system for selecting genotypes with desirable qualities e. g. tolerance to toxins, adverse temperature and other stresses. Stored pollen can be used as a subject for manipulations like DNA transformation (Wilms and Keijzer, 1985; Wilms and Keijzer, 1988).

Further, with advancement of new technologies in genetic engineering and hybridization, sperm cell biotechnology has become relevant during the past few years. And for sperm cell isolation, DNA transformation etc. it is necessary to have viable and functional pollen in store. The pollen or isolated sperm cells when stored may serve as 'gene-banks' for various applications including preferential fertilization (Wilms and Keijzer, 1988). The work on storage of pollen was initiated toward the end of nineteenth century when viability of pollen of more than 80 species stored at low humidity was tested (See Stanley and Linskens, 1974; Shivanna and Johri, 1985; Heslop-Harrison, 1987). The pollen of different plant species can be stored for specific durations after which they start losing their viability. Sufficient literature is available on the methods of pollen storage and determination of their viability (Johri and Vasil, 1961; Shivanna and Johri, 1985). The cause of losses of pollen vitality during storage has been a subject of only a few detailed investigations.

Rarely methods of storage, tests for determining viability and causes of loss of viability during storage of pollen, are discussed at one place. The present review is an attempt in this direction.

METHODS OF POLLEN STORAGE

Different methods have been attempted to store pollen for short or long durations. Pollen are collected in small glass or plastic unsealed vials, and stored in desiccators containing suitable dehydrating agents, such as saturated solutions of different salts to maintain desired relative humidity (RH). In fact, temperature and humidity are the most important factors essential for maintaining pollen viability during storage. Liquid nitrogen and dry ice are generally used for maintaining low temperature. Other techniques of pollen storage include vacuum drying, freeze-drying and organic solvents, etc. (Stanley and Linskens, 1974; Shivanna and John, 1985).

Temperature and Humidity

The optimal temperatures and RH during storage of pollen varies from species to species (Holman and Brubaker, 1926; Visser, 1955; Johri and Vasil, 1961; King, 1965; Stanley and Linskens, 1974). Pollen of several taxa stored under variable temperature and/or humidity conditions have been listed by King (1965). Shivanna and Johri (1985) have also discussed the results of successful storage of pollen of a number of taxa under sub-freezing, ultralow-temperatures and by the manipulations of temperature and humidity. In general low temperature(s) and low RH are favorable for successful pollen storage except in graminaceous taxa. Pfundt's (1910) studies on pollen viability conducted on 140 plant species using 0, 30, 50 and 90 per cent RH at 17°C-22°C indicated that longevity was maximum at low RH;(0-30 per cent). Holman and Brubaker's (1926) results on 52 plant species under similar conditions supported these inferences. In general, the longevity of pollen is negatively correlated with relative humidity required for optimal storage (Visser, 1955).

But the Gramineae pollen retains its viability only at high RH (80-100 per cent RH) and that also for a short period. One exception is that of *Pennisetum typhoideum* in which pollen remained viable for 186 days at-16-35°C under 0 per cent RH (John and Vasil, 1961). Hall and Farmer (1971) have shown that pollen *of Juglans nigra* retained viability for three months when stored at 4°- 30° and -196°C. Pollen stored for one year at -196°C was effective in inducing fruit-set (Griggs *et al*, 1971). Pollen grains of many taxa have been successfully stored at ultra low temperature using *dry* ice (-80°C) and liquid nitrogen (-196°C) (Bajaj, 1987). Date palm pollen stored in liquid nitrogen retained viability for 435 days (Tisserat *et al*, 1983). According to

Barnabas and Rajki (1981), water content of pollen is critical for the success of ultra-low temperature storage. Ganeshan (1986) reported that onion *(Allium cepa*) pollen retained viability and fertilizing capacity up to 360 days during storage in liquid nitrogen (-196°C), Hecker *et al*, (1986) have successfully stored the pollen of sugar beet containing about 12 per cent moisture in liquid nitrogen up to one year.

The pollen of *Broccoli*, when stored in liquid nitrogen, retained its viability but the seed produced using this pollen rapidly lost its germinability (Crisp and Grout, 1984). However, the plants raised from this seed and their progenies gave no indication of genetic damage resulting from the low temperature treatment of pollen. It may be considered that storage of pollen in liquid nitrogen is a safe method of genetic conservation. Collins *et at.*, (1973) reported storage of pollen in liquid nitrogen of alfalfa *(Medicago sativu)*, soybean *(Glycine max)*, cotton *(Gossypium hirsutum)*, corn *(Zea mays)*, oats *(Avena sativa)*, rye *(Secale cereale)*, sorghum *(Sorghum bicolor)* and wheat *(Triticum aestivum)*.

Freeze-Drying

Freeze-drying and vacuum-drying techniques have been successfully used for preservation of fungi (Fennel *et al*, 1950; Sharp and Smith, 1957), bacteria (Fry, 1954; Proom and Hemmons, 1949), bacteriophages (Annear, 1957) and viruses (Dyxtra and DuBuy, 1942; Hollings and Lellicott, I960) and some of these were preserved for several years with little or no loss in viability. Pollen from several different species has also been effectively preserved by freeze-drying or vacuum-drying methods (Hanson, 1961; King, 1959, 1960; 1961; Visser, 1955). In this technique freezing of pollen is done at -60° to -80°C for varying periods in a freezing bath followed by gradual removal of water under vacuum by sublimation (Shivahna and Jphri, 1985). Freeze-dried pollen can be stored at room temperature under nitrogen or in vacuum. Pollen of different species varies in their ability to withstand exposures to freeze drying or vacuum drying. Visser (1955) successfully preserved pear and apple pollen by freeze-drying while Pfeiffer (1955) has shown moderate success with *Lilium* pollen.

Hesseltine and Snyder (1958) did not succeed in preserving pine pollen by freeze-drying, King (1961) found that freeze-dried pine *(Pinus taeda)* and onion *(Allium cepa)* pollen remained viable up to 379 and 191 days, respectively, at room temperature, but the respective controls were non-viable after 31 and 5 days. For successful freeze-drying of

pollen its initial moisture status, period of freeze-drying and rehydration after storage need indepth attention. Akihama *et al*, (1978) reported that pollen of peach and Japanese pear having moisture content less than 8 per cent only show satisfactory freeze-drying. Layne and Hagedorn (1963) have shown that in pea pollen freeze-drying for 15-20 min is satisfactory but gives negative results when extended to 6 hours. After freeze-drying, the rehydration of pollen has to be gradual, may be for hours (King, 1965).

Organic Solvents

Storage of pollen in organic solvents avoids the problem of maintaining a specific RH and is a useful technique for transporting pollen without dry ice or refrigeration (Iwanami, 1962, 1971)."Many organic solvents such as benzene, benzyl alcolol, butanol, methanol, isopropanol, petroleum ether, ethanol, acetone, diethyl ether and chloroform are found suitable to store pollen grains of several taxa (Iwanami, 1972; 1972a; 1973; Iwanami and Nakamura, 1972; Jain and Shivanna, 1988). In some organic solvents pollen grains lose viability within a few weeks or months, while in others the pollen grains remain viable for many years} (Iwanami 1984). Pre-soaked *Petunia* pollen in organic solvents is found to be effective in inducing seed set following pollination" *Lilium* pollen stored in acetone for three months was effective in inducing fruit-set, (Iwanami, 1972). Kobaylishi *et al.* (1978) showed that pollen of *Citrus* species maintained viability in various organic solvents for three months.

Organic solvents have also been effectively used in studies on seed biology. Seeds treated with organic solvents show increased germination (Milborrow, 1963; Rao *et al.*, 1976). Many inhibitors which do not affect germination but inhibit subsequent growth of the seedlings are reported to be present in the organic solvent leachate following soaking of lettuce seeds (Rao *et al.*, 1976). This is in agreement with the suggestion of Iwanami and Nakamura (1972) that organic solvents may remove some inhibitors of tube growth to explain the production of longer tubes in pollen grains soaked in organic solvents when compared with tubes from fresh pollen. Acetone and dichloromethane have been used to introduce hormones and other chemicals-in dry seeds (Meyer and Mayer, 1971; Tao and Khan, 1974). Thus there is ample scope for the use of organic solvents in basic studies on pollen biology.

Pollen Viability Tests

The most critical morphogenetic event in pollen toward fulfilling its ultimate function of discharge of male gametes is germination. Pollen must be living and capable of germinating at the time of pollination for seed set to occur (Knox *et at.*, 1986). Therefore, it is very essential to know the germination potential of stored pollen before usirtg for hybridization purposes. According to Gay *et al.* (1987) pollen quality and vigor of stored pollen samples has to be monitored at each step of fertilization programme which starts with pollination followed by hydration, germination, style penetration, tube growth and fertilization. Several tests have been standardized from time to time for assaying viability of pollen. These are described below in brief with each test having some limitations.

Germination Assays

(i) *in vivo germination*

It involves; application of pollen to a compatible stigma on micropyle and observations are made on pollen germination in style. Different staining techniques are used to trace the pollen tubes in the stylar tissues (Nair and Narasimhan, 1903). A more sensitive and easily applied method of detecting germinating pollen tubes *in vivo* is based on the use of florochrome dye, which reacts with callose that occurs in germinating pollen tubes (Linskens and Esser, 1957). Shellhorn *et al.*, (1964) used acridine orange dye which stains pollen tube cytoplasm and does not require the presence of callose. Except the fluorescent method, all other methods work with only a few selected taxa and are not entirely satisfactory (Esser, 1955; Dionne and Spicer, 1958; Pandey and Henry, 1959). Ockendon (1974) has demonstrated that pollen of *Brassica oleracea* is considered to be viable which produces about 70 pollen tubes in the style. In some tax a shrivelling of pistil following pollination is correlated with good seed set (Dejong and Kho, 1982) and can thus be considered as an effective indicator of pollen viability.

(ii) *Fruit and seed set*

The pollen sample to be tested is hand pollinated on a compatible stigma after emasculation which leads to seed and fruit set. In this method flowers of male sterile lines are preferred as it eliminates the possibility of contamination from self-pollination. Other ways to avoid contamination of the stigma are by bagging or keeping the plant in an insect-proof glass house. This test of pollen viability was first carried out by Crandally (1912) and this is one of the most authentic and accurate tests. Heslop-Harrison *et al.*, (1984) have listed a few

limitations of this test. It is time consuming and laborious since it takes weeks before seed set can be assessed. This test can be used for a limited time period varying from species flowering season. It is more of a quantitative test.

(iii) *In vitro germination*

This is a simple and fully quantitative test for assessing pollen viability. The ability of the stored pollen to set seeds following pollination is correlated with *in vitro* germination percentage (Akihama *et al.*, 1978; Janssen and Hermsen, 1980). For three- celled taxa this test is not applicable because of difficulty in achieving *in vitro* germintion. It is quite common that stored pollen samples which fail to germinate *in vitro* induce satisfactory fruit and seed set (King, 1965; Ghatnekar and Kulkarni, 1978). Thus stored pollen which lose germinability cannot be considered as completely non-viable. On the other hand, pollen showing moderate *in vitro* germination may not induce seed set. According to Stanley (1982) pine pollen showed germination even after 15 years of storage but was unable to induce seed set.

Non-germination Assays

Starring of pollen grains with specific dyes is often used as indices of viability. Dye tests are based on the presence of functional enzymes which are closely related to the metabolic capacity of pollen to respire and grow (Bartels, 1960). Some of them are based on the ability of enzymes to transfer protons and electrons to a colourless substance to change it into a coloured form.

(i) Redox dyes

***(a) Tetrazolium salts*:** This test determines the activity of dehydrogenases mainly. In the presence of enzyme dehydrogenase there is reduction of soluble colourless tetrazolium salt to reddish insoluble formazan (Smith, 1951). The commonly used tetrazolium salts are 2, 3, 5-triphenyl tetrazolium chloride (TTC) and nitroblue Tetrazolium (NBT). Tetrazolium reducing activity can be determined by the method of Burrows and Carr (1969). Pollen of several taxa has shown a positive response to tetrazolium test (Shivanna and Johri, 1985). Hauser and Morrison (1964) reported positive respones of pollen of 16 taxa including *Lilium, Helianthus* end *Oenothera* with NBT which is specific to succinic dehydrogenase. Norton (1966) assessed the viability of plum pollen with 12 different tetrazolium salts and found 3(4,5-dimethyl thiazolyl 1-2) 2,5-diphenyl to show.the best results. There was significant

correlation between germination percentage and pollen viability shown by tetrazolium test.

In some taxa this does not give reliable results. For example stored pollen of *Simmondsia* showed poor *in vitro* germination but 95 per cent of this stored pollen gave positive results with tetrazolium test (Beasley and Yermanos, 1976). A rimilar situation has been observed in some varieties of peach, pear, apple and grapes (Obrele and Watson, 1953).

(b) *Benzidine*: The response of viable and non-via|ble pollen grains to benzidine test vary with different taxa (King, 1960). This test is based on the oxidation of benzidine by peroxidase in the presence of hydrogen peroxide (Shivanna and Johri, 1985). Viable pollen takes blue color, while non-viable remain colorless. However, this test is unreliable for testing the viability of pollen of some plant species (Layne and Hagedorn, 1963; Janssen and Hermsen, 1963; Janssen and Hermsen, 1980). For example, viable pollen becomes blue. Khan *et al,* (1971) observed a close relationship between peroxidase activity in pollen and seed-set in wheat

(ii) Fluorochromatic reaction (FCR) test

FCR test is rapid and accurate for assessing pollen viability and was introduced by Heslop-Harrison (1970). Fluorescence microscope is a must to conduct this test. By this test the integrity of plasma membrane of vegetative cell and esterase activity capable of cleaving the fluorescein ester, fluorescein diacetate (FDA) are assessed. The pollen to be tested is mounted in FDA solution which penetrates the plasmalemma of the vegetative cell and enters the pollen cytoplasm. Enzymatic cleavage within the cell cytoplasm releases fluorescein which gives bright fluorescenee under fluorescence microscope. This test has shown satisfactory results in genera of some family's viz. Compositae, Crucif-erae, Gramineae (Heslop-Harrison, 1970; Heslop-Harrison *et al,* 1984). Pollen grains which lack intact plasmalemma and enzyme activity do not show positive results with this test.

(iii) Estimation of macromolecules

(a) *Prolin*: It has been found that proline contend of amino acid extracts from pollens of many species is very high and is indirect ratio to Its vitality (Linskens and Schrauwen, 1960; Ahokas, 1978; Zhang *et al,* 1982). Palfi and Mihalik (1985) have developed a new isatin staining method by which pollen grains exhibit different colours according to their proline concentration, isatin is known to give an extremely good reaction with proline. In the

pollen grains with high proline concentrations pollen wall stains dark blue or black and when its content is lower, the walls of the^pollen grains assume blue, dense blue or greenish blue colour. When the pollen grains have proline in quite 'samall quantities or in traces, their walls turn brownish yellow or do not get stained at all and retain their original pale yellow colour. According to Palfi and Mihalik (1985) isatin reagent can be used only for species with a proline concentration exceeding 1.0 per cent of the pollen dry matter.

(b) ***ATP:*** The level of ATP is used as a measure for monitoring activities of microorganisms in the food, beverage, drug industries, in medical diagnosis, etc. (Seitz and Neary, 1974). ATP content is correlated significantly with germinability of pollen (Ching *et al,* 1975). Because of simplicity and rapidity of assay procedure (Ching and Ching, 1972) ATP content can be easily used as a good biochemical index to predict pollen viability. ATP is best estimated with luciferase-Luciferin system. Ladyman and Taylor (1988) suggested to use phosphorus (^{31}P) NMR for estimating pollen viability. This provides information on the amounts of organic phosphorus (e. g. ATP) available for metabolism in the pollen.

(iv) Inorganic acids

Koul and Paliwal (1961) have shown that inorganic acids like hydrochloric acid, sulphuric acid and nitric acid induce pollen rupture. This bursting of pollen with acid treatment is due to the movement of ions into the pollen leading to increase in osmotic pressure or rapid swelling of membrane colloids in acid medium (Stanley and Linskens, 1974). Linskens and Mullaneers (1967) demonstrated the formation of instant pollen tubes in response to acid treatment. Pollen was pretreated in the germination medium for 5-35 min before acid treatment (sulphuric acid 4%). This resulted in the formation of instant short pollen tubes. It **is** believed that pretreatment results in weakening of intine, especially at the site "of germpore. The acid treatment results in stretching of the intine to a great extent and leads to the formation of instant pollen tubes. Instant, pollen tubes are different from tube like structures obtained by acid treatment (Koul and Paliwal, 1961). This test shows positive response only in viable pollen and is reliable in assessing pollen viability.

(v) Biophysical test

T_2 measurement: It is a normal feature that the transverse relaxation time (T_2) of protons decreases with a reduction in water content in biological tissues (Kaku *et al,* 1984). Pulse H'-NMR (Nuclear magnetic resonance) measurement of transverse relaxation time of protons

is used to study proton mobility in pollen grains during dehydration (Kerhoas and Dumas, 1988). The T_2 Value increases when the fertilization capacity of pollen grains decreases. So change of proton mobility can be correlated to fertilizing capacities of pollen samples. T_2-measurement is an indirect but non-destructive test of pollen viability and cannot be used as a routine test.

The rationality of methods available for evaluation pollen of viability has been discussed by several workers (Stanley and Linskens, 1974; Heslop-Harrison *et al*, 1984; Knox *et al*, 1986). The various tests used for pollen viability permit testing the capacity of pollen grains to achieve some or all of the sequences of fertilization programme (Gay *et al*, 1987). Most of the tests used for pollen viability do not evaluate the ability of pollen to achieve any particular step of fertilization programme. The only method available so far to test the fertilization capacity is seed set. But this test involves a considerable investment of time and depends not only on pollen viability but also on receptivity and viability of female partner. The ability of pollen to respond to a particular test is dependent upon the inherent chemistry of the pollen. A viability test has to be selected according to the species to be studied and the aim of experiment.

CAUSES OF LOSS OF VIABILITY

Storage of pollen under various conditions leads to reduction in germination capacity and ability of pollen to set seeds. For reduction in germination capacity of pollen during storage can be the result of inactivation of enzymes, reduced availability of metabolic substrates essential for germination, or accumulation of secondary metabolic products such as organic acids, etc. Extensive biochemical studies have been carried out in seeds related with loss of viability (Bewley and Black, 1982). Comparatively a few experiments have been conducted on pollen to delineate parameters that affect pollen viability. Based on the available reports the following have been suggested to be the major factors for loss of pollen viability during storage.

Moisture Status

The water content of pollen at the time of dehiscence is substantially lower than a normal somatic cell. Pollen are usually shed under dehydrated state (water content < 20 per cent), except in members of Gramineae in which the pollen has about 30-60 per cent water content. The environmental factors like temperature and humidity influence the water content of pollen and their viability, for instance in maize, pollen shed in the morning has

about 20 per cent higher water content than those shed at noon (Walden, 1967; Barnabas and Rajki, 1976). Most of the water present in desiccated pollen is in a bound state. Free water content increases in pollen following hydration. The essential prerequisite for pollen germination is rehydration. Uptake of water is governed by a positive feedback mechanism acting to facilitate further ingress of water (Heslop-Harri-son, 1979). The flow of water towards the pollen is governed by the variations in water potential of pollen. A considerable hydrostatic pressure is developed in the pollen with the uptake[1] of water and is relieved either by bursting or by the emergence of tubes.

Pollen increases in size following incubation in the germination medium due to ingression of water. Grewal (1988) observed that stored pollen samples of pea, tomato, maize and sponge-gourd (under different conditions) failed to attain size comparable to that of fresh pollen when incubated in the germination medium. Further, the hydrostatic pressure developed as a result of ingression of water was not sufficient for the emergence of pollen tubes. Grewal (1988) used two parameters, namely per cent germination and tube growth to measure the effects of storage on pollen vigor. Thus, increase in volume (pollen size) during incubation seems directly correlated with pollen viability. The ability of stored pollen to develop internal hydrostatic pressure may be reduced as a consequence of the deterioration of membranes, thereby lowered capability for maintaining required turgor. Moisture content of cells/tissues is a critical factor governing the permeability of membranes (Simon, 1974). But the retention of comparatively high moisture content in pollen during storage at high humidity levels did not help into maintaining pollen viability beyond specific period (Grewal, 1988). This suggests that loss of viability is of an intrinsic nature.

Membrane Permeability

Based on the studies on desiccated seeds, it was suggested that membranes lose their lamellar structure and permeability properties when the moisture level of membrane falls below 20 per cent (Simon, 1978). Rehydration of seeds results in restoration of the membrane integrity. Shivanna and Heslop-Harrison (1981) studied the membrane state in pollen of several taxa exposed to desiccation (5-10 per cent RH), for various periods and correlated it with germinability *in vitro*. The ability of pollen to withstand desiccation varied among different taxa. Pollen grains of grasses are highly sensitive to desiccation. Controlled

hydration provides suitable conditions for restoration of membrane integrity and permits normal germination.

Setia *et al,* (1989) used measurement of electrical conductivity of pollen steep water to evaluate the vigor difference among pollen samples of pea stored at various levels of humidity. There was an enhance leakage of electrolytes and various metabolites from stored samples as compared with fresh pollen. Similar observations were made in pollen of tomato, sponge gourd and maize (Grewal, 1988). Inorganic ions, amino acids, organic and fatty acids, nucleotides, organic phosphates, etc. contributed towards total conductivity. Simple sugars do not contribute to conductivity since they do not carry any electric charge. These results suggested that storage exerted its effect on the functional integrity of membrane. A significant negative relationship between loss of pollen viability and electrolytes leakage has been observed (Setia *et al,* 1989). The differential degree of leakage from pollen stored at different humidity levels might be due to different rates of water uptake and hydration of cellular compartments (Dumas *et al,* 1986; Grewal, 1988).

Hoekstra and Roekel (1985) reported a close relationship between loss of pollen viability and increase in potassium leakage during storage at 22°C irrespective of the widely divergent longevities of pollen species concerned. Ching and Ching (1964)'observed increased leaching of carbohydrates, phosphorus and amino acids from freeze-tdried pollen. Similar increase in leaching of various metabolites into culture medium from freeze-dried pollen of *Lilium* was observed by Davis and Dickinson (1970). Jain and Shivanna (1988) studied the effect of pollen storage .in organic solvents, pollen stored in organic solvents with low dielectric constant (a measure of their non-polar nature) showed high degree of germinability and very little leaching of metabolites. The storage of pollen in organic solvents with high dielectric constant (a measure of their polar nature) exhibited extensive leaching and little germination. According to these workers organic solvents resulted in loss of pollen viability by exerting their effect on lipid composition which in turn affects membrane integrity. Membranes constitute the principal boundary between living organisms and their environment. Many sequential processes such as protein synthesis and electron transport (in respiration) require a high degree of structural order. The increased leakage of electrolytes and metabolites following storage might be due to low availability of metabolic energy for membrane transport mechanism and maintenance of cellular integrity (Abu-Shakra and Ching, 1967; Anderson, 1970; Priestley and Leopold, 1979).

Mckersie and Stinson (1980) showed that the activation energy of a cell reduces markedly when the water content of the tissue falls below a critical level. Thus, definite water content is required to maintain the normal architecture of the membrane. Biophysical experiments on dehydrated membranes have shown that lipid bilayer conformation is not adopted by the membranes when their water content is below 20 per cent (Simon, 1978). In the dry state, membrane is in hexagonal shape and it changes to lamellar phase upon hydration (Pollock, 1909). Further, due to changed composition of lipids, the membranes fail to adopt a lamellar configuration upon hydration. According to Tappel (1965) lipid peroxidation is a primary cause of membrane deterioration. Pammenter *et al*, (1974) could very successfully extend seed viability by providing a source of electrons which could pair with unpaired electrons of free radicals. The usefulness of iodine treatment prior to storage of sponge-gourd pollen has been demonstrated by Grewal *et al*, (1987).

Iodination improved germination as well as tube elongation of pollen over the stored non-iodinated pollen. This treatment also influenced membrane behavior as reflected by decreased electrical conductivity of pollen steep water. The role of iodine vapors in improving germinability of pollen might be the result of its reactions with the accessible C-C double bonds (Tappel, 1965). The usefulness of iodine treatment for maintenance of vigour and viability in seeds of several crops has been demonstrated by Basu and Rudrapal (1980). It appears that for the successful storage of pollen which loses viability rapidly, attempts should be made to maintain the stability of membranes during storage.

Biochemical Changes

During germination and tube growth of pollen an active array of metabolic reactions is initiated to bring about the mobilization of, endogenous substances. The pollen tube generally emerges as extension of intine which is probably achieved partly by hydration resulting in the weakening of hydrogen bonding between microfibrils and partly by the activity of wall held hydrolases. Several physiological and biochemical processes are initiated after landing of pollen on stigma or when pollen is put in the germination medium. These include ingression of water, initiation of respiration, aggregation of ribosomes into polysomes, activation of some enzymes, initiation of protein synthesis, and nucleic acid metabolism, etc. Soon after tube emergence, new wall materials have to be inserted in an orderly fashion for the continued growth of the tube (Heslop-Harrison, 1979).

Recently some detailed studies have been carried out in this laboratory on changes in the content of sugars, amino acids, proteins and activity pattern of some enzymes in fresh and stored pollen of pea, sponge-gourd and tomato with a view to -relating these intrinsic changes with the loss of pollen viability (Grewal, 1988). Following is a summary of the changes in the levels of various metabolites and enzymes that occur in pollen during storage.

***Sugars*:** The level of sugars becomes low during storage. Identification of various sugars in fresh and stored pollen samples by paper chromatography indicated that in stored pollen fructose was comparatively high. The level of these sugars decreased as a result of their utilization in respiration process during storage (Grewal, 1988). It is known that stored pollen of various plant species requires higher concentration of sugars for normal germination (Johri and Vasil, 1961). Grewal *et al,* (1989) observed increased germinability of stored pea pollen on supplementation of medium with glucose, and sucrose proved ineffective. The increased sucrose concentration required to obtain optimal gremination has been attributed to a decrease in pollen permeability (Kuhlwein, 1937). Stanley and Poostachi (1972) carried out analysis of carbohydrates and organic acids in the fresh and stored samples of *Pinus* pollen. It was observed that pollen which maintained better germinability had a higher level of low molecular weight sugars and organic acids.

Amino acids: Significant changes in the amino acid composition in pollen following storage have been reported by various investigators (Linskens and Pfahler, 1973; Nielsen, 1956; Gerwal, 1988; Grewal *et al,* 1989). Dashek and Harwood (1974) correlated loss of pollen germinability upon storage' with decrease in endogenous level of proline. However, Shellard and Jolliffe (1968) did not find any relation between loss of pollen viability and changes in amino acid composition during storage in wheat (Palfi and Mihalik, 1985). Decreased level of various amino acids following storage shows poor availability of organic nitrogen. Supplementing the germination medium with amino acids like glutamic acid, aspartic acid and proline caused significant improvement in germinability and tube elongation of stored pollen (Grewal *et al,* 1989, Grewal, 1988; Dashek and Harwood, 1974).

The precise mechanism(s) associated with changes in amino acid composition during storage is not clear. But from the alterations in amino acid composition of stored pollen it is apparent that metabolic changes continue to occur during storage. The presence of transaminases has been established in. pollen (Sawada, 1960; Stanley and Linskens,

1974). Since varying responses are obtained for amino acid composition in pollen of different plant species during storage, it seems that the content and composition of amino acids could not be used as an accurate indicator of pollen viability.

Proteins: Biochemical studies have shown that protein synthesis is initiated during early stages of pollen germination before tube growth (Mascarenhas, 1975; Malik *et al*, 1979). Changes in level of total soluble proteins in fresh and stored pollen samples of pea and tomato during germination have .been investigated by Grewal (1988). She observed that the magnitude of increase in level of soluble proteins remained low following germination of stored pollen samples as compared with that of fresh. Studies on the banding pattern of proteins in fresh and stored pollen samples using acrylamide gel, electrophoresis has revealed variations in number and intensity of bands (Grewal, 1988). According to Bingham *et al*, (1964) age and prior handling of the pollen can modify the protein pattern. The poor ability of stored pollen samples to synthesize proteins might be the result of biochemical lesions in the components of RNA and protein synthesizing systems.

***Lipids*:** Since pollen grains become leakier following storage, therefore, information on the changes in lipid metabolism appears fundamental to understand the loss in viability. The viability of seeds of many plant species has been linked with losses of phospholipids especially phosphatidyl choline (Priestley and Leopold, 1979). But information on the changes in lipids during pollen storage is scanty. Lipids, particularly phospholipids, play an important role in molecular organization of the membranes (Simon, 1974). Any change in phospholipid composition will influence the maintenance of membrane fluidity (Chapman, 1975), the activity of membrane associated enzymes (Sandermann, 1978) and ion permeability and its selectivity (Hall and Baker, 1975; Kupier, 1975). Lomonova *et al*, (1977) studied the lipid composition of *Pinus sibirica* pollen and found a relationship between its lipid content and viability. Hoekstra and Barten (1985) reported accumulation of free fatty acids prior to loss of vitality in Pollen of *Narcissus* and *Typha* during storage.

The use of antioxidants and local anaesthetics could not prevent these changes. Jain and Shivanna (1988) observed considerable leaching of pollen lipids into organic solvents during storage. They pointed out that organic solvents affect the viability of pollen by exerting their effect on phospholipid composition which in turn affects membrane integrity. Grewal (1988) observed decline in the level of total phospholipids and glycolipids and increase in sterol: phospholipid ratio in pollen of pea stored at various humidity levels.

Studies on changes in the lipid content of senescing tissues have been frequently concentrated on phospholipid and sterol composition (Chia *et al*, 1981; Mckersie *et al*, 1979). Most of the phospholipids of the cell are located in the membranes (Singer and Nicolson, 1972). Therefore, a loss of phospholipids must affect the membranes. In seeds, ageing is said to be the result of deteriorative changes in the membranes probably via peroxidation reactions involving unsaturated fatty acids (Parrish and Leopold, 1978; Stewart and Bewley, 1980) similar changes might be taking place in pollen during storage.

Enzymes: Pollen contains large number to enzymes to metabolize external and internal substrates essential for germination and tube growth. The ultimate role of different enzymes is to facilitate tube growth (through, the stylar tissue (Stanley and Linskens, 1974). The storage substances such as lipids, starch, sugars, phenolic glycosides, proteins, etc. are hydrolyzed and utilized for respiration, pentose phosphate pathway, building of wall materials of pollen tube, etc. (Malik, 1985). Loss of viability of pollen during storage also appears to be the result of changes in activities of various enzymes and very little information is available on this aspect. Haeckel (1951) reported decrease in activity of some amylases and phosphatases in pollen of several taxa stored for one year. Grewal (1988) assayed the activities of different enzymes in ungerminated and germinated fresh and stored pollen samples of pea, tomato and sponge-gourd and correlated their changes with loss of pollen viability.

There was reduction in the activity of invertase, acid phosphatase, glucose-6-phosphate dehydrogenase, malate dehydrogenase, peroxidase and catalase following storage and very slight increase was observed during germination of stored pollen as compared with fresh pollen. The low invertase activity indicated poor utilization of carbon source. Acid phosphatase is one of the representatives of the lytic compartment of cells. Mascarenhas (1975) ascribed the role of this enzyme to facilitate the penetration of pollen tubes in the stigma. The low activity of malate dehydrogenase indicated reduced respiration. This v/as also supported by decreased tetrazolium reducing activity in stored pollen of all the tested plant species (Grewal, 1988; Stanley and Linskens, 1974). Respiratory activities reflect several complex and interrelated metabolic processes that take place during pollen germination.

Respiration in relation to pollen vigor can be considered as a useful tool fOr evaluating pollen viability. Reduced activity of glucose-6-phosphate dehydrogenase (a key enzyme of

pentose phosphate pathway) in stored pollen indicates poor source of reducing power and various pentose phosphates needed for biochemical reactions (Grewal *et al,* 1980; Setia *et al,* 1989; Setia *et al,* 1990). There were also reduction in-activities of peroxidase and catalase in stored samples. These enzymes are involved in defense of aerobic cells against H_20_2, a product of partial reduction of oxygen. In pollen of certain plant species, a short exposure to temperature below freezing temperature is found to increase its germination capacity. This has been attributed to an increase in or release of endogenously bound enzymes (Stanley and Linskens, 1974). The decreases in activities of various enzymes during storage are difficult to explain as several factors are involved in regulating the activity pattern of enzymes (Schopfer, 1977).

Growth Hormones

Loss in viability could also be the result of loss of ability of pollen to provide essential hormones that govern the germination and tube growth, of pollen. The occurrence and role of various hormones in regulating pollen germination and tube growth are , well established (Stanley arid Linskens, 1974; Chhabra and Malik, 1978; Malik and Bhandal, 1983). The improvement of germinability of stored pollen samples of pea (Grewal *et al.*, 1989), tomato and sponge gourd (Grewal, 1988) with exogenously supplied IAA, GA_3 and cytokinins suggests that various storage conditions also affect the endogenous level of hormones. However, there are no reports available on the endogenous levels of various hormones in stored pollen. Information on the ultra structural aspects of viability losses in stored pollen is completely lacking. It appears that loss in viability is accompanied by damage to various sub-cellular organelles. One of the effects of storage is clearly visible that is membrane damage as shown by increased leakage of cell constituents.

CONCLUSIONS

Different storage conditions affect the viability and quality of pollen. In fact pollen quality is a parameter which is rather tedious to evaluate. By and large pollen viability and longevity are monitored through cytologic (FCR), physiologic (measurement of water content through NMR, *in vitro* germination), biophysic (^{32}P-NMR) methodologies. In most studies any one of the above tests are preferred to assess viability of pollen after specific period of storage which is usually in months or years. It is highly desirable to employ multidisciplinary assaying for evaluating pollen quality regularly at defined intervals.

There is a overlapping of gametophytic and sporophyt.c gene expression up to 60 per cent (Ottaviano *et al*, 1988). But the relationship between physio biochemic levels is not vivid. Is it that pollen storage is associated with some sporophytic trait(s)? It is pertinent to investigate the relationship between gametophytic and sporophytic generations especially in systems like maize where pollen is exposed to intense pollen genotype selection during their development. Pollen selection might attain a high proportion for production of high quality true potato seed (TPS) since TPS appears to be viable alternative to low quality seed tubers in many regions. The high level of sporophytic heterogeneity in the tetraploid potatoes generally causes undesirable phenotypes in a TPS progeny. Modifications of genotype frequencies through pollen selection would be highly useful for progeny performance. In potato, hence, different techniques have been developed and applied as pre-storage treatments e. g. dried before freezing and freezing without drying.

Following pollen storage under various conditions several physiological, biochemical and structural changes occur. Pollen membrane becomes leakier as evidenced by enhanced leaching of electrolytes and metabolites. If pollen is subjected to humid air treatment, prior to incubation in the germination medium, germination is improved considerably. The composition of fatty acids of phospholipids of stored pollen does not differ significantly from the fresh pollen. However, it is reported that the content of phospholipids decreases and free fatty acids (FFAs) increase in pollen during storage. Recently we have shown that iodination of pollen prior to storage can prolong pollen viability. It will be interesting to evaluate whether FFAs accumulate prior to loss of viability and that antioxidants could prevent and/ or delay the process. The role of anaesthetics in prolonging the pollen viability should also be examined. Generally several organic solvents have been successfully used for pollen storage. We need to know whether organic solvents can be used for penetration of antioxidants and other chemicals into pollen for improving longevity and quality.

FURTHER READING

1. CSIR, New Delhi. *Plant Embryology.*
2. Council, New Delhi. *Plant Embryology: A Symposium.*
3. Berry A. k, *An Introduction to Embryology*. Emkay Publications, New Delhi.
4. P. Maheshwari. *An Introduction to Embryology of Angiosperms*. Tata Mc. Graw. New Delhi.
5. Johri, B.M. Bhojwani. *Embryology of Angiosperms*. Vikas, New Delhi.

6. B.P. Panday. *Embryology of Angiosperms*. S. Chand, New Delhi.
7. Bhojwani, S.S. Bhatnagar, S.P. *Embryology of Angiosperms.* Vikas Publishing House Pvt. Ltd. New Delhi.
8. Johri, B.M. Bhojwani, S.S. *Embryology of Angiosperms*. Springer, New Delhi.
9. B.M. Johri. *Experimental Embryology of Vascular Plants*. Narosa, New Delhi.
10. Johri, M. *Experimental Embryology of Vascular Plants*. Springer Verlos, New York.
11. Varghese, T.M. *Introduction to Experiments and Applied embryology of Angiosperms.* Oxford, IBH. Publishing Co. Pvt. Ltd. New Delhi.
12. P. Maheshwari, *Introduction to Embryology of Angiosperms.* Tata Mc. Graw Hill Publication Co. Pvt. Ltd. New Delhi.

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