Chapter 1 Introduction

Plant embryology is a basic science， which studies the morphogenesis in the process of embryonic development and sexual reproduction of plants.

Researches on the sexual reproduction of higher plants have been lasting for more than 100 years since 1824，when G. B. Amici found the germination of pollen on stigma，the growing behaviors of pollen tubes and their functions in fertilization. It had formed a complete scientific system by the first half of the 20th century， including descriptive embryology， comparative embryology and experimental embryology .

Since the 1960s， significant changes on the subjective characters have taken place in the researches of plant embryology. Research of plant embryology has gone beyond the traditional morphological category and gradually became an inter-disciplinary and comprehensive branch of science，because it has combined with its related branches of science，especially with cell and molecular biology，and because of the rapid improvement in research for its technology，such as the employment of various methods in cellular biology.The modern plant embryology is also designated as plant reproductive biology，which can further embody its connotation.

In China，researches on plant sexual reproduction began comparatively late .There were rare researches in this field in the first half of the 20th. Since 1950s， plant embryology， as an important branch in the system of plant sciences，has attracted more and more attention .In a period of over a decade after that，a special contingent of Biologists was gradually formed for both research and teaching on the plant embryology.

In the 1980s，the Chinese plant embryologists had kept in step with the developing trend from plant embryology to reproductive biology in the world and had carried out many researches on the advanced subjects of this branch .At present，the research work on plant embryology and reproductive biology in China has gained some repercussion in the world. *Acta Botanica Sinica* is an important journal in issuing botanical research achievements in China.

The developmental process of plant embryology in China can be roughly divided into three stages: the initial stage，the establishment of plant embryology as a branch of science，and developing from plant embryology to reproductive biology and its vigorous development.

1 The Initial Stage of Plant Embryology

During the first half of the 20th century，botanical researches on the morphology of sexual reproductive development were quite rare. Up to 1950， there were only about 10 original papers on this subject .In 1930，Li published *The Early Embryology of Hosta sieboldiana* and may be considered today as the pioneer Chinese scientist in plant embryology. Later，several other papers were issued and mainly concerned the experimental embryology.

The embryological researches at their initial stage in China were mainly targeted to gymnosperms. Those on the embryo cultures belonged，at the world advanced level，to the

area of experimental embryology.

2 The Stage of Plant Embryology Establishment

In the beginning of the 1960s， plant embryology was gradually established as a branch of botanical science in China. Here I should particularly mention the pioneer Chinese plant embryologist，Professor WANG Fu-Xiong who devoted his life to the research work on plant embryology since the 1940s .

In the 1950s，the Chinese academic circle was influenced considerably by the science and technology from the former Soviet Union. At the early developmental stage of plant embryology，the Russian scientists had done much excellent works. For example，the double fertilization in angiosperms，which was taken as an important milestone，was found in 1898 by the known Russian botanist S .G. Nawaschin .

At this stage，a rapid development marked experimental researches on the plant embryology (mainly in anther culture) as well as in descriptive researches， including gametophyte development， fertilization， embryos and endosperms development. Investigations on gymnosperms were mainly done by WANG Fu- Xiong and his collaborators.

At the same period， researches on angiosperms were mainly focused on the economically important plants. These embryological data constitute valuable references to plant cultivation and breeding.

Researches on some specific issues and new applicable techniques are highlighted as follows. (1) Researches on sexual crossing process. (2) Researches on the polyembryogeny. (3)The dynamics of starch accumulation in the process of fertilization and grain development of wheat，and the variation of DNA and RNA in the process of sexual cell fertilization，using the method of cytochemistry of polysaccharides，DNA and RNA.

These researches initiated the application of cytochemical methods to the studies on the variation of storage of substances and genetic materials.

In short，a foundation was laid，from training of personnel to the development of research work， for plant embryology as a branch of botanical science in China by the efforts made in more than 10 years after 1950s.

3 The Stage for Evolving Plant Embryology to Reproductive Biology and Its Vigorous Development

The last few years of 1970s were characterized by the normalization of all works after the disaster of the Great Cultural Revolution .In this short period，a number of new achievements were scored on the research of plant embryology.

In the 1980s，researches developed rapidly in plant embryology，which was embodied by the increase of research personnel and the improvement of research techniques. Cytochemistry and electron microscopy were extensively applied to the researches of plant embryology.

In the recent two decades or more，researches were conducted on many subjects and a huge

number of papers were published in this period which became a historical stage in the development of plant embryology in China. Because there is a considerable difference in the sexual reproduction of angiosperms and gymnosperms， and researches on them are distinct from each other，it is easier to introduce those data separately as follows.

3.1 Researches on the angiosperm embryology

In the recent stage of researches on angiosperm embryology，efforts have been focused both on the basic research at the optical microscopic level and further advanced studies on descriptive and experimental embryology of the essential reproductive process， employing new techniques in cell biology .In addition， many basic researches related to plant breeding have also been carried out.

3.1 .1 Researches on double fertilization at microscopic level

Researches on double fertilization， even at the microscopic level， were very few before 1970s.

3.1.2 Researches on the correlation between structures and functions in the process of reproduction

Based on the descriptive embryology and starting with fine structural studies，much research work on the correlation between structures and functions has been achieved. Global researches in this subject began in the 1960s， mainly with electron microscopic observations and other techniques of cell biology， had unveiled quite a number of phenomena in a period of more than 10 years .

The achievement has indeed gained new cognizance on the relationship between the structures and functions of the reproductive process .In China， this scope of research started around 1970s and since then it has been undergoing continuously as a quite active subject .The followings are brief introductions to some of the intensive research among a large number of research subjects.

3.1.2 .1 Researches on developmental structures of microspores and male gametophytes

(1) Dynamics of vegetative nucleus and generative cells.

(2) The ultrastructures of cytoplasm in the process of microsporogenesis. It was confirmed that significant changes took place in the morphology of both chloroplasts and mitochondria during meiosis.

There were many significant achievements in the researches on the ultrastructures in the development of microspores and male gametophytes. For examples， (1)the analysis on the relationship of cell organelle to morphogenesis based on the Dynamics of cell organelles at a specific developmental stage.

(3) The analysis on the relationship of cell wall structures and properties with cytoplasm differentiation of microgametophyte based on the changes of cell structures and properties of generative cells during its formation and development;(3) the understanding of the structural characteristics in the pollen wall of orchids explaining the mechanism of pollinium formation by pollen adhesion;

(4) The analysis on the functions taking place in the tapetum during pollen development based on the cytochemical and ultrastructural changes of the tapetum.

3 .1.2 .2 Ultrastructural researches on fertilization

Taking the fertilization process as the central link in sexual reproduction， a large amount of researches in the world since the 1960s have been focused on the ultrastructures of male and female gametophytes， pollen germination and pollen tube growth， gamete fusion and zygote formation， etc.

Many phenomena，which could not be seen under the optical microscope，were visualized at an ultrastructural level. In the recent 20 years， quite a few researches on this aspect in China had reached the advanced level of the world . And some of them will be briefly mentioned.

(1) Sperm cell and male germ unit (MGU)

The concept of MGU was put forward in the 1980s. It means that a pair of sister sperm cells maintain a close linkage with each other， and keep physical association with the vegetative nucleus .

It is believed that their function is to ensure a pair of sister sperm cells in a synchronous action when they are moving in the pollen tubes and released in the embryo sacs. The structures of MGU were well understood through the ultrastructural researches of sperm cell.

Based on the results from observations under scanning electron microscope， the role in preventing fusion among sister sperm cells was attributed to the MGU.

(2) Matured embryo sacs and female germ unit (FGU)

The term of FGU was put forward simultaneously with the MGU. In matured embryo sacs， there was a typical cell wall at the end of micropyle while there was a relatively large area exposing plasmic membrane in the bordering part of chalazal end， between synergid and central cell.

By this way， the egg apparatus (egg + synergids) and central cell formed a structural unit designated as FGU. The structures of FGU showed that there was no cell wall in the target area of fertilization， which can be taken as the guarantee for the fusion of male and female gametes in the condition of protoplasts. In the recent 10 years， many researches were conducted on the ultrastructures of matured embryo sacs in China.

It was pointed out that the FGU was a temporary structural unit， which disintegrated after the completion of fertilization. Thus， the function of FGU was further clarified.

(3) Fusion of gametes and its influence on the cytoplasmic inheritance pattern

In the 1960s， a model， stipulating that the fusion of nuclei of male and female gametes occurs through the fusion of membrane ， was established based on the results from ultrastructural observations.

By the 1980s， a preliminary study revealed that the contact of cytoplasmic membrane of male and female gametes was a prerequisite to the cell fusion. However， this subject， in China， recorded very few investigations. The ultrastructural changes in the fusing process of sperm nucleus and egg nucleus were limitedly described in wheat fertilization.

At present， whether male gamete cytoplasm participates in gamete fusion is still debatable.

Nevertheless，whether the male cytoplasmic transmitted in gamete fusion influences the modes of cytoplasmic inheritance.

In view of the characteristics of the significant differences in the morphological structures of plastids and mitochondria in sperm and egg cells of *Pelargonium hortorum*， these organelles from both sperm cell and egg cell could be identified after fertilization .Thus， this proved that the sperm cytoplasm has participated in fertilization and transferred its cell organelles containing DNA into the zygote. This result provides cytological evidence of the biparental inheritance.

The biparental inheritance is an inheritant pattern found in relatively few species of angiosperms.

The cytological mechanism in the different modes of cytoplasmic inheritance has recently been a new research subject for more than 10 years. Many researches on this aspect have been conducted in China using electron microscope and DNA fluorescence methods， and provided important data on cytological evidence supporting the cytoplasmic maternal inheritance， which is dominant in angiosperm， and the rather few biparental or paternal inheritance in angiosperms.

In addition，there were also some enzymological studies on the DNA degrading mechanisms in male plastids， and the determination of the cytoplasmic inheritance pattern by RFLP method.

(4) Researches on the pollen tube passage through pistil

In the programic phase of fertilization， the process revealed that cells of transmitting tissue possessed vigorous secretory activity，in which secretion was transported to large intercellular space or to the style canal. The intercellular space and the style canal were filled with matrix， that provide materials essential for pollen tube growth.

The micropyle had always been just considered as a simple aperture through which the pollen tube gets into an ovule. However， ultrastructural studies had confirmed that the micropylar is a complicated structure. The cells on both sides of the micropyle are physiologically active，showing their functions in providing essential conditions for the growth of pollen tubes. It seems that the micropyle， like the style， includes two types of opened and closed ones.

3.1.2.3 Researches on cytoskeleton in reproductive system

The cytoskeleton is involved in the realization of many functions in plant reproduction. Researches on this aspect started in the 1970s in the world， mainly on pollen tube with the focus on microtubule and microfilament. In China， researches in this field were carried out in the recent 10 years or so， with works at the world advanced leve1.

3 .1 .2 .4 Researches of transfer cells in reproductive system

The transfer cell was reported in 1969 as a new type of plant cells， which is mainly characterized by its wall ingrowth. Several years later， many researches confirmed that the transfer cell is a cell type adaptable to apoplasmic transport .

Their structure is favorable for transportation of solute between cells and their external

environments. In the reproductive cycle of higher plants， it is highly significant that the transfer cells garantee the effective transportation of nutrients between parent and progeny generation， which had no structural connection with each other， at a certain stage during generation transfer of sporophyte and gametophyte.

In the recent 10 years or more，many researches on the ultrastructures in sexual reproductive system have provided examples describing the extensive distribution of transfer cells. The earliest observation of the stylar canal cells revealed wall ingrowths on the secretory face of the canal cell.

In addition， it confirmed that the transfer cell is a cell of typical secretory type by its organelles and characteristics activity.

All these researches have cleared the functions of transfer cells in short distance transportation in the reproductive system on the one hand， and that they are helpful to understand the pathway in transportation of material during reproductive process on the other hand.

3 .1 .2 .5 Researches on the cell degeneration in ovule and endosperm

As normal phenomena， the nucellus tissues degenerate continuously during the developmental process of embryo sac and the surrounding tissues (mainly endosperm) of embryo degrade gradually as well.

Many researches have been engaged in the significance of those normal phenomena in the recent 20 years.

Such data are extremely valuable in the description of the changes of the supply-demand relationship of nutrients during the developmental process of embryo sac and embryo. In addition，there are some researches on the ultrastructural changes and the variations of ATPase and acid phosphatase in the process of nucellus degeneration. These data supported the viewpoint that the nucellus degeneration belonged to the programmed cell death， which is genetically controlled.

3.1.3 Researches on several physiological activities in reproductive processes

3 .1.3 .1 The functions of Ca^{2+} in fertilization process

The Ca2+ plays an extensive role in plant physiological activity. Yang had reviewed the intimate relationship Ca2+ with fertilization and its current research trends. In China， researches on this aspect were carried out for more than 10 years.

In the recent years， the characteristics of time and spatial distribution of CaMmRNA and CaM protein in the developmental process of rice anthers and pistil were examined by in *situ* hybridization and immunohistochemical localization， respectively. It is proven that the CaM gene expression intensity varied with the different developmental stages.

In the researches of the Ca^{2+}distribution in embryo sac before and after fertilization in *Nicotiana tabacu*， a specially significant phenomenon was recorded，i .e.，there appeared a temporary zone with rich CaM between egg apparatus and central cell before fertilization. This zone was similar to the location of actin corona that had been observed.

The actin corona was believed to have a role in the translocation and fusion of gametes.

Thus，it is inferred that the CaM also is involved in this process. It is highly significant for further investigations on the relationship between the expressions of CaM and CaMmRNA and some physiological activities in the process of fertilization.

3.1.3.2 ATPase participating in the transrport of materials in ovules

ATPase，as a carrier in organisms，participates in the process of energy conversion，in metabolism，transportation of substances and transmission of information，etc.

Researches on the involvement of ATPase in metabolism of biological substances in the reproductive process were focused on the distribution of ATPase in ovule and a mbryo sac for further understanding the ways of transporting maternal materials into embryo sac. Some Chinese workers have put forward the possible ways for transporting nutritional substances into embryo sac in several plants based on the observations by ultracytochemical localization of ATPase activity.

It is believed that the hypostase，the intercellular space of embryo sac wall and the micropyle passage join together，forming a complicated apoplasmic channel transporting nutrients to the egg apparatus in *Antirrhinum majus*. In *Helianthus annuus*，the whole embryo sac surface was found to enable absorption of nutrients，especially at the surface of the central cell with wall ingrowth. In *Vicia faba* , the chalaza is the most important site for transporting nutrient material to the embryo sac .

In addition，in *situ* studies on the localization of ATPase in stigma cells of *Populus lasiocarpa* revealed the presence of ATPase in the pellicle. Thus，it is believed that the ATPase plays a role in the interaction between pollen and stigma. In the fertilization process of wheat，ATPase activities were noted in cells of the mature embryo sac，and it varied before and after fertilization which is believed to be associated with the variation of metabolic conditions of embryo sac cells.

3.1.3.3 Researches on the recognition proteins of stigma pellicle and plasma membrane of sperms

In the fertilization process of angiosperms，incompatibility is dependent first on the recognition reaction between pollen and pistil，and then，on the recognition between gametes.

Researches on recognition mechanisms in fertilization of angiosperms were carried out in the 1970s in the world，but，in slow progress .In China they have been conducted since the 1980s，and focused on identification and analysis of the recognition substances .

As for the relationship between pollen and pistil，the glycoprotein isolated from pollen wall and stigma pellicle，and its compositions，were analyzed. Lectin was extracted from pistil，which could probably be involved in the rejection of distant pollen by the pistil. In addition，some distant hybridization experiments have been conducted on exploring the interaction between pollen and stigma .

The cross compatibility of stigma was estimated based on the reaction of stigma to various ways of pollination. For the research on gametes recognition，isolation of cytoplasmic membrane protein of sperm cell，and detection and purification of specific protein were conducted in several species .

Analysis of their compositions proved that the sperm plasma membrane contained multiple glycoproteins，possibly including some lectin recipients. These data were valuable for further research on the role of glycoproteins in adhesion and conjugation induced by gamete recognition.

3.1.4 Researches on embryology related to plant breeding

3.1.4.1 Male sterility

The male sterility can be expressed in several different forms. At present， mainly the cytoplasmic and nuclear male sterility is applied to plant breeding. Researches on male sterility have been carried out on many aspects in order to tap its potentiality in plant breeding.

(1) Cyological studies on male sterility

In the 1970s， when researches on the mechanisms of male sterility was vigorously undertaken in the world，a scientific research group in Peking University conducted optical and electron- microscopic observations on the microsporogenesis in male sterile and its maintainer lines of wheat for the first time.

Some abnormal phenomena were revealed in the development of sterile anthers and pollen .Under the electron microscopic observations， abnormal activities were found in vacuome system of microspores，which showed the destination of degeneration in microspores at a relatively early stage.

After that， the ultrastructures in Taigu nuclear male sterile wheat was reported， which revealed an association of pollen abortion with endoplasmic reticulum. Later on，there were many reports on optical and electronic microscopic observations on pollen abortion in sterile lines of many crops.

The abnormal phenomena of various kinds in the performances of anthers and pollen of male sterile plants were all not beyond the scope of abnormal development of the structures of filament or anther vascular bundles， tapetum or endothecium of anther wall， blocking of microspore meiosis，and other blocking of development in microspores or pollen . All those abnormal phenomena were actually resulted from the expression of male sterility gene(s).

(2) Physiochemical researches on male sterility

The abnormal physiological activity is expressed in various forms in male sterile plants .The researches on the cytoplasmic male sterile *Brassica napus* confirmed a disordered active oxygen metabolism in *vivo*. High Ca^{2+} concentration was found in cytoplasm of abortive pollen in photoperiod sensitive cytoplasmic male sterile wheat.

The increase of organic free radicals was noted in anthers during the abortion of microspores in male sterile rice. The free proline concentration was low in anthers of nuclear male sterile Taigu wheat. There were many researches on photoperiod sensitive genic male sterile rice .Such abnormal physiological phenomena were described in detail in the review by Tong，in which he particularly described that the male sterility is related to the concentration of endogenous auxin.

It may be concluded that the metabolism confuse is controlled by the male sterile gene(s).

(3) Molecular biological and genetic researches on male sterility

Based on the fact that cytoplasmic male sterility was expressed as one of the characteristics of maternal inheritance，researches on the mechanism of male sterility have been focused on DNA of mitochondria and chloroplast since the 1980s in the world.

A large amount of work has confirmed that the DNA in mitochondria is associated with male sterility. In recent years，the Chinese researchers have started researches on this subject from various aspects，such as the relationship of male sterility with mitochondrial genome at molecular level，and comparative analysis on mitochondrial DNA in different types of cytoplasmic male sterile lines.

The researches on the relationship of male sterility with its inheritance in photoperiod sensitive genic male sterile rice have confirmed that the expression of photoperiod sensitive male sterile gene(s) was dependent on the expression of photoperiod sensitive gene(s)，and the fertility was affected by the interaction of non-allelic gene(s).The sterile gene(s) was localized on the third chromosome shown by RFLP method.

More researches were focused on the expression of specific photoperiod sensitive genic sterile gene(s) by means of purification and sequencing analysis of specific proteins related to sterility. Significant results have been obtained in this aspect.

In addition，creation of male sterile lines and restorers by genetic engineering was also attempted .Ste rile gene has been successfully integrated into *Nicotiana tabacum* and *Brassica napus*.It is proven that the exogenous gene-specific expression is resulted from an early degeneration of tapetum in sterile plants of trans-genic *N .tabacum*.

3.1.4.2 Apomixis

Apomixis is present in sexual reproductive process.

The genotype of progenies produced by this reproductive way of non-fertilization is completely the same as which of the maternal parent.

Since the 1970s，the research on apomixis was oriented to its application to genetic breeding based on an attempt to use this characteristic in searching for a simpler heterotic breeding system. In the late 1980s，Chinese plant breeders suggested to use apomixis as a way of fixing heterosis，which attracted great attention of rice breeders.

Large amount of researches were carried out on searching of apomictic materials (resources)，which becomes the most vigorous activity in the research on apomixis recently in China.

(1)Embryological studies on polyembryonic seedling of rice lines

It is reported that the polyembryos and polyseedlings of rice lines had different origins，i.e.，egg cell，egg-like cell and synergid. They were either fertilized or nonfertilized. It might result from the variation of the embryo sac. The presence of more than one embryo is often resulted from fertilization of more than one egg，in the embryo sac and is by no means from apomixis.

In addition，anatomical observations on the polyembryonic seedlings of rice lines showed that the polyseedlings were composed of a main seedling and its auxiliary shoots developed from one embryo. From observations on polyembryos and/or polyseedlings in several plants with apomictic characteristics，it is concluded that polyembryos and polyseedlings can be used

as an indicator in searching for apomictic materials.

(2) Embryological studies on spontaneous and induced apomixis

Apomixis can be induced by culturing non-fertilized ovaries or ovules. Cytological data were obtained on the initiation of apogamy and parthenogenesis from embryological researches when those successful experiments were conducted.

It was recorded，long ago， that *Allium tuberrosum* had a high frequency in parthenogenesis and apogamy of antipodal cell. Electron microscopic observations on its ultrastructures showed that its egg and antipodal cell could spontaneously develop into embryo. In addition， embryological studies were also conducted on the species or lines with apomixis， such as *Sorghum bicolor*， *Pennisetum squamulatun and Boehnrria Silzestrii*. Their apomictic types were also determined.

All those basic embryological data are valuable for promoting the application of apomixis to plant breeding.

(3) Embryological studies on distant hybridization

In recent years，experiments on distant hybridization were stressed on transferring beneficial characteristics (including apomixis) of wild species into cultivated plants and on inducing haploids.

Sun and his collaborators have established a complete set of effective methods for producing haploids from wheat egg cells in wheat X maize. This method adopted the characteristics of insensitivity of maize to the hybridizable gene(s) of wheat and spontaneous elimination of maize chromosome in zygotes.

New homogenous dormant genic Taigu wheat germplasms which do not exist in the nature were obtained by hormone treatment， rescue and doubling of chromosomes to the haploids from the experiments on the hybridization between Taigu sterile wheat and maize.

Embryological observations on the hybridization between *Avena muda* and maize confirmed a certain frequency of fertilization and elimination of maize chromosome in zygotes. Embryological observations on the hybridization between wheat and *Leymus secalinus* also showed a possibility of distant hybridization for transferring beneficial genes.

3.2 Gymnosperms

The embryological researches on the gymnosperms started relatively early in China's plant embryology .It is unique and significant to China， because China has abundant resources of gymnosperms， with comparatively more specific genera and species. WANG Fu-Xiong and CHEN Zu-Keng have made a great contribution to this subject. The researches on this group of plants are mainly focused on the following aspects.

3 .2 .1 Embryological studies on specific gymnosperms

Researches have been devoted to sexual reproduction process in many genera and species unique to China using traditional methods. Many research data provided embryological evidence to，and have discussed on their taxonomical positions and genetic relationships.

3.2.2 Researches on fertilization

Cytological observations on the fusion of nucleus from male and female gametes showed

two different patterns of fertilization，i .e.，the pattern of premitotic and postmitotic syngamy.

(2)It is proven that a neocytoplasm is formed around the zygote nucleus after fusion.

The electron microscopic observation on fertilization and embryonic development at early stage confirmed that male cytoplasm is involved in fusion and provided cytological evidences on the mitochondria in heritance from both parents and the sole paternal plastid inheritance.

(3) Ultrastructures of sperm cell.

Firstly，the ultrastructures of flagellated sperm of *Ginkgo biloba* were described in detail，including the multilayered structure of the flagellar apparatus，the typical 9+2 characteristics of flagellar microtubules， and the structure of blepharoplast in the sperm developmental process.

These data are highly significant in the studies on the relationship of the structures of the flagellated sperms with functions.

Secondly，a pair of sperms surrounded by cytoplasm was studied. Fluorescent and electron microscopic observation revealed the presence of DNA in plastids，mitochondria and other cell organelles， providing evidence that the sperm of *Pinus tabulaeformis* possesses cell structures，other than the“male nucleus" as conventionally believed.

In addition，researches related to the reproduction in gymnosperms are also conducted using molecular Biological methods. For example，the pollen actin of *Ginkgo biloba* is analyzed and compared with that in angiosperm *Zea mays* in order to understand the role of actin in systematic development and evolution of plants. The pattern of inheritance of chloroplast and mitochondria in *Cunninghania lanceolata* using PCR technique was further reported.

4 Conclusion

Studies on the sexual reproduction in higher plants targeted more and more obviously to the mechanisms of reproductive development. The introduction of molecular Biology， both in theories and techniques to plant reproductive biology studies， is obviously a trend for advancement，while great attention is also paid for providing basic theories and methodologies to plant breeding.

The 18th International Conference on Sexual Plant Reproduction will be held in Beijing in 2004，which marks that China has her own contribution to sexual plant reproduction parallel to that of the world， and has a substantial research capability at a relatively high level. It is believed that the conference will surely accelerate the development on biology of plant reproduction in China.

Chapter 2 Sexual Reproduction

And Alternation of Generations in plant

1 Sexual Reproduction in Angiosperms

Angiosperms are the flowering plants (today the most abundant and diverse plants on earth). Most are terrestrial and all lack locomotion. This poses several problems. Gametes are delicate single cells. For two plants to cross-fertilize, there must be a mechanism for the two gametes to reach each other safely. There must also be a mechanism to disperse their offspring far enough away from the parent so that they do not have to compete with the parent for light, water, and soil minerals (fig 2.1).

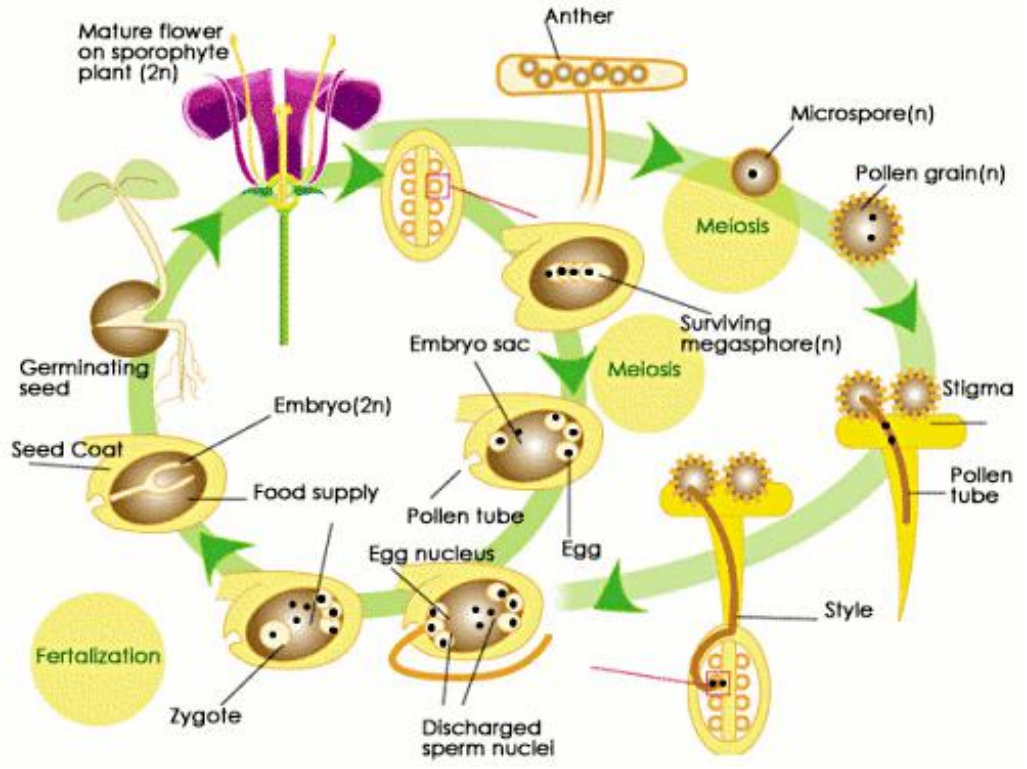

Fig 2.1 The life cycle of angiosperm (www.sparknotes.com/.../lifecycle/section1.html)

The functions of the flower solve both of these problems.

1.1 The flower and its pollination

In angiosperms, meiosis in the sporophyte generation produces two kinds of spores: the microspores, which develop in the microsporangium and will germinate and develop into the male gametophyte generation; the megaspores, which develop in the megasporangium and develop into the female gametophyte generation. Both types of sporangia are formed in flowers.

In most angiosperms, the flowers are perfect: each has both microsporangia and megasporangia. Some angiosperms are imperfect, having either microsporangia or megasporangia but not both. Monoecious plants have both types of imperfect flower on the same plant. Dioecious plants have imperfect flowers on separate plants; that is, some plants are male, some female. Examples include willows, poplars, and the date palm.

Flowers develop from flower buds. Each bud contains 4 concentric whorls of tissue. From the outer to the inner, these develop into a whorl of sepals (collectively called the calyx); a whorl of petals (collectively called the corolla); stamens in which the microsporangia form.

1.2 Stamens

Each stamen consists of a lobed anther, containing the microsporangia and supported by a thin filament. Meiosis of the diploid microspore mother cells in the anther produces four haploid microspores. Each of these develops into a two-celled pollen grain. The two cells are the tube cell and the generative cell

1.3 Carpels

Carpels consist of a stigma, usually mounted at the tip of a style with an ovary at the base. Often the entire whorl of carpels is fused into a single pistil. The megasporangia, called ovules, develop within the ovary. Meiosis of the megaspore mother cell in each ovule produces 4 haploid cells: a large megaspore and 3 small cells that disintegrate.

1.3.1 Development of the megaspore

The nucleus of the megaspore undergoes 3 successive mitotic divisions. The 8 nuclei that result are distributed and partitioned off by cell walls to form the embryo sac. This is the mature female gametophyte generation.

The egg cell will start the new sporophyte generation if it is fertilized. It is flanked by 2 synergids. In corn, and probably other angiosperms, they secrete a sperm attractant which guides the sperm through the micropyle into the embryo sac.

The large central cell, which in most angiosperms contains 2 polar nuclei, will after its fertilization develop into the endosperm of the seed.

In addition, there are 3 antipodal cells.

1.4 Pollination

When a pollen grain reaches the stigma, it germinates into a pollen tube. The generative nucleus divides by mitosis forming 2 sperm nuclei. These, along with the tube nucleus, migrate down the pollen tube as it grows through the style, the micropyle, and into the ovule chamber.

In Arabidopsis, at least, the pollen tube follows a gradient of increasing concentration of gamma amino butyric acid. There is also evidence that a gradient of nitric oxide (NO) can guide pollen tubes to their destination. The pollen tube with its contents makes up the mature male gametophyte generation.

1.5 Double fertilization

The pollen tube enters the ovule through the micropyle and ruptures. One sperm nucleus fuses with the egg forming the diploid zygote. The other sperm nucleus fuses with the polar

nuclei forming the endosperm nucleus. Most angiosperms have two polar nuclei so the endosperm is triploid (3n). The tube nucleus disintegrates.

1.6 Self-incompatibility

Most angiosperms have mechanisms by which they avoid self-fertilization.

1.7 Seeds

After double fertilization, each ovule develops into a seed, which consists of a plumule, made up of two embryonic leaves, which will become the first true leaves of the seedling, and a terminal (apical) bud. The terminal bud contains the meristem at which later growth of the stem takes place. One or two cotyledons which store food will be used by the germinating seedling.

Angiosperms that produce seeds with two cotyledons are called dicots. Examples: beans, squashes, Arabidopsis. Angiosperms whose seeds contain only a single cotyledon are monocots. Examples: corn and other grasses.

The hypocotyl and radicle, which will grow into the part of the stem below the first node ("hypocotyl" = below the cotyledons) and primary root respectively. The development of each of the parts of the plant embryo depends on gradients of the plant hormone, auxin.

In addition to the embryo plant (derived from the zygote), each seed is covered with protective seed coats derived from the walls of the ovule.

The food in the cotyledons is derived from the endosperm which, in turn, received it from the parent sporophyte. In many angiosperms (e.g., beans), when the seeds are mature, the endosperm has been totally consumed and its food transferred to the cotyledons. In others (some dicots and all monocots), the endosperm persists in the mature seed. The seed is thus a dormant embryo sporophyte with stored food and protective coats. Its two functions are dispersal of the species to new locations (aided in angiosperms by the fruit)
survival of the species during unfavorable climatic periods (e.g., winter).

"Annual" plants (e.g., beans, cereal grains, many weeds) can survive freezing only as seeds. When the parents die in the fall, the seeds remain alive — though dormant— over the winter. When conditions are once more favorable, germination occurs and a new generation of plants develops.

1.8 Fruits

Fruits are a development of the ovary wall and sometimes other flower parts as well. As seeds mature, they release the hormone auxin, which stimulates the wall of the ovary to develop into the fruit.

In fact, commercial fruit growers may stimulate fruit development in nonpollinated flowers by applying synthetic auxin to the flower.

Fruits promote the dispersal of their content of seeds in a variety of ways.

Wind. The maple "key" and dandelion parachute are examples.

Water. Many aquatic angiosperms and shore dwellers (e.g., the coconut palm) have floating fruits that are carried by water currents to new locations.

Hitchhikers. The cocklebur and sticktights achieve dispersal of their seeds by sticking to the

coat (or clothing) of a passing animal.

Edible fruits. Nuts and berries entice animals to eat them. Buried and forgotten (nuts) or passing through their g.i. tract unharmed (berries), the seeds may end up some distance away from the parent plant.

Mechanism. Some fruits, as they dry, open explosively expelling their seeds. The pods of many legumes (e.g., wisteria) do this.

2 Alternation of Generations

Sexual reproduction involves the two alternating processes of meiosis and fertilization. In meiosis, the chromosome number is reduced from the diploid to the haploid number. In fertilization, the nuclei of two gametes fuse, raising the chromosome number from haploid to diploid. Whatever variation in details there may be from one organism to another, these two activities must occur alternately if sexual reproduction is to continue. In most plants meiosis and fertilization divide the life of the organism into two distinct phases or "generations".

The gametophyte generation begins with a spore produced by meiosis. The spore is haploid, and all the cells derived from it (by mitosis) are also haploid. In due course, this multicellular structure produces gametes — by mitosis — and sexual reproduction then produces the diploid sporophyte generation.

The sporophyte generation thus starts with a zygote. Its cells contain the diploid number of chromosomes. Eventually, though, certain cells will undergo meiosis, forming spores and starting a new gametophyte generation.

Two points revealed by plant life cycles:

Mitosis can occur in haploid cells as well as diploid ones.

A haploid set of chromosomes, and hence a single set of genes (one genome), is sufficient to control cell function in these organisms (but not in most animals).

In fact, the gametophyte generation is the major stage in the life of mosses and an independent plant in ferns.

However, the gametophyte is only an inconspicuous structure in angiosperms and other "higher" plants. Many protists and fungi have a haploid dominated life cycle. The dominant phase is haploid, while the diploid phase is only a few cells (often only the single celled zygote, as in Chlamydomonas). Many protists reproduce by mitosis until their environment deteriorates, then they undergo sexual reproduction to produce a resting zygotic cyst.

2.1 Life Cycle of an Alga

The sea lettuce Ulva grows on rocks and other surfaces in shallow seas worldwide. It follows a reproductive pattern called alternation of generations, in which it takes two generations—one that reproduces sexually and one that reproduces asexually—to complete its life cycle (fig 2.2).

Although mature members of both generations look the same to the naked eye, microscopic chromosomal differences distinguish one from the other.

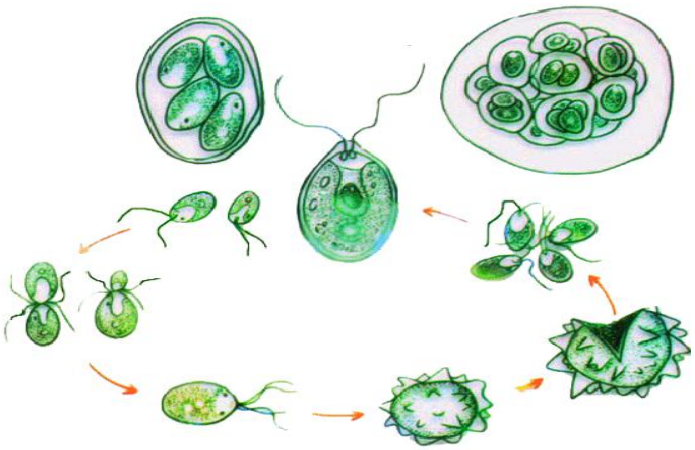

Fig 2.2 The life cycle of *Ulothrix*

2.2 life cycle of fungi

The life cycle of fungi can follow many different patterns. For most of the molds indoors, fungi are considered to go through a four-stage life cycle: spore, germ, hypha, mature mycelium.

Fungi can have very complicated life cycles but the standard fungus life cycle includes a multicellular or colonial haploid stage. This stage is in the form of a fibrous mass called a mycelium and each of the fibers is called a hypha.

Fungus life cycles also include the presence of genetically different mating strains. These look alike but the cells of the hypha， can tell them apart. What's critical for mating is that unlike mating strains (- and+) get together and their nuclei fuse to form the diploid zygote.

The zygote often develops into a resistant thick walled structure often called a zygospore. When conditions are ripe the cell in the zygospore produces sexual spores by meiosis. These are haploid and are dispersed through the air. When they land in a suitable spot such as bread in your bread box, the spores germinated and develop into new hyphae.

Also, the hyphae, will develop specialized structures that produce asexual spores by mitosis. The key thing is that there is no true diploid multicellular stage in fungi. Even in mushrooms, the fruiting body that we see above ground is not diploid. The mushroom forms when hyphae of different mating strains fuse to form a special type of cell with two separate nuclei one from each of the original mating strains. These cells are called not diploid by dikaryotic since the nuclei don't fuse (fig 2.3).

Mushroom spores are produced by specialized dikaryotic cell in which the nuclei now fuse, produce a standard diploid zygote which then undergoes meiosis to produce spores.

2.3 Mosses and Liverworts (Bryophyta)

Mosses and liverworts are traditionally classified together in the Division Bryophyta on the basis of their sharing:

a similar life cycle (alternation of generations)

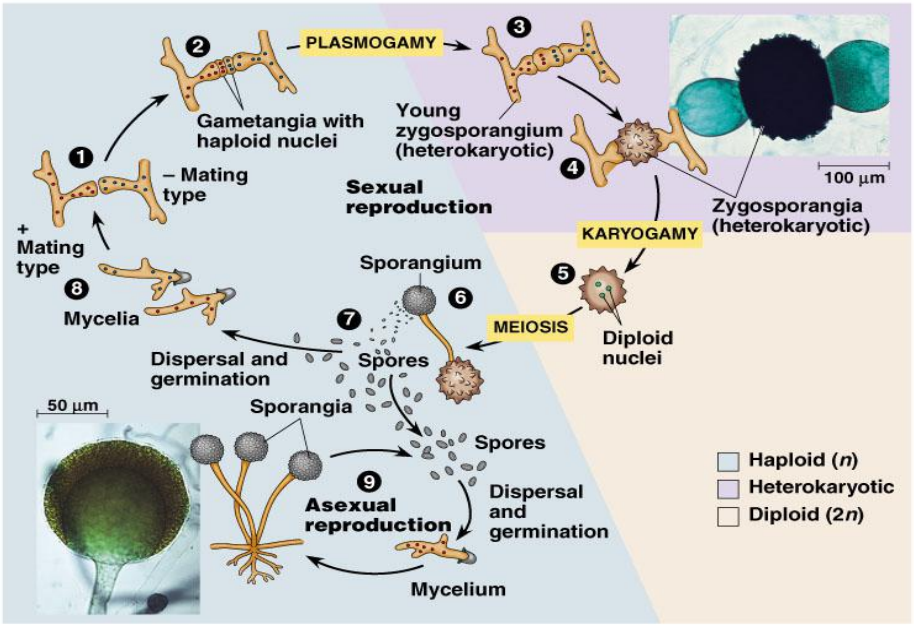

Fig 2.3 The life cycle of the zygomycete Rhizopus (black bread mold)
(microbewiki.kenyon.edu/index.php/Zygomycota)

similar reproductive organs (antheridia and archegonia)

lack of vascular tissue (xylem and phloem)

Some 23,000 species of living mosses and liverworts have been identified. These are small, fairly simple, plants usually found in moist locations.

Liverworts have a thin, leathery body that grows flat on moist soil or, in some cases, the surface of still water.

Mosses have an erect shoot bearing tiny leaflike structures arranged in spirals.

Neither mosses nor liverworts have any woody tissue so they never grow very large. They have neither xylem nor phloem for the transport of water and food through the plant (fig 2.4).

2.3.1 The Gametophyte Generation

The leafy shoot of mosses is haploid and thus part of the gametophyte generation. In the common haircap moss, Polytrichum commune (shown here), there are three kinds of shoots:

Female, which develop archegonia at their tip; a single egg forms in each archegonium.

Male, which develop antheridia at their tip; multiple swimming sperm form in each antheridium.

Sterile, which do not form sex organs?

In early spring, raindrops splash sperm from male to female plants. These swim down the canal in the archegonium to the chamber containing the egg. The resulting zygote begins the sporophyte generation.

2.3.2 The Sporophyte Generation

Mitosis of the zygote produces an embryo that grows into the mature sporophyte generation.

It consists of:

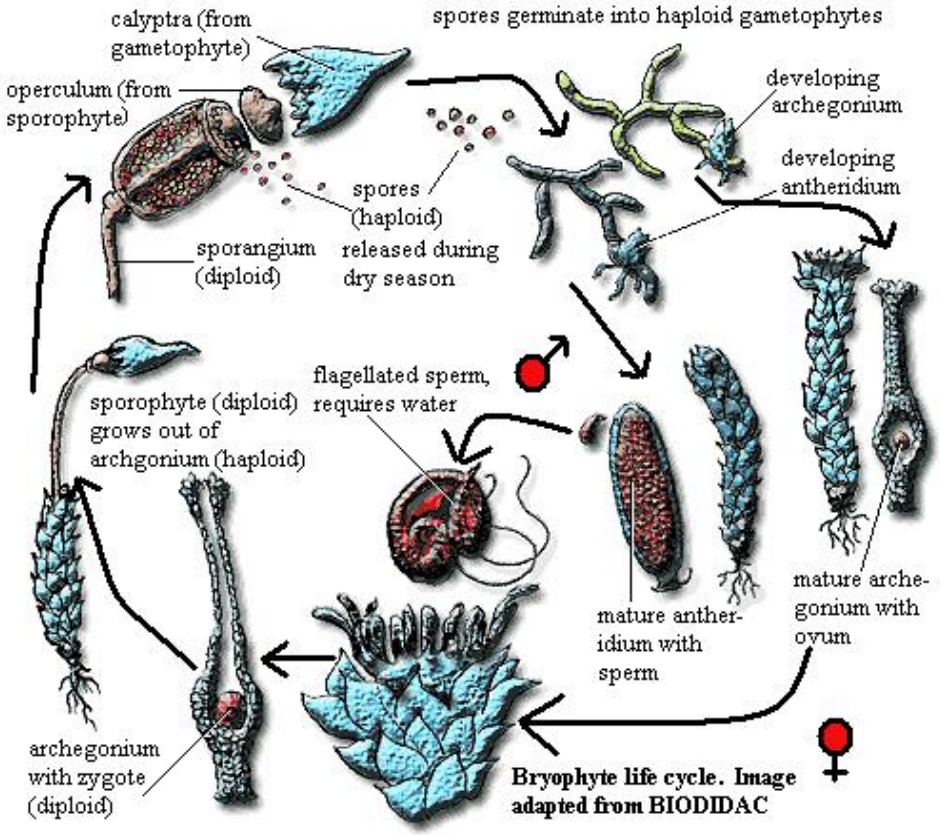

Fig 2.4 The life cycle of the Bryophyta (www.palaeos.com/Plants/default.2.htm)

a foot, which absorbs water, minerals, and probably some food from the parent gametophyte.

a stalk, at the tip of which is formed a

sporangium.

The sporangium is

filled with spore mother cells

sealed by an operculum, and

covered with a calyptra. The calyptra develops from the wall of the old archegonium and so is actually a part of the gametophyte generation. It is responsible for the common name ("haircap moss") of this species.

During the summer, each spore mother cell undergoes meiosis, producing four haploid spores - the start of the new gametophyte generation. Late in the summer, the calyptra and operculum become detached from the sporangium. Low humidity causes the ring of teeth within the opening of the sporangium to pop outward ejecting the spores.

These tiny spores are dispersed so effectively by the wind that many mosses are worldwide in their distribution.

If a spore reaches a suitable habitat, it germinates to form a filament of cells called a protonema. Soon buds appear and develop into the mature leafy shoots. So, the gametophyte generation is responsible for sexual reproduction; the sporophyte generation is responsible for dispersal.

2.4 Ferns

Ferns are over 10,000 species. Most are found in the tropics where tree ferns — with their above-ground stems — may grow as high as 40 feet. In temperate regions, the stems of ferns — called rhizomes — grow underground. The leaves — called fronds — grow up from the rhizome each spring (fig 2.5).

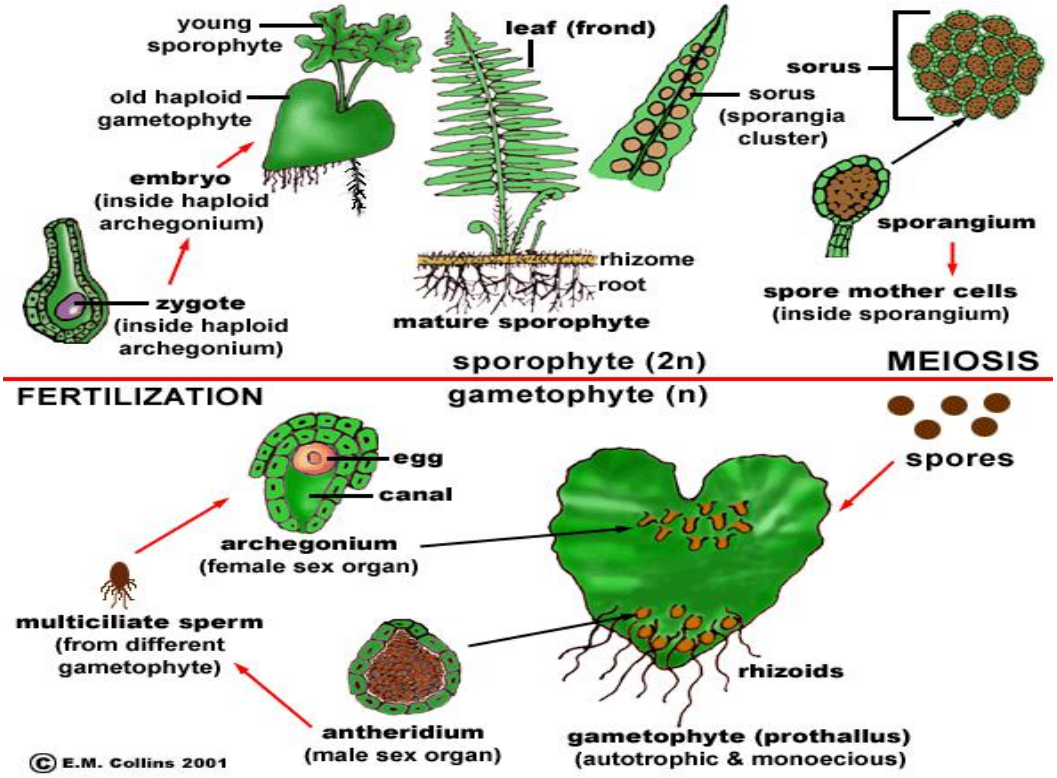

Fig 2.5 The life cycle of the ferns (waynesword.palomar.edu/lmexer8.htm)

2.4.1 The Sporophyte Generation

The plant we recognize as a fern is the diploid sporophyte generation. Sori form on the fronds. Each contains many sporangia mounted on stalks. Within each sporangium, the spore mother cells undergo meiosis producing four haploid spores each.

When the humidity drops, the thin-walled lip cells of each sporangium separate; the annulus slowly straightens out; then the annulus snaps forward expelling the spores.

2.4.2 The Gametophyte Generation

If a spore is blown to a suitable moist location, it germinates into a filament of cells. This grows into a prothallus with:

rhizoids, which absorb water and minerals from the soil;

archegonia, which produce a single egg (by mitosis);

antheridia, which form swimming sperm (again, by mitosis).

2.4.3 Fertilization

If moisture is plentiful, the sperm swim to archegonia — usually on another prothallus because the two kinds of sex organs generally do not mature at the same time on a single prothallus.

Fertilization restores the diploid number and begins a new sporophyte generation. The embryo sporophyte develops a foot that penetrates the tissue of the prothallus and enables the sporophyte to secure nourishment until it becomes self-sufficient.

Although it is tiny, the haploid fern prothallus is a fully-independent, autotrophic plant.

2.5 Pine (gymnosperm) life cycle

The dominant generation of the gymnosperm life cycle is the long lived sporophyte, and the gametophyte generation is inconspicuous. The male gametophyte, the pollen grain, is not dependent on water for sperm transfer. The female gametophyte, the ovule, is nutritionally dependent on the sporophyte. Fertilisation of the sperm and egg cell produces a woody naked seed (fig 2.6).

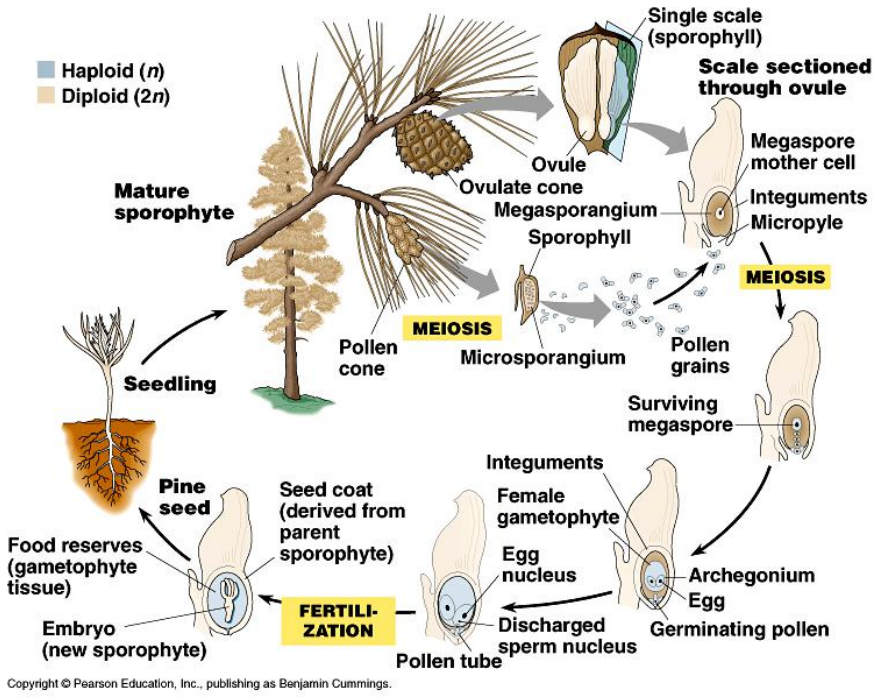

Fig 2.6 The life cycle of the pine (www.lifesci.utexas.edu/.../bio213/plants.html)

2.5.1 Mature sporophyte (2n)

A mature tree of Pinus elliottii is the diploid sporophyte generation and bears the reproductive structures in cones. Each tree carries both male and female cones, but they are produced in different positions on the tree.

The female cones (2n) are larger than the male cones and take two years to develop. The spirally arranged ovuliferous scales each bear two ovules. Meiosis always produces 4 non-identical cells with half the ploidy of the parent cell. It occurs in the production of gametes in animal and spores in the plant sporophyte.

The female cones are larger than the male cones and take two years to develop. The spirally arranged ovuliferous scales each bear two ovules.

Within the ovule four haploid megaspores are produced by meiosis but only a single megaspore is functional. It undergoes a series of mitotic divisions, which result in the formation of haploid gametophyte tissue.

The further development of the male and female cones is synchronised so that the male cones release the pollen when the scales of the female cones have moved apart. The micropyle of the ovule, through which the pollen will gain access to the egg cell, is located at the base of the scale close to the cone axis. Wind-borne pollen is swirled around the cone and filters down towards the micropyle.

Male cones are small and occur in groups at the ends of leafy branches. Each cone has a series of spirally arranged scales, bearing the microsporangia. Meiosis occurs within the microsporangia to produce haploid microspores, which develop into the pollen grains. Meiosis always produces 4 non-identical cells with half the ploidy of the parent cell. It occurs in the production of gametes in animal and spores in the plant sporophyte.

The microsporangia(n) containing developing pollen grains.

At the time of release each pollen grain consists of four cells, which together constitute the haploid gametophyte. Pollen grains have the two external air sacs.

Once pollination has occurred the female cone scales close up again. Within the pollen grain one of the four cells, the generative cell, divides to produce a sterile cell and a spermatogenous cell.

The grain germinates and the tube nucleus controls the growth of the pollen grain into the female gametophyte.

Little further growth and development of the male and female gametophytes occurs until the following year. The female gametophyte within the ovule undergoes further divisions to produce 1-5 archegonia, each containing an egg cell.

The pollen tube continues growing towards the archegonium. The spermatogenous cell, divides to produce two sperm nuclei. When the pollen tube enters the archegonium the sperm nuclei are released. One nucleus fuses with the egg cell nucleus to form the diploid zygote. All other nuclei disintegrate.

Mitotic cell division occurs in the zygote to produce a multicellular embryo. Embryo (2n) is surrounded and nourished by the remains of the female gametophyte (endosperm).

The endosperm is a food reserve formed from the female gametophyte tissue. It increases the embryo's chances of survival.

The seed is 2n. A hard seed coat encloses the whole structure. The winged seeds, which are released when the cone scales open again, are carried away from the parent tree by the wind.

Following germination a primary root is established and a whorl of cotyledonary leaves expand. The plant will grow for many years before becoming reproductively mature. It then starts producing male and female cones and the life cycle will be repeated.

Chapter 3 The Microsporangium

1 Morphology and Structure

In most angiosperms the typical stamen comprises an anther inclusive of microsporangia and intervening connective, and a filament. The primitive stamen, unlike the advanced type, lacks any distinction between filament and anther. In the putative primitive angiosperms, the stamen is laminar, i.e. a broad expanded structure with scanty or no differentiation in the sterile and fertile parts. The microsporangia are not protuberant, but are embedded abaxially (Degeneriaceae, Annoaceae, Himantandraceae), or adaxially (*Austrobaileya, Magnolia*). Such a primitive stamen has undergone reduction in the extensive sterile lamina, accompanied by retraction of margins. The connective tissue, which separates the microsporangia, is massive in the laminar stamen and undergoes progressive reduction. A similar reductional trend has been observed for the distal connective appendages, frequently met with in Magnoliaceae, Cercidiphyllaceae, Eupteleaceae, and Nymphaeaceae.

The angiospermous anther normally comprises four microsporangia, but it may be bisporangiate as in Adoxaceae, Circeasteraceae, Epacridaceae, Malvaceae, Philydraceae, and Restionaceae. Bixaceae have eight sporangia. Multisporangiate anthers of Rhizophoraceae, Gentianaceae, and Loranthaceae result from apparent partitioning of the sporogenous tissue by sterile septae. Both bi- and tetrasporangiate anthers co-occur in about 4 monocotyledonous and 12 dicotyledonous families, such as Araceae, Asclepiadaceae, Celastraceae, compositae, Cucurbitaceae, Lauraceae, Lemnacesae, Moringaceae, and Piperaceae. In *Cucumis sativus* and *Echinocystis lobata* (Cucurbitaceae) bi- and tetrasporangiate anthers are met with in the same flower. The morphology of the stamen in *Arceuthobium* (Viscaceae) has been variously interpreted as unisporangiate, or multisporangiate.

2 Wall Layers

In most angiosperms the anther wall consists of the epidermis, endothecium, middle layer(s), and tapetum. In primitive angiosperms the anther wall is massive, which is primarily due to a larger number of middle layers (Magnoliaceae, Degeneriaceae, and Ranunculaceae) and, occasionally, bilayered tapetum as in Magnoliaceae, Trochodendraceae, Schisandraceae, and Illiciaceae. Contrarily, in advanced families the anther wall is thin, chiefly because there is only one middle layer. The wall layers, except the tapetum, are only casually referred to as regards the presence or absence of fibrous bands in endothecium, and the ephemeral nature of the middle layer(s).

2. 1 Epidermis

The single-layered external covering of the sporangial wall may remain intact as in Amaryllidaceae, Anacardiaceae, Araceae, Balanophoraceae， Begoniaceae, Canellaceae, Degeneriace, Gramineae, Lauraceae, Liliace, Magnoliaceae, Trochodendraceae, and

Winteraceae. The epidermal cells may become stretched, compressed, scattered, or completely sloughed off (the cells could also be missed when compressed) during the course of maturation of anther as in some members of Cannabinaceae, Moraceae, Ul-maceae, *Dorstenia*, and *Broussonetia*. In *Aristolochia*, *Calycanfhus,* and *Ckelone glabra* the anthers are covered all over by hairs of epidermal origin. In *Arceuthobium* the epidermis develops fibrous bands like that of endothecium, and is termed exothecium. In *Zeuxine longilabris* the epidermal cells simulate tapetum, and even become binucleate. In *Trochodendron ara/ioides* and *Triticale* the epidermal cells develop cuticular fibrillar projections.

2. 2 Endothecium

During the maturation of anther, the endothecial cells acquire thickenings which are present even in reduced aquatic plants such as *Utricularia* and *Wolffia*. The fibrous bands, which arise chiefly along the inner tangential walls, extend outward and upwards terminating near the outer tangential wall. In *Trochodendron aralioides*, however, the fibrous thickenings appear to continue along the outer tangential walls as well resulting in complete annular or ring-like bands. In *Lens* (Biddle 1979) bulbous thickenings develop in the endothecial cells. Such an interpretation can also perhaps result from sections having been cut at right angles to the fibrous bands which have thinner bases. In fact, the best way to study the thickenings is from the whole-mounts of endothecial cells. Eames has reviewed the structural variation in fibrous thickenings. The cytoplasm before it senesces, at the mature pollen grain stage contains RER in long rope-like strands, polysomes, and plastids, some even with starch. Multilayered endothecium is common in relatively more primitive families which indicates that it is a primitive feature, and there is a phylogenetic trend towards reduction of endothecial tissue. The endothecium is limited to the protuberant part of microspotangium, and originates from the parietal layer. The endothecium-like tissue, whenever present, towards the inner side, is always contributed by the connective tissue. In *Triticale*, however, Bhandari and Khosla have shown a complete ring of endothecial layer surrounding the tapetum and sporogenous tissue. This originates exclusively from the parietal layer. In *Triticale* the cellulosic nature of thickening has been confirmed by a strong PAS reaction. The endothecium brings about the dehiscence of anthers.

2. 3 Middle Layers

Depending upon whether outer and inner primary parietal layers, or anyone or none of them, contribute to the formation of middle layer(s), their number varies from none to two. The middle layers are transient or ephemeral, and become compressed, crushed, or obliterated even before the microsporangium is fully mature and ready to dehisce. In *Nigella damascena* and *Lilium* two, or two to five, middle layers persist until the dehiscence of anther. In *Lilium* these even contribute towards the development of pollen. Sometimes, the middle layer lying immediately below the endothecium may also develop fibrous thickenings as in *Agave*, *Amyema, Argemone, Crinum, Olax, Oxychloe,* and Zingiberaceae.

2.4 Tapetum

The tapetum is the innermost layer of anther wall and surrounds the sporogenous tissue.

Because of its strategic position. Currently, it is generally assumed that the tapetum may be involved in three different aspects of pollen development, namely: nourishment of microspores, formation of exine, and synthesis and release of materials which take part in the deposition of tryphine and "Pollenkitt"

In angiosperms the tapetum is of two types: glandular or secretory in which the tapetal cells remain intact and persist in situ, and the periplasmodial or amoeboid type when the walls of tapetal cells break down, and the protoplasts protrude into the locule and fuse to form a coenocytic plasmodium.

2.4. 1 Types and General Organization

Davis points out that of the 231 families of angiosperms where the information on tapetum is available, 81 families show glandular type, 29 amoeboid types, and 21 both glandular and amoeboid types. A survey of subsequent literature revealed that about 200 species spread over 68 dicotyledonous and 11 monocotyledonous families have been studied. Of these, 176 show glandular type, 7 amoeboid types, while in the remaining plants precise information is not available. In 17 families, subsequent to Davis' compilation, additional information is now available, and all of them, including three monocots, show glandular type.

According to Davis, the tapetum in Pandanaceae is of the glandular type. Cheah and Stone, on the other hand, report that in *Pandanus parvius* the tapetal cell walls disintegrate releasing the nuclei and protoplast into the loculus, and the tapetum is of the amoeboid type.

Generally, the tapetum comprises a single layer; exceptionally it may divide and become biseriate throughout as in *Pyrostegia*, *Tecoma*, some Bignoniaceae, *Magnolia*, and *Buckleya lanceolata*. A multiseriate condition is known in *Combretum grandiflorum* (Combretaceae) and *Oxystelma esculentum*. The tapetum is single-layered towards the epidermis, and multilayered towards the connective in some Rubiaceae, Vacciniaceae, and Oleaceae. The reports of origin of the entire tapetum from the parietal layer were chiefly due to the fact that the investigators confined their attention to the protuberant (outer) region of microsporangium. Ontogenetic studies confirming the dual origin of tapetum are fewer because of the morphological similarity after alignment of tapetum derived from the parietal and connective tissues, and because of the rapid differentiation along the connective region. In *Anemone rivularis*, however, because of slow development, it was possible to demonstrate the origin of tapetum towards the inner side from the connective tissue. Plants with dimorphic tapetum such as *Salvia mellifera* and some species of Acanthaceae, Scrophulariaceae, and Labiatae are ideal materials to confirm the dual origin. In *Alectra thomsoni* and *Celsia coromandelina* the tapetal cells which differentiate from the connective tissue are not only structurally different, but also differentiate and develop precociously. That the tapetum is of dual origin in most angiosperms is a logical conclusion. In some instances, however, it could arise from the sporogenous tissue as well. In *Triticale* the tapetum originates as a concentric layer around the sporogenous tissue, solely from the parietal tissue, and is not of dual origin. They presume a similar mode of origin of tapetum in the other graminaceous members, although early ontogenetic studies are wanting.

Concurrent with meiosis in pollen mother cells, the synthetic activity in the tapetum increases and certain food reserves, such as starch, lipids, tryphine, etc., are stored. These are degraded or released as such in the anther locule. Rarely, calcium oxalate raphides, or oval to spherical discs, or prismatic crystals have been reported in plasmodia and secretory tapetum.

2.4.2 Ultrastructure

Periplasmodial Tapetum. Of the three studies on the plasmodial tapetum in *Tradescantia bracteata*, *Helianthus annuus*, and in *Mahonia aquifolium*, the first one furnishes considerable information. In *Tradescantia* and *Helianthus* the tapetum is uniseriate, while in *Mahonia* it has three layers, which release their cellular contents into the locule, one after the other. In *Tradescantia bracteata* the tapetal cells, preceding meiosis, show prominent population of organelles. The plastids have dense matrix, pro-lamellar bodies in variable organizational state, and different inclusions like plastoglobuli, large polysaccharide granules, and bodies of moderate electron density and characteristic granular appearance. Raphides, without any connection to membranous structure, accumulate and grow abundantly. Styloid-like crystals have been observed in *Helianthus annuus*. The peripheral tapetal cells become extremely vacuolate and large dictyosomes are formed which produce numerous small, single membrane-bound vacuoles. Many such vacuoles become enclosed by an element of ER, and result in multivesiculate bodies, which also show doublemembraned vacuoles. The outer membrane of such multivesiculate bodies becomes confluent with the plasmalemma, and discharges the contained vesicles between the plasmalemma and cell wall. Also, the dictyosome-derived vesicles are similarly extruded individually. In *Ipomoea purpurea* and *Helleborus foetidus*, which show secretory tapetum, similar polyvesicular bodies have been observed but have not been conclusively shown to bring about the lysis of tapetal cells.

The periplasmodial cytoplasm shows signs of reorganization by the reappearance of rough ER, dictyosomes and formation of microtubules. Prior to callase synthesis in the tapetum which degrades special callose wall, the dictyosome-derived electron-lucent vesicles fuse with the membranes surrounding the tetrads. In *Helianthus*, at the tetrad stage, the tapetal cells have dense cytoplasm and organelles are difficult to distinguish, but mitochondria, plastids, and vacuoles are quite evident. There are numerous dictyosomes with associated vesicles.

Vacuolation of plasmodium increases owing to increased hydration which brings about·a close contact between tapetal cytoplasm and pollen grains. Such a high degree of vacuolation has also been observed in *Helianthus* and *Mahonia*. Consequently, tryphine - polysaccharide granules, flattened lipid globuli, and other cytoplasmic debris - is deposited as a superficial layer on pollen exine.

Secretory Tapetum. The glandular tapetum has been studied in a much larger number of taxa: *Poa annua*, *Silene* and *Cannabis*, *Helleborus foetidus, Lilium longiflorum*, *Allium cepa*, *Beta vulgaris*, *Citrus limon*, *Sorghum bicolor*, *Capsicum annuum*, *Antirrhinum majus, Gentiana acaulis*, *Pelargonium zonale, Kalanchoe obtusa*, *Avena sativa*, *Lycopersicum peruvianum*, *Olea europea*, *Pisum sativum* and *Lens culinare*, and *Lilium*.

Tapetum Before and During Meiosis. In *Avena* the newly formed tapetal cells show plasmodesmatal connections between the adjacent cells, and also between the sporogenous cells. Microtubules run parallel to the long axis of anther along the tangential walls and tangentially or radially along the radial walls. However, prior to nuclear division, which results in binucleate tapetal cells, aggregates of such microtubules are formed along the radial and tangential walls. Even after the primary wall is completely dissolved, microtubules are still present beneath it. Similar microtnbules have also been reported (at this stage) in *Olea* and *Helleborus*. The microtubules are normally concurrent with the orientation of cellulose fibrils during early stages of pollen-wall formation or its subsequent modification, owing to differential thickenings. However, it is rather enigmatic that the microtubules persist beneath the plasmalemma of tapetal cells even when their walls have dissolved, and when no fresh synthesis of cellulose is expected. In *Helleborus*, at the sporogenous stage, organelles in the tapetal cells are recognized with difficulty, though mitochondria, plastids, and a number of "grey bodies" or pro-Ubisch bodies can be identified. Some dictyosomes with peripherally associated vesicles, ribosomes, and associated profiles of SER are also present. In *Helleborus* and *Beta*, however, a large number of vacuoles occur interspersed in the tapetal cytoplasm. Some protein inclusions have been observed in plastids in *Lycopersicum*. In *Olea*, because of similar ribosomal density, the tapetal cells are difficult to distinguish from the sporogenous cells. However, at the leptotene stage, the density of ribosomes in the tapetal cells is higher than that of the microspore mother cells. In *Lycopersicum peruvianum*, Avena, and *Helleborus* the ribosomes are fewer in the tapetal cells. In *Helleborus* and *Avena* the walls of tapetal cells are thin, and comprise the middle lamella, and a small amount of cellulosic primary wall composed of numerous fine, lightly stained fibrils. Plasmodesmata connect the adjoining tapetal cells and to the microspore mother cells in *Avena, Beta, Citrus, Helleborus, Lens, Lycopersicum, Olea, Pisum*, and *Sorghum*. In *Lens* and *Pisum* such connections were not observed between the tapetal and sporogenous cells.

In *Sorghum* the tapetal cytoplasm contains distinct dictyosomes, vesicles, mitochondria, and an extensive and enlarged membranebound tubular system of ER, as observed earlier in *Helleborus*. The amplification of tapetal endoplasmic reticulum, both smooth and rough, during meiotic divisions in microspore mother cells appears to be of universal occurrence, as in *Avena, Beta, Citrus, Lens, Lycopersicum, Olea*, and *Pisum*. The cytoplasm presents a denser appearance because of the increase in ribosomal population and the formation of pro-orbicular bodies which are extruded. At the tetrad stage the dictyosomes, so far relatively inactive, produce a large number of vesicles which become included in larger vesicles, the polyvesicular bodies. The membrane of such polyvesicular bodies becomes confluent with the plasma membrane and, thus, releases numerous vesicles into the space between plasmalemma and tapetal cell.

The structural changes in the tapetal cytoplasm and organelles, narrated briefly, should serve as an introduction since the origin of pro-orbicules and their extrusion, synthesis of sporopollenin and its deposition on Ubisch bodies, formation and release of callase, origin and

development of orbicular wall (tapetal membrane), and the role of tapetum in the formation and deposition of sporophytic proteins, Pollenkitt and tryphine on the pollen surface have been dealt with separately.

2.4.3 Cytology of Tapetum

Concomitant with the onset of meiosis the tapetal cells exhibit significant cytological variations. The single nucleus may undergo one or two mitotic cycles resulting in two to four nuclei which may fuse so that a polyploid nucleus is formed. Or, through endomitosis and restitution, the tapetal cells become polyploid. The cells may remain uninucleate throughout, or become multinucleate, and 13 nuclei are reported in *Hepatica acutiloba*. They often fuse resulting in various levels of polyploidy.

2.4.4 Synthesis of Sporopollenin: Deposition

Sporopollenin is a highly resistant chemical substance(s) in the pollen exine and exosporium of a large number of spore walls.

The synthesis of sporopollenin occurs both in the tapetum and in the cytoplasm of young spores. In the microspores it is deposited in an orderly fashion to form the delicate patterns on the wall of pollen grains. The capacity to synthesize sporopollenin is also present in the tapetum of some plants; the controlling mechanisms are either absent or non-functional.

The deposition of sporopollenin (whether of tapetal or microspore origin) is invariably extracellular, and there are not many well-documented instances of sporopollenin being deposited within the cytoplasm. *Allium cepa* is an exception.

Site of Sporopollenin Precursors in Microspores and Tapetum. In anthers with secretory tapetum electron-dense bodies surrounded by a limiting membrane, the pro-Ubisch bodies are primary sites of sporopollenin polymerization.

It is most likely that, during early stages of pollen-wall development, the sporopollenin is formed from precursors located within the pollen grain cytoplasm.

The cellulosic primexine forms a template on which the sporopollenin is deposited. The control for this template of pattern determination may reside in (1) interaction between sporophytic tapetum and developing microspores, (2) sporophytic control from relict information possessed by diploid mother cells and passed on to the haploid microspores, (3) gametophytic control through information inherited and segregated at meiosis, and (4) haplophytic control. Current evidence favours sporophytic control of wall patterns, but the mechanism is unknown.

Transfer of Sporopollenin Precursors from Tapetum to Microspores. It is presumed that any material passing from the tapetum to the microspores would be a precursor of unpolymerized sporopollenin, and three pathways are postulated by which this material could find its way into the anther cavity: (1) solubilized precursor could be deposited on pre-existing sites such as thin lamellae or tapes on and within the maturing exine; (2) the precursor could be deposited or polymerized in the form of Ubisch bodies; (3) the precursor could be in the form of small granules or accretions laid down onto a pre-existing site.

Process of Polymerization. In *Lilium henryi,* proorbicules formed in the tapetum consist of a

solution of carotenoids and carotenoid esters in a fat and/or hydrocarbon solvent, stabilized presumably by an emulsifying agent, perhaps of a protein origin.

When the pro-orbicules leave the tapetal environment, they come in contact with an aqueous phase, which contains a specific enzyme system and a source of molecular or chemical oxygen. The enzyme operates as an ionic catalyst since an alternative free radical oxidation seems unlikely. Co-polymerization of the carotenoid mixture occurs in what appears to be a fine suspension polymerization process in *Lilium henryi*.

Site of Sporopollenin Deposition. The deposition of sporopollenin starts on membrane-like lamellae. These structures are described as an universal mode of sporopollenin deposition, and their origin is related to elements of RER or vesicles. The origin of the membrane-like lamellae outside the microspore is not known, but they may be elements of the plasmamembrane of tapetal cells.

Apart from the lamellae, an electron-transparent layer on the surface of the exine is present when it becomes more compact. This means that the lamellae are dependent on the presence of sporopollenin.

In *Lilium* the lamellae are formed by the undulated plasma membrane, and not by ER or vesicles as reported in other cases. Outside the plasma membrane a special surface is formed as a glycocalyx.

In relation to the deposition of sporopollenin, the receptor surfaces are described as ordered polymerization surfaces, and are related to the glycocalyx of the plasma membrane.

Mainly after release from plasma membrane, the membrane-like lamellae are sites of sporopollenin deposit. They are sometimes visible around the orbicules as well as around the sexine.

Role of Endothecium and Middle Layers in Formation of Sporopollenin. Because of the increase and decrease of starch in the middle layer(s), and endothecial cells, its breakdown products are considered as the main source of nutrition for the development of microspores and pollen. An intermediate product in the loculus could be active acetate as a common precursor of lipids, carotenoids, and probably sporopollenin. The breakdown of starch can be related to the formation of such a product.

2.4. 5 Functions of Tapetum: Cause of Sterility

Irrespective of the origin and type of tapetum, together with the temporal variation displayed in its disintegration, it is evident that this tissue maintains a very delicate balance vis-a-vis the differentiation of sporogenous tissue. Any factor(s) which upsets this harmony between the two tissues results in asynchronous development and, finally, degeneration of pollen.

Numerous investigations on the cytoplasmic male sterile (CMS) lines indicate that tapetum, through altered physical or physiological factors, initiates the process of abortion of pollen.

Apart from tapetum, endothecium or conducting strand of the filament may also influence the abortive process.

In *Sorghum* and *Zea mays* the male sterile and fertile lines show a gradual increase in the

reducing sugars in an identical way. The starch, however, persists in endothecium and parenchymatous cells of the connective in fertile line, but disappears in the CMS line by anthesis. In *Raphanus* young anthers in sterile lines had less fructose than fertile anthers, while older stages showed more sucrose. Fructose, together with glucose, continued to be much less.

3 Ubisch Bodies/Orbicules

The secretory tapetum, in many angiosperms, in contrast to the periplasmodial, is characterized by the development of Ubisch bodies or orbicules along the tangential surface facing the anther locule.

The Ubisch bodies may remain individually separate or, during the course of development, fuse to form larger aggregations or compound bodies.

In Helleborus foetidus, at the sporogenous stage numerous membranebound "grey bodies" or the pro-Ubisch bodies appear in the tapetal cytoplasm, prominently aggregated towards the anther locule. ER cisternae and ribosomes are in close association with the developing pro-Ubisch bodies at the tetrad stage. The limiting membrane is discontinuous at sites of radiating out rays of ribosomes. The similarity of material present in ER and pro-Ubisch bodies as well as their close association with ribosomes indicate that ER and ribosomes are the organelles involved in the synthesis of pro-Ubisch bodies.

Risueno et al. (1969) observed dense bodies aligned along the plasmalemma in Allium cepa. The ER running parallel to the latter widens at places, and becomes filled with material of similar electron density as in pro-Ubisch bodies, revealing its positive role in their origin. No ribosomal association was observed. Heslop-Harrison and Dickinson (1969) were unable to find any relationship of ER and ribosomes with the origin of pro-Ubisch bodies, but commented upon their resemblance to spherosomes (see also Echlin 1971).

In *Lilium* a persistent stalk connecting the Ubisch bodies with the tapetal cytoplasm. The stalks are actually portions of plasmalemma entering the base of orbicules. This contact continues until the formation of the tapetal wall.

The presence of larger "grey bodies" in *Helleborus* in tapetal cytoplasm, subsequent to extrusion of pro-orbicules, more or less of similar dimensions, casts doubt on their being the actual progenitors of orbicules.

4 Tapetal Membrane: Structure, Origin, and Significance

Structurally, the tapetal membrane comprises three layers - fenestrated, reticular, and orbicular. The Ubisch bodies get attached to the tapetal membrane, and it reveals the imprint of the tapetal cells, which act as a template. In *Helleborus foetidus, Lilium longiflorum,Citrus limon,* and *Olea europaea*, with secretory tapetum and orbicules, there is no tapetal membrane. In some members of composite, which have plasmodial tapetum in the form of a complete sac surrounding mature pollen. At the late vacuolate microspore stage, and subsequently, polymerization of additional sporopollenin in inter-orbicular areas produces a reticulum, which together with Ubisch bodies constitutes the tapetal wall. Beneath the reticulum develops

a layer of fibrillar material. At the engorged pollen-grain stage, the tapetum degenerates but the distinct massive orbicular wall persists. In the anthers of *Lilium* after the dissolution of tapetal cell walls at the tetrad stage a layer of fine fibrillar material, staining weakly for polysaccharides, develops between the orbicules and tapetal cell to which the orbicules stick. This thin layer with frequent electron-dense spots persists until dehiscence without much change.

The precise role of tapetal membranes is rather enigmatic. One possible function may be to act as a "culture sac" which surrounds the maturing pollen and labile periplasmodium, or the contents released by the disintegration of secretory tapetum. It might also help to prevent a quick loss of water from within the anther locule, which could indirectly affect Pollenkitt deposition on exine.

5 Pollenkitt and Tryphine

The general term Pollenkitt is applied to various substances responsible for imparting stickiness to the pollen. A similar plastidial origin of Pollenkitt coating is reported in *Lilium*. This, eventually, is deposited on the surface of pollen and, possibly, forms a part of Pollenkitt. In *Artemisia mutellina*, soon after degeneration of the tapetum, lumps of Pollenkitt material composed of lipid droplets of various shape and size, together with other cellular organelles, float in the anther locule. At the time of anthesis, underneath an electron-transparent Pollenkitt crust the lipid droplets become entrapped and adpressed to the pollen exine. The Pollenkitt's role in attracts insects, protects against UV radiation damage, and adheres to insect body because of its sticky nature. In most insect-pollinated species the Pollenkitt is electron-dense and homogeneous, and forms a complete coating on the exine, rendering the pollen sticky. In anemophilous species, on the contrary, it is electron-transparent and not homogeneous; its quantity is also much smaller. Consequently, it either becomes inactive in the loculus, or sinks to the bottom of the exine perforations. The pollen is, thus, non-sticky and powdery.

In *Acer* various species show differential depositions: (1) inside the exine cavities (*A. negundo*), (2) inside the exine cavities, with only small drop-lets adhering to the tectum (*A. campestre*), (3) average amount of granular material deposited both inside the cavities and outside as a slender film (*A. pseudoplatanus, A. opalus*), and (4) a good amount of granular material filled in the cavities of exine and deposited as a thick layer over the surface of tectum (*A. platanoides*). Thus, the pollen is (l) powdery, (2) moderately sticky, (3) sticky, and (4) very sticky which can be correlated to anemophilic, amphiphilic, and entomophilic modes of pollination.

Distinction is made between the Pollenkitt and tryphine, though these are similar in many respects. The former is a synthetic activity, while the latter is formed from the remains of degenerated tapetum. These two differ in containing hydrophobic or hydrophilic lipids, respectively. The latter also includes some cytoplasmic structures such as ER, and pro-Ubisch bodies in Helleborus, which are tansitory. However, the existence of Pollenkitt in H. foetidus is not certain. It should be interesting to find co-occurrence of both in any taxon.

6 Sporogenous Tissue: Ultrastructure

Concurrent with meiosis, conspicuous changes occur in the cytoplasm and organelles of microsporocyte. The sporophytic-gametophytic transition, although ultimately an expression of genetic potential, must depend upon extranuclear factors. The continuity of plastids and mitochondria through somatic cell lineages and microsporogenesis, and other changes in the cytoplasm, are no doubt connected with the sporophytic-gametophytic transition.

6.1 Cytoplasmic Membranes and Ribosome Population

In Lycopersicum, that before meiosis the ribosomes per unit area are more numerous in microspore mother cells than in tapetum, but the contrary is true after the beginning of meiotic prophase. In microspore mother cells of *Lilium* large amounts of cytoplasm are generally invested in double membranes; concentric multimembrane inclusions are also formed quite regularly. Similar structures have been observed in *Pisum* and *Lens*. As the meiocytes enter prophase, there is an abundance of free ribosomes and an appreciable amount of ribosomal endoplasmic reticulum. The process of encapsulation of the cytoplasm commences in late leptotene to early zygotene, just prior to elimination of cytoplasmic ribosomes. The outer membranes lose the major part of their surface polysome population, form cup-shaped profiles and, finally, come to envelop a portion of the premeiotic cytoplasm. This process appears completely non-selective, in that lipid droplets, organelles, or even other membranes may also become invested. A substantial fall in ribosome number occurs during the zygotene-pachytene interval, and by mid-prophase about 15% of cell cytoplasm becomes included in double - or multimembrane systems. During the process of degradation the ribosomes enclosed in these membrane-bound bodies are far less affected by agents affecting their eradication/disintegration in the general cytoplasm. The ribosomes are usually concentrated in the centre, because those on the surface of investing membranes are degraded. Granular deposits persist; however, suggesting that only one component of the ribosome is eliminated. By late prophase, the ribosome level is lowest. There is practically no further change until the breakup of these membranes, which begins at the end of meiosis. Some ribosomes become associated to form polysomes. In *Lilium* they persist until early stage of microspore.

Scheer and Franke (1972), in MMCs of Canna generalis, describe annulate lamellae of irregularly spaced interconnected cisternae bearing ribosomes, and continuous with rough endoplasmic reticulum (see also Kessel 1968). They consider annulate lamellae as a "degenerate form of rough endoplasmic reticulum" which suggests that these do not necessarily have any particular function, such as storage of ribosomal or messenger RNA.

6.2 Nucleus

The post-pachytene nuclear envelope generates numerous double membranebound inclusions by sacculation of the inner nuclear membrane and complementary blebbing of the outer membrane of the nuclear envelope. It is also coupled with inwardly directed activity associated with the inner nuclear membrane. This development is a general one in plants. In *Lycopersicum* vacuole formation coincides with the completion of synaptonemal complex

development. The nuclear pores are evidently involved in nucleo-cytoplasmic interaction.

It is possible that the accumulation within the vacuoles represents nuclear material, which is undergoing degradation or reorganization. It is pertinent to point out that such reorganization in the nucleus assumes meaningful importance, since contemporary cytoplasmic reformation is being conducted by elimination of ribosomal population. The restandardization of the non-genetic components of nucleus in preparation for the gametophytic generation might, therefore, involve elimination of nucleoplasmic structures associated specifically with sporophytic growth.

6.3 Nucleolar Cycle

Meiocytes enter prophase with one, two, or more nucleoli, depending upon genotype. At the time of synapsis, when there is more than one, the number is reduced; subsequently there is usually an increase in volume. Often, there is a change in shape due to the flattening of nucleolus towards one pole.

In *Lilium* the spherical shape is restored at diplotene, and during pachytene supernumerary nucleoli are formed at the nucleolar organizing sites of the nucleolar-organizing chromosomes. When the microspore mother cell nucleolus is losing basiphilia and becoming vacuolate, they frequently lie in chains, the smallest showing the highest basiphilia. Late diplotene nuclei contain supernumerary nucleoli, which are not associated with chromosomes, and probably these get detached from nucleoli organizing regions of chromosomes. The accessory nucleoli are released after dissolution of the nuclear membrane at the end of diakinesis. There are instances, however, where the nucleolus of mother cell does persist through the meiotic divisions. The zygotene nucleoli still in association with the nucleolar organizer part of chromosome show the two components: the granular and fibrillar regions. The two segregated regions, quite distinct by diplotene, undergo fragmentation and, eventually, the fibrillar masses disappear. Concurrently, the granular masses become looser and increase in number. Large areas of nucleolar envelope, however, remain intact. During metaphase I numerous pre-nucleolar bodies coalesce on to the nucleolar organizer to form the main nucleolus. The cycle is repeated during meiosis II as well.

Bodies cytochemically and structurally similar to nucleoli become visible in the spindle region during anaphase. They do not appear to be formed at the nucleolar-organizing regions of the chromosomes. Most of these are released into the cytoplasm as nucleoloids. The residue enclosed within the envelope of dyad nuclei is released into the cytoplasm during anaphase II.

6.4 Cytoplasmic Organelles during Meiosis

During the premeiotic period profiles suggestive of the division of plastids are frequent. All through prophase the number of divisional configurations decline. The starch grains disappear, while the lamellar system regresses and the ribosomes present in the centre of the plastid get degraded. Towards late zygotene, the plastids assume isodiametric form, dedifferentiate completely, and become almost indistinguishable from the double-membrane structures; only the osmiophilic globules remain intact. Plastids may also be ellipsoidal, with few or more discernible ribosomes, and a severely reduced lamellar system. Subsequently, and through

metaphase I, the plastid population remains relatively unaltered.

An unusual, and possibly unique, structural feature appears at interphase consisting of an association between a membrane component, in the form of a flattened tubule or ribbon, and a cluster of particles of diverse size. The granules measure 15-60μm and may be interspersed with a small number of osmiophilic bodies or droplets. Diffuse masses of fibrils are also present. This membrane-particle association is probably present in all plastids from the dyad to the late tetrad stages. With rare exception, only one membrane-particle association is present in each plastid at the dyad stage. Its significance cannot be specified, but its consistent occurrence suggests that it may be concerned with the redifferentiation of plastids, which occurs from late tetrad onwards. During this stage, divisional figures of plastids are invariably accompanied by synchronous division of the membrane-particle association.

The mitochondria show regular changes, which closely synchronize with nuclear, and other cytoplasmic events. During the premeiotic period the cells entering meiotic prophase have normal mitochondria (1.0μm) much like the somatic cells. In the leptotene stage, however, the mitochondria begin a phase of division followed by reduction in size (maximum diameter about 0.3μm). They are spherical and retain a small number of cristae, but the matrix is rather electron-dense, or opaque, and rich in ribosomes. In the young spores, an enlargement and recovery of the normal structure is common.

This cycle of dedifferentiation and redifferentiation in plastids and mitochondria is presumably connected with the sporo-gametophytic transition.

7 Initiation and Control of Meiosis

7.1 Duration of Meiosis

The duration of meiosis decreases with the increasing temperature. Dehydration and physical damage have either no effect, or may bring about complete cessation of the meiotic process.

There is a positive correlation between nuclear DNA content and decrease in the duration of meiosis. There is no effect of the diploid chromosome number. A comparison of diploids and polyploids with more or less the same DNA content indicates that the polyploids have shorter meiotic duration. However, it increases in a series of related polyploids, e.g. an octoploid showed maximal increase over the related diploid than its tetra - and hexaploid.

7.2 Synthesis of Callose: Deposition and Significance

During meiotic divisions a distinct, refractile wall, "special callose wall" is secreted around the microspore mother cells. This wall is composed of callose -- β -l, 3-g1ucans,. This is generally present in small quantities in structurally different plant tissues, and is a substance which has special physical and physiological properties, e.g. it is rapidly synthesized and degraded with equal ease.

During prophase 1, the callose deposition is initiated between the plasmalemma and the original cellulosic cell wall, particularly along the corners of the PMCs, and extends laterally until a continuous layer is laid down. In some graminaceous members, soon after some space

develops by separation of PMCs, the callose is first deposited along the inner tangential cell walls towards the centre of the anther locule, as small pegs and, subsequently, extends towards the outer tangential walls. The callose wall is not of uniform thickness.

In *Helleborus foetidus* the plasmalemma is convoluted and, at places, distinct gaps have been observed between the plasmalemma and wall of pollen mother cell which contains certain electron-transparent material. A material of similar electron density is reported in discrete vesicles distributed within the cytoplasm of meiocyte and, it could be callose. However, it has not been ascertained if the callose is formed in the dictyosomes or ER.

At the termination of meiosis, and at a time when the tapetal cells have become capable of producing sporopollenin, the callose wall is rapidly broken down and the microspores are released into the anther locule. The dissolution of callose in *Lens* and *Pisum* is generally centripetal; in tetrahedral quartets, however, the dissolution begins at the corners. There is a positive relationship between pH, callase activity, and breakdown or degradation of callose. In fertile *Petunia hybrida* anthers, pH during meiosis is 6.8 to 7.0, and the activity of callase cannot be detected. However, at the tetrad stage, the pH drops to 5.9-6.2, and there is a sharp increase in callase activity resulting in the digestion of callose around the tetrads and release of microspores in the anther locule. On the other hand, in the anthers of male-sterile *Petunia*, the drop of pH and callase activity is precocious and, consequently, the development of microspores is arrested. In other sterile genotypes, the pH of locule remains high; callase activity is not detected at the end of tetrad formation with the result that the callose wall remains intact until a very late stage. Certainly, there is a precise time during microsporogenesis when callose dissolution should occur to ensure normal development of pollen.

7.2.1 Functions of Callose

The development of callose at pollen mother-cell stage, and its degradation a little after the completion of meiosis suggests that this callose wall layer performs some special function(s). The callose wall not only isolates the sporogenous tissue from the somatic tissue, but it also isolates the individual microspores. Some well-established functions of callosic wall, and their direct role in pollen ontogeny, may be enumerated. It gives mechanical isolation to the developing microspores, thereby preventing cell coherence and, by their rapid and total dissolution, set the microspores free.

The callose layer also functions as a kind of chemical isolation, establishes a selective barrier between genetically different haploid cells, which must pass through their developmental stages unexposed to the influence of their sister spore, or of the adjoining spores and somatic tissue. Subsequently, the tracer was excluded from the mother cell until the dissolution of tetrads. On their release the young spores take up the radioactivity readily. The incorporation of thymidine occurs mostly in premeiotic and early leptotene period initially, and at the disappearance of call0se walls. This suggests that the callose wall is resistant to the passage of labelled thymidine derivatives. Therefore, these authors suggest that callose acts as a barrier or "molecular filter" to the exchange of at least some macromolecules, and also

provides genetic autonomy to each developing sporocyte.

It has also been suggested that the callose wall protects the developing sporocytes from the harmful hormonal and nutritional influence of the adjoining somatic cells.

Premature dissolution of callose around the tetrads may contribute to steriliity. The published reports show that abortion can occur at almost any time during microsporogenesis, and that probably several phenomena and mechanisms are involved. The behaviour of callose is more or less similar through the early meiotic stages. Later, however, the central callose mass in fertile anthers splits into sectors along the plane of the original microsporocyte walls, and forms a covering that isolates the microsporocytes and young microspores. This callose wall then dissolves to free the microspores from the tetrads. In sterile plants, on the other hand, callose starts separating from the developing microsporocytes, and begins to accumulate in the form of an amorphous mass in the centre of locule. This mass becomes fibrous and diffuse, and disappears during early meiosis. As the callose wall is dissolved early, the microspores do not get physical and physiological isolation. These studies offer a new insight into the role of callose in pollen abortion in malesterile lines.

Callose wall also seems to play an effective role in the establishment of the very first pattern of exine. Various hypotheses have been proposed to explain the role of callose in laying down the pattern of primexine:

The callose wall supplies carbon compounds, like glucose, for the development of cellulosic primexine which furnishes a basic framework of the future exine. It acts like a template, or mould for the future pattern exine. The inner face of an empty spore chamber shows delicate, negative replica of the primexine. Further, callose is formed as a normal and universal constituent of the special wall at the tetrad stage and important developmental stages occur when the callose wall is still present. This indicates that the special wall plays an essential role the initial steps of pollen development.

7.3 Cytokinesis

The four products of meiosis become separated from each other through the process of cytokinesis accompanied by formation of cell plates. Two basic types have been recognized: (1) the successive type in which the two cell plates are laid down in centriflugal manner immediately after the first and second meiotic division, and (2) the simultaneous type where the isolation curs by concurrent centripetal furrows.

Successive centrifugal cell plates at the end of meioses I and II, e.g. *Zea mays.*

Simultaneous centripetal constriction furrows at the end of meiosis II, e.g. *Nicotiana*. There are three variants:

A heterotypic furrow appears at the end of meiosis I. A homeotypic furrow, however, initiated at the end of meiosis II reaches the centre and quartet formation occurs in a simultaneous manner, e.g. *Magnolia kobus*.

Heterotypic furrowing completes the division of PMC into dyad before homeotypic furrowing, e.g. *Magnolia liliflora*.

There is a partially deposited cell plate (*Zygogynum*), or evanescent cell plate precursor

(*Pseudowintera, Magnolia*).

Simultaneous centrifugal cell plate formation at the end of meiosis II, e.g. *Helleborus*, *Illicium, Schisandra* and *Kadsura.* Apparent reconstruction of telophase I phragmoplast, and appearance of secondary spindles at the end of meiosis II, e.g. *Laurelia.*

Cytokinesis in PMCs of *Helleborus* involves almost simultaneous occurrence of granulose cell plates across the equators of six spindles. In *Laurelia,* however, cytokinesis occurs by formation of four cell plates. In the successive type there are three plates and their structure varies with the species (e.g. *Sansevieria, Canna, Lilium)*. That differences in structure may be due to (1) velocity of embedding process, (2) direction of growth of the cell, and (3) stage at which the cell plates become embedded. They concluded that in *Lilium* the embedded cell plates present obvious chemical and structural analogy with the cell plate formed during somatic cytokinesis.

7.4 Cellulosic Wall of Microspore Mother Cell

A number of LM and TEM studies indicate that the pectocellulosic wall of the MMC disintegrates before the end of meiotic prophase and concurrent with the deposition of callosic wall. In *Helleborus foetidus* and *Olea europaea* it may be observed at places in a highly reduced form. In *Allium tuberosum*, tuberosum both the callose layer and primary cellulosic wall dissolve simultaneously to release the young rnicrospores. In *Cyclamen persicum*, on the other hand, at the end of telophase II, the wall becomes tenuous, breaks down on account of flocculation and, as a result thereof, remnants of original wall adhere to the callosic wall in the form of numerous globules.

Chapter 4 The Pollen Grain

1. Introduction

The pollen grain is the carrier of the male gametes or their progenitor cell, in higher plants, in a single unit, each grain contains all the genetic information required to specify an entire haploid plant organism (for example, pollen embroils in tissue culture), or to unite with the female gamete at fertilization and form a diploid zygote and, hence, a new saprophytes. The male gametes, the sperm cells (or their progenitor, the generative cell) are housed entirely within the cytoplasm of the vegetative cell. This is dehydrated like a seed at maturity, and is filled with storage reserves. The vegetative cell is surrounded by a complex, intricately patterned outer wall, and its nucleus controls at least the initial growth and metabolism of the pollen tube following germination.

Such a structure represents one of the simplest, isolated cellular systems in flowering plants. Each grain is either bi- or tricellular at maturity (see Fig. 4.1 A-C), and is the product of only two rounds of cell division following microspore formation (as opposed to five rounds of mitosis necessary for pollen formation in gymnosperms). Thirty years ago, Maheshwari declared that pollen grain development in angiosperms is remarkably uniform, referring to the cytological process of nuclear division. During the last 10 years it has become evident that while pollen grains at dispersal may show superficial similarities, nevertheless pollen grain development and wall structure proceeds along different routes in different groups of flowering plants. Each is distinctive, presumably an adaptation to its cellular environment and mode of pollen dispersal by wind, water or animal vectors.

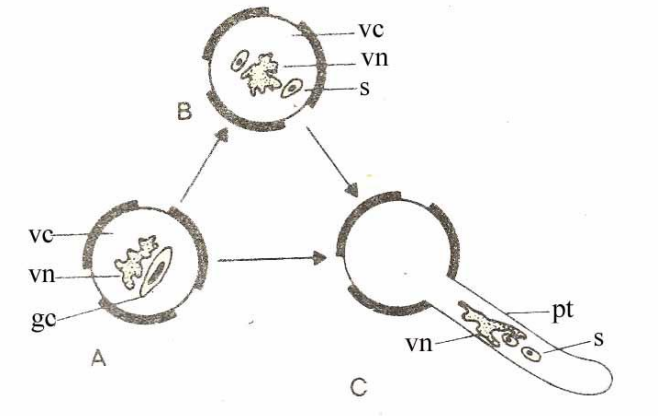

Fig. 4.1 A Mature bicellular. B Tricellular pollen grains. C Bicellular grain becomes tricellular after germination. VegetatIve cell *vc,* generation *g,* vegetativc nucleus *vn*, sperm celis *s,* pollen tube *pI*

2. Pollen Structure and Cytochemistry

The pollens of most species of flowering plants are free, cellular structures, each developing from a single microspore. In some families variation on this theme occurs, with the formation of composite grains. These may be tetrads, containing all four products of a single microporocyte linked together, or multiples of four forming polyads of 8, 16、32, or 64 grains, or more complex masses, or pollinia. Among tetrad types, the grains may have 0, 1, 2, 3, or all 4 grains fertile, with the sterile aborted grains remaining attached, i.e., they may be nullads, monads, dyads, triads, or tetrads. Each microspore is walled off, and regulates its own destiny. However, in one family, the Cyperaceae, each grain forms from one of the four microspores, the others aborting with or without wall formation.

2.1 Pollen Shape

The form of the pollen of angiosperms is a genetically determined character, and is established in three ways:

1. By siting of the microspores within the meiotic tetrad.
2. By the number and disposition of germinal apertures, determined within the meiotic tetrad.
3. By differential cell expansion during microspore and pollen development.

The first factor may control shape mechanically. The shape of the grains is established within the thick, rigid callose special wall, which shows little or no expansion during tetrad period, acting like a mould or template for initial pollen wall growth. The second factor is also determined within the callose wall since the sites of apertures are pre-determined to specific areas within the tetrad. For example, in many monocot pollen grains the single aperture is sited on the outer tangential surface of each microspore.

The third factor is manifested in grains with an unusual shape. Most pollen grains are spherical, and essentially do not change their gross shape after spore release period. However, in filiform grains of some sea-grasses, as in *Amphibolis* and *Halodule*, the tetrad of microspores shows little elongation until release when each grain shows characteristic tip growth - resulting in the long filiform grains that may reach 5 mm in length in *Amphibolis*.

2.2 Pollen Cytology

In most families of flowering plants, the pollen grain is bicellular. The largest cell, the vegetative cell, contains a lobed central nucleus with pores in the nuclear membrane communicating with a cytoplasmic endomembrane system. The cytoplasm is also packed with organelles: mitochondria, plastids, golgi, and ribosomes; and storage organelles: starch grains, P (polysaccharide) particles, lipid droplets and proteins stored within membrane vesicles of the endoplasmic reticulum. Stacked endoplasmic reticulum is an unusal ultrastructural feature of the cytoplasm of the vegetative cell of pollen grains.

The generative cell is usually bounded by a plasma membrane and distinct cell wall, often containing callose, e.g., in orchids. The cell is located entirely within the cytoplasm of the vegetative cell, is usually markedly elongate (Fig.4.2), and contains a limited range of

cytoplasmic organelles.

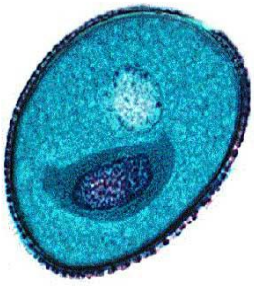

Fig.4.2 The mature pollen grain

Division of the generative cell to form the pair of sperm cells most commonly occurs within the pollen tube shortly after its emergence and penetration into the stigma. In tricellular types of pollen it occurs precociously into the maturing grains.

First, what specific processes are involved in sperm cell differentiation? Chu and co-workers at Peking University, Peking (Beijing), China, have demonstrated that there are four developmental periods in the tricellular pollen of wheat:

1. Naked cell stage: After mitosis the sperm cell is enveloped in a discontinuous plasma membrane.
2. Walled cell stage: The cell is enclosed within a plasma membrane surrounded by a callose wall and the plasma membrane of the host vegetative cell.
3. Cytoplasm-increasing stage: Period of organelles and cytoplasmic differentiation.
4. Mature sperm cell: Wall becomes discontinuous, surrounding the sperm with an elliptical nucleus at one end and organelles (especially mitochondria) concentrated in the remainder of the filiform cell.

A range of organelles occurs in the sperm cytoplasm including mitochondria, ER, Golgi bodies, ribosomes, small vacuoles, and plastids with reduced lamellar structure, In addition, microtubules, and numerous microfilaments are arranged parallel to the longitudinal axis of the cell.

2.3 Pollen-wall Structure

There are two domains in the pollen wall: the exine, the outer patterned layer made of sporopollenin, and the intine, the inner smooth polysaccharide layer. These layers are chemically, morphologically, developmentally, and genetically distinct. The terminology applied to the wall of pollen grains is daunting, especially as it has been developed from early light microscope work, and then transposed to the images seen in the transmission and scanning electron microscopes (Fig.4.3).

The exine is made of sporopollenin--a wall polymer remarkable for its resistance to biodegradation, and considered to be formed by the oxidative polymerization of carotenoids and carotenoid esters. The exine has many properties in common with lignin and cutin. The pollen wan may contain about 1 % elemental silicon, as determined by X-ray microanalysis. In

morphological terms, there are two types of exine:

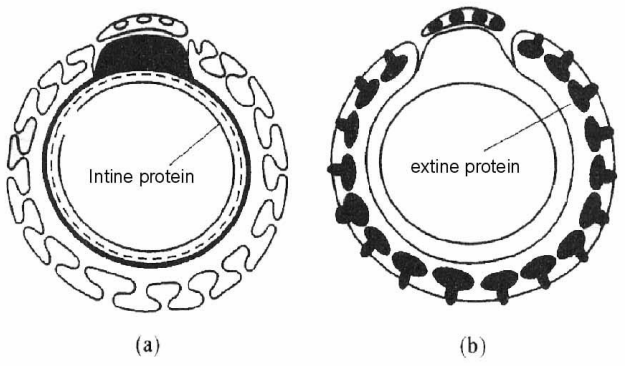

Fig. 4.3 The pollen-wall structure

1. Where the patterned surface layer contains arcades covered by a roof or tectum, supported by rod-like bacula (or columellae) - the "tectate" type (Fig. 4.4 A, 4.5 A, B).
2. Where the patterned surface layer is open, the pattern comprising the bacula (pili) which may be ornamented or fused together at their tips - the "pilate" type (Fig. 4.4).

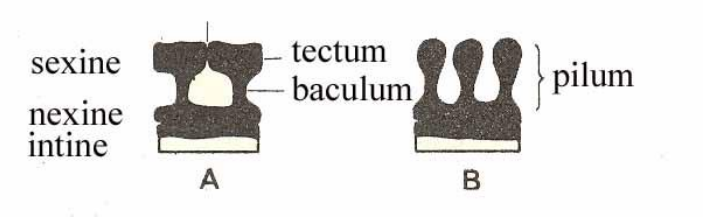

Fig. 4.4 Stratification of pollen walls. A Tectate exine, B pilate exine.

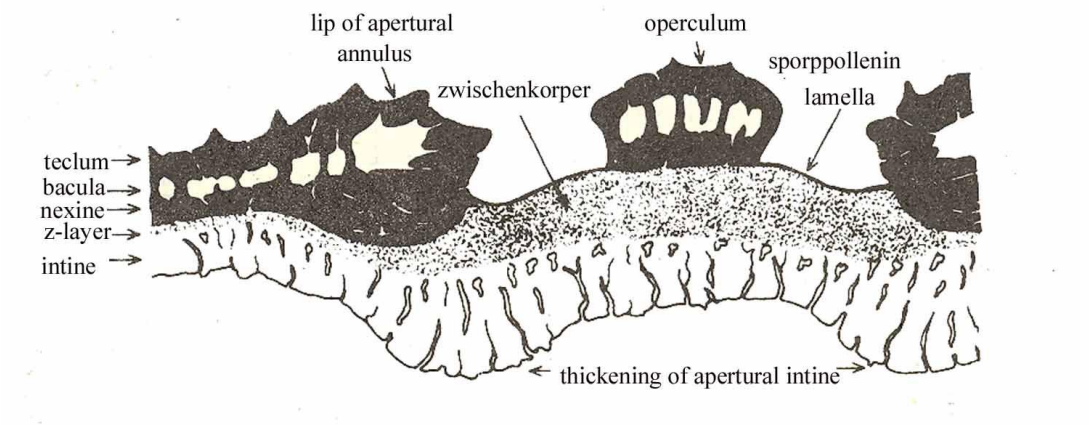

Fig. 4.5 Aperture region of a grass pollen grain to identify various structural features of the wall.

The exine layer is complex and comprises several substrata: (1) the outer tectum, when present, (2) the bacula (singular: baculum; may be called bacules), (3) the foot layer or nexine,

and (4) the innermost nexine-2. Layers 1 and 2 are often termed the sexine, while 1, 2, and 3 are called the ektexine; layer 4 has been termed the endexine. In the Compositae the two nexine layers may be separated by spaces, the cavea.

In tectate exines, the tectum is perforated by micropores, communication channels which lead to the ciypt-like arcades within; in some specific pollen types, microchannels traverse the nexine layer linking with the nexine-2 or intine, e.g., in grass pollen. In pilate exines the spaces between the pili are open to the exterior. In both these types of grains it is usual for the, surface cavities and exine arcades to be filled with pollen coat substances.

The intine forms the surface of the grain at the germinal apertures. Here, the exine layer is absent, greatly reduced or forms a cap-like operculum. In many pollen types it comprises a thick polysaccharide layer, often with radially or tangentially oriented tubules, and is considerably thicked at the germinal aperture. In other types it may consist of more than one layer, differentiated by their characteristic appearance by transmission electron microfibrillar appearance, while the outer tubular zone stains with ruthenium red, indicating a high content of acidic poly saccharides.

The chemical and morphological identity of the two strata has been defined by chemical tests in which the layers can be differentially solubilized in organic solvents, and acidic (especially acetolysis) or alkaline solutions. The stratigraphy can be most readily demonstrated by cytochemical test. These are based on the affinity of the exine for dyes binding lignin, phenolic compounds, lipids and negatively charged groups, and of the intine for cytochemical tests for polysaccharides, acidic polyanions, and proteins. The chemical differences between the exine sublayers-tectum (~ektexine) and nexine (~endexine)-may be distinguished by their differential affinity for negatively charged dyes (e. g., basic fuchsin) and for lipids (auramine O fluorescence).

The inner exine layer, the nexine-2 (or endexine) appears to very variable in its chemical composition and ultrastructural appearance in different pollen systems. It is frequently present as an electron-dense lamellated layer, e.g., in the monocot *Clivi*, and the dicot *Saintpaulia*. This intermediate layer between exine and intine frequently shows differential solubility by acetolysis, while its ultrastructural appearance suggests it may be composed of both sporopollenin and polysaccharides.

The complexity of wall structure revealed in these free pollen types (monads) are also reported in composite pollen. Composite grains are of two types:

1. "Claymmate" grains united together by a continuous layer of sexine (i. e. tectum, bacula, and foot layer).

2. "Acalymmate" grains not united by continuous layer of sexine, but by other means.

In calymmate tetrads, for example in Asclepiadaceae and Goodeniaceae, a common tectum links the grains together. In acalymmate complexes the tectum is not united between grains and the cohesion of the polyad or pollinium has been assured by other means; for example wall bridges or by a special adhesive layer. The development of composite pollen is considered later. Its ultimate expression is seen in the large pollinia and massulae found the

higher Orchidaceae..

2.4 The Pollen Tube

The pollen tube is produced after germination in vivo on the stigma, or during suitable incubation in vitro. For most angiosperms it is the vehicle for the male gametes, since it is within the tube that the generative cell divides to form the sperm cells. Both the generative cell and vegetative nucleus migrate into the tube soon after its formation, and move towards the tip of the tube. In *Lycopersicon peruvianum* they remain in the nuclear zone, the largest of the four regions of the growing pollen tube, and division to form the sperm cells occurs immediately. Unfortunately, there is little information on the early events of pollen-tube formation and extension. Within the pollen tube the sperm cells are closely associated with the vegetative nucleus, and in some recent studies there is electron microscopic evidence that they may indeed be structurally linked, e.g., in *Gossypium* and *Plumbago*. Apart from *Lycopersicon*, scanty data are available on the events within the pollen tube cytoplasm, although ultrastructural studies have been carried out on the monocots *Lilium*, *Clivia, Nymphea*, and the dicots *Petunia* and *Lobelia*.

The nature of the pollen-tube wall has shown that the tube wall is thick and multilayered. The developing tube wall of *Lycopersicon* is bi-layered, comprising an outer pecto-cellulosic wall continuous with the intine, and an inner layer of callose, detected by its fluorescence with decolorized aniline blue. A similar organization has been observed in *Nicotiana* pollen tubes. Four zones, reflecting functional specialization, have been detected by electron microscopy in *Lycopersicon*: (l) and (2) Apical and subapical zones: cytoplasm with abundant Golgi bodies and vesicles; thin fibrillar cell wall. (3) Nuclear zone: houses vegetative nucleus and elongate generative cell surrounded by two membranes, often with fibrillar material between them; callose layer in inner tube wall. (4) Zone of vacuolation and callose plug formation: very thick callosic wall ending with callose adjacent to pollen aperture. The callose wall deposition in *lycopersicon* tubes is similar to that described for *Petunia* and *Nicotiana*. The participation of golgi vesicles in secretion of the tube wall polysaccharides, especially callose, has been suggested in several studies.

Plugs of callose and related polysaccharides contain the living protoplast at the pollen tube tip. Ultrastructural studies of the plugs have been carried out several dicots, e.g., *Lychnis*, *Lycopersicon*, *Oenothera*, *Petunia*, and *Prunus*, and *monocots*, e.g., *Lilium*, and *Secale*. Presumably, the plugs seal the protoplast, permitting renewed tip growth, although considerable controversy exists concerning their chemical nature.

During its subsequent development, the pollen tube has its unique characteristics expressed both in its chemistry and structure. Structural differences occur in pollen tubes of *Rhododendron* during interspecific matings. Pollen tubes of species exhibited markedly different phenotypes during growth through the styles of different species. At the same time, pollen tube tips showed a range of characteristic cytological abnormalities associated with inter-specific incompatibility during in vivo growth on the stigma, and in the style and ovary. Studies need to be directed towards determining the nature of gene expression in the pollen

tube tip--as this structure provides the cellular interface with much of the pistil during the interactions that determine the fate of the sperm cells.

2.5 Structural Adaptations of Pollen for Dispersal

The pollen grain forms only a small part of the flower whose entire structure is subject to selection pressures for adaptation to mode of pollination, especially potential interactions with animal pollinators. Nevertheless, it is the haploid gametophyte generation, and therefore might be expected to reflect genetic changes more rapidly and directly than the surrounding diploid parental tissue. Until recently, the evidence from pollen morphology has not supported this concept. The pollen grain has been seen as a conserved structure: it has species group-specific exine patterning, and intraspecific variation has seldom been demonstrated. Together with the evidence of genetic experiments, this conservatism suggested that exine form and structure is under sporophytic control.

The adaptations that were first observed were correlations between exine structure, and mode of pollination in families with both wind-pollinated and animal-pollinated genera. Characters such as exine thickness and extent of sculpturing, type of aperture, and the presence of different adhesive components in the surface pollenkitt have also proved of adaptive significance. In colpate and sulcate types the exine has specific mechanisms for dehydration, including unfolding. In porate types, e.g., grass pollen, the whole grain can act in water-flow control as this is regulated both through the operculum and through microspores which traverse both sexine and nexine wall layers, and there is no extensive lipid seal covering the grain surface. The dry grains are wrinkled while the germinated grains are spherical.

In polyporate grains the exine is usually massive, e.g., in *Malvaviscus* and *Ipomoea* pollen, and a thick lipid seal is present in mature pollen. In colporate or triapertutate types, e.g., pollen of *Eucalyptus*, *Amyema*, *Ricinus*, *Datura*, *Cosmos*, and *Crossandra*, there is a combination of the mechanism shown by colpate and porate pollen types. The grains may be infolded or have shutters or plates. The grains of *Crossandra stenostachya*, an African species of Acanthaceae, have a most unusual shape, long and narrow. The grains have a thick exine covered by a tectum, and a thick underlying nexine layer.

Finally, there are pollen types without preformed apertures, the inaperturate. Here, the exine may be fragile and much reduced, forming a surface reticulum in *Populus* or isolated spinules in *Cinnamomum*. The intine is usually thick and prominent in these types. The surface of the grains may have a prominent lipid seal or be covered with mucilage (in aquatic plants with hydrophilous pollination). The unique filiform pollen of many sea-grasses, e.g., *Amphibolis*, has no exine.

The structure and components of the pollen wall will now be examined in relationtion to their function in different modes of pollination.

2.5.1 Pollen Transport by Animals

Animal vectors of pollen range from insects to mammals, and a characteristic feature is that a reward or attraction is provided. This may be in the form of nectar, a sugary secretion produced by special floral glands, the nectaries, usually containing up to 15 % sugars,

especially sucrose, fructose, and glucose with traces of amino acids. Nectaries are usually, but not always sited within the flower and are structurally distinct from the anthers. Sugary udates are a feature of some types of stigmas, and may serve as an attractant licking insects, as well as an adhesive and germination medium for the pollen. In addition, pollen itself may be employed as an attractant - its high carbohydrate and protein content making a suitable food for animal pollinators. In this special food anthers filled with sterile cells resembling pollen, or simply extra anthers, attract the pollinator. These lures are often supplemented by the presence of a scent or odour attractive to the pollinator. While the scent-gland cells are usually located in the petals, there is evidence that the anthers may also participate as in Winteraceae.

Pollen grains of entomophilous plants usually have a greatly thickened exine in which the inner nexine layer provides solid foundation for the long bacula and elaborately sculptured and ornamented tectum or pili, for example, the pollen of lily, *Lilium*, in monocots, and of *Cosmos*, *Hibiscus* or *Malvaviscus* among dicots. The surface ornamentation apparently assists grain adhesion both to the pollinating insect and to the stigma surface. The grains may large (> 300μm) and are often spherical in shape.

A most conspicuous feature is that the grains are usually covered by a sticky adhesive pollen-coat (lipid seal) so that they adhere together in masses-almost an agglutination reaction, e.g., in *Eucalyptus*. This is achieved by means of a kind of surface pollen cement ("Pollenkitt") that forms a thick viscous coating over the grain surface, and may confer a characteristic stickiness, odour, and colour to the grains. This is termed pollen-coat materials. The pollen-coat includes carotenoids or flavonoid pigments which are common in 80% of pollen grains. The sticky nature of the coating is due to the presence of glycoproteins, lipids, glycolipids, and monosaccharides which all contribute to the adhesive properties.

These surface coatings are produced in the tapetal cells that line the anther cavity, and transferred to the surface of the pollen grains late in pollen development just before tapetal dissolution. This was first shown for *Lilium* pollen, and has since been confirmed for pollen of many genera of dicots.

The type of pollen-coat present in various genera of Euphorbiaceae has been correlated with the development of "secondary" entomophily in *Euphorbia* by its ultrastructural appearance. The effectiveness of the pollen-coat, as an adhesive for insect pollination has been compared with viscin threads in sveral angiosperms. The pollen-coat agglutinates the grains together because it is a sticky surface component. Viscin threads, in contrast, are themselves nonsticky, thin, and non-elastic but flexible fibres of sporopollenin which link grains together. In the Onagraceae the viscin threads may originate from within the composite grains of *Epilobium*, as also in certain Caesalpinioideae. In the Ericaceae the viscin threads are tached to the surface of the grains and may tie them like ropes to insect hairs bristles.

The surface appearance of these composite pollen grains which possess viscin threads is comparable to other composite types that have other means of an ensuring adhesion of the pollen to visiting insects. The tetrad pollen of *Leschenaultia* and the, polyads of *Acacia* have sticky pollen-coat terials. In orchids various configurations of grains occur, from tetrads to

complex masses of grains (massulae) and pollinia. Pollen-coat materials are present in and on the exine that surrounds the massulae of Dactylorchis.

The structural adaptations of pollen grains for pollinations by birds and mamals are less well known, although some principles are emerging from current search. There are two main sites of deposition of pollen on birds: on the beak, and on the head feathers (the cap). As with insect pollination, the site is determined in the placement of the anthers in the flower, deposition of pollen on cap feathers requiring a much longer distance between the nectaries and the anthers. This is often achieved by means of a pollen presentation organ, usually pistil which, as it uncoils during flower opening, squeezes the pollen onto its tip, a feature seen in many Proteaceae. The pollen is usually light in colour (white pale yellow) and powdery. In Loranthaceae and Proteaceae the grains are often markedly three-lobed, or may be covered with spines which enable the grains to fit into barbules of the head feathers.

In contrast, in beak-pollinated pollen as in Myrtaceae, the pollen is usually and spherical or triangular in shape, and is copiously covered with a thick layer of pollen-coat. Occasionally, it may be dark-brown, black, or blue in colour. There is a conspicuous absence in the literature of structural information concerning the pollen of species pollinated by mammals, including bats, rats, mice, and marsupials. The pollen is carried on scales or bristles on the snout, and we may expect adaptations of shape, ornamention, and pollen-coat materials. This gap in our knowledge is maximal in tropica species.

No account of pollen structure would be complete without mentioning the unpollen wall found in certain families of monocots, in the Scitamineae-where the intine is greatly thickened and the exine reduced sometimes to a few spinules, presumably an adaptation to the mode of pollination in the tropical rain. In *Tapeinochilos* (Costaceae) the exine consists of a single outer layer formed by fusion of radially rod-like units, so that there are no arcades. In the *Heliconiaceae, Heliconia* pollen has an even more remarkable exine which is confined to surface spinules linked by a thin zone of sporopollenin lamellae at the surface of the intine, which is greatly thickened and differentiated into four layers. These authors consider that this unusual pollen-wall may be related to pollination by humming birds.

A greatly reduced exine is also present in several tropical Lauraceae. Ultrastrucural studies of pollen of avocado *Persea american*a show that the exine is reduced to scattered surface spinules overling thick intine. Studies of these types of pollen have been important in tanding development of the exine, and the great reduction ine must presumably be viewed as an adaptation to pollen transfer in the humid tropics. Such pollen adaptations to the environment may now be added to the list compiled by Tomlinson for the Scitamineae. It may also be analogous to the reduction in the exine in aquatic angiosperms such as sea-grasses.

2.5.3 Pollen in Air Currents

Grass pollen is one of the commonest types found in air currents. Each grain is very light and pollen grains are spherical, with a relatively thin wall and a powdery, non-sticky surface. The grains have a single aperture. The exine is tectate with the tectum raised upon short bacula from the foot layer, forming an intricate patten of arcades. The tectum surface carries many

short spinules giving a roughened surface.

Ultrastructural investigations show that the tectum and foot layer are perforated by numerous channels 14 to 25 nm in diameter. The intine is well developed, and markedly thickened at the apertures.

In grasses each species has a similar appearance by light microscopy, and only slight differences in surface pattern are evident by scanning electron microscopy. Most other windborne pollen has low sculpturing, e.g., ragweed (*Ambrosia* spp.), which has low spines. Other features of windborne pollen include their seasonal periodicity and often high concentration in the atmosphere. While 99% of pollen grains are deposited within 1 km of their source, it is possible for pollen grains to remain up to 3 days in the upper atmosphere before deposition. It is also for pollen to be deposited and redeposited by air currents several times.

2.5.3 Pollen in Water Currents

Most aquatic plants flower above water in the air, and use animal vectors pollination. A few are completely hydrophilous; flowering when submerged, and uses water currents for pollen dispersal. The largest are probably the sea-grasses which have some remarkable adaptations for marine pollination.

The extraordinary pollen of the sea-grass, *Posidonia oceanica*, was fist described and illustrated by F. Cavolini at Naples in 1806. He observed that the pollen was "different from that of other plants, being oblong likes little eels, with a sudden and brisk motion exploded, and scattered their sperm in the twinking of an eye". He noted its cottonwool-like appearance when the anthers dehisced in the sea, forming a cloud of pollen in the water. Later, in 1826, the French botanist C. Gaudichaud reported on the thread-like pollen of the sea *nymphphilobis antarctica*, from Shark Bay, Western Australia. He noticed its extrusion in rope-like masses from the anthers. He also observed that the pollen of wrack (*Halophila*) had spherical grains released into the sea in long, sticky threads.

The exceptional appearance of the pollen of eelgrass, *Zostera*, which also formed long thread-like tubes tightly filled with granular cytoplasm resembling the pollen tubes developed by other pollen types at fertilization: Fritsche also recorded the presence of slime at the surface of pollen of *Najas, Caulinia, Zannichellia*, and *Zostera*. The pollen grains of the Mediterranean eelgrass were coiled in a spiral arrangement within the anther.

The pollen grains of sea-grasses display three different adaptations to the marime environment:

1. Spherical grains, 100 x 150 μ m in diameter, are embedded in spherical droplets of slime in the family Hydrocharitaceae, [turtle grass *Thalassia,* and *Enhalus* (Fig. 4.6A)]. The filamentous shape is presumably an adaptation to transport in sea currents, as is the surface coating of slime.

2. Ellipsoidal grains (40 x 80μm) released in linear tetrads within a tube of slime, in the family Hydrocharitaceae, sea wrack, *Halophila* (Fig. 4.6 B).

3. Filiform, thread-like grains occur in the families Zosteraceae (*Zostera*, *Heterozostera*),

Posidoniaceae (*Posidonia*), Cymodoceaceae [Amphibolis where each grain may be up to 5 mm long, 30 μ m in diameter (Fig. 4.6 C)].

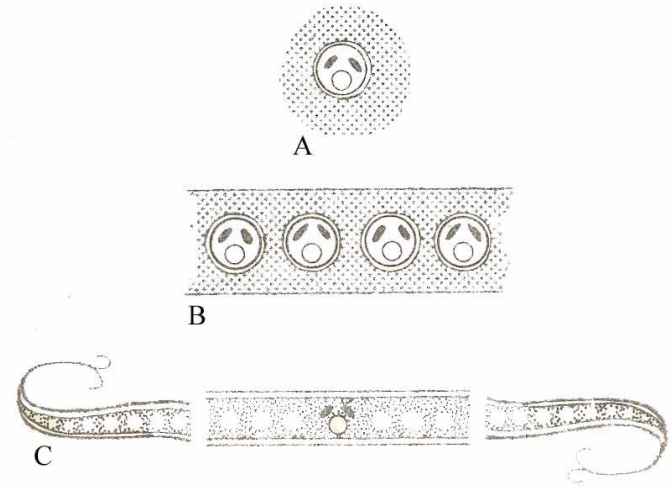

Fig. 4.6 Three different adaptations in morphology or sea-grass pollen. A Spherical grains or Tholassi. or Enhalus, embedded in a droplet of slime. B Ellipsoidal grains of Halophila held within a mucilaginous tube. C Filiform grains of Amphibolis.

A further remarkable adaptation for hydrophilous pollination occurs in the cell wall that encases the pollen of sea-grasses. Extreme differences exist in sea-grasses that have filiform pollen, and these are reflected, to some extent, in the other species with spherical grains. The wall in filiform types is essentially reduced in structural complexity, when compared with terrestrial pollen. The outer exine layer is absent, so that the pollen wall resembles the intine of terrestrial pollen. This structural difference occurs in filiform pollen of *Amphibolis* and *Zostera,* and in the string of spherical grains of *Halophila*. The pollen wall of these two kinds of sea-grasses is, therefore, unique among flowering plants. The wall resembles the intine of terrestrial pollen in two characteristic respects: (1) Its microfibrillar, electron-lucent appearance in thin sections viewed by transmission electron microscopy; this is consistent with its likely chemical composition of cellulose microfibrils embedded in a matrix containing acidic polysaccharides, suggested by the cytochemical evidence. (2) If fresh mature grains are freeze-sectioned, the pollen wall reacts positively for a marker enzyme, acid phosphatase, that is characteristically stored within the polysaccharide matrix of the intine of terrestrial pollen. In the ellipsoidal grains of *Thalassia* and *Enhalus* a rudimentary exine is, however, present.

3. Formation of Pollon

3.1 Cytology of Development

The microspore, the first cell of the gametophyte generation, is the determinative cell of pollen development. After meiosis the tetrad of microspores is formed within the callose special wall. Microspore determinants are responsible for the design and initial construction of

the complex outer pollen wall, the exine, and for the siting of the apertures, when present. The life of the microspore is terminated by the first mitotic division, an asymmetric division forming the vegetative and generative cells of the pollen grain.

The longevity of the microspore period is shortest in tropical plants: *Tradescantia refllexa* 4 days, *Styrax obassia* 7 days, *Himantoglossum nircinum* 14-21 days. In the arctic species, *Uvularia sessilifolia*, *Empetrum nigrum*, and *Betula odorata*, may extend throughout the winter period. In the canary grass, *Phalaris tuperosa*, the microspore period lasts for 6 days out of 13 cays required for pollen development.

The microspore cytoplasm is non-vacuolate when the spores are first released from the callose special wall at the end of tetrad period (spore release period), but vacuolation begins soon after. In grasses the vacuole expands as the volume of he pollen grain increases. This young spore period is the main focus of cell expansion in the entire pollen development, termed the vacuolate period. During this period, the diameter of the pollen grain of grasses more than doubles to about three-quarters of its final size. Towards the end of the vacuolate period, the amount of cytoplasm increases and the vacuole becomes resorbed bout the time of the microspore division.

The first pollen grain mitosis is the division that heralds the end of the microspore period. In the grasses it occurs about half-way during pollen development. In the sea-grasses Halodule and Amphibolis antarctic, the first pollen mitosis occurs almost immediately after microspore release from the tetrad, just as the grains begin to elongate. In *Najas*, the division is even more precocious, occurring within the tetrad period. A pattern is manifest in this first mitosis. It is an unequal cell division, and is important for two reasons:

1. Partitioning of the cellular organelles into the vegetative and generative cells.
2. The geometric precision of its siting.

A relationship has been demonstrated in many pollen types between the siting of the aperture and the future position of the generative cell. Huynh embodied this in his "law of the longest distance", in which he showed that the generative cell will be cut off at a point furthest from the aperture. He considers this siting might ensure that the vegetative nucleus would enter the pollen tube first. Some exceptions to this relationship have been noted by Sampson, but he considers that the law is of general validity.

Pollen-grain mitosis is often synchronous within the anther, e.g., in orchids. An interesting observation of synchronous pollen mitosis in the permanent tetrad grains of the Winteraceae has led Sampson to associate synchrony with the presence of cytoplasmic connections in the proximal walls of the tetrad of microspores. In tetrads with asynchronous division connections are absent.

After the microspore mitoses, pollen development is under way, and is characterized by the accumulation of storage reserves-protein, carbohydrate, and lipid within the cytoplasm of the vegetative cell. This seems to be a major function of the vegetative cell, which becomes progressively dehydrated as grain matures, and is ready for dehiscence. At this time, the pollen of most angiosperms remains bicellular. In a few families, notably the Compositae, Cruciferae,

and Gramineae, the generative cell divides precociously forming a pair of elongate sperm cells, which are present in the mature grain--so that the grains are tricellular when released.

The development and differentiation of microspores and pollen grains can be meaningfully described:

1. Tetrad period: four microspores within callose special wall.

2. Spore-release period: microspores are released from callose special wall within exine. Considerable distortion may occur during fixation.

3; Pre-vacuolate period: grain spherical, with exine and pore clearly visible; dense non-vacuolate cytoplasm.

4. Early vacuolate period: spherical vacuole present, with diameter up to half that of the pollen grain.

5. Mid-vacuolate period: grain spherical with the vacuole filling the grain, giving a "signet-ring" appearance.

6. Late-vacuolate period: grain distinctly ovoid in shape, showing buildup of cytoplasm so that vacuole is reduced to one-half to one-third of grain volume.

7. Early maturation period: first pollen mitosis has occurred; cytoplasm fills the grain, further reducing the size of vacuole.

8. Late maturation period: second pollen mitosis has occurred, grains are tricellular, and cytoplasm with starch grains completely fills the grain.

The complex development schemes of earlier workers are based on the use of chromic or alcoholic fixatives. The use of buffered aldehyde fixatives, whose osmolarity can be adjusted to that of the pollen grains, should prevent many of these artifacts. More recent schemes are all similar to that used in this chapter. This is based on the use of fresh pollen, with the fluorochromatic reaction which provides a rapid evaluation of pollen development, based on the appearance by fluorescence microscopy of the characteristic vacuoles and cytoplasm of the microspore and vegetative cell.

Having established the criteria for the development periods, it is practicable to compare these with anther length, and then to follow the sequential changes in pollen development with time. This has been done for canary grass, *Phalaris luberosa*. As might be expected, the major periods- vacuolate period and maturation period - are the longest, although it is surprising to find the maturation period of 7 days. This period, however, is important since the storage reserves are synthesized, and also the pollen becomes partly desiccated. Schemes such as that used here are of great value in assessing the developmental origins of pollen sterility in cereals and grasses.

3.2 The Primexine

These primexine elements are synthesized by the protoplast of the microspore while enclosed within the callose special wall. Rodlike probacula or flat trilaminar plaques are initiated at the outer face of the microspore plasma membrane, in the future wall zone. Structures, termed lamellations or white lines, become inserted below the probaula providing an additional site for accumulation of protosporopollenin to complete the basic elements of

microspore wall pattern.

The development of the exine from the primexine matrix that has been described in *Silene* is typical for the pollen of many other angiosperms. Among monocotyledons, this differentiation system has been observed in *Zea, Endymion, Sorghum, Lilium, and Tradescantia.*

The basic features of microspore exine formation are shown in Table 4.1 and involve sporopollenin synthesis at two periods in development: (1) at tetrad period within the callose special wall; and (2) during the young spore period, with deposition of sporopollenin synthesized by the tapotal cells (elaborating the foot layer, bacula, and tectum), and by the microspore protoplast (forming the nexine). Significant variations from this theme have been discovered in two groups of monocotyledons: in one only tetrad period synthesis occurs and, in the other, sporopollen synthesis does not (apparently) occur at all.

Finally, the pollen of certain sea-grasses, especially the Australian sea nymph, *Amphibolis antarctica*, has been shown by cytochemical and ultrastructural studies to completely lack an exine layer at maturity.

We can conclude from this evidence that the presence of a primexine is essential for exine differentiation. The pollens of angiosperms appear to have utilized many variations in producing their exine structure.

3.3 Establishment of Apertures

While some pollen types in both monocotyledons and dicotyledons do not have germinal apertures (inaperturate or omniaperturate types), most possess an area of wall devoid of exine, or covered by a cap or operculum at which pollen tube mergence may occur in a successful pollination.

There are two main types of aperture: simple furrows or pores; and complex types comprising an outer and inner aperture superimposed on each other. Huynh investigated the structure and arrangement of the furrow types of aperture, and considers there are two different kinds: (1) "sulcate" pollen in which the tangents to the furrows, each lying along one furrow on its mid-point, are parallel with the equatorial plane (Fig. 4.7 A, B); (2) "colpate" pollen in which the tangents to the furrows are borne perpendicularly on the equator (Fig. 4.7 C, D). The arrangement of the apertures is determined by their siting within the tetrad, which can be observed by staining with a dye specific for the exine, for exmple, congo red and maleic hydrazide), or Calberla's stain. The apertures are distinguished by the absence of staining. Huynh has identified four main patterns of aperture arrangement, based on the siting of the furrow in relation to the equator of the microspore in the tetrad (Fig. 4.7 A-D).

In all cases the aperture is defined during tetrad period, and ultrastructural, studies have revealed that the site is frequently designated by the endomembrane system, ER being adpressed to the plasma membrane.

An inventory of the families which possess inaperturate pollen is given by Erdtman. Among monocotyledons this type of pollen is frequent among the Helobiae, and in the Scitaminae, including the Musaceae, Zingiberaceae, Cannaceae, and Marantaceae. Among dicotyledons, the condition is frequent in the Polycarpiae, especially in the following families:

Nymphaeaceae, Ceratophyllaceae, Annonaceae, Gomortegaceae, Monimiaceae, Lauraceae, Hernandiaceae, and Aristolochiaceae. These are mostly regarded as primitive groups, and generally possess monosulcate pollen. Exceptions are known in the Linaceae, Salicaceae, Icacinaceae, and Euphorbiaceae-Crotonoideae. These families possess some genera with inaperturate pollen, but accompanying related genera with normal three-colporate structure.

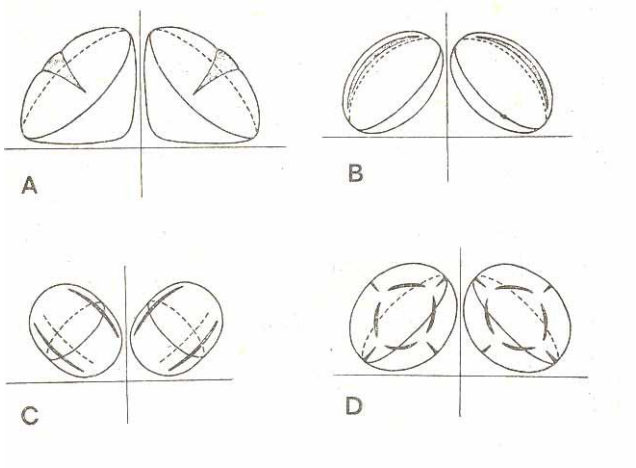

Fig. 4.7 Four types of microspore arrangement at tetrad period. Each diagram shows the upper pair of sister microspores of a tetrad. A Monosulcate microspores. B Monocolpate microspores. C Tricolpate microspores D Pentacolpate. Explanation of *perspective lines:* The more or less *elliptic lines.* each of which encicles one microspore, represent the equators. The *horizontal line* and the *vertical line* represent, in each figure, the two planes, perpendicular to the plane of the figure and to each other, that run through the tetrad and touch both the microspores. All of the apertures are *dotted,* except for C where the third aperture is represented by an *interrupted line;* in D only the apertures on the upper faces of the microspores are represented

3.4 Exine Differentiation and Maturation

The tectum, bacula, and foot layer (nexine) are laid down by the deposition of sporopollenin on the surface of membrane-like lamellae, a lipoid substrate, or polymer already deposited. The major and minor spinules that may decorate the tectum are also determined. These events occur within the tetrad. Thereafter, at microspore release, subsequent development is dependent on the special circumstances peculiar to each pollen system. It usually involves the development of the inner nexine-2 or endexine layer (made of sporopollenin), and of the intine (made of polysaccharides).

Both these layers develop during the vacuolate period, e.g., in *Vida*. While these have been studied in several systems, two cases are especially interesting: *Lilium*, in which both the wall layers develop simultaneously, and, in certain Compositae, *Artemisia* and *Cosmos*, where there is a degree of separation.

Within the tapetal cells, sporopollenin is produced which will coat the young developing sexine. Sporopollenin accretion takes place upon a lipoid former or substrate. This lipoid material either coats the walls of the loculus or appears as droplets in the inward facing or

radial walls of the tapetum. Sporopollenin is deposited on these structures, forming the peritapetal membrane lining the loculus and the orbicules present in many anther types. Sporopollenin accretion ceases about the time of the first mitotic division of the microspore. Dickinson associated sporopollenin production with vesicles of the endoplasmic reticulum which is conspicuous in the tapetum. The amount of polymer produced in the microspore at tetrad period is considered to be too small to produce a conspicuous change in the microspore cytoplasm so that the organelles involved there are unknown. The tapetally synthesize exine polymer is, therefore, characteristically deposited on a lipoid substrate although the mature orbicules and peritapetal membrane are said to have no remaining lipophilic material. This seems unusual in view of the affinity of the exine, and orbicules of many pollen grains for lipophilic dyes such as Scarlet R, Auramine O, etc., and autofluorescence characteristic.

The layers of the exine can be distinguished from one another cytochemically by the use of certain dyes. Especially useful are toluidine blue, which generally stains the exine layers blue or green, and auramine O gives a brilliant yellow fluorescence, while the nexine-2 may not be fluorescent, for example, in Acacia polyads. The nexine-2 in some types is stained by decolorized aniline blue, giving a bright yellow fluorescence, for example, in suntlower, *Helianthus annuus*.

Rowley and co-workers employed chemical etching techniques to dissect away the sporopollenin, revealing the existence of the underlying glycocalyx work. Results have been interpreted in terms of a model of exine structure and a development of an early model.

Finally, the surface of the exine must be considered as it is the site, which first makes contact with the stigma at pollination. Here, signals may be generated and exchanged during the biocommunication that is an essential prelude to pollen-stigma interactions. Exine surface coatings have been observed in many pollen types: *Clivia*, *Populus*, *Aegiceras*, and *Gladiolus*. The most convincing demonstrations have resulted from the work of Rowley and co-workers, who have used cations (such as ferric iron, thorium, and lanthanum) or cationic dyes (such as ruthenium red or alcian blue) to stabilize polysaccharides. In Populus the exine coating after traditional buffered glutaraldehyde fixation, is a simple trilamellate structure (Rowley and Erdtman 1967). However, after addition of alcian blue or ruthenium red to the glutaraldehyde fixative, the exine surface coating consists of a fine reticulum.

3.5 Intine Synthesis and Deposition

The intine is the final wall to be synthesized in the microspore. Often it does not reach its final thickness until the maturation period after the microspore mitosis. The intine is the essential wall layer of pollen grain since none has been observed, in which this layer is absent. The intine is a smooth layer, with a microfibrillar ultrastructtiral appearance, although it may possess several distinctive zones differentiated by their granularity, electron density, other features including the presence of tubular or lamellar structures. Chemically, the intine is readily solubilized by acetolysis, by treatment with EDTA, ammonium oxalate, and monoethanolamine. Cytochemically, it shows a positive reaction with carbohydrate and protein stains.

The intine is synthesized from the haploid microspore or pollen protoplast. In some pollen types, Golgi bodies are frequent during intine synthesis, for example Ranunculaceae, *Olea europaea*; while others, endoplasmic reticulum and polyribosomes are abundant, for example *Cosmos*. The intine may contain proteins glycoproteins stored in its polysaccharide matrix, either through microvillus-like extensions of the plasma membrane or by a process of exfoliation in which ribbons or leaflets or tubules containing the proteins appear within the wall. In *Heliconia* radically aligned channels are apparently dissolved in the intine. In the mature *Heliconia* intine these channels are sealed by a thin coating of exine on the external side, and by a thin layer of radially oriented microspore fibrils on the inner surface.

An important function of the intine, in its broadest sense, is to form the wall of the germinal apertures. It is at these sites that the intine reaches its greatest complexity, for example in *Malvaviscus*. In the Compositae the aperture development is of especial interest, as the oncus develops at late tetrad period, while the microspore is within the callose special wall.
This has been noted in several Compositae, including *Artemisia* and *Helianthus*

Indeed, aperture development in *Artemisia* occurs from white line centred braneous lamellae which form at the site at which the lens-shaped oncus develops. At spore release period, a biconvex orate lens differentiates under the pore. Ultrathin sections show that the oncus is formed from the nexine (endexine) is a complex lamellar structure, 2-3μm in diameter. Later in pollen developemt it regresses, and is absent by the maturation period. Rowley and Dahl (1977) show that concurrently with oncus regression, an ellipsoidal disc of intine forms ler the apertures, providing the tip zone of the putative pollen tube.

In grass pollen grains the aperture structure is also remarkable. The first ultrastructural studies, for example, of *Poa annua* pollen, revealed the existence of lens-shaped oncus beneath the operculum, and above the intine layer. The layer had a distinctive ultrastructural appearance-homogeneous without the tubules that traversed the intine proper-and had a higher electron density.

4 Pollen Germination and Pistil Interactions

Pollen germination is triggered by hydration, either in vitro or in vivo, on the moist surface of the stigma. The pollen tubes may grow extremely rapidly and can be observed within 1 min in wheat.

4.1 Pollen Quality

Pollen quality is dependent on many factors, including the genetic background of the material, the environment in which the plants were grown to flowering, and the methods of pollen collection and storage in breeding experiments. Pollen of high viability is needed for breeding purposes and, often, scanty information is published on this topic, in many crop plants, not to mention specific experimental systems. The methods available will now be described and their advantages and disadvantages outlined, where known.

For storage of pollen, pollen must generally be dried, at least partially, over a desiccant before storage in vials at low temperatures. Bicelluar types of pollen retain their viability

longer in storage than tricellular types. Temperature and relative humidity are considered to be the major factors controlling successful storage. Storage had the effect of greatly weakening pollen tube growth. Thawing of the pollen, even once, during storage, resulted in complete inviability.

Accordingly, what methods are available to determine pollen quality? Three classes of techniques are currently available, but none are completely satisfactory.

1. *Direct Staining of Pollen.* This method gives the percentage of filled normal grains produced by the anthers. It is assessed after staining pollen with, e.g., cotton blue in lactophenol, or 0.5% lissamine green, and viewed by bright-field microscopy, and is suitable for field conditions, It distinguishes between sterile or partially sterile plants, but does not give indication of loss of pollen viability in storage.

2. *Enzyme-based Methods.* Fluorochromatic reaction, FCR test. This microscopic test is applied to samples of pollen, and gives an estimate of the percentage of viable grains in the sample. The principle of the test is that a non-fluorescent ester enters the grains through the plasma membrane, is broken down by enzymic action to fluorescein, a highly fluorescent compound which is retained within the protoplast in viable grains, but lost from inviable grains. Its advantage is that it gives results within a few minutes; but its disadvantages are that it must be assessed at the time of preparation (no storage of slides) and requires fluorescence microscopy.

Other enzyme-based methods are available which produce coloured reactions visible in the light microscope. The most widely used is the TTC topographical enzyme test, based on the use of triphenyl tetraolium chloride (TTC). The principle of the test is the acceptance of H^+ions from enzymes in the pollen by TTC through flavoprotein nucleotides, which are intermediate H^+ion acceptors for dehydrogenase enzymes. Unfortunately, not all pollen is equally sensitive to this test, giving false positive results. The reagents in the TTC test are stable at 5℃ for 3 months if stored in the dark.

3. *Direct Pollen Germination.* There are two methods available. Hanging Drop Test: pollen germinated in a small droplet of sterile medium (sucrose and salts solution) adhering to the lower side of microscope cover-slip raised above the surface of microscope slide by plastic O ring. Agar Slide Test: pollen germinated on surface of a thin film of solidified agar (containing a carbon source such as sucrose, together with boron and Ca^{2+} on a microscope slide.

In both tests, the slide is sealed within a moist Petri dish, and the percentage pollen germination and tube length are assessed after a standard period (e.g. 2 to 6 h). Incubation at standard temperature is required. An interesting modification of the agar method is "David's Bread Loaf ", a solid agar medium which is employed as 2 mm thick, slices. For use, pollen is dusted onto the newly cut surface with a camel hair brush. Pollen that is readily, germinated in liquid media, e.g. of *Lilium,* or *Petunia*, may be sprayed on TLC plates for detection of growth inhibitors.

These germination tests can also be used to assess the vigour of pollen tube growth from pollen of different genotypes. There is some evidence that vigour of tube growth varies, and

that the most rapidly growing pollen tubes produce the most vigorous progeny. A general principle of all these in vitro germination tests is that a viability rating of 40% or higher is generally satisfactory for field pollinations.

A fourth test, involving NMR spectroscopy has recently been developed, and is non-destructive of the pollen sample.

4.2 Pollen Germination and Tube Development

When pollen hydrates, the pollen tube emerges from one of the previously differentiated germinal apertures in the wall of aperturate grains. In *Lycopersicon peruvianum*, the grains have three semi-spherical apertures which are extruded at hydration. On germination "the pore opens like a port-hole door; the thicker portion of the pore, protruding through the corresponding part of the exine, breaks off along a large part of its edge forming a kind of door that opens towards the outside. The portion of the edge that remains attached to the intine functions as a hinge".

In many types of pollen, the pollen tube tip is preformed in the aperture, which may account for the structural complexity of the apertural intine, and the enzymes laid down within its walls. The emergence of the plug-like tip or oncus of pollen tubes has been reported in *Lychnis*and in various Compositae: *Cosmos*, sunflower, *Helianthus*, and *Artemisia*. In *Artemisia* ultrastructural studies have demonstrated that the emergent tube is covered by a bubble of coarse fibrillar material through which the tube brows, surrounded by a modified intine-like wall. In germinating pollen of rye, *Secale cereale,* the oncuslike, Zwischenkorper"layer at the aperture forms a gel that swells displacing the sporopollenin cap and the tube emerges within the dispersing gel.

In a study of pollen germination in 12 genera with inaperturate grains, Muller- Stoll has outlined three common conditions: (1) in *Canna* and *Hedychium* the exine remains intact on hydration and the aperture, on germination, virtually punches the exit pore through it; (2) in *Arum* and *Aristolochia* slits appear in the exine at hydration, and the pollen tube exits through the slits; (3) in *Tulipa* and *Populus* the exine is brittle and tears into small portions at hydration, so that the germinating grains are almost naked , covered by the intine .

In inaperturate pollen types, the tube is formed by a wall that appears continuous with the intine, for example in *Populus*. There are differences in two cases. In avocado, *Persea Americana*, the pollen tube at germination may be surrounded only by the plasma membrane, wall deposition does not occur until the pollen tubes have entered the stigma. In the filiform pollen of the sea-grass, *Amphibolis antarctica*, pollen tubes are formed by dissolution of the intine-like pollen wall by a process of localized digestion, focal autolysis. The position of the aperture may be detected cytologically by a marked loss of staining affinity of the wall, indicating that the wall has apparently undergone dissolution. This may occur at one or more sites adjacent to the stigma. A tube-like outgrowth of the plasma membrane subsequently emerges within a mucilaginous bubble. The tube wall develops soon after emergence, and is cytologically distinct from most of the pollen grain wall. In these cases extracellular secretions of slime, containing carbohydrates, are involved in pollen germination, especially the early

stages of tube emergence and growth.

Growth of the pollen tube occurs at the tip, as detected in *Lilium* by the charcoal powder technique. Pollen tube cytology and physiology may differ between artificial and natural germination. Tubes growing in vivo on the pistil developed transfer cell wall ingrowths at the tube tip, while those germinating in vitro in growth medium had only a thin wall at the tip. On the stigmas, pollen of *Lycopersicon* hydrates within 15 min, and germination begins after 3 h 30 min. In contrast, in germination medium hydration occurred in seconds, and germination followed in 45 min. Bicellular types of pollen may show two phases of growth: an initial period of growth, followed by a second period of more rapid growth. In contrast, the tricellular types of pollen show only a single growth period. Questions that need to be answered concern the relationship- between the vegetative cell and the germinating tube. Is the tube an extension of the vegetative cell, capable only of its limited gene expression? Is the tube wall simply an extension, of the intine or one of its layers? It is well known that a callose wall is laid down, secondarily in the pollen tube in many systems, but is the pollen-tube wall unique in its physical and chemical composition?

4.3 Diagnostic Landmarks of Pollination

Fertilization in flowering plants involves interactions between the pollen grain or its pollen tube, and the pistil. The pistil comprises several diploid sporophytic tissues which surround and protect the embryo sac, the female gametophyte. The pollen grain alights on the receptive cells of the stigma, where germination may occur. This involves hydration and swelling of the dry grains, so that the pollen tube may emerge, penetrate the stigmatic surface, and grow through the apoplast system of the stigmatic zone. Pollen-tube growth is always intercellular, through the gel matrix of the transmitting tissue of the style, or the stylar canal mucilage. Ultimately, the tubes pass into the ovary, usually growing through a track of mucilage leading them directly to the micropyle of an ovule.

The growth of the pollen tube, or even pollen germination itself, may be halted in incompatible pollinations at several points. Such incompatible matings are of two types: foreign pollinations where there are genetic or physiological barriers preventing fertilization; self-incompatibility where a genetically determined system operates to prevent selfing and m thus, inbreeding. The system is commonly based on a major gene S, which has many alleles (usually 30-40); although as many as four major genes controlling self-incompatibility have been recorded. Pollen grains, that are otherwise quite fertile, are unable to effect fertilization on their own pistil. The system operates quite simple; when pollen and pistil carry the identical S-allele, incompatibility ensues; where the S-alleles differ, compatible pollination occurs. The incompatibility reactions of pollen grains, therefore, depend on mutual interactions with the pistil, so that the S-gene can be considered as a supergene controlling mutual recognition.

It is hardly surprising to find that self-incompatibility responses are of two different types: (1) Sporophytic systems where the pollen grain response is governed by the phenotype of the parent sporophyte, when dominance of one allele over another is exhibited, and as a consequence al the pollen grains within any one another behave alike in their incompatibility

response. (2) Gametophytic systems in which the behaviour of in dividual pollen grains follows that of their own S-genotype, and there is no dominance and, consequently, in heterozygous situations the pollen grains in any one anther will be equally divided in their S-allele type.

In sporophytic self-incompatibilty systems, such as those in the Cruciferae and Compositae, the site of pollen arrest is usually on the surface of the stigma, and experimental tests with proteins extracted from the surface of the pollen grains have demonstrated that pollen-held determinants control sporophytic S-gene action. These proteins are laid down in the exine along with the pollen-coat after transfer from the diploid sporophytic tapetal cells.

In contrast, the gametophytically derived fraction in the pollen wall is held within the intine and in the protoplast itself, and the site of polle-tube arrest is usually within the style. To date, it has not been possible to test experimentally whether the intine proteins are implicated in the control of gametophytic self-incompatibility because the interaction occurs deep within the style. Immunobiological tests have revealed the existence of an antigen in one pollen system-*Oenothera organensis*, those presence correlates precisely with the S-allele type of the pollen. Unfortunately, this antigen has not been characterized, nor has its presence been investigated in the stigma of *Oenothera.* In other stigma system, while S-specific antigens have been detected, they have shown no immunological identity with pollen fractions from the same system.

The inhibition of pollen-tube growth follow self-incompatible mating has recently been investigated in *Primula spp*. Which have a different control system, known as heterostyly. Here both pollen and pistil are morphologically different in the two forms of pin and thrum flowers. The system is di-allelic, and pin pollen is inhibited on pin stigmas, and the reciprocal. Compatibility follows outcrossing between the two forms. The pin extracts inhibited pin pollen germination in vitro, not thrum pollen, and the converse, in *Primula obconica*. The system in P. *vulgaris,* dialysates of high molecular weight fractions from stigma and style exert some differential effect on pollen tube growth. Those from thrum pistils retarding thrum tubes while having a lesser effect on pin tube, and the converse. These experiments suggest that control is exerted by the stylar S-gene product, but give no indication of the nature of the interaction with the pollen, pollen tube, or their putative receptors.

An interesting response to pollination is the production of callose in the wall of the pollen grain and pollen tube as a result of incompatible matings. The "rejection" callose deposits in the wall are detected cytochemically by fluorescence after staining with decolorized aniline blue. Callose contains a 1,3 β-glucan, and may be considered as an active rejection response. These authors have shown experimentally that extracts of the exine proteins will induce a callose response in stigma cells of Cruciferae in self-incompatible but not compatible systems. In other cases, as in the grasses, which have a gametophytic self-incompatibility system, callose is induced only in the inhibited pollen grain and tube. Evidence concerning these sites of origin of the proteins and glycoproteins active in these responses will be given later.

In order to explore the role of pollen grains, and their tubes, in pollination, specific mutants

of structural or regulatory genes are needed. Regrettably, only a few such pollen mutants have been detected:

1. Wall-shape mutant in sweet pea, *Lathyrus*: sporophytic inheritance.
2. Waxy mutant ill maize, *Zea*; gametophytic inheritance.
3. Alcohol dehydrogenase in maize, *Zea mays*; gametophytic inheritance.
4. β-galactosidase in *Brassica*; gametophytic inheritance.

The use of such mutants in controlled pollination experiments will provide a precise tool to explore the role of the specific gene products in the events of reproduction.

4.4 Role of Pollen in Fertilization and Seed-setting

The essential role of the pollen grain is in sexual reproduction; it is crucial to the survival of flowering plants. The structural adaptations of the pollen wall, especially for pollinators, wind and water currents, have been stressed in this chapter-but are there any direct co-adaptations between pollen and stigma?

The remarkable co-evolution between the pollinia of orchids, containing several thousand pollen grains, and the development of adhesive mechanisms for attachment of the pollinia in specific parts of the body of pollinating insects, and later on the stigma. The cell biology of the adhesive mechanism for attaching the pollinia of asclepiads to visiting pollinators has been investigated by Schnepf et al.. An unique translator organ, composed from lipids and mucilage, carries out this function.

Morphological adaptations have recently been noted in the family Leguminosae, subfamily Mimosoideae, whose species may have compound pollen grains or free grains (monads). In the Mimoseae, the different genera possess an unusual stigma morphology. The stigma cup varies from funnel-shaped in *Dinizia,* tubular in *Entada* and *Prosopis*, to narrow porate in *Adenanthera*, *Mimosa*, and *Piptadenia*. Polyads are recorded in genera with the two extremes-broader funnel-shaped stigmas, and very narrow porate types. Indeed, *Mimosa* has probably the smallest stigma, together with the smallest polyad recorded in the angiosperms. A similar correlation between stigma size and polyad size appears to operate in the Acacieae.

In different species of Acacia there is a polymorphism in polyad grain number - 4, 8, 12, 16 grains in Australian species, and 16, 32, 48, and 64 in African species. The significance of these variations is that there is a parallel matching in seed number per pod. In species with 4-grain polyads the maximum pod seed number is 3; with 8-grain polyads, 8; with 16-grain polyads, 12-16; with 32-grain polyads, 21-24 seeds per pod. The relationship is further supported by data from two genera of Mimoseae with free grains, which have 20-25 seeds per pod.

5 Pollen-wall Proteins and Allergens

One of the most remarkable features of the angiosperm pollen grain is the complexity of its wall which, as we have seen in this chapter, is usually a product of gene expression of parental determinants, the microspore, and tapetal cells. The role of this wall in protecting the male gametophyte during pollen dispersal has been emphasized, and the pollination strategies that

might influence the evolution of pollen shape have been featured. However, the existence of the intricate, cryptlike arcades with their microchannels within the pollen wall and the complex and specific patterning all suggest that the exine plays a crucial role in recognition and other early events of pollen germination on the stigma surface. The exine arcades are filled with proteins (for example, in *Iberis*) glycoproteins, carbohydrates, lipids, and pigments, to ensure both efficient pollen adhesion to pollinator or stigma surface, and also to initiate macromolecular contact with the stigma leading to pollen hydration and germination. Evidence for the presence of wan proteins will now be reviewed, together with the available experimental evidence concerning their functions.

Cytochemical methods, using freeze-sectioned anthers and pollen grains, have established that both the pollen wall and cytoplasm contain a wide range of enzymes. These tests depend on the production of coloured products on the hydrolysis of insoluble artificial substrates. Using such methods, the presence in the pollen wall of hydrolases, transferases, dehydrogenases, oxidases, ligases, and lyases has been demonstrated. Many of these enzymes are apparently located within the polysaccharide matrix of the intine, especially the hydrolases. Others are present in the arcades of the exine especially the dehydrogenases; some of these enzymes are present in both sites. Wall-held enzymes occur in the spores of several lower plants.

The presence of allergenic proteins and glycoproteins in wind-borne pollen types led to the first immunocytochemical studies of flowering plant antigens being carried out on pollen, with the aim of locating the sites of allergens in the grains. In pollen that is dispersed by air currents, and so is able to make contact with the upper respiratory tract of man, allergens are released causing seasonal asthma and hay fever. Immunocytochemical studies have revealed that the allergens of ragweed responsible for autumnal hay fever in North America are located in the outer wall of the pollen grain. Immunofluorescence studies of pollen, freeze-sectioned within the anther to minimize diffusion of antigens, showed that Antigen E (the principal allergen, a protein of MW 38,000) is concentrated in the intine, and also in the exine, where it occurs in both the arcades and at the surface. Birch pollen allergen is also present at the exine surface.

These immunofluorescence studies revealed that when the pollen is moistened, even only momentarily, the allergens would be released to coat the surface of the grains. The two principal routes for protein emission are shown in Table 4.2. When ragweed pollen was freeze-sectioned by preparing slurry in 15% gelatine, and frozen within 30 s of pollen contact, all the antigens were found in the gelatine surrounding the grains. This extreme mobility of the antigens has been confirmed by making pollen prints of fresh pollen, to observe the routes and time of antigen emission. The pollen was sprinkled on adhesive tape, placed in contact with a thin agarose gel on a microscope slide, removed after varying intervals of time, and the film dried rapidly and processed for Antigen E activity by indirect immunofluorescence. The pollen prints revealed that Antigen E is present in both the, exine, from which it is immediately released, and intine at the apertures from which it is released more slowly during germination. Freeze-substitution followed by paraffin embedding with minimum exposure to high

temperatures produced sections which gave the same localizationin mature dry pollen as had been obtained with freeze-sectioned pollen within the anther, but without the hazard of diffusion artifacts. The structure of the pollen wall of ragweed is adapted for rapid release of its wallheld proteins, and for wind-pollination

These observations on the kinetics of allergen release suggest that the antigens play a role in pollination: in male-female recognition at the stigma surface. An interesting adaptation of immunofluorescence has provided circumstantial evidence for this in both ragweed and grasses: pollinated stigmas were placed into specific antipollen diffusate sera in order to fix the antigens in their sites during the pollination process. The pollen antigens were shown to be present both on the surface of pollen grains and tubes, but also bound to the stigma surface.

Grass pollen is dispersed in air currents, and is the major source of seasonal asthma and hay fever in spring and early summer in temperate climates. The allergens of grass pollen have been recently located by immunocytochemistry using antiserum to purified pollen components. The pollen of rye grass, *Lolium perenne*, contains four groups of allergens, named Groups I-IV. Group I allergen is the major allergenic fraction: it is a glycoprotein, MW 32,000, existing in multiple electrophoretic forms which differ only slightly in amino acid composition. A similar fraction has been isolated using ion exchange chromatography and gel filtration, and has all the properties of Group I allergen including similar behaviour by starch gel electrophoresis, antigenicity, and allergenic activity.

A post-embedding staining technique has been developed to overcome the difficulties of antigen mobility in aqueous-media. Anthers of ryegrass just prior to anthesis are either briefly fixed in cold methanol or freeze-dried, and placed directly in a water-soluble methacrylate resin, which has the advantage of short infiltration and embedding time at 2 °C, and of being anhydrous so that there should be little possibility of the antigens showing diffusion artifacts. In immunofluorescence studies, the specific fluorescence is observed in the outer wall layer, and also in the cytoplasm of the sectioned pollen grains. In order to precisely determine the sites of these antigens within the grains, immuno-electron microscopic methods have been developed. The indirect method is used, with the anti-antibody labelled with ferritin. The results are spectacular: using specific antisera, ferritin molecules are observed bound to spherical material within the exine cavities, to wisps of material at the exine surface, to vesicles within the intine, and at the periphery of the cytoplasm.

Chapter 5 The Ovule

1. Ovular Morphology

The ovule is the forerunner of the seed. Therefore, studies of the ovule are of vital importance for the understanding of the structure and function of the seed. The ovule consists of nucellus, integuments, chalaza, raphe, and funicle (Fig. 5.1).

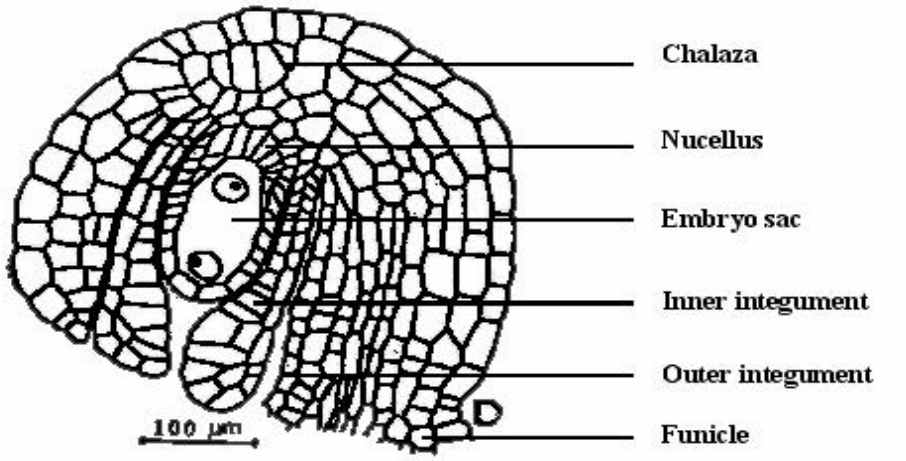

Fig. 5.1 The structure of ovule

The nucellus represents the homolog of the megasporangium of the progenitors of the seed plants, i.e., the organ in which the process of megaspores takes place. Since, in the spermatophytes, the megaspores are mot liberated from the megasporangia any longer, it is also the site of megaspore development (megagametigenesis). Within the megagametophyte (embryo sac) endosperm and embryo are formed after fertilization.

The nucellus is enveloped by one or two integuments. They overgrow the nucellus and arch over its apex to form the micropyle which acts as a passage for the pollen tube. The integuments protect and nourish the nucellus and its contents. In the mature seed the integuments, together with the chalaza and raphe, form the seed-coat.

The most commen type of ovule is the anatropous one. During its development this ovule undergoes a curvature of 180º, so that it becomes completely inverted and the micropyle comes to lie close to the place of attachment of the ovule.

The ovule is attached to the gynoecial placenta by a stalk, the funicle. In most ovules the funicle is provided with a vascular bundle. The part of the funicle that runs parallel with the nucellus is called the raphe. The chalaza is the region between raphe and nucellus, where the integuments are inserted. In fact, funicle, raphe, chalaza, and nucellus form a continuous tissue, the central axis of the ovule, and no sharp demarcation lines can be drawn between them. The structure of the ovule can become complicated by curvature as in campylotropous and

amphitropous ovules.

Already before fertilization special structures, such as appendages (arils and arilloids), operculae, hypostases, and epistases, may start their development.

2. Ovule Initiation

Our knowledge about ovular initiation has not advanced much since Warming's publication of 1878. Floral morphologists usually limit their study to carpel initiation, and embryologists start with the ovule primordium without paying attention to its initiation. In most publications ovule primordia are described as mere bulges arising from the placenta, and no more precise information is given about its early histogenesis.

Ovule primordia are initiated by periclinal divisions in the seconds or third layer of the placenta, so that we can distinguish two-or three-zonate primordia, respectively. In transverse sections they always exhibit a radial symmetry. Kordyum states that the histogenetic organization of the ovule primordia is similar to that of the shoot apex (according to the concept of tunica and corpus). This may be true to some extent, but it is more appropriate to use the neutral terminology of two- and three –zonate (or di – and tri- zonate). Ovule primordia, since we do not have a fair cognizance of the continuity of the independence of the tunica layers during the transition from the vegetative apex to the floral primordium, and the consequent formation of the carpellary, placental, and ovular meristems.

3. Nucellus

The nucellus develops out of the apex of the ovular primordium. During early stages one cell, lying directly below the nucellar epidermis, differentiates into a primary archesporial cell, which is conspicuous by its larger size, denser cytoplasmic contents and more prominent nucleus. In the majority of the angiosperms the archesporial cell divides periclinally to form the primary parietal and primary sporogenous cells. The former may undergo a variable number of further periclinal and anticlinal divisions to form the parietal tissue which, ultimately, is one to ten or more cells thick (up to 40 in Vochysiaceae). The primary sporogenous cells usually do not undergo further mitotic divisions, and functions as the megaspore mother cell. In more derived forms the archesporial cell does not form a parietal layer but directly act as the megaspore mother cell.

In some taxa regularly, in others more occasionally, more than one megaspore mother cell may be present. This is brought about either by the differentiation of several archesporial cells, or by the mitotic division of the primary sporogenous cell. Multicellular archesporia occur in many taxa, they are common in Casuarinaceae, Rosaceae, Compositae, Rubiaceae, U mbelliferae, and Liliaceae. They play a role in polyembryony and apomixes.

The differences in nucellar morphology and size are caused by a number of characters, viz., the structure of the apex of the ovule primordium, the number of archesporial cells and whether or not the latter form parietal cells, the amount of parietal tissue, the presence of cells adjoining the archesporiumm the mitotic activity of the adjacent and underlying cells of the

integument, and others.

Since the nucellar morphology is determined by so many characters, exhibiting reduction as well as specialization trends, no classification can ever adequately reflect the great diversity in nucellar structure and dimensions, In spite of the restrictions of the customary classification in crassi- and tenuinucellate ovules, it appears to be the best available and has proved to be of much practical value. Ontogenetically, the nucellus develops from the apex of the ovule primordium. Accordingly, the structure of this apex is the point of departure, and also the basis for description of the nucellus.

Generally, the dermal layer of the nucellus undergoes only anticlinal divisions, thus remaining one layer thick. However, in many cases the dermal cells of the nucellar apex divide periclinally to form a plurilayered epidermis or nucellar cap. Massive nucellar caps are known to occur in Aceraceae, Bombcaceae, Burseraceae, Combretaceae, Meliaceae, Petiveriaceae, Rhamnaceae, Rosaceae, Simaroubaceae, Vitaceae, and Agavaceae. In the extreme case the nucellus becomes beak-shaped. The nucellar beak protrudes from the micropyle or even extends beyond both integuments finally to reach the obturator ouside the ovule (Fig. 5.2A). Nucellar beaks are known in Aextoxicaceae, Cucurbitaceae, Euphorbiaceae, Malpighiaceae, Polygonaceae, Rhamnaceae,and Trapaceae.

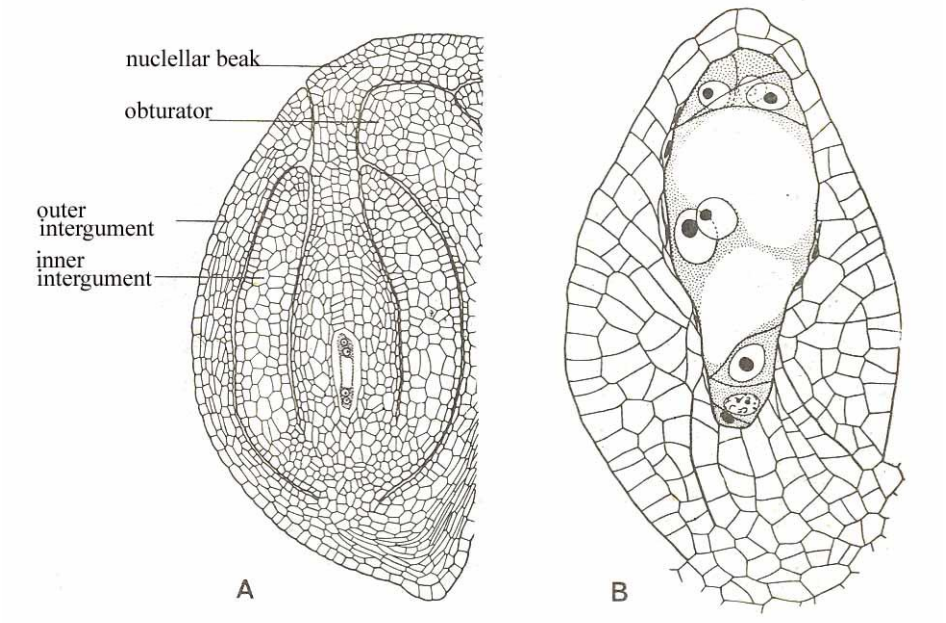

Fig. 5.2 A Ovule of *Chrozophora obliqua* with nucellar beak protuding from the micropyle. B Nucellus of *Medeola virginiana,* showing multilayered epidermis laterally.

In liliaceae and Gramineae the nucellar epidermis remains one – or few-layered at the apex, but becomes plurilayered around and below the middle of the embryo sac, thus contributing toward the bulk of the nucellus (Fig. 5.2B). In Zingiberaceae, N ymphaeaceae, Centrolepidaceae, and some Liliaceae the cells of the nucellar epidermis undergo a

pronounced radial stretching, sometimes followed by cell wall thickening (the nucellar pad).

In an attempt to construct a more detailed classification of the nucellus, Davis introduced the term pseudocrassinucellate for tenuinucellate ovules provided with a plurilayered epidermis. Although this term may be useful for embryological descriptions, it seems incorrect to consider it as equivalent to crassi-and tenuinucellate. The incidence of periclinal divisions in the nucellar epidermisis far less essential than the presence of parietal cells If the term is to be used at all, it must be employed for a subdivision of both the tenui-and crassinucellate categories.

The destiny of the nucellus in the mature seed may be quite different. In small ovules the nucellar tissue can already be resorbed in the mature ovule, in most bigger ovules it is completely or almost completely consumed during embryo and endosperm development, whereas in, e, g., Piperaceae, Nymphaeaceae, and Znigiberaceae it undergoes a pronounced development and forms a persistent storage tissue, the perisperm.

4. Megasporogenesis

The megaspore mother cell (megasporocyte or meiocyte) (Fig. 5.3A) divisions, and gives rise to four haploid megaspores whose mutual arrangement varies. The first or heterotypic division is always transverse and results in two dyad cells (Fig. 5.3B). Usually, the micropylar dyad can also divide longitudinally to form a T-shaped tetrad (Fig. 5.3C,D) Frequently, the micropylar dyad can also divide longitudinally to form a T-shaped tetrad (Fig. 5.3E,F). According to the data collected by Davis, 213 angiospermous families are characterized by having exclusively linear tetrads, while in 56 families T –shaped tetrads occur next to linear ones. Especially within this group also tetrads of an intermediate type occur, in which the wall between the two micropylar megaspores lies at an angle of 45º. Other configurations as ⊥ -shaped (Musaceae), isobilateral (Crassulaceae), and tetrahedral (Hydrocharitaceae) ones are rare, and mostly found in combination with linear and T-shaped tetrads. Meiosis II may be synchronous or heterochronous. The division of one of the dyad cells can be further suppressed in such a way that no separating cell wall is formed, and the dyad becomes binucleate, or that the division is abortive. Complete suppression results in a row of three cells, of which only two are megaspores (Fig. 5.3G, H). Such triads have been described in Amaranthaceae, Araliaceae, Cactaceae, Caryophyllaceae, Orchidaceae, Petiveriaceae, and Phytolaccaceae.

The above –mentioned suppression of the development of the nonfunctional dyad may be interpreted as a trend toward further reduction. However, failure of wall formation in the functional dyad, or even already in the meiocyte, represents a trend to specialization. By this elimination of cytokinesis, binucleate, or tetranucleate coenomegaspores are formed, respectively (Fig. 5.3 I-N). These conditions will lead to a bisporic and tetrasporic development of the embryo sac, which is more extensively discussed in Chapter 6.

In the majority of angiosperms (268 families according to Davis 1966), the three micropylar megaspores degenerate and only he chalazal one develops onto megagametophyte (embryo

sac). Only in exceptional cases does the subchalazal (Caealpiniaceae, Cunoniaceae, Symplocaeae), the micropylar (Balanophorscese, Calenduleae, Onagraceae, Rosa,Furcraea) or any given megaspore (Casuarina, Gloriosa, Ostrya, Poa,and Rubus)becomes functional.

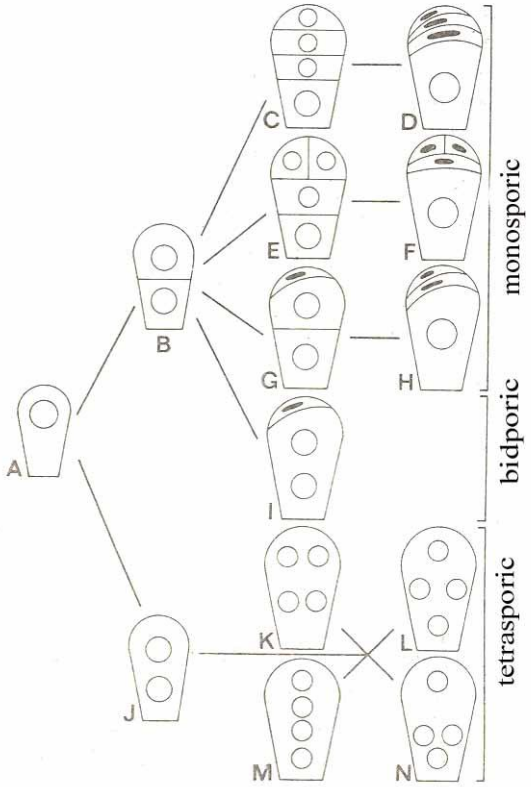

Fig. 5.3 Different types of megasporogenenis. A Meiocyte. B, J Dyads. C, D Linear tetrads. E, F T-shaped tetrads. G, H Reduction of the micropylar dyad. I Bisporic development. K-N Tetrasporic development

The degeneration of the nonfunctional megaspores is accompanied with a high density of ribosomes, appearance if dictyosomes and lipid granules, vesicles and autophagic vacuoles, and results in a loss of turgescence and a low cellular activity. The products of degeneration of the megaspores and surrounding nucellar cells can be used by the functional megaspore.

By applying the aniline-blue fluorescence method to ovular squashes of Orchis maculata, Rodkiewicz was the first to demonstrate callose formation during megasporogenesis in angiosperms. Subsequently, a fair number of observations have been made. In the course of megasporogenesis callose appears transiently in the cell walls of plants with a mono- or bisporic type of embryo sac, but the polysacchride has till now not been observed in species with a tetrasporic type. The formation of callose in the walls during megasporogenesis is similar to that in microsporogenesis. Callose always appears in the early meiotic prophase.

The presence of callose seems to be more than just an archaic feature. It is assumed that the callose wall forms a molecular filter decreasing the permeability of the cell wall. In this way

the generative cells become temporarily isolated, which enables the cells to embark upon an independent course of development.

Besides, the uneven distribution of callose in megasporogenesis is clearly connected with the strong polarization of the cells. The beginning of callose deposition and its disappearance, correspond with the localization of the active megaspore in the tetrad.

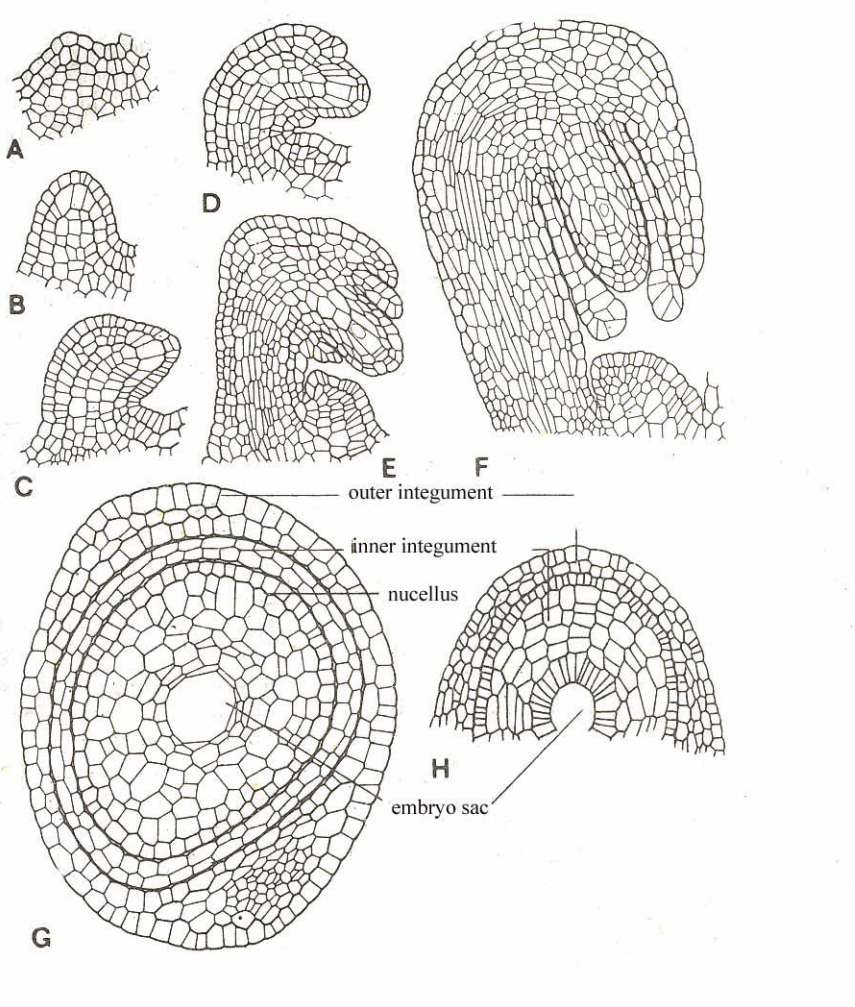

Fig.5.4 lntegument ontogenesis. A-F Nigella damascena, ovule and integument initiation. G Polygalavulgaris, cross section of an ovule showing partly subdermal outer integument. H Lunaria annua ,cross section with subdermal part of the outer integuments laterally, inner integument multiplicative, nucellus resorbed; the layer of radially-elongated cells around the embryo sac is endothelium

5. Integuments

Angiospermous ovules may have two, one or no integument, and are consequently called bitegmic, unitegmic, and ategmic. The initiation and early histogeny of the integuments have been studied inadequately. The inner integument is usually initiated before, sometimes

simultaneously, with the outer integument. In Podostemonaceae and some Malvaceae the outer integument is initiated first. Other reports in which the outer integument is said to appear first need confirmation. The inner integument is almost always of fully dermal derivation. It is initiated by periclinal or oblique divisions in a circular zone around the ovular primordium mostly two to three cells high. Successive longitudinal divisions give rise to an integument two to three cells thick. The family Euphorbiaceae is the only to one in which a subdermally initiated inner integument is reported.

The outer integument is initiated in either the dermal or subdermal layer of the ovular primordium. If dermal, the development is comparable to that described for the inner integument. The subdermal outer integument consists of a middle layer one to several cells in thickness, covered with a true outer and inner epidermis (Fig 5.4A-F). At the apex the dermatogen mostly divides periclinally to form the micropylar part of the outer integument. The type of initiation of the outer integument is generally a taxonomic character at the family level. The outer integument in Magnoliaceae, Ranuculaceae, Rosaceae, and Papilionceae is subdermal, and Geraniales dermal. Dermalization of the outer integument must have taken place several times during the phylogeny of the angiosperms. Both dermal and subdermal outer integuments are in a number of families: Cruciferae, Malvaceae.and Rutaceae. A partly dermalized, asymmetric, outer integument occurs in *Polygala vulgaris* (Fig. 5.4G) and *Lunaria annua* (Fig.5.4H).

Bitegmy is the more common, and primitive condition. The evolutionary changeover from bitegmy to unitegmy must have taken place many times. Well-known families with both bitegmic and unitegmicspecies are: Corylaceae, Cytinaceae, Fabaceae, Fagaceae, Menispermaceae, Monimiaceae, Myrtaceae, Nyctaginaceae, Piperaceae, Ranunculaceae, Rosaceae, Salicaceae, Saxifragaceae, and, among Monocotyledons, Amaryllidaceae, Orchidaceae, and Poaceae. Next to families with bitegmic and unitegmic ovules, the category of families showing ovules with a structure intermediate between bi-and unitegmy (by having a bifid integument)can yield indications about the process of unitegmization. There seem to have been three different pathways to unitegmization. The simplest is the elimination of one of the two integuments by retardation or complete suppression of its development (Fig. 5.5 A, B). Examples of a probable loss of the outer integument are some Piperaceae, Hydnoraceae, and Rafflesiaceae. A very weakly developed inner integument is described in *Populus*. A second pathway, the fusion of integumentary primordia, is only possible if the two integuments are initiated in the same fashion (Fig. 5.5C). Such a fusion between a dermal inner and a dermal outer integument gives rise to a single, massive, dermal integument as in Anacardiaceae and Balsaminaceae. A third pathway, integumentary shifting (Fig.5.5D), has recently been described in Ranuculaceae. It takes place in ovules with a dermal inner, and a subdermal outer integument. Integument shifting a complicated ontogenetic process involving (1) a fusion of primordia in the sense that the initials of the two integuments give rise to a common structure, (2) a shifting of the inner integument, and (3) an arrested grow of the latter (Fig.5.5A-D).

The unitegmic condition must have been achieved several times, and along different

ontogenetic pathways during the evolution of the angiosperms. This means that the condition of unitegmy is not necessarily homologous throughout the angiosperms. Detailed studies on the initiation of the integument in unitegmic families are scanty. Most data still date from Warming who concluded that the integument of sympetalous taxa, in spite of is thickness, is mainly of dermal derivation. Independent evidence of the dermal origin of the single integument was obtained by Satina from observation on cytochimeras of *Datura*, and by Bhandari et al. in *Scrophularia*. However, Bouman and Schrier showed that the single integument can also be of subdermal derivation (e.g., Convolvulaceae and Cuscutaceae), and that in *Gentiana* the sequence and position of the dermal and subdermal initials render it plausible that this single integument has been derived from a dermal inner and a subdermal outer integument by integument shifting.

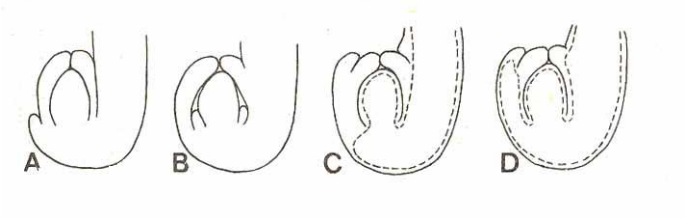

Fig.5.5 Intermediate stages in unitegmization. A Reduction of the outer integument. B Reduction of the inner integument. C Integumentary fusion. D Integumentary shifting

Integuments often increase in thickness during ovule and seed development. Such integuments are called multiplicative.The mode of multiplication depends on the type of integument. A subdermal (outer) integument can become plurilayered by periclinal division of (1) the outer dermal layer (Cucurbitaceae), (2) the subdermal, middle layer (most common), or (3) the inner dermal layer (Magnoliaceae, Vitaceae). Two-layerd (i.e., dermal) integuments can become three-layered by periclinal divisions of the inner layer. In many cases only the middle layer divides further periclinally, thus giving rise to a plurilayered integument: e. g., the inner integument of Cruciferae (Fig. 5.4), Geraniaceae, Linaceae(Fig 5.6A,B),the outer integument of Oxalidaceae and Liliaceae. However, in primary or secondary three –or more –layered dermal integuments both the inner and middle layer can participate (Dichapetalaceae).The growth in length and thickness of the integuments can be successive, or proceeds simultaneously. In the latter case the analysis of the precise course of ontogeny is rendered much more difficult.

One of the more remarkable phenomena in the histogenesis of integuments is the differentiation of an integumentary tapetum of or endothelium. This has been recorded in about 65 families of dicotyledons and seven of monocotyledons. Both the terms and criteria, and also the putative function, have long been much debated. The endothelium is difficult to define, because it's morphological, cytological, and functional characteristics show much variation. It forms a new limiting layer of the embryo sac in many ovules, where the nucellus disorganizes at an early developmental stage. As such it is especially present in tenuinucellate or weakly crassinucellate ovules. The endothelium originates from either the inner layer of the single

integument as in unitegmic ovules of sympetalous families, in which group it is best known, or from the inner layer of the inner integument as in bitegmic ovules of Celastraceae, Elaeocarpaceae, Erythroxylaceae, ecythidaceae, Linaceae, Primulaceae, and Zygophyllaceae. It often surrounds the embryo sac almost completely, but may also be confined to a part of it as in Ericaceae, Gesneriaceae, and Scrophulariaceae. In most families it remains uniseriate but in Compositae it becomes bi-or multilayered. The cells become radially stretched and they contain prominent nuclei which often behave aberrantly. The endothelial cells can become bi-or multinucleate, or polyploid by endomitosis and nuclear fusion.

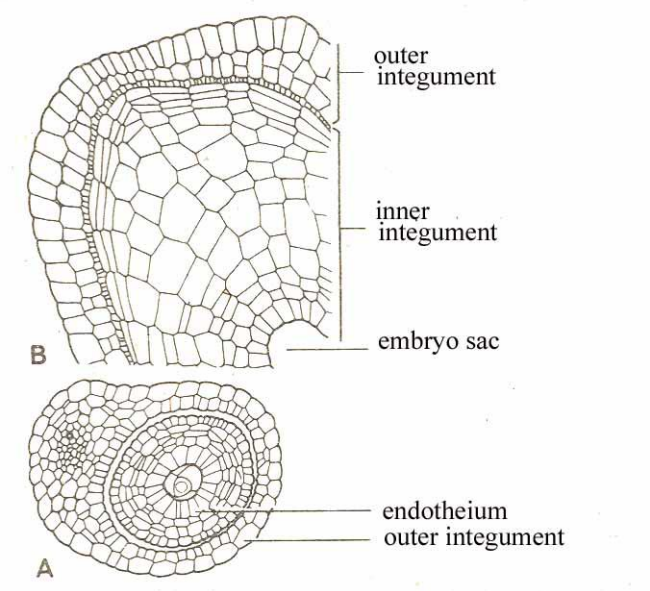

Fig 5.6 *Linum usitatissimum.* developmcnt of the multiplicative inner integument. A Cross section of young ovule. B Cross section of immature seed.

6. Types of Ovules

The ovule may have various forms, depending on the shapes and position of the different ovular parts. We can distinguish between ovules with straight nucelli (anatropous and atropous ones) and those with curved nucelli (campylotropous and amphitropous ones) (Fig. 5.7).

Anatropy is of most common occurrence among the angiosperms. According to Davis, 204 families are exclusively anatropous. The anatropous curvature starts shortly after the initiation of the ovule. It is, in fact, an intercalary growth of the funicle. This growth takes place on the convex side of the ovular primordium in the zone below the attachment of the integument (s), and on the concave side in the zone between the outer and inner integument (in bitegmic ovules), or between the integument and nucellus (unitegmic ovules). By this process the nucellus turns over 180º and the micropyle shifts toward the future hilum. In anatropous ovules the outer (or single) integument is less strongly developed at the raphal side.

Occasionally, the indication of its presence is restricted to an enlargement of a few epidermal cells; in extreme cases there is not a single trace of an integument in that region.

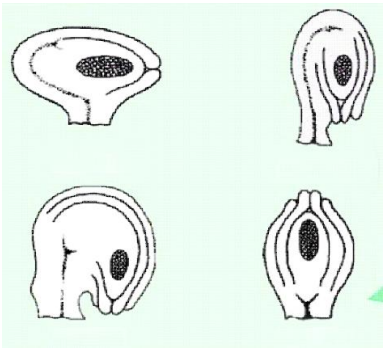

Fig 5.7 The types of ovules

After a study on the anatropous curvature, Bor concluded that the ovule consists of two separate developmental "units": (1) the funicular unit which gives rise to raphe, chalaza, and outer integument, and (2) the nucellar unit from which the inner integument is derived. This hypothesis does not seem to be in accordance with the ontogenetic facts.

Orthotropous or atropous ovules occur in 20 families (e. g., in Juglandaceae, Polygonaceae, Urticaceae, Najadaceae). Funicle, chalaza, nucellus, and micropyle lie in a direct line. Owing to its common presence in the gymnosperms, several authors consider the orthotropous ovule to be primitive. It is probable, however, that in at least several angiospermous families the orthotropous condition has been derived from the anatropous one, and may be correlated with the trend to mono-ovuly. The changeover to orthotropy has consequences for the pathway of the pollen tube. Orthotropous and anatropous ovules are connected by a complete range of transitional stages. If the nucellus forms an angle of about 90º with the funicle, the ovule is called hemi (ana) tropous. On the other hand, in the circinotropous ovule the curvature of the funicle exceeds 180º and it encircles the ovule more or less completely (Cactaceae, Plumbaginaceae)

Ovules with curved embryo sacs occur in many families of the Centrospermae, Cruciferae, Leguminosae, Alismataceae, Butomaceae and others. In most textbooks a distinction is made between campylotropous ovules characterized by a slightly curved, kidney-shaped embryo sac, and amphitropous ovules with a more strongly curved, horseshoe-shaped embryo sac. Since these do not provide clearcut criteria and do not reflect ontogenetic aspects, other workers use the term campylotropous for all ovules with curved embryo sacs. According to Bocquet, and Bocquet and Bersier, the bending of amphitropous ovules is accompanied by the formation of a so-called basal body which extends into the arch of the nucellus. They clearly demonstrated that campylotropy and amphitropy are independent processes in respect of anatropy. Combining the basic series of ortho-, hemi-, and anatropy with campylotropy and amphitropy, they distinguish nine different types of ovules (Fig. 5.8). This classification has

not come into general use, and does not seem adequate to cover the great diversity. For instance, the basal body may be of different ontogenetic origin. It is sometimes formed by the nucellus or raphe, sometimes by the inner or outer integument, or by two or more in combination. Moreover, a curved embryo sac may also develop secondarily from a straight nucellus as a result of the resorbing activity of embryo sac or endosperm. Curvature of the embryo sac is clearly a derived character, and has the advantage that the embryo may become twice as long as the seed.

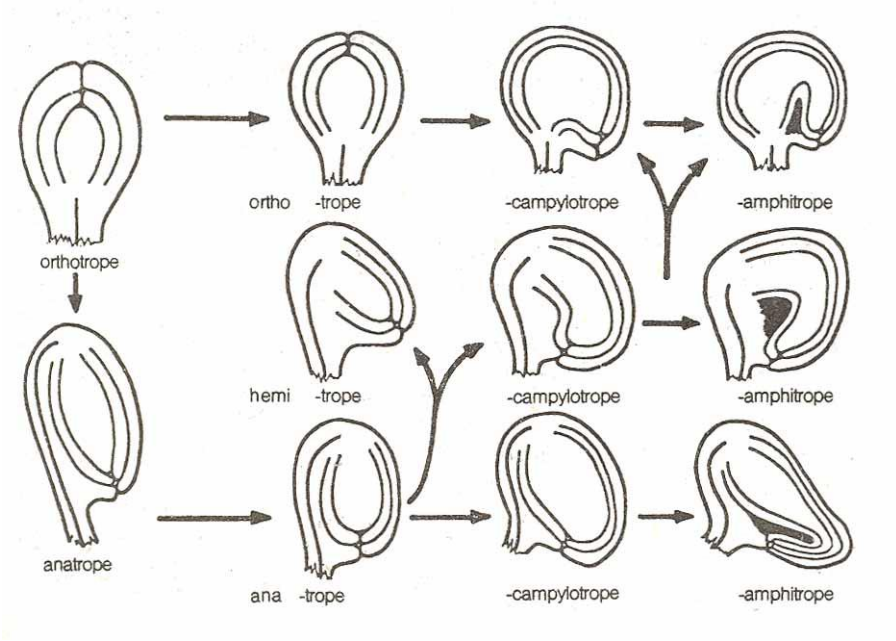

Fig. 5.8 Relations between orthotropous, anatropous, campylotropous, and amphitropous, and amphitropous ovules.

The great diversity in the distribution and structure of ovules with curved embryo sacs renders it plausible that they have developed more than once, and along several developmental pathways during the evolution of angiosperms.

7. Vascular Supply of the Ovule

Most angiospermous ovules have a very simple type of vascularization: the funicle and raphe contain a single bundle which ends blindly or fans out in the chalaza. But little attention has been paid to the structure of the funicular and raphal bundle. Collateral bundles seem to be most common but, probably, amphicribral ones are not rare (e.g., Cruciferae, Malvaceae, Pittosporaceae, Rutaceae, Styracaceae, Zingiberaceae). Multiple raphal bundles occur in Erythroxlaceae, Juglandaceae, Myricaceae, Myristicaceae, Papilionaceae, and Polygonaceae. Orthotropous ovules show a tendency toward a more concentric arrangement of vascular elements.

In a fairly large number of families the outer or single integument may also become

vascularized and, in rarer cases, even the inner integument or the nucellus. By analogy with floral morphology, the importance of the ovular vascular supply is not consistently appraised in current literature. Some authors consider integumentary bundles as original and as representing vestiges of more extensive vascular patterns in the progenitors of the angiosperms, whereas others suppose, from a more functional point of view, that vascular bundles simply differentiate “where they are needed ”, so that their presence does not have any phylogenetic implications. There seems to be a general relation between the size of ovules and seeds and the degree of vascularization. Large ovules and seeds mostly have a more extensive vascular supply, whereas small ones show reductions. Xylem elements become differentiated only in later developmental stages, or not at all, and the raphal bundle remains procambium-like (Begoniaceae, Gentianaceae). In the smallest ovules (Orchidaceae) there is not even a trace of any provascular tissue in the raphe.

Vascular bundles in the outer integument are present in at least 80 families (e. g., Annonaceae, Cucurbitaceae, Euphorbitaceae, Leguminosae, Magnoliaceae, Myristicaceae, Rosaceae, Sterculiaceae, and Vitaceae). The vascular pattern varies from a single bundle in the antiraphe to an extensive network of bundles. In monocotyledons integumentary vascularization is more incidental.

Vascularization of the single integument occurs in, e.g., Alangiaceae, Boraginaceae, Compositae, Convolvulaceae, Juglandaceae, Myricaceae, and Oleaceae.

Vascularized inner integuments are far less common; and have only been reported in at least some species of Dipterocarpaceae, Elaeocarpaceae, Euphorbiaceae, and Myristicaceae.

The problem of nucellar vascularization of the angiosperms has received much attention, especially in connection with the presence of vascular bundles in the nucellus of several extinct gymnosperms (Trigonocarpales and Cardiospermales). Vascular elements in the nucellus have been reported in taxa belonging to about 14 angiospermous families. The records vary from isolated tracheidal cells around the embryo sac (Asclepiadaceae, Casuarinaceae), or a subepidermal sheath of tracheid-like cells in *Luffa* to distinct vascular bundles connected with the vascular supply of the raphe as in Thymelaeaeceae and Euphorbiaceae. Except for the monogeneric Casuarinaceae, no other angiospermous family shows nucellar vascularization as a general character. Many recorded cases are poorly documented or have been contradicted. The whole subject needs a fresh reappraisal, especially since it is doubtful whether the reported structures are indeed true xylem elements or simply cells with reticulate cells thickening, comparable with those sometimes found in integuments (“tracheidal seeds”).

8. Special Structures

The nucellar and chalazal tissue facing the antipodal end of the embryo sac may differentiae into a “hypostase”. Both structure and function of the hypostase are poorly known and, in recent embryological literature, the term hypostase is used in a rather loose sense. The features of the hypostase are rather diverse in different taxa: dense cytoplasm, accumulation

of tannin-like substances, impregnation, and thickening of cell walls with cutin, suberin, lignin, or callose. The hypostase is situated above the chalazal vasculature and, depending on the form of the nucellar base; it may be present as a cluster of cells or a disk-or cuplike cell plate. It may be extant only in the mature ovule, and in certain stages of seed development, but may also persist in the mature seed. The hypostase is known in many families, e.g., Amaryllidaceae, Cyperaceae, Liliaceae, Zingiberaceae, Anacardiaceae, Bixaceae, Euphorbiaceae, Lauraceae, Sapindaceae, and Theaceae. Many functions have been attributed to the hypostase: it may act as a barrier tissue for stopping the encroachment of the embryo sac; it may connect the vascular supply with the embryo sac and, thus, facilitate transport of nutritional material; it may be responsible for the production of certain enzymes or hormones; play a role in the water balance of dormant seeds, or have a protective function in mature seeds. In the mature seeds of Celastraceae, Chloranthaceae, Clusiaceae, Flacourtiaceae, Staphyleaceae, Violaceae, and Vitaceae the chalazal cells become clearly sclerified, and plug the opening in the endotestal or exotegmic layer. Undoubtedly the hypostase has a variable structure and is multifunctional. Much detailed research is needed to elucidate its precise characters in different taxa.

The “podium” and “postament ” are structures more or less comparable to the hypostase in that they consist of nucellar tissue resistant to the absorbing activity of the embryo sac. A podium is a cuplike remnant of the nucellar base, which looks like a pedestal for the embryo sac. When a postament is present, only the axial part of the nucellar base persists, surrounded by the embryo sac and, often, having at its apex the antipodals (Ranunclaceae), or the chalazal endosperm chamber (several monocotyledonous families).

Also, at the micropylar end of the embryo sac nucellar remnants may persist. This is called “epistase”, which plugs the micropyleand is mostly formed by the nucellar cap or pad. It is known only in a few families: Zingiberaceae, Nymphaeaveae, and in some representatives of the Myrtales.

In *Ornithogalum* the cells of the nucellar cap and inner integument secrete an exudate, which fills the micropyle. After fertilization the micropyle is occluded by a plug of flocculent material, which probably serves to preclude pathogen invasion and prevent desiccation of embryo and endosperm

In the direct vicinity of the micropyle often a special structure, the “obturator”, is present, which has a function in the guidance and growth of the pollen tube. This obturator varies appreciably in structure and origin. In ovules with a distinct funicle the obturator is generally of funicular origin, while in more sessile ovules it is a derivative of the placenta or of a combination of placenta and funicle. It can be a prominent, often subdermal, bulge covered by secretory hairs, or simply a region with a papillate epidermal layer. On the basis of the presence of deeply staining cytoplasm, thick outer cell walls with electron-dense wall ingrowths, secreted material outside the cell walls, and numerous dictyosomes, they characterize the cells as transfer cells. The transfer cells are supposed to be involved in the short-distance transport of metabolites and /or the secretion of chemotropic substances for the

growth of the pollen tube. The surface of the obturator opens on to the micropyle, or may fill the micropyle, and is mostly continuous with the pollen-guiding tissue which covers the stigma, style, and locule cavity of the ovary. After pollination, the obturator degenerates.

9. Ovule Reduction

The complexity of the process of ovule reduction in combination with superficial or incomplete studies has resulted in many misinterpretations and confusion in terminology. Fundamentally, the reduction of the ovule can be analyzed as the reduction of the nucellus, the integument, or of the entire ovule.

The process of nucellar reduction to a smaller or greater extent, can be observed in a number of sympetalous families. The nucellus tends to become smaller and, ultimately, consists of only an archesporial cell partly immersed in the tissue lying between the attachment of the integument, and partly extending –covered by some epidermal cells –between the integuments proper.

The reduction of the integument, ultimately, results in ategmic ovules which lack a micropyle. Taxa showing a progressive range of integumentary reduction not preceded by or concomitant with nucellar reduction have so far not been recorded. The amaryllidaceous genus *Crinum* may provide an example.

Several allegedly ategmic ovules show rudiments of the integument in the form of a locally dividing epidermal layer, and had better be defined as “undifferentiated ovules”. In most cases the ovular reduction is a complex process involving nucellar reduction, integumentary reduction, and reducrions of the funicular and raphal tissues combined with the loss of anatropous curvature. The distinct reduction processes may start at different developmental stage, and operate at different rates. This is convincingly demonstrable within the Santalales. Indian embryologists have substantially contributed to our knowledge of the families of this order.

Chapter 6 The Female Gametaphyte

1. Introduction

The female gametophyte develops from the megaspore formed in the nucellar tissue of the ovule. The megaspore enlarges and after some mitotic divisions a small coenocyte differentiates. In general, a megagametophyte consists, after cytokinesis, of an egg apparatus, a central cell, and antipodal cells.

The development of this highly specialized structure is characterized by a clear polarity from micropyle to the chalaza. At the micropylar side an egg cell and normally two synergids develop, while at the chalazal part three antipodal cells are formed. The largest portion is occupied by the central cell with two polar nuclei which, after fertilization, differentiates into endosperm.

During the development of the megagametophyte, nutrients are supplied by and transferred through the ovular tissue. When the megagametophyte is fully differentiated, its development becomes arrested. The duration of this resting stage depends on the moment of pollination and arrival of the pollen tube. The arrival of the pollen on the stigma activates the ovule which permits fertilization. Fertilization, in turn, triggers further development of the ovule into a seed.

2. Types of Megagametophyte Development

During megasporogenesis the formation of a cell wall after the first and second meiotic division usually results in a linear row of four megaspores. As a rule, three megaspores degenerate and only one remains functional. This megaspore is the onset of the megagametophyte. This type of development occurs in most of the angiospermous, and is called the monosporic type of megasporogenesis. In a number of plant species the cell-wall formation after the second meiotic division is absent, leading to the formation of a dyad of which each cell contains two nuclei. Deganeration of one cell of the dyad results in the formation of one functional dyad with two nuclei as starting point of the megagametophyte. This type is called the bisporic type. Finally, there are species in which cell wall formation does not occur after the meiotic divisions. Then a coenomegaspore with four nuclei is the onset of the megagemetophyte, called the tetrasporic type (see Fig.5.3). These three types of megasporogenesis can be followed by variable number of nuclear divisions. Based on these criteria, several types of embryo sac development are distinguished (Fig. 6.1). Because of further, criteria, even more types have been proposed. Other criteria for the differences in embryo sac typology may be a sequence of cellular events.

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So changes in cytological steps in megasporo- and megagametogenesis give rise to a different cell pattern in the embryo sac. Based on the high number of cytological configurations leading to the polygonum type, and the high frequency of this type in plants, this process can be considered as basal program in the development. The use of typology is still of great value for comparative embryology.

Many alternatives in development can be observed within a distinct type of development. For instance, in *Oenothera* different pathways have been observed in relation to the selection of the megaspore after meiosis. In *Allium* a delay of postmeiotic mitoses and variation in number can take place, and in Ranunculaceae many types and variations during all developmental stages are present.

Aberrations are very common during the development of the megagametophyte. The causes for such events in *Ophris* are the variability in megasporogenesis, the behavior of the nuclei of the embryo sac, and insufficient nutrient supply. The formation of twin embryo sac originating from two megaspore mother cells indicates that during the meiotic induction also an aberration can start. All these examples of variation illustrate the variability in megaporo- and megagametogenesis.

Samy and Krishnamurthy (1975) have simplified the typology of embryo sac development by focusing attention on the exclusive features of the megagametophyte. The attainment of haploidy, the establishment of polarity, an elaboration phase to the formation of the egg apparatus, and a mature organization are recognizable in all types. The micropylar pole of the embryo sac is very regular in structures, whereas the chalazal pole shows a great variability. Of causal nature are factors like the wall types formation during meiosis, the number of mitotic divisions after formation of the megaspore, cell –wall formation after mitotic divisions, and the fusion of nuclei. With the time of establishment of polarity as the guiding factor, four types of embryo sacs are distinguished. The Polygonum type is as representative for the “Supra-Homeotypic” category, the Allium type as representative of the “Homeotypic” category I, and the Penaea type and Fritillaria type as representatives of the “Homeotypic” category II.

Compared with the other more elaborate typologies, no new characteristics are added, but several fixed point in the development of the megagamentophyte are taken into account. Primarily the establishment of polarity is chosen as a guiding factor. Indeed, polarity is a factor which acts from the start of megasporogenesis and determines the existence of the micropylarchalazal axis.

Meiosis, polarity, and the number of postmeiotic mitoses mainly characterize the typology, but there are other factors also which determine the development of the embryo sac. The developing embryo sac is dependent on the surrounding tissue for the supply of nutrients. In a sterile ovule an embryo sac is formed, but lack vascular tissue. The nuclei of nucellar cells can

also migrate into the developing embryo sac as observed in *Pandanus*, and suggested in *Costus*. Seasonal influences are influences are reported in the ovule of *Capsicum.* In winter, a preference of the bisporic type of megasporogenesis has been observed, whereas in summer the monosporic type dominates.

The process of megasporgenesis and the formation of the megagametophyte show distinct phases of development. Such a sequence is genetically determined. The aberrations visible in the nucellar tissue and the effect of an environmental factor show that the process is a result of genetic as well as environmental influences. The induction, probably the duration of the process, show the genetic base; nutrition and probably temperature are influences from outside, acting on the sequence of embryo sac development.

3. Development of the Megagametophyte

3.1 The Megaspore

The cellular content of the mature functional megaspore is prepared mostly during the meiotic prophase. Because of unequal divisions, the cell generally contains more than a quarter of the cell contents present just before divisions. There are differences in storage between the functional megaspores of different species. The plastids of *Impatiens* contain starch, in *Lilium* and *Allium* no starch but lipid bodies are present. In the developing megaspore of *Gastera* both lipid and starch are present. In *Conium* the production of starch takes place when the megaspore starts growing. There is also a difference in the degree of vacuolation at the onset of the development of the megagametophyte.

The selection of one functional megaspore is based on a prepared polarity. This is visible, for instance, in the position of the nucleus, plastid, probably elements of the membrane system, the contact with the nucellar cells by plasmodesmata, and the position of a callose wall. But polarity is the result of a genetical plan modified by external factors like nutrition and ovular development.

In general, this onset of polarity along the micropylar – chalazal axis results in the selection of one megaspore, but is expressed again in the following development. In *Lilium*, a tetrasporic type, the position of the four nucler is initially related to the shape of the cell. Polarization at the four-nucleate stage is achieved by the movement of three nuclei to the chalaza pole. Polarity exists also in the position of plastids, and the endomembrane system. In *Allium* the plastida prefer a chalazal position. In Impatiens a bisporic type has plasmodesmatal contact with the chalaza nucellar cells; there is no clear polarized position of cell organelles at the onset of the four-nucleate stage.

In the central part of the function megaspore the vacuolation will start only after an augmentation of cell organelles has taken place in the preceding stages.

The just-formed functional megaspore may have storage products and may get nutrients from its dissolving callose wall, if present, through open plasmodesmata from the chalaza nucellar cells. During this stage the nucellar tissue is still differentiating, a structure like S-cells, a hypostase, is still developing or still absent. Obviously, in this situation of early

differentiation, the functional megaspore partly depends on the nucellar tissue for nutrients. Therefore, the start of the megagametophyte development might be partly autonomous.

Till the stage of the functional megaspore, the subcellular and functional activities during maga- and microsporogenesis are comparable. When growth of the functional megaspore starts, fewer similarities remain.

3.2 The Coenocytic Megagametophyte

In the polygonum type from the formation of the functional megaspore to the cellular stage of the embryo sac, three mitoses take place. The duration of this differentiation is very variable, and depends on different conditions. In *Lycopersicum* it takes about 50 h, and in *Gasteria* about 80h.

The functional megaspore has a higher osmolarity than the degenerating megaspore(s), and enlarges. The maturing megaspore of *Pisum* has small vacuoles which coalesce during the formation of the unequal partition of the cytoplasm.

Normally, the vacuole is situated in the micropylar half of the cell. The nucleus of the enlarging megaspore lies near the cell center at the chalazal end. So a larger micropylar vacuole is present. With the formation of the two-nucleate stages In *Pelargenium*, the two vacuoles fuse and form one central vacuole. In *Crepis* and *Brodiaea* the micropylar vacuole increase and forms a large central vacuole. In *Stipa* and *Triticum* a central vacuole as well as chalazal vacuole is present. This variation in the process of vacuolation can result in another composition of the cytoplasm. But it may also be an indication of the preferential place of cellular enlargement. In relation to the nucellar structure, the micropylar part permits an enlargement because of a lower resistance. Such growth occurs in *Oryzopsis* and *Jasione* but can be followed as in *Dipcadi* by an enlargement in the direction of the chalaza. A sign of the direction of enlargement is the degeneration of nucellar cells. In *Juglans* the position of the vacuole and nuclei is variable. The most frequent situation is a central vacuole with, after the mitoses, the nuclei located at the micropylar and chalazal part of the cytoplasm. However, it is possible that all the nuclei are formed and positioned in the micropylar part of the cytoplasm. Some of these nuclei will move to another position before the ultimate formation of the cellular megagametophyte. An accumulation of cytoplasm is always present at the micropylar pole. Before formation of the various cells, the cytoplasm is reorganized followed by the movement of the nuclei. The ultimate position of the nuclei depends on the organization of the cytoplasm. Polarity in the coenocytic megagametophyte is determined by the distribution of the cytoplasm as well as in its organization. The position of the vacuole or vacuoles is an indication for the cell polarity. In some Commelinaceae and Zingiberaceae the functional megaspore has only small vacuoles, and an increase of cytoplasm takes place during its enlargement.

In *Gasteria* an increase of the number of plastids, mitochondria, lipid granules, dictyosomes, and ribosomes takes place in the megaspore preceding the nuclear divisions. In the vacuolated four-nucleate stage only polysomes increase in number. So, in general, the main production of cell organelles occurs before vacuolation.

In the four-nucleate stage, in the micropylar part many dictyosomes are present. In *Allium, Lillium*, and *Impatiens* the number of organelles remains unaltered up to the four-nucleate stage.

4. The Relation with the Nucellar Tissue

The formation of the coenocytic megagametophyte depends on its surrounding ovular tissues, especially the nucellar tissue. The megagametophyte is surrounded by a thin cell wall containing PAS-positive material and, in combination with the cell walls of the nucellar cells; it becomes a thick covering composed of thin, appressed lamellae. Plasmodesmata are not frequent, or can be closed.

In the chalazal region nucellar tissue forms the intermediate link between the embryo sac and the transport tissue. In some cases enlarged cells with specific structures are present in the nucellar tissue, forming an epistase or hypostase. The hypostase is more commonly situated at the chalazal part of the megagametophyte. These morphological structures can be interpreted as a possible pathway of nutrients. Also, haustoria or extensions of the megagametophyte indicate the presence and location of a possible way of transport

Considering the various changes in the nucellar tissue, the degeneration of nucellar cells by enlargement of the coenocytic megagametophyte, the storage of reserves in the nucellus, as well as the continuous differentiation of the nucellar tissue during the coenocytic stage of the embryo sac, a special nutritive function of the nucellus is not probable. Moreover, the changes in the developing megagametophyte indicate more or less an uptake of water and, in some cases, a use of its storage products. There is a minor uptake of nutrients. The development of the coenocytic megagametophyte does not show, in this stage, a strong dependence on the surrounding nucellar and other ovular tissues.

In models of a possible pathway in the ovule of spinach (Fig.6.2), Wilms stated a shift from young stage to mature and postfertilization stage. After the young stage, the chalazal tissue proliferates and the embryo sac does not receive any more nutrition. After fertilization, however, this tissue and the original chalazal tissue, as well as degeneration products, supply nutrients to the now activated embryo sac.

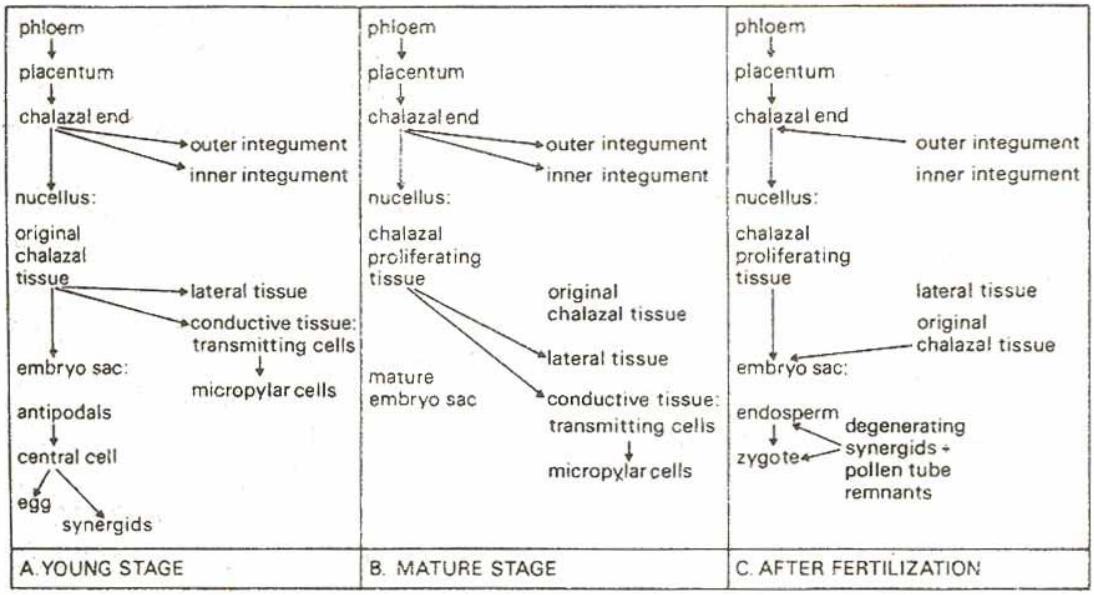

Fig.6.2 Possible changes in the nutritive pathway in the ovule of *spinach*.

5. Organization of the Embryo Sac

In most angiosperms the embryo sac consists of seven cells: the egg cell, two synergids, the central cell, and three antipodal cells. This type of embryo sac is referred to as "Normal" or "Polygonum" type. In the remaining angiosperm species other embryo sac types are reported, characterized by being composed of more or fewer cells, and by aberrant ploidy-level of some of their nuclei. Different cell numbers can result from the absence of one or both synergids, and a reduced or increased number of antipodal cells.

In the normal embryo sac type all nuclei, except that of the central cell, are haploid. The central cell contains two haploid nuclei, called the polar nuclei which, eventually, fuse and form the diploid central cell nucleus. Haploidy is a common feature for the nuclei of both egg cells and synergids, if present, in all types of embryo sacs. This is in contrast to the ploidy level of the nuclei of the central cell and antipodal cells, which varies greatly among the different embryo sac types.

The egg cell and the synergids are large, elongated or pear-shaped cells, which are located at the micropylar pole of the embryo sac. These three cells show a triangular arrangement and share common surfaces, forming in this way the structure known as egg apparatus. The egg apparatus is attached to the embryo sac wall bordering only at the extreme micropylar pole, and the major portion of the cells is surrounded by the central cell. Usually, only the synergids can be in direct contact with the micropyle. The apex of the egg cell lies some microns below the apices of the synergids at one side of the embryo sac. Since all cells of the egg apparatus have approximately the same length, the egg cell extends somewhat further into the central cell than the two synergids. Around the cells of the egg apparatus walls can be formed. The

thickness of these walls diminishes strongly when vacuolation begins and the cells elongate. The remaining wall can be irregular or disappear locally. For some species the synergids are reported to develop haustoria into and beyond the micropyle, as in *Ursinia*, *Calendula*, *Cotula*, *Quinchamalium*, and *Cortaderia*.

The central cell occupies the largest portion of the embryo sac, and ranges from the micropylar pole to the chalazal pole, where it borders the antipodal cells (Fig. 6.3). Usually, the antipodal cells are the smallest cells of the embryo sac, and in many species of the dicotyledons they are ephemeral. In monocotyledons, however, especially in grasses, the antipodal cells undergo a number of divisions which results in the formation of a mass of antipodal cells in the mature embryo sac.

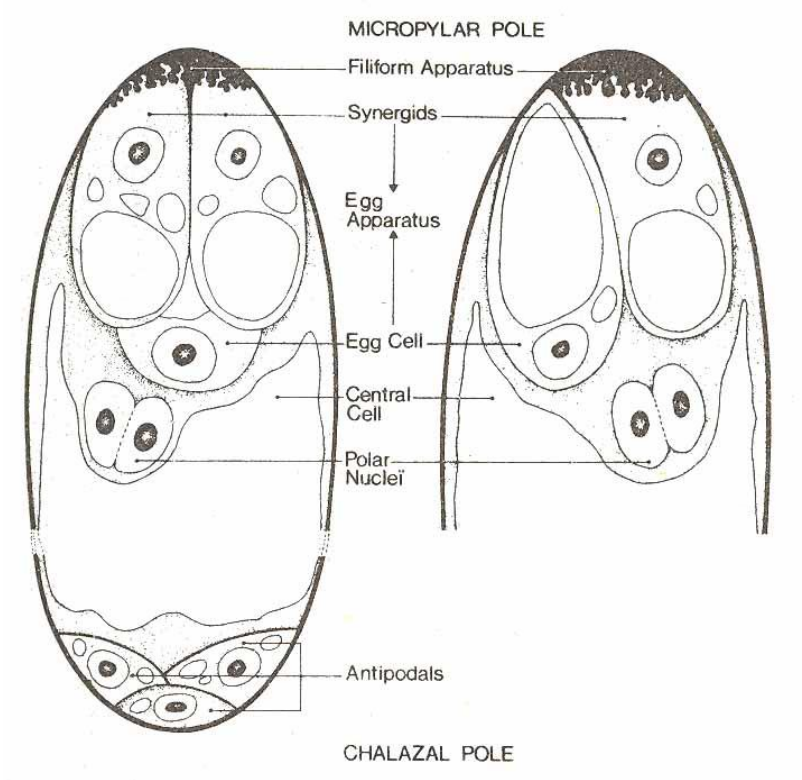

Fig. 6.3 Diagrammatic survey of the "normal" type of embryo sac in angiosperms. If the cell wall is distinctly present, the line is *thickened* and includes the plasma membrane

All cells of the embryo sac show a specific morphology and structural organization. One of the main characteristics of the cells is polarity, expressed by polar distribution of the cytoplasm, and specific position of the nuc1ei and some cell organelles.

5.1 The Synergids

In most species the synergids are only partly surrounded by a cell wall. Besides, this cell wall shows considerable variation in thickness. At the extreme micropylar pole the wall is strongly thickened, forming the structure known as "Filiform Apparatus" (FA) (Fig. 6.4). From

the FA toward the base of the synergids the wall gradually thins. The chalaza1 half of the synergid is surrounded by the plasma membrane only. The plasma membrane lies close to the plasma membrane of the neighboring cells although, locally, the two membranes can be separated by electron-translucent spaces. There are only a few exceptions to this rule. Schulz and Jensen showed the local presence of PAS-positive material at the cha1azal region of the synergids in *Capsella*. The partial cell wall in this region appears to have a honeycomb structure. In *Epidendrum* the synergid cell wall is even more complete. Several authors proposed that the absence of a cell wall at the chalazal region of the synergid plays an essential role in the fusion process of male and female gametes. After the penetration and discharge of the pollen tube into one of the synergids, the plasma membrane of that synergid disintegrates and disappears. The absence of the plasma membrane and the absence of a cell wall in the chalazal portion of the synergid enable the male gamete, transferred through the synergid, to come in direct contact with the plasma membrane of the adjacent female gamete. In *Capsella*, where cell wall material is present in between the synergid and the female gamete, frequently a pore has been observed in this cell wall after successful fertilization. This indicates that the presence of a cell wall is not necessarily in contradiction with the proposed fusion mechanism.

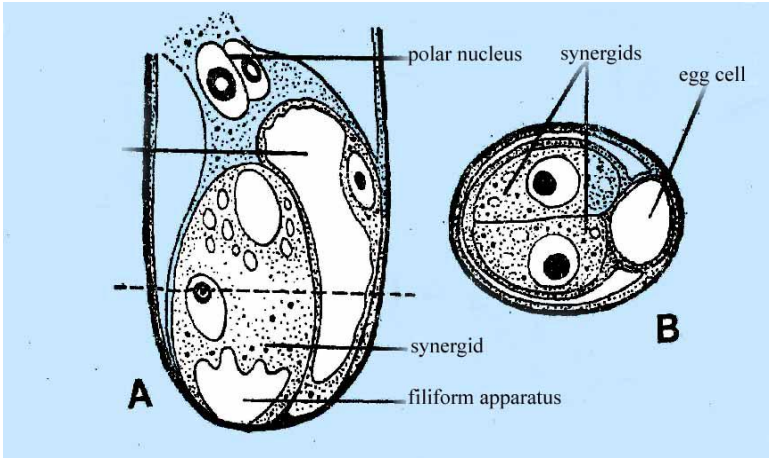

Fig. 6.4 The egg apparatus of cotton

The morphology of the FA is highly variable among species. In *Torenia* it is spherical, in *Petunia* and *Helianthus* it is wedge-shaped and located between the tips of the synergids, while in most other species it forms a broad layer of long, irregular cell-wall projections on top of the synergids. In *Petunia* and *Torenia* the FA exhibits a relatively homogeneous structure and smooth surface. The FA of *Petunia* contains a loosely organized network of cellulosic microfibrils, embedded in a matrix of pectic nature. At those places where the FA is connected to the lateral wall of the synergid, it is impregnated with an electron-dense material. A similar situation has been observed in *Jasione* where the lateral walls of the synergids are cutinized.

However, in most species studied, thus far, with the electron microscope, the FA shows a very complex organization and heterogeneous structure. It usually consists of a mass of wall projections extending deep into the cytoplasm, and vice versa cytoplasmic tunnels penetrating

deep into the FA. As a result of this organization there is a tremendous increase of the plasma membrane surface in this region. The wall projections themselves show two structural phases. They consist of a central electron-dense core surrounded by an electron translucent layer.

In a few species the presence of plasmodesmata has been reported in the cell walls of the synergids, except for those parts which are in contact with the surrounding sporophytic tissue.

Most of the cytoplasm of the synergids is located in the micropylar half of the cell. Invariably, the chalazal part of the synergid is occupied by one large or several smaller vacuoles, surrounded by a thin layer of cytoplasm. In *Gossypium*, the vacuoles of the synergids are rich in ash (after microinciner ation), in contrast to the vacuole of the egg cell which is negative in ash content. The synergid vacuoles give a negative reaction after staining for carbohydrates, nucleic acids, and proteins. Although the chemical nature of the ash could not be established, Jensen assumed that it may be calcium, which has been shown to be important in the directional growth of pollen tubes.

The cytoplasm of the synergids shows a complex organization, and is rich in organelles. Common features of synergid plasma are the presence of a large number of mitochondria and dictyosomes, extensive endoplasmic reticulum, and abundant ribosomes. In some genera, as *Gossypium* and *Capsella*, there is a tendency for mitochondria to be concentrated near the FA, although mitochondria are present throughout the cytoplasm. In other genera, as *Petunia*, *Spinacia*, and *Quercus*, the mitochondria are uniformly distributed. The ribosomes are both free and bound to membranes. Frequently, long ER profiles are found running parallel to each other, and are oriented parallel to the long axis of the cell. Although dictyosomes are reported to be numerous in the mature synergid, they are thought to be more active in the premature stage. Especially during the formation of the FA, the presence of active dictyosomes associated with numerous Golgi vesicles and dilated ER has been described for the synergids of *Helianthus* and *Gasteria*. After the formation of FA, the numerous dictyosomes, their associated vesicles, and the dilated ER are no longer present. In other taxa, as *Aquilegia*, *Capsella*, *Nicotiana*, and Spinacia there are numerous dictyosomes and they actively produce vesicles.

Gossypium and *Nicotjana* have numerous plastids and contain starch. However, in most other taxa mentioned before plastids are less frequent and are devoid of starch grains or contain only a few small ones. In *Paspalum* starch is present in the immature synergid, but disappears in the mature, cells. The main reserve storage in this taxon is lipid, which is present as numerous small bodies near the FA. Concrete lipid bodies are also found in the cytoplasm of the synergids of *Stipa* and *Spinacia*.

In a number of species degeneration of one of the synergids prior to the arrival of the pollen tube has been observed: *Gossypium, Hordeum vulgare, Linum, Paspalum, Stipa elmeri, Nicotiana, and Spinacia.* The second synergid retains its original constitution. The degeneration results in a decreased volume of the synergid, disappearance of the vacuole, and an increased stainability and density of the cytoplasm. The start of the degeneration process is usually marked by the deposition of osmiophilic material on the membranes of the

mitochondria. Subsequently, the nucleus, dictyosomes, plastids, and mitochondria lose their identity by the disintegration of their membranes. Simultaneously, the plasma membrane disappears. Frequently, the ER remains intact until the final stage of degeneration. There are only few exact data concerning the possible relation between the moment of pollination, and synergid degeneration. Usually, data are collected as part of a study of the process of fertilization, and degeneration is observed after previous pollination.

In other taxa, as *Petunia*, *Helianthus*, *Capsella*, and possibly *Quercus*, both synergids remain healthy until the moment of pollen tube penetration into the embryo sac. However, when the pollen tube has penetrated and ejected its content, the penetrated synergid shows degenerative changes. These postpenetration changes are strikingly similar to those during prepenetration degeneration as described above.

Generally, the synergids are considered as highly active cells. The presence of numerous organelles, especially mitochondria and dictyosomes, abundant ribosomes, and extensive RER indicate an intense metabolism. Histochemical studies show that the synergid cytoplasm is rich in protein, and RNA. Malik and Vermani observed activity of a number of enzymes in the mature synergids of *Zephyranthes* and *Lagenaria*. There was intense cytochrome oxidase and succinate dehydrogenase activity, together with acid phosphatase, ATPase, alkaline phosphatase, phosphorylase, and lipase activity. Shortly before fertilization they observed an increasing peroxidase activity. These data also suggest the synergids to be a seat of highly active metabolism.

Because of the polar distribution of the cytoplasm, most of this metabolic activity is confined to the micropylar region of the synergid. In the same region we find the filiform apparatus. The FA shows all characteristics of a transfer cell wall, and because of its convoluted nature it represents a strongly increased area of plasma membrane. Gunning and Pate related this type of cell wall to places of intense exchange, absorption, and secretion of substances. Since the FA borders the micropylar nucellar tissue in crassinucellate ovules, and the integuments and micropyle in tenuinucellate ovules, a transfer function of the FA and synergid must involve these tissues as well.

According to Jensen, the synergids in cotton play an important role in the nutrition of the embryo sac. Through their FA they are supposed to take up nutrients from the metabolite-laden nucellus. The observed close association of mitochondria with the plasma membrane of the FA strongly suggests that the energy necessary for absorption is being supplied by the mitochondria. The presence of many plastids, containing large amounts of starch in the synergid near the FA, also points to an absorptive function of the FA and endocytosis of the synergids. The extensive ER of the synergid, with its characteristic distribution and orientation, parallel to the long axis of the cell, is thought to function as an internal transport system, through which nutrients are transported toward the egg cell and central cell. The transfer of material to the latter cells may be facilitated by the absence of a cellulosic cell wall, at the chalazal end of the synergid. A nutritionsl function is also proposed for the synergids in the taxon *Cortaderia*. In *Cortaderia,* the tip of the synergids elongates and grows along and out of

the micropyle (Fig. 6.5). Ultrastructural studies show the presence of strongly developed transfer walls in the haustorium, especially at the tip, and a dense ER parallel to its long axis.

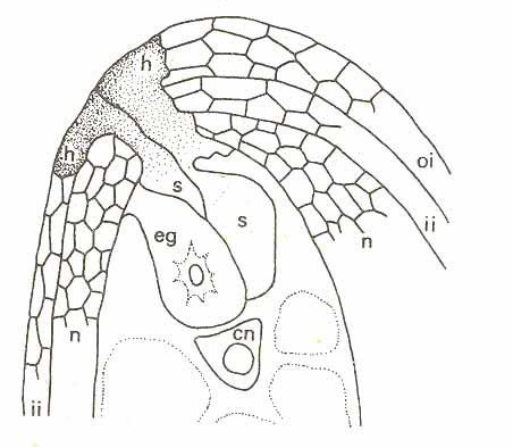

Fig. 6.5 Synergid haustoria in *Corladeria* projecting through the micropyle. *cn* central ce11 nucleus, *eg* egg ceII, *h* haustorium, *ii* inner integument, *n* nucellus, *oi* outer integument, *s* synergid.

Some authors doubt a nutritional function of the synergids. In many species the FA of the synergids is in direct contact with the micropyle only, whereas the integuments are covered with a cuticle. Only in a few species the plastids of the synergids contain starch.

Most of the features usually mentioned as indicative of absorption can also be interpreted as indicative of a secretive function. In this respect it is interesting to mention the close resemblance between synergids and the secretory cells lining the stylar canal of the lily pistil.

The secretory function of the canal cells is well established. The stylar canal cells show a similar polarity both in structure and function, as do the synergids. At their secretive surface they have a highly convoluted and thickened cell wall; this structure closely resembles that of the FA. They also contain numerous mitochondria, abundant ribosomes, and extensive rough ER, as do the synergids. Most of the canal cell cytoplasm is located at the secretory pole of the cell, while the opposite pole is usually filled with vacuoles.

In *Petunia* the synergids are in direct contact with the micropyle. The synergids become mature and develop their specific ultrastructure within a very short period, just prior to anthesis. Only after the synergids have reached maturity the micropyle becomes filled with densely stained material, suggesting its synergidal origin. In *Spinacia* Wilms observed that the middle lamellae of the nucellar tissue between the embryo sac and the micropyle are altered when the ovule becomes receptive. Dissolution of the middle lamellae starts near the FA, suggesting that the enzymes involved are secreted by the synergids.

A secretory activity of the synergids is usually considered to be related to a chemotropically directed growth of the pollen tube in the ovary. Although, for a number of species, chemotropic activity of ovules has been established, there is no direct evidence that synergids are involved. On the contrary, according to several authors, the absence of synergids in a

number of taxa, as *Plumbago*, indicates that they are not involved in the directional growth of the pollen tube. It was, however, proved by Cass and Cass and Karas that at least in *Plumbago* the absence of synergids is compensated by the presence of a filiform apparatus and synergid-like cytoplasm in the egg cell.

Well-established and documented is the role of the synergids in the entrance and discharge of the pollen tube into the embryo sac, and the subsequent transfer of the sperm cells to the egg cell and the central cell. In all species-examined thus far with the electron microscope, the pollen tube enters one of the synergids through the FA. After its arrival in the cytoplasm of the synergid, the growth of the tube ceases and an opening is formed in the tube wall. Opening of the pollen tube can occur by simple rupturing at the tube tip, as proposed for *Petunia*. In other species, as *Gossypium*, a well-defined pore is formed; the shape and position indicate that its formation is well regulated and active.

Opening of the pollen tube leads to the effusion of an amount of tube cytoplasm into the synergid, including the vegetative nucleus and the sperm cells. The vegetative tube cytoplasm can be ·usually recognized by the presence of specifically structured organelles, and the presence of numerous small PAS-positive spheres. The amount of tube cytoplasm and its position in the synergid indicate that the ejection happens with force, and that there must be considerable、 differences in internal pressure between pollen tube and synergid. Apparently, the pollen tube and synergid cytoplasm do not merge. The tube cytoplasm forms a distinct mass in the center of the synergid, reaching from the FA to the base of the cell, whereas the synergid cytoplasm is forced to a peripheral position. Since the plasma membrane of the penetrated synergid has already degenerated, and the synergid cell wall is only partial, the sperm cells probably come in direct contact with the plasma membranes of the egg cell and central cell. The subsequent fusion of the plasma membrane of one sperm cell with the plasma membrane of the egg cell results in the formation of a bridge through which the sperm nucleus and, possibly, sperm cytoplasm can enter the egg cell. In the same way the second sperm cell is believed to fuse with the central cell.

5.2 The Egg Cell

In most species and embryo sac types the egg cell shows a similar organization, characterized by distinct polarity. Only a limited region of the egg cell is in contact with the embryo sac boundary, most of the cell is surrounded by the synergids and the central cell. Usually, the egg cell is only partially surrounded by a cell wall. Its chalazal one-half to one-third portion is limited by a plasma membrane only. Exceptional, in this respect, is the egg cell of *Capsella* which in its chalazal region is surrounded by a thin and irregular PASpositive cell wall.

The egg cell wall is thickest at the micropylar region of the cell, and gradually thins toward the chalaza1 region. Cell- wall thickenings and protuberances, as usually reported in the other cells of the embryo sac, are completely absent in the egg cell. Different in this respect is the egg cell of *Plumbago* which has a filiform apparatus-like structure in the micropylar region. In *Plumbago* the synergids are absent, and it is concluded that the egg cell in the Plumbago type

of embryo sac also partly functions as a synergid.

In most species the egg cell is highly vacuolate. The micropylar two-thirds of the cell is almost completely filled by a single large vacuole, while the cytoplasm is restricted to a thin layer along the plasma membrane. The chalazal one-third is filled with cytoplasm containing the egg nucleus and, sometimes, some small additional vacuoles. In *Stipa elmeri* the egg cell is also vacuolate, but there are many small vacuoles randomly distributed in the cytoplasm. According to Jensen, the vacuole of the *Gossypium* egg cell shows no ash residue after microincineration. In this respect the egg cell vacuole is completely different from the synergid vacuole which leaves a considerable amount of ash.

The mature egg cell nucleus gives only a faint reaction after Feulgen staining. The reduction in stainability during maturation of the nucleus is ascribed to the large increase in nuclear size and consequent dilution of the DNA. Frequently, a large vacuolate nucleolus is present in the egg nucleus. According to Schulz and Jensen the structure of the nucleolus in *Capsella* suggests that ribosomes are not being produced. However, in *Helianthus* the presence of a large nucleolus is considered to be indicative of a high rate of ribosome synthesis. The ultrastructure of the egg cell cytoplasm varies considerably among the various species studied. In *Gossypium* the egg cell contains a large amount of ER, numerous ribosomes, numerous mitochondria with only few cristae, and only few dictyosomes and plastids. In *Petunia* ER is scarce, and polysomes, mitochondria, plastids, and dictyosomes are few. The egg cell of *Zea mays* contains many mitochondria and plastids, whereas there are only few dictyosomes, and a small amount of ER. A similar condition has been observed in *Helianthus*. In *Spinacia* the number of mitochondria increases considerably and they mainly become clustered in the micropylar part of the egg cell, whereas large plastids accumulate starch in the chalazal part.

A common feature of egg cell cytoplasm is the presence of only a limited number of relatively inactive dictyosomes. In all other cytoplasmic aspects egg cells are very specific. Egg cells seem to be rich in protein; whereas some react strongly after RNA-staining while others give only a faint reaction. Most authors assume that the egg cell is in a relatively inactive state, on the basis of the cytoplasmic constitution. The ultrastructural changes that have been observed in the cytoplasm after fertilization underline this assumption. However, substantial· biochemical or physiological evidence for this assumption is lacking.

As already indicated previously, a specific situation has been observed in *Plumbago*, where synergids are missing. Nearly all of the *Plumbago* egg cell is surrounded by a cell wall. At the micropylar pole where the egg cell is in contact with the embryo sac wall, a filiform apparatus is present. The egg cell contains one large vacuole which occupies most of the cell volume. There are two accumulations of cytoplasm: one at the micropylar pole associated with the FA, and the other at the chalazal pole containing the egg nucleus. The micropylar cytoplasm has many mitochondria and dictyosomes. The chalazal cytoplasm contains many plastids, mitochondria and polysomes, and conspicious RER. The *Plumbago* egg cell is considered as a highly metabolic active cell which combines both gametic and syncrgid functions.

5.3 The Central Cell

The central cell usually occupies the largest portion of the embryo sac, bordering and surrounding the major portion of the egg apparatus at the micropylar pole, and reaching to the antipodal cells or nucellus at the chalazal pole. The lateral cell wall of the central cell, therefore, forms the main part of the embryo sac wall in contact with the surrounding tissue.

The central cell is highly vacuolate, and its cytoplasm is confined to a thin layer along the embryo sac wall and accumulates near the egg apparatus and the antipodal cells. The micropylar accumulation contains the two polar nuclei, or the fusion nucleus. The time of fusion of the polar nuclei varies from species to species. In *Capsella* and *Helianthus* the fusion is completed before fertilization. In *Gossypium* and *Spinacia* fusion starts in the mature embryo sac, but is only completed after the arrival of the sperm nucleus. In *Spinacia* the polar nuclei form large protrusions before the fusion. The polar nuclei and fusion nucleus are the largest nuclei observed in the embryo sac. They generally show very little heterochromatin, and contain a large nucleolus. In *Helianthu* the nucleolus has a large vacuole, while in *Gossypium* the nucleoli are relatively massive. Ultrastructural details of nuclear fusion in plants were first given by Jensen (1964) in his description of the fusion process of polar nuclei in *Gossypium*. In this taxon the nuclear envelopes are continuous with and connected by cisterns of the ER. Fusion of the nuclei starts with the contact and fusion of the outer nuclear membranes, followed by the contact and fusion of the inner membranes. In this way a number of small bridges are formed which, gradually enlarge and coalesce. After the nuclear fusion the nucleoli also fuse, indicating a completion on chromatin level.

Like the cells of the egg apparatus, the central cell is surrounded only by a partial cell wall. The cell wall is absent at the micropylar pole near the base of the egg apparatus at those places where the neighboring cells also lack cell walls. The wall against the nucellus or integuments is usually thick and regular. In *Helianthus*, *Euphorbia*, Jasione, *Capsella*, and *Spinacia* the micropylar part of the lateral wall shows many fingerlike projections. In *Capsella*, *Helianthus*, and *Euphorbia* similar projections also occur along the chalazal part. The wall projections strongly resemble the FA and, similarly, greatly increase the plasma membrane surface in that area. According to Newcomb and Steeves, the presence of embryo sac wall projections suggests that the central cell plays an important role in the absorption of metabolites from the surrounding tissue. In *Jasione* the embryo sac is surrounded by a thick cuticle. However, in the region with the central cell-wall projections, the cuticle consists of small plaques with discontinuities. This configuration also suggests an absorptive function of this part of the central cell.

Most central cells contain complex organized cytoplasm which is rich in organelles. There are numerous mitochondria with well-developed cristae; frequently there is extensive RER; there are a large number of dictyosomes appearing active in the production of vesicles; there are numerous ribosomes, both as monosomes and polysomes. The number and ultrastructure of plastids is variable. In *Helianthus* and *Linum* the plastids do not contain starch, whereas in *Gossypium*, *Capsella*, and *Spinacia* some starch is present, but less than in the egg cell. There is abundant starch in the central cell of *Nicotiana rustica* before fertilization. In *Capsella* the

plastids contain a well-developed thylakoid system, including grana, indicating that the cell is partly autotrophic. The plastids in the central cell of *Gossypium* are reported to contain phytoferretin. Besides starch, usually lipids are present in the central cell as reserve substances. The presence of lipids is accompanied by the presence of numerous microbodies. The microbodies possibly represent glyoxisomes containing the enzymes for β-oxidation of fatty acids, and the enzymes of the glyoxylate cycle for the conversion of acetyl-CoA to succinic acid.

In *Petunia* the central cell has a poor cytoplasmic constitution. The number of mitochondria and dictyosomes is low, and the ER is only poorly developed. The plastids are large and contain high amounts of starch. Free ribosomes are very abundant and, likely, they are present as monosomes. The mature central cell is probably in a state of low metabolic activity. In this species triple fusion causes a dramatic change in the cytoplasmic ultrastructure. The number of mitochondria and dictyosomes, and the amount of RER rapidly increase, whereas the starch quickly disappears. Simultaneously, the ribosomes become organized as polysomes. Apparently, the newly formed endosperm cell becomes metabolically very active.

5.4 The Antipodal Cells

The antipodal cells show much variation among angiosperms. In many taxa of the dicotyledons they degenerate before or during the maturation of the embryo sac. In other species they persist even during embryo and endosperm formation, although their condition can be highly variable. Finally, in monocotyledons, especially in grasses, they can proliferate into a multicellular tissue consisting of up to 100 cells. The great variability makes it impossible to draw general conclusions on the structure and functions of antipodal cells.

In *Capsella* the antipodals are small, inactive cells. They are surrounded by cell walls of uniform thickness, invariably containing plasmodesmata. So the antipodals are interconnected as well as connected to the nucellus and central cell. This indicates the presence of a pathway for metabolites from the nucellus through the antipodals to the central cell, and vice versa. A similar condition is reported for *Helianthus* and *Jasione*.

Frequently, the antipodal cell walls bordering the nucellus have finger-like wall projections, similar to those observed in the central cell and the, filiform apparatus. In *Stipa*, *Helianthus*, *Zea*, and *Spinacia* the presence of these transfer wall-like projections is accompanied by the presence of very active cytoplasm. The cytoplasm contains abundant organelles as mitochondria, dictyosomes, plastids, and ribosomes. The ER is extensive, and consists of parallel and partly concentric cisterns.

Histochemical tests also indicate the presence of high metabolic activity. Malik and Vermani established enzyme activity, as peroxidase, succinic dehydrogenase, phosphorylase, cytochrome oxidase, and lipase in the antipodal cells of *Zephyranthes* and *Lagenaria*. These cells also contain high amounts of protein, polysaccharides, lipids, and RNA. During maturation of the embryo sac there is an increase of RNA and protein. Similar results have been obtained for the antipodal cells of *Paspalum*. In this taxon lipids are transferred from a solid to a liquid state during maturation. In many species the antipodal cells contain starch,

which is used up during fertilization and subsequent development of endosperm, and embryo.

Variability has also been observed in the cellular organization of the antipodal tissue. In *Helianthus*, instead of three antipodals conforming to the Polygonum type of embryo sac, only two antipodal cells are present. One of the antipodal cells is mononucleate, the second antipodal cell contains two nuclei. In *Linum* the antipodal cells are only partly separated by walls. These incomplete walls subsequently dissolve, and a syncytium is formed. Especially in species with proliferating antipodals, cells can develop with more than one nucleus as in Oryzopsis and Stipa.

Invariably, the antipodal nuclei stain intensely for DNA. This might be partly due to the largely heterochromatic condition of the nucleoproteins. However, for many species a strong increase in the amount of DNA per nucleus has been reported. The enhancement in DNA content can be due to karyokinesis followed by nuclear fusion, endomitosis, and polyteny. In *Scilla* the level of endoploidy reaches as much as 1,024 C.

Generally, three main functions can be attributed to antipodal cells. Firstly, they may be involved in the transfer of nutrients and serve as a pathway for metabolites from the nucellus to the central cell. This conclusion is based on the presence and location of plasmodesmata, and the presence of transfer cell-like walls, in combination with cytoplasm which appears active.

Secondly, they may serve as storage for the developing endosperm and embryo. Many antipodals contain abundant starch, lipid, and proteins which are used after fertilization. Degeneration and breakdown of antipodal cells after fertilization can serve as an additional food supply. Thirdly, the antipodal cells may have a secretory function. This is indicated by the presence of extensive rough ER and abundant ribosomes. The antipodals possibly produce and secrete growth-controlling substances which regulate the development of the adjacent endosperm.

6. Female Gametophyte Development

The ovule has, from its onset, an axis of polarity from micropyle to chalaza. Along this axis evolves the development of the megagametophyte. This process is programmed by the genome present in the cell, and later the cells of the megagametophyte. But, considering the shape of the nucellus, the megaspore selection, the cellular contact and type of vacuolation, also influences of the surrounding tissue are very likely.

Megagametophyte development is the result of an interaction between sporophytic tissue and gametophytic cells. The developing megagametophyte gets its nutrition from the surrounding tissue. The flow of these nutrients comes from the chalazal part, but after formation of the embryo sac the flow passes possibly the basal part and penetrates along the side of the embryo sac. Depending on the structural elaboration of the nucellar tissues, more pathways are possible. Nutrition flow and megagametophyte development are still not fully understood. Ultrastructural, histochemical, quantitative, and physiological approaches can elucidate, also, the relation of the megagametophyte development and its surrounding tissue.

The function and appearance of special structures like the hypostase and integumentary tapetum, and the different outgrowths of embryo sac cells, are to be included in this process of interaction.

The differentiation patterns of the embryo sac result in a polarity with respect to the programmed receptivity of the micropylar part for the pollen tube. In this receptivity and acceptance the function of both synergids needs more attention together with the possible resting period of the embryo sac. About the function of the antipodals different opinions still exist.

Elucidation of the relation of megagametophyte development to pollination and seed formation will further add to our understanding of the early stages of ovular development. Starting with this background, abortion or deviation in development and polyembryony are linked to the process of ovular development.

Chapter 7 Fertilization

1 Introduction

In angiosperms a reduction of the gametophyte is manifest. After meiosis small gametophytes are formed with a low number of cells on a high level of differentiation. The microgametophyte produces two sperm cells and, in the macrogametophyte, one egg cell is formed.

The development and differentiation of the gametophytes are influenced by the sporophyte. The nutrition of the gametophytes and the duration of their development are governed by the sporophyte. The same holds good for the seed development, and the initial development of the new sporophyte. The reduction of the gametophyte, and the process of seed formation are strictly related to the higher differentiation and development of the sporophyte. Due to the dominarice in the stigma, style, and ovule during the interaction with the pollen and pollen tube, as well as in the process of fertilization, the sporophyte is established. Only the specific interaction between gametophyte and sporophyte in a sequence of distinct stages results in a successful fusion of the gametes. This interaction is expressed in structural and functional changes in pollen, stigma, style, ovary, ovule, and embryo sac during the progamic phase.

Because of the limited number of species examined, the recent investigations can only give an incomplete view of data, and generalization is disputable.

2 Stigma and Style

2.1 Function and Structure of the Stigma

The stigma is the receptive surface for pollen, and consists of specialized surface cells which are in connection with the stylar tissue. The morphology of the Stigma and style shows great diversity and depends on the composition of the flower and, in some cases, is related to the type of pollination.

The function of the stigma is to accept the pollen and to permit germination. The stigma can provide nutrients to the pollen and direct the pollen tube growth; it is also involved in the regulation of the flower metabolism.

A stigmatic surface, wet or dry, needs a correct physiological condition for the pollen, i.e., a balanced osmolarity and a sufficient water supply.

Because of the changing environmental factors, pollination can be delayed. Stigma and style, therefore, need good vitality to retain their function for some time. In recognition and receiving signals or stimuli both the pollen coating and the stigma coating are involved.

For the further development of flower, the presence of a stigma and changes in metabolism after pollination seem to be necessary. Also, without pollination in wilting plants the stigma functions as a regulating component.

The morphology of the stigma shows great diversity. Some comparative studies, mainly

several ultrastructural ones, present detailed features of some plants, Based on the morphology of the stigmatic surface, on the amount of secretion, and the nature of the surface cells of almost 1,000 species of about 900 genera of 250 families. The stigmas are divided into two categories, those with copious fluid secretion - the wet stigmas - and those with limited surface secretion - the dry stigmas.

The dry stigmas have dispersed receptive cells on multiseriate branches (group 1, e.g., most Gramineae, as *Zea, Sorghum,* and *Hordeum)*, or concentrated in distinct ridges, zones or heads (group 2). The second group can be subdivided into stigmas with nonpapillate surfaces, as in *Potamogeton* sp., *Cyperus* sp., and stigmas with papilla. The last type bears unicellular (mainly Liliaceae as *Allium*, *Tulipa*, and Iridiaceae, as *Crocus* and *Iris*, and Juncaceae, as *Luzula*), or multicellular papillae. The multicellular papillae can be uniseriate, as in *Caliphruria*, or multiseriate, as in. *Limnobium*.

The wet stigmas have a receptive surface with small-to-medium size papillae (group 3), as in some Liliaceae (*Aloe Gasteria*), Orchidaceae (*Epipactis, Ophrys*), and other plants, as *Canna*; or a nonpapillate surface, as in *Dracaena, Alpinia*, and the cells are often necrotic at maturity (group 4).

With these parameters, a relative taxonomic uniformity in some families can be estimated. Additionally, binucleate pollen seems to be correlated to wet and dry stigmas, whereas the trinucleate pollen prefers a dry stigma.

Dumas, basing on the position of the papillae, gives a better morphological classification in terminal and lateral stigmas. A further classification can be based on the function and nature of the secreted products. Stigmas may have an abundant secretion of lipophilic or polysaccharide substances, or a less abundant secretion with the same products but also with polyphenols, tannins, or flavonoids; they may have no liquid secretion at all. These stigmas are covered with waxes or proteins. This classification can be combined with the stylar structure. Hollow styles of monocotyledons produce an exudate of mainly polysaccharides. Dicotyledons with a solid style mostly produce a lipophylic exudate. Orchidaceae, Scrophulariaceae, and Solanaeeae excrete a lipopolysaccharide substance.

This last classification includes the abundancy and nature of the stigmatic exudate. It should be of interest to involve the nature and morphology of the pollen covering in its function of sticking pollen onto the stigma.

In Commelinaceae there is extreme variability in stigma morphology. Not all surface ornamentations, as blisters, or in case the style is splittering, as in Cyanotis, can be correlated with pollination. In Boraginaceae a correlation exists between pollen and stigma size, preventing some types of pollination.

A particular morphological aspect of the stigma is the recurved lips, branches or lobes, which are sensitive to tactile stimulation and can close. In pollinated stigmas, after the primary closure, of the branches, a reopening takes place followed by permanent closure. This occurs only when pollen is present, and depends on the amount and the species of pollen. The agent is heat-sensitive and present in the pollen. The mechanism of movement depends on changes in

water permeability of the plasma membrane

2.2 The Nature of the Stigma Covering

The receptive surface of the stigma is formed by surface cells with a more or less glandular nature. This layer is bordered by a transition zone of "stigmatoid" tissue without glandular cells. The stigmatoid tissue, also called the "neck" of the transmitting tissue, in turn borders on the transmitting and cortical tissue of the style. Earlier, the term stigmatoid tissue was reserved for the whole tissue into which the pollen tube penetrates. The term transmitting tissue is now more commonly used.

The dry stigma has a cuticle covered with a pellicle. The cuticle of *Silene* has discontinuities. Obviously, a route for water and a relation to the pellicle exist. Both the pellicle and the surface of the stigma remain hydrated. When the cutinized pellicle shrinks, the cuticle is closed by the rodlets.

The pellicle is an extracellular product originating from the stigmatic cells of a hydrated protein film. The pellicle is a product of the outer cytoplasmic zone of the developing papillate cells. Here microbodies are present which release their granular and fibrillar content. In older papillate cells numerous esterase-containing bodies are present in the cytoplasm. The pellicle also shows an esterase activity. A wet stigma surface can also show an esterase activity). By gel electrophoresis the pellicle protein of *Hibiscus* can be separated into 7 bands, of which two show an esterase activity.

The exudate of wet stigmas has been studied in some species. The main components are amino acids, lipids and antioxidants, and proteins, whereas alkaloids show a variation. In relation to pollination, the exudate may serve as a nutritive for birds, bees, beetles, bats, and moths, as well as a nutrient for providing boron for some pollen.

The exudate of wet stigmas differs in quantity. Lipophilic substances and polysaccharides are present in general. Additionally, polyphenols, tannins or flavonoids can accumulate in the exudate. The composition of the exudate shows many differences, and can be considered as a complex liquid. These products are formed by the papillate cells, and transported through the cell wall and pass the cuticle by ruptures of interstices. The glandular cells usually function till, or long after pollination, but in the wet stigma type of group 4, some papillate cells are necrotic before pollination.

In the hollow style of *Lilium* the stigmatic exudate has the same composition as the product in the canal. It is an aqueous solution with a mixture of acidic polysaccharides, glycoproteins and oligo-saccharides as galactose, arabinose rhamnose, glucuronic acid and galaturonic acid. The exudate resembles in its composition the gum exudate of plant origin, In *Trifolium* the composition of the stigmatic fluid is comparable with the fluid in the hollow style and contains glucose sucrose, and traces of galactose and arabinose, some proteins and esterases. However, the stigma fluid contains two more glycoproteins, and the stylar fluid has one specific glycoprotein.

Fresh exudate of *Phaseolus* contains lipids, proteins, amino acids, as tyrosine, proline, alanine, and cysteine; sugars, mainly glucose, reducing acids, and phenols. Alkaloids are

absent. The presence of sugars can be related to the stimulation of pollen germination. The lipids as a waxy component prevent desiccation and can be an attractive element for insects. The reducing acids prevent oxidation of the lipoidal substances. The protein can function in recognition, and the amino acids as nutrient for in-sects. Phenols are considered as chemical protection against chewing insects and pathogens, but they inhibit pollen germination too and might be involved in the barrier of self-pollination. Primarily, the exudate regulates the water supply to the pollen.

2.3 Function and Structure of the Style

The morphology and anatomy of the style are very variable. During the development many changes take place. In relation to its transmitting function the style has either a canal lined with a glandular epidermis, the open type, which is mostly present in monocotyledons; or it has a solid core of transmitting tissue, the solid type, mostly present in dicotyledons. In some plants there is a half-closed type in which the transmitting tissue can be limited to one side of the stylar canal. In Petunia showed three phases in stylar development: a young style (5 mm) with strong protein and RNA increase and cell divisions in the basal meristematic zone. The next stage is 5-15 mm where cell elongation is induced by the anther. The third phase leads to the mature style, and is triggered by the vegetative system and/or floral parts, such as calyx and corolla. This classification is based on the study of stylar development.

The transmitting tissue in a solid style is either loosely arranged or has a more compact structure. This depends on the amount and quality of the intercellular substance. In ultrastructural studies of stylar transmitting tissue the composition of the intercellular substance has been studied. In a short style, as in *Spinaeia*, it is scanty. In other types there is a large amount. In general, the long cells of the transmitting tissue show a thick cell wall with a small lumen, or a thin cell wall with a large amount of intercellular substance, secreted by the stylar cells and present in the intercellular spaces. The composition of these intercellular substances in *Diplotaxis* is of pectine-containing substances, and should be considered as a wall-like structure. But in *Petunia*, in between the cells of the transmitting tissue, a secretion product is presented comparable with the mucilage of the canal cells in the open style of *Lilium*. The intercellular substance in the stigmatic zone of *Petunia* is also sensitive to protease and contains esterase. Along the transmitting tissue only carbohydrates, peroxidase, and acid phosphatase have been demonstrated. The carbohydrate consists of pectic substances and low molecular acid carbohydrates, as galacturon and glucuronic acids. In *Gossypium* all cell walls of the transmitting tissue have a thick-layered structure. The innermost part consists of pectin and hemicellulose, in the second layer hemicellulose dominates, and the third layer is again rich in pectic substances and poor in hemicellulose and cellulose. The middle lamella, the fourth layer, is fibrous and the pectic substance is very dominant while hemicellulose is also present. The latter two layers react positively on staining for proteins. The pollen tube grows through these walls.

In *Lycopersicon* the cell wall is thin and fibrillar. The intercellular substance contains, in an early stage of development, polysaccharides of pectic nature; in the mature stage proteins are

added. Comparable is also the situation in tobacco, *Malus*, and *Prunus*. After irradiation of the style of *Prunus*, empty areas in the intercellular substance and necrotic cells indicate an inhibited or altered secretion during the development. The cells of the transmitting tissue are different from the stylar cortical cells. They are elongated and have plasmodesmatal contact in the transverse walls. The cell has a large vacuole, plastids, well-developed mitochondria, and an elaborate membrane system. Depending on the state of development, rough ER and polysomes are present. The rough ER is related to the formation of proteins in the intercellular substance during a later stage of development, as demonstrated in *Lycopersicon*.

Some studies report presence of special cellular structures or compounds, for Instance, phytoferritin related to senescence, calcium as mineral supply, and crystal-containing bodies perhaps related to abscission. In the ontogeny of the style of Petunia, a change in protein pattern has been reported but there is no qualitative change in isoenzyme pattern of phosphatases, esterases, and peroxidases. During cell degeneration a callose deposition in the pit fields or, in extreme situation, along the whole surface of the wall takes place. In *Persea,* it is suggested that the reduced pollen tube growth in the pistil is associated with the presence of callose.

In an open type style as *Lilium*, secretion appears in the stylar canal. The canal cells have dense cytoplasm and are rich in organelles. The secretion product is a layered wall structure of pectin, a nonesterified pectin complex with protein and cellulose. This wall shows some similarities with the products in the canal. The cell membrane of the canal cell contains proteins and glycoproteins in paramural bodies. The cell can have more nuclei, and has a polar localization of organelles. A relation between a specific cell organelle and the secretion product is evident. Probably the bordering parenchyma cell produces the secretion, and the canal cell serves as transfer cell.

In *Gladiolus* the canal is covered by a cuticle which prevents the dehydration of the mucilaginous excretion. This cuticle remains intact, and the pollen penetrates the cuticle and grows through the secretion. In *Lilium*, at mature stage, the cuticle collapses.

The open style canal cells provide elements for pollen tube growth and, in this function, their secretion products can be compared with the intercellular substances of a solid style. However, the few but detailed data and differences suggest many more implications. To these different situations the condition for pollen tube growth should be worked out and compared. In stylar structure and function, an interesting field of research is the study of stylar development and growth.

3 Pollen Germination

3.1 Sticking and Hydration

Pollens are transported to the stigma by wind, animals, and water or directly by the contact between open anther and stigma. The pollen dimension, mass, and sculpture reflect the mode of transport. The species-specific exine pattern can be covered by tryphine consisting mainly of the Pollenkitt and remnants of the locule fluid.

The first interaction between pollen and stigma is sticking. Adhesion results from the high viscosity and sticky nature of the exudate. Factors in sticking in sequence of priority are: the surface tension, wind force, electrostatic force, electrodynamic force, gravity, and inertial force. Mainly, the extent of wetness of pollen, or stigma, determines the sticking. In such a situation the rate of sticking hardly depends on the composition of the fluid, the volume, mass or morphology of the pollen. Drying out of stigmas during aging causes the fall of pollen because of loss of surface tension and action of, for instance, a wind force.

In plants with two different pollen and stigma types, as in *Armeria maritima*, the shape of both determines successful pollination.

In *Brassica* incompatible pollen shows a less firm bond in sticking than compatible pollen. The duration of stay of incompatible pollen is shortened by a change in mobility of the proteinaceous component of the pollen coating.

Connected with sticking is the uptake of water by colloidal inhibition and endosmosis. All pollens swell in the presence of water indicating its rapid uptake. The water passes through the exine, and is taken up by the intine and the cell. Because of the desiccated condition of mature pollen, this rehydration takes place mainly along the contact site with preference of the colporal or poral zone, or through the canals in the exine. A hydrodynamic phenomenon based on the study of pollination in rye, and an early exudation of liquid by the pollen, have been observed. This outward movement is important for the release of products present in the intine. This exudation has a short duration and is localized in the apertures or pores. This type of water transport is not in accordance with normal membrane transport, or diffusion of water. It is suggested that a special plasma membrane condition as a nonstable bilayer is present. Immediately after rehydration, the plasma membrane is rebuilt. In the meantime, the exudation takes place because of the loss of an effective control by a normal plasma membrane, and a constant presence of stigmatic fluid. In this condition the pollen, without a normally functioning plasma membrane, swells quickly to a maximum water capacity and a further passage of water from the stigma to the grain than results in exudation. This hydrodynamical process strongly depends on the condition of the cytoplasm of the vegetative cell, and the thickness of the intine because of its imbibition capacity. However, the full hydration of pollen is not necessarily a prerequisite for germination. The uptake depends on the relative humidity of the surrounding atmosphere. The uptake of fluoresceine diacetate in pollen affirms a dissociation of the membrane of the vegetative cell.

When pollens stick and are rehydrated, the fluids of the pollen and stigma surface get mixed. Depending on the nature of these substances, the possibility of a separation of different products exists.

3.2 Pollen Tube Formation and Growth

In vivo as well as in vitro pollen germination depends on several internal and external factors. Mature pollen shows significant differences in structure and function. From comparative studies it is known that the pollen development can reach various end stages. The tri- or binucleate pollen condition of mature pollen represents such a variation. But there are

also the differences in the rate of vacuolation, and the kind and quantity of storage, such as starch or lipids, which lead to a classification of mature pollen in several groups. Important is the state of the mitochondria, especially their number of cristae. Few cristae are indicative of a low metabolic activity. Even more important is the condition of the protein-synthesizing system, and the presence of the mechanism for pollen tube formation which includes production of plasma membrane and wall.

On this condition of the mature pollen depends whether a quick or slow germination on the stigma will follow. If the pollen is fully developed, as in the case of most trinucleate pollen, germination will start quickly. In less-developed pollen the stigma can provide an additive condition for germination. In all cases water or a high relative humidity is necessary; in some cases even a food supply, mostly carbohydrates, and minerals should also be present.

After sticking, the pollen germination starts with the imbibition phase, which is usually followed by a lag phase, tube initiation and elongation. From pollination experiments on young stigmas it is concluded that germination depends only to a low extent on the condition of the stigma.

Compiled from in vitro studies, pollen activation precedes tube formation. This includes an initial high respiration, the development of mitochondria to an active state, the utilization of the pollen storage products, and an enlargement of the membrane system as dictyosomes, for the formation of the plasma membrane and pollen tube wall.

In vivo, the lag-time period before germination in *Lycopersicon* involves activation of dictyosomes, development of RER cisterns and formation of polysomes, both related to the proteins. The activation of dictyosomes leads to production of small and large vesicles. The large vesicles are related to callose formation and deposition at the nonfunctional pores and, subsequently, against the new cell wall. Near the germinal pore both large and small vesicles are present. The small ones are related to the first formation of the pollen tube wall which opens the pore as a door. This process depends on the developmental state of the pollen, since differences in developmental state are related to lag-time phase, as observed in *Oenothera*.

The formation of the pollen tube involves a growth zone, vacuolation, and plasma streaming. The growth zone is the tip where new plasma membrane is formed, and a wall with the characteristics of a primary wall appears. This tip contains many organelles, mainly dictyosome vesicles which fuse with the plasma membrane excreting wall precursors and proteins. In some species the ER is involved in this process. This zone is also characterized by the presence of ribosomes and polysomes. Microtubules are absent. This type of growth is comparable with that of the fungal hyphae. Here the tip zone permits the excretion of proteins and enzymes. Germinating pollen also excretes enzymes, among other products, probably at the tip zone mainly. The pollen tube wall consists of one outer pectic layer and an inner callosic layer, which originates in *Lilium* from the polysaccharide particles in the pollen tube.

The cytoplasm in the remaining part of the pollen tube can be divided into different zones as observed in cotton, *Impatiens*, *Liliurn*, *Lycopersicon*, and *Prunus*. Directly behind the tip zone dictyosomes and, in some pollen tubes, SER are present. Upon this zone mitochondria and,

sometimes, a second type of vesicles become visible. The presence of vacuoles marks the following zone; here the vegetative nucleus and the generative cell or sperm cells are also present. The vacuole is formed by the second type of vesicles, tonoplast is produced. The rest of the pollen tube contains a large vacuole and a thin layer of cytoplasm. In this part the streaming is usually manifest,

The transport of the vegetative nucleus and the generative cell is related to the growth of the tube and the cytoplasmic streaming. This streaming is caused by microfilaments. The direction of the streaming is the result of interconnection between microfilaments, microtubules, and endoplasmic reticulum. In transport of the vegetative nucleus and generative cell fibrillar material near these structures is probably responsible for the movement.

The tube growth can start after imbibition and activation-within a very short period. This includes the possibility to form a new plasma membrane and a cell wall, and to start vacuolation. In quickly germinating pollen these processes are due to the presence of vesicles for the tip growth, and another type of vesicles for the formation of the vacuoles by fusion and uptake of water.

In wheat observed the beginning of germination and initiation of the pollen tube. The sperm cells lay near the germinal pore and emerged before the vegetative nucleus. In growing pollen tubes the vegetative nucleus normally lies near the tip, and the generative cell or sperm cells follow. This means that in the first stage of tube formation the position of the vegetative nucleus is reversed and, probably, the stratification of the tube is not yet complete.

Closely behind the tip zone an additional cell wall layer is formed, containing callose. Depending on the length of the pollen tube and the rhythmic growth, callose plug formation takes place. The region of callose plug formation is marked by electron density of the ground plasm, absence of plastids and dictyosomes, and presence of lipid bodies. The RER is involved in the synthesis of callose. Vesicles or spheres containing callose fuse with the plasma membrane. The plug formation shuts off part of the pollen tube. During pollen tube growth the cytoplasmic position and the number of cell organelles remain fairly constant. Pollen germination and pollen tube growth are processes of a free gametophytic plant. As can be concluded from in vitro experiments, the metabolism involved is complex and species-specific. The order in the pollen tube represents the zones of functions which seem independent of the surrounding tissues.

3.3 The Sperm Cell

The generative cell forms two sperm cells. The generative cell, as the result of an unequal division of the microspore, has few organelles. In some plants plastids are absent in the sperm cells. The generative cell wall is initially continuous with the intine, but in most pollens the cell is set free in the cytoplasm of the vegetative cell.

In *Secale*, during the cytokinesis, the generative cell is attached to the pollen wall and cell plate formation is clearly visible. The two sperm cells have large nuclei. The nucleus is surrounded by a layer of cytoplasm with mitochondria, dictyosomes, endoplasmic reticulum, and parallel arrays of microtubules. A cell wall around the round cell is absent. In *Heleocharis*

the sperm nuclei are fusiform and, after cell division, two fusiform cells appear but with a flat ending in the zone of separation. In *Gossypiu*m the nuclei are small with a small nucleolus. In the cytoplasm is conspicuous dictyosomes which produce vesicles and numerous polysomes. In *Plumbago* an unequal distribution of mitochondria and plastids is present in the generative cell. After division, the association with the nucleus of the vegetative cell persists. The sperm cell connected with the nucleus of the vegetative cell contains majority of mitochondria, the other the plastids. In studies of the movement of sperm cells, the cellular connections of the sperm cells and with the nucleus of the vegetative cell should be included.

Only a few reports deal with the division of the generative cell in the pollen tube. The division is slow and, in some plants, a spindle seems to be absent. The sperm cell can change its shape in response to conditions of squash preparations. In *Beta* microtubules are present oriented parallel to the long axis of the sperm cells. In *Hordeum* these microtubules are considered as elements of a cytoskeleton which preserve the cell shape.

The more or less fusiform shape of the cell permits them to move into the pollen tube. The movement of the generative cell or sperm cell is a result of cytoplasmic streaming. The direction of the movement depends on the existence of two opposite cytoplasmic flows. The streamlined shape of the generative cell permits to move toward the tip with the central streaming because of its position in the tube. The small end of the cells is directed toward the pollen grain, and the other broader end toward the tip.

Based on observation of living pollen of *Zea*, which germinate and form a tube in vitro, suggests that the independent movements of the vegetative nucleus and the sperm cells are related to a shift of the dynamic center of the cell. Because of the cytoplasmic movements, the forces which fix the nucleus in the cell are continuously changing. The position of the nuclei and sperm cells should be considered as dependent on, or a result of, various cellular forces.

The movement of the sperm cells is a cytological problem, on the same level as the more or less fixed position of a nucleus in a cell. In this respect the microtubules and filaments, and the band of fibrils in the cytoplasmshould be considered in a model of movement. But the influence of the current from base to the tip, as is present in the pollen tube of *Lilium*, should not be excluded. The mechanism of movement is still not clear.

4 Pollen-Pistil Interaction

4.1 Pollen Recognition, Acceptance, and Rejection

After pollination of the wet stigma of Cosmos, the pollen is covered by a proteinlipid and carbohydrate coating. In this situation products held by the exine of the pollen mix with the stigmatic exudate. In dry stigmas the pollen sticks on the pellicle which makes contact with the pollen covering. As has been demonstrated in *Silene*, the papillate cells react on pollination with a change of permeability, plasma streaming, and plasma membrane.

In rye, after the initial contact, a penetration of pollen wall proteins could be proved by fluorescence microscopy. After 10 min the tips of the papillate cells are reached, after 24 h the cytoplasm of these cells also shows an intensive fluorescence. In response to pollination of

Brassica the proteins in the exudate show a high turnover. Some proteins that are released from the pollen wall can be involved in immunological reactions. These types of proteins, the antigens, are considered as functional in recognition and may also be present in other related species

In the recognition system the presence of some complementary products is usually involved. Enzymatic removal of the pellicle proteins in *Agrostemma*, and *Raphanus* prevents pollen tube entry. Also, concanavalin A binding compounds in the pellicle function as an adhesive for pollen. These results support the idea that an inactive precursor present in the pollen is activated by a factor in the stigma. Another quick reaction after pollination is the enhancement of the esterase activity of the stigma.

The recognition is a stage necessary for pollen acceptance and germination. The recognition of acceptable pollen is followed by pollen activation. The pollen starts to germinate and produces enzymes for penetration, as cutinase, and for nutrition. The presence of such enzymatic activity is demonstrated by the prints made by the pollen on the papillate surface. In rejection the pollen germination or tube growth is blocked. Several factors are involved in the recognition reaction.

4.2 Pollen Incompatibility

Pollen incompatibility reactions after pollination in angiosperms are manifest either in stigma, style, or ovule. The pollen does not germinate; tube growth is blocked on the stigma, in the style, in the ovary. Or, the pollen tube stops near the embryo sac, and fusion of sperm cell does not take place.

The incompatibility is genetically based on one S-locus with many alleles, or has two (S and Z) or more genes. The expression of the gene activity can be manifest in the sporophyte or the gametophyte.

In the sporophytic incompatibility the products, probably of proteinaceous nature, are formed in the tapetum and added to the exine of the pollen grain. These products are set free on the stigma, and are involved in a complementary reaction.

In the gametophytic incompatibility the products involved are formed in the pollen during their development. These products are stored in the intine and are released during pollination on the stigma and act during or shortly after pollination. The incompatibility reaction is still under study, which means that it cannot be excluded that other sporophytic tissues Such as stigma, style or ovule, as well as the embryo sac, are involved in the production of incompatibility substances.

In many families with gametophytic incompatibility the wet stigma dominates, and pollens are mostly binucleate. In sporophytic incompatibility the dry and papillate stigmas are more common but this is not true for *Gramineae*. The pollens are mostly trinucleate.

In plants with a wet stigma and solid style the recognition of pollen takes place in the stigma. In species with a wet stigma and hollow style the recognition is confined to the style. In Poaceae with a dry stigma the contact with the pollen tube tip and stigma seems necessary, and there the recognition takes place. In grasses the tip of the pollen tube is a very important

place in the recognition reaction. The inhibition of the pollen tube growth occurs with a strong or weak reaction after contact with the pollen tube tip and the papillar cell. After the strong reaction the primary effect is a dislocation of the wall materials on the tip. The weak reaction is comparable with those in gametophytic systems where inhibition occurs in the transmissing tissue of the style.

The role of the pollen-coat is not clear. Washing out of the pollen-coat proteins of *Lilium, Pyrus,* and *Vicia* does not affect pollen germination. But in *Brassica* different types of washing have a clear influence. A function of the *Brassica* tryphine, which contains many lipids, in recognition can be restricted to a preparation of a nonpolar environment for an interaction process. In *Lilium*, the recognition and response occurs in the style, but this is not a result of the inability to take up materials from the style. The suppressed growth of the tubes may be caused by the loss of the turgor pressure within the tube. Glycoproteins in the stylar fluid are possibly the major source to offer osmotically active molecules, which is inhibited in incompatible tubes.

In dry stigmas the pellicle in contact with the pollen coating creates the condition for the recognition reaction. In pollen-wall extracts of *Iberis* the proteinsor glycoproteins are supposed to be involved in the incompatibility reaction. In *Brassica*, a glycoprotein in an extract of papillae of mature stigmas. This glycoprotein is absent in the immature stigma, which does not show an incompatibility reaction. The pollen-coat factor should react with the stigmatic glycoprotein, and a suppression of the pollen development takes place. The nature of the pollen-wall factor in *Brassica* is unknown, and there are no differences in the pattern between compatible and incompatible pollen-wall proteins. The presence of a special stigmatic glycoprotein has been reported earlier in studies on *Brassica*. The production of the stigma factors concerned with incompatibility, is not always synchronous with the factors concerned in germination.

In dry stigmas the rejection reaction results either in failure of pollen to germinate, or the occlusion of short pollen tubes with callose, or the failure to penetrate into stigma and style. The stigmatic papillae react with cell activation and production of callose in the papillate cells near the pollen or pollen tube.

The deposition of callose in *Raphanus* is possibly related to the perforation of the cuticle by the pollen tube, and can be compared with a wounding effect. The protein fraction of the tryphine causing the callose reaction consists of a protein with low molecular weight.

In wet stigmas with gametophytic incompatibility the pollen tube growth is blocked in the stigmatoid, or in the transmitting tissue. In the transmitting tissue the pollen tube growth ceases and callose is deposited at the tip, or the tip swells and bursts. The place of this reaction can be near the stigmatic tissue, as in *Oenothera* and *Alopecurus*, or on the upper half of the styles, as in *Petunia* or *Lycopersicum*. During hydration of the pollen on the stigma proteins are released. The proteins present in the intine are considered to function in the recognition system of gametophytic incompatibility. On ultrastructural level, the cytoplasm of the pollen tube of Petunia shows concentric ER, indicative of a block in protein synthesis, few

polysomes, and numerous amyloplasts with phytoferritin. After incompatible pollination the stylar tissue shows more or less no change in reserve substances, and is comparable with stylar tissue after pollination prior to passage of the pollen tubes. In *Lycopersicum* the tube tip bursts reaching the zone of blocking, and an accumulation of particles appears in the tube, which consists of a mixture of proteins and substances for wall production. In these tubes concentric ER has also been observed. The ultrastructure of gamma-irradiated compatible pollen tubes shows a great similarity.

For the stigma-pollen interaction several hypotheses have been proposed to specify the mechanism of recognition. The products of the S-genes are considered as complementary or opposed to each other.

4.3 Entry of Pollen Tube into Stigma and Style

After recognition and acceptance, the pollen tube is directed to the stigmatic surface and penetrates the stigma and stigmatic tissue. This ingrowth involves the ex- cretion of cuticle and cell wall-affecting enzymes. In contact with the stylar tissue a nutritive relation develops. In open styles the pollen tube does not penetrate the stigmatic tissue.

Before pollination of *Petunia* the stigmatoid or neck cells show a special type of wall contact, formed by ridges of walls which fit in a complementary furrow. These cells contain abundant ER and ribosomes. Between the stigmatoid cells the presence of carbohydrates and proteins, as demonstrated by protease digestion, and also enzymes as acid phosphatase, peroxidase and especially esterase, could be established. After pollination, not dependent on the incompatibility reaction, the stigmatoid cells contain an increasing number of polysomes. For a short period cytoplasmic inclusion, the embayments, associated with the plasma membrane, are added to the intercellular sub- stance. Near the pollen tubes the cells can become necrotic, and start to degenerate after the passage of tubes. In this region, initially, the amount of starch is high, but one day after pollination it decreases, especially after a compatible pollination caused by the passage of the pollen tube. The same holds good for the lipid drop- lets. Before pollen tube arrival a synthesis probably of polypeptides and carbohy- drate starts in the stigmatoid cells, and these products are excreted in the intercel- lular substance. The starch in these cells is used up during the passage of the pollen tube. Starch degradation provides free sugars for the pollen-wall formation and respiration. In incompatible crossing the latter process is blocked.

During pollination and pollen tube growth three phases of synthesis are distinct in the stigmatoid zone, the first before pollination, a second stimulated by pollination involving cellular excretion and, finally, a phase of transfer of nutritive substances to the pollen tube. During pollen germination the nutrition comes from internal as well as external sources. It should be emphasized that the pollen storage products can also function in animal transport, because of their use as nutrition by insects.

During pollen germination in *Oenothera* the pollens are bound with threads of viscin to the stigmatic surface. This surface has a saturated lipid and droplets of proteins and carbohydrates in a layer of viscous fluid on papillate secretory cells. A region of alveolar parenchyma lies

beneath the secretory cells, and the subjacent layer has loosely packed parenchyma cells mated with a fine film of carbohydrates and protein. These cells have very little starch content. The pollens contain carbohydrates as well as different types of membrane-bound bodies and vesicles. During tube formation a change in the membrane-bound bodies and the vesicles, in association with the mitochondria, takes place and vacuolation starts. During pollen tube growth the carbohydrate content remains constant. Compared with the incompatible pollen tube growth less carbohydrates are present, a difference in wall formation and change in membrane-bound bodies and vesicles occurs, callose plugs are formed and the growth is slow and finally stops within 24 h. The lack of free carbohydrate in the compatible pollen inhibits its growth. The role of surrounding tissue and the exudate is not clear. In *Persea*, before pollination, the stigma and stigmatoid cells begin to become necrotic.

In *Petunia), Crocus, Oenothera* and many other plants; the pollen tube grows intercellularly.

In *barley* and *Spinacia*, on the dry stigmas the pollen tubes grow at first along the surface of the hairlike cells, and then penetrate its wall. In barley the pollen tube utilizes its own reserve substances for penetration. Following penetration of the cell wall, a thickened and looser texture of the wall is visible after the excretion of enzymes by the tube tip. In *Spinacia* the sticking of the pollen-coat to the pellicle is stable till germination elevates the pollen grain. The pollen tube penetrates the pellicle and cuticle, and grows either in the outer cell wall layer or along the inner side of the cell wall. In *Zea mays* the penetration of the silk via the multicellular hairs is restricted to two opposite sides of the silk, near the place of the vascular bundles and the transmitting tissue.

Pollen tube growth is a directed growth. On the stigmatic surface the tube grows to the transmitting tissue, and in this tissue to the ovary and then to the ovule.

In experimental studies on attraction, the major techniques used are the surface test, the angle test, and the depression test. In *Lilium* a chemotropic activity of the papillate cell to the pollen tube has been observed. In Oenothera mixtures of organic components are involved. In *Antirrhinum* the presence of calcium is presumed to be a factor in pollen tube attraction, although this mechanism is not active in some other plants. Besides, a gradient of calcium also exists in the pollen tube, with the highest concentration at the tip. The tip zone should be altered or corrected during the growth. This part of the pollen tube is also a major zone of excretion, and probably, of uptake of nutrition. Mascarenhas suggests that a missing component can result in a chemotropic reaction. A shift in the direction of growth of the tip center should be related to an uneven distribution factor. This influences the tip metabolism. In *Begonia* the change in surface influences the pathway of the pollen tube. The cells around the micropyle have a smooth surface in contrast to the other cells which are covered with deposits of waxy substances.

Also, the situation is complex in *Spinacia*, near the nucellar tissue. Here the mass of pollen tubes branches and ceases to grow further; only one, or a few tubes penetrate the nucellar tissue and a selective attraction of one tube takes place. Such a reduction in the number of penetration of tubes also occurs in *Persea*. The ovary has one ovule which is reached by only a

few pollen tubes. The rest remain arrested, and the tips of the tubes swell.

In the chemotropic attraction of the pollen tube a significant role depends on the genetical background, the nutrient level in pollen and the transmitting tissues of the pistil, as well as gradients in pollen and pistil. Before and during entrance of pollen tubes, the interaction of pollen and stigma is determined by processes of nutrition and penetration. Both are mainly a feature of the pollen. The stylar and stigmatic nutrition, however, can be present in the exudate and stigmatoid and stylar tissue with its starch content. Carbohydrate nutrients seem to be very common. A lack of regulation in nutrition is observed in incompatibility crossings. So the redistribution of metabolites is a very important reaction on pollination.

4.4 Pollination Effects

Wilting of flowers of Cucubalus can be related with inter- and in-traspecific pollinations. Wilting of the corolla was accelerated by cutting off the stigma or the style halfway down in *Petunia*. In young plants the rate of wilting is faster. The wilting takes place in early morning, except for cross-pollination which seems to be independent of a day rhythm.

Pollination with foreign pollen or killed *Petunia* pollen does not influence the wilting pattern of unpollinated flowers. These experiments indicate the presence of a sense organ. The process of wilting is a factor in the communication of the plant with its environment involved in pollination.

In the style the pollination leads to a signal for the regulation of floral development. Also, an activation of the ovary is induced. In translocation experiments with labelled glucose in *Petunia*, during cross-pollination, the amount of labelled carbohydrates decreased in corolla and ovary. After self-pollination, this amount remains at a higher level. After cross- pollination an accumulation of carbohydrates takes place in the ovary when the pollen tubes have reached the base of the style. For labelled amino acids compatible pollination is marked by a more direct increase of label in calyx and corolla compared to an incompatible crossing. The more intense label in anther and style after self-pollination is remarkable. These data are indicative of a redistribution process, related to pollination. The style becomes an attraction center, and the ovary a sink after cross-pollination. A reaction on pollination is also the increase in metabolic activity after about 12 h, expressed in changes of the ribosomal protein and incorporation of C-14 leucine in the polysomes of the ovary of Petunia. Depending on different types of pollination, the pattern of protein synthesis changes. These reactions in the ovary show the existence of a long-distance trans- mission of information. This information communicates the fact of pollination and selection in the type of pollen.

Pollination changes the metabolic constitution of the flower, mainly in stigma, style, and ovary. The complexity and sequence of reactions should be related to the process of flowering, fertilization, and seed formation.

5 Entrance and Discharge in Embryo Sac

In angiosperms the pollen tube has to enter the embryo sac for successful fertilization. The pathway of the pollen tube from the placenta to the embryo sac is highly variable, related to

the structure and organization of the ovular tissues. In most species the embryo sac is surrounded by one or two integuments, leaving a narrow opening called the micropyle. In between the integuments and the embryo sac there can be a varying amount of nucellar tissue, which has to be passed by the pollen tube.

5.1 Course of the Pollen Tube

Usually, the pollen tube enters the ovule through the micropyle, called porogamy. Exceptionally, the ovule is entered at the chalaza (chalazogamy), or laterally (me- sogamy). In a number of species the embryo sac protrudes through the micropyle prior to fertilization, and can be reached by the pollen tube directly, as in *Torenia fournieri*. For the passage of the pollen tube from the placenta to the micropyle several situations have been de- scribed. In a number of species special structures, such as obturator and papillae, formed by either the placenta or integuments, are present and serve as a pathway for the growing pollen tube and facilitate its entry into the micropyle. Usually, the obturator is a swelling of the placenta which grows to- ward the micropyle. In other cases cells of the conducting tissue of the style, or cells of the inner integument, elongate and form a well-defined pathway for the pollen tube to reach the apex of the ovule.

However, in most species, the pollen tubes grow along the surface of the placenta, funiculus, and ovules in order to reach the micropyle. It is assumed that the growth of the pollen tube toward and into the micropyle is directed, although the nature of this directed growth is poorly understood. In *Impatiens glandulifera*, ectotrophic growth on the funiculus, and directed growth through a slime canal on the margin of the funicle and ovule toward the micropyle. Numerous authors assume that the growth of the pollen tube is directed chemotropically by substances produced by the ovule, and secreted through the micropyle.

Chemotropic activity of ovules, especially in their micropylar-halves, has been established in bioassays for a number of species. In *Antirrhinum* directional growth is achieved by the presence of calcium gradients.

5.2 Entry into the Embryo Sac

Based on a large number of light microscope observations, it was presumed that the pollen tube could enter the embryo sac by one of the following main routes: 1. In between the cells of the egg apparatus, i.e., the synergids and egg cell. 2. Between the cells of the egg apparatus and the embryo sac wall. 3. Into one of the synergids, after previous degeneration or accompanied by the destruction of that synergid.

Since the light microscopic studies show so many different routes, it is even more noticeable that all electron microscope studies, so far, show the same out-standing pattern of penetration and discharge of pollen tubes in embryo sacs with three-celled egg apparatus (Fig. 7.1A-C). Only for species which lack synergids, as *Plumbago*, a different pattern of pollen tube entry and discharge has been described. The mature megagametophyte consists of five cells: the egg, the central cell, and three antipodal cells. The mature egg is a highly polarized cell. The nucleus, numerous mitochondria and the majority of plastids are located at the chalazal end, while the micropylar end of the egg shows wall ingrowths, strongly resembling a

filiform apparatus. The pollen tube penetrates the female gametophyte through the "filiform apparatus" and grows for another 80 um between egg and central cell. The pollen tube discharges through a terminal aperture, resulting in release of male gametes, vegetative nucleus, and a limited amount of tube cytoplasm. The discharged male gametes remain cellular during their presence between egg and central cell.

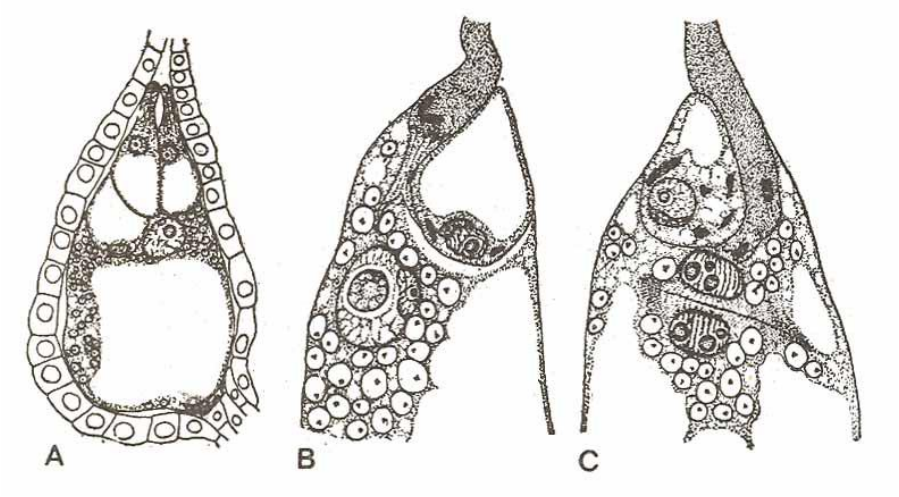

Fig. 7.1 Stages in fertilization in *Petunia.* A Mature embryo sac showing two synergids, egg cell, and central cell, surrounded by integumentary tapetum. B Upper portion of embryo sac after entrance of pollen tube. The pollen tube is presumed to form two branches through which the two male gametes were discharged. C Up portion of embryo sac showing the zygote, and division in the primary endosperm cell. Note abundant starch grains in A-C.

5.3 Growth Through the Filiform Apparatus

The ultrastructural studies clearly show that the synergids play a vital role in the pollen tube entry. Penetration of the pollen tube in the embryo sac starts with the growth of the pollen tube into and through the filiform apparatus (FA). Essentially, the FA is an extension or elaboration of the micropylar synergid wall. Observations on living material have shown that the entry of the pollen tube and the release of the tube content cause considerable and very sudden changes in the organization of the embryo sac.

The changes in organization and the interaction of male and female material also result in drastic changes in staining properties of the cytoplasm, and these, in turn, interfere with the light microscopic observations and interpretation of stained sections. Furthermore, some of the structures involved are very near the limits of the resolving power of the light microscope, and it is possible that some conclusions based on light optical research are based on misinterpretations.

A well-established example is the conclusion of Cooper that in Petunia the pollen tube enters the embryo sac in between the cells of the egg apparatus (Fig. 7.1A-C). Later electron microscopic studies have revealed that in Petunia the pollen tube actually penetrates into the

synergid.

For this reason, in this chapter, we focus attention on observations based on electron microscopy and their interpretation. In most species the cytoplasmic surface of synergid is highly convoluted, resulting in a strongly increased plasma membrane area. Histochemical studies reveal that the FA is PAS-positive indicating the presence of polysaccharides, merely of pectic and hemicellulosic nature. In *Petunia* the presence of a very loosely organized cellulosic fibrillar network has been' shown. Although the chemical composition and ultrastructure indicate that FA does not represent a solid barrier to the pollen tube, it is clear that it offers some resistance to tube growth which must be overcome. In *Epidendrum,* the pollen tube forms a cap over the micropylar part of the embryo sac, then an extension develops through the FA. Especially, the latter observation indicates that the final part of pollen tube growth is invariably directed, rather than being merely accidental. The growth pattern suggests that the pollen tube is guided toward the synergid.

5.4 Entry into the Synergid

After the passage of the filiform apparatus the pollen tube grows into one of the two synergids (Fig. 7.2 A-D). In several species, as *Gossypium, Zea, Epidendrum, Hordeum, Linum, Lycopersicum, Spinacia,* and *Quercus,* one of the synergids starts degenerating before the arrival of the pollen tube. It has been stated that, in fact, pollination triggers the beginning of the degeneration process. However, in cultured, nonpollinated and unfertilized ovules of cotton Synergid changes strikingly similar to those after pollination in vivo. Apparently, in this case synergid degeneration is not triggered by pollination, but as a part of the common embryo sac development. In *Torenia, Petunia, Helianthus,* and *Capsella* both synergids remain unchanged, until the pollen tube has penetrated one of them. The penetrated synergid then undergoes cytoplasmic changes that closely resemble those of the preentrance degeneration of synergid. Both degeneration processes are marked by decrease of cellular volume, collapse of the vacuoles, disintegration of the plasma membrane, and disorganization of the cell organelles.

In those species which do not show structural differences between the two synergids before the arrival of the pollen tube, it is not clear whether any of the synergids can be penetrated or not. Chemical or physiological differences between the two synergids, regulating the penetration event cannot be excluded.

Shortly after the entrance of the pollen tube in the cytoplasm of the synergid, pollen tube growth ceases. Cessation can take place immediately after the passage through the filiform apparatus, as in *Petunia*, or the pollen tube can extend into the synergid for some distance, as in *Gossypium* and *Linum*.

It appears that the degeneration of the synergid, the entrance of the pollen tube, and cessation of tube growth are closely related. In a few cases entrance of the pollen tube in the persistent synergid, or the entrance of additional pollen tubes in that synergid, has been reported. In these cases pollen tube growth does not cease in the normal way. Growth is continued until the degenerated synergid is reached and then, eventually, it stops. This

indicates that the degenerated condition of the synergid cytoplasm is needed, or is responsible for tube growth cessation. It also indicates that in species in which both synergids apparently remain healthy until fertilization, the degeneration of cytoplasm of the penetrated synergid observed afterward might have actually taken place shortly before the arrival of the pollen tube and been triggered by the latter.

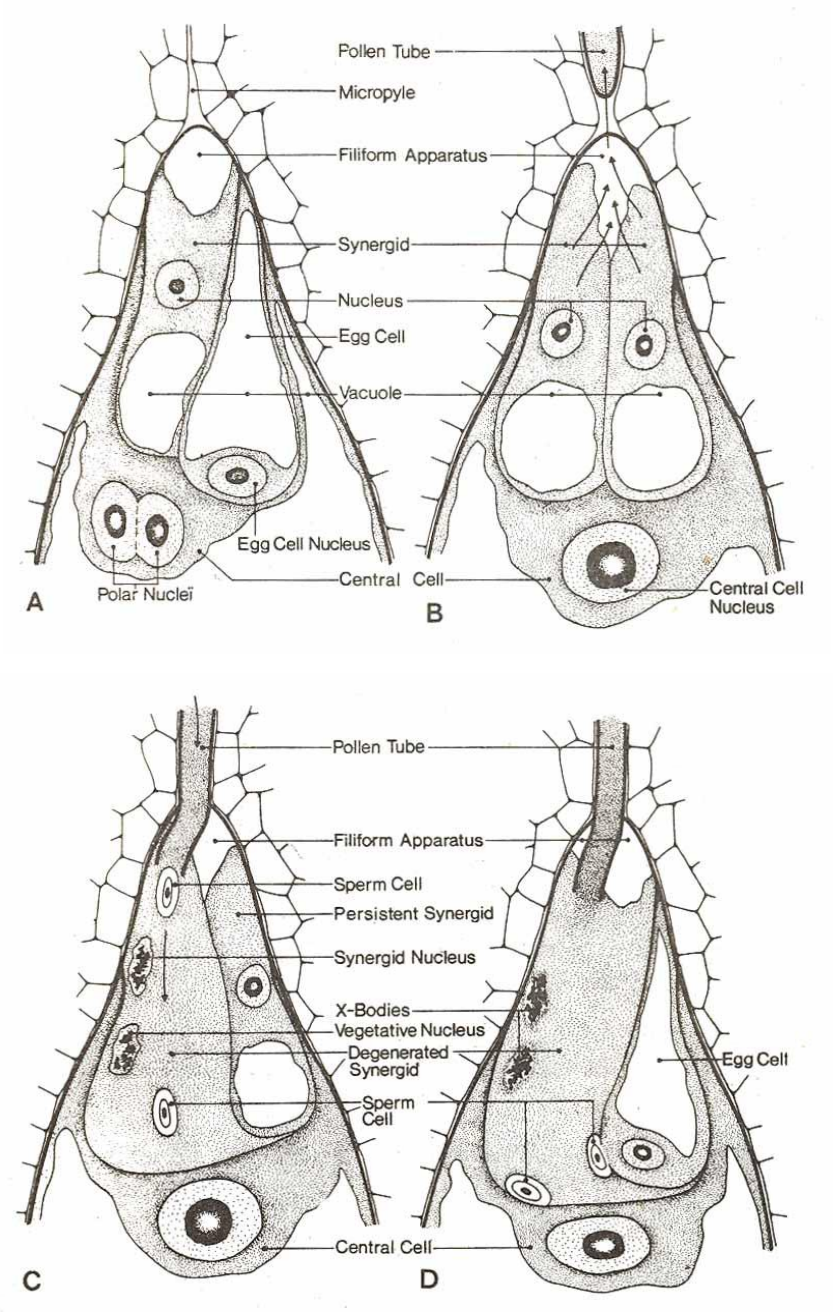

Fig. 7.2 Diagrammatic summart of pollen tube entrance, tubed discharge, and gametic transfer in angiosperms. A Micropylar part of mature embryo sac. B Proposed chemotropic activity of synergids, and directed growth of pollen tube. C Discharge of pollen tube. D Proposed way of gametic transfer and fusion

After arrival inside the synergid, discharge of the pollen tube content takes place. In *Gossypium*, a subterminal pore develops on the side of the pollen tube toward the egg cell. The position of the pore and its regular diameter indicate the presence of a highly ordered process rather than a simple rupturing of the pollen tube. In Petunia, the pollen tube opens at its tip, and it was concluded from the morphology of the opened tube that the opening merely occurs by bursting of the pollen tube.

5.5 Transfer of Tube Content

After the opening of the pollen tube a fair portion of the tube content, including the two sperm cells and the vegetative nucleus, is released into the synergid. The tube cytoplasm can easily be recognized, in spite of the degenerated appearance of both tube and synergid cytoplasm. The tube cytoplasm contains numerous small PAS-positive spheres. The pollen tube discharge forms a roughly cone-shaped mass spreading from the pore toward the chalazal end of the synergid. Not all of the tube cytoplasm is injected into the synergid. In cotton, soon after the release of the pollen tube cytoplasm, a plug is formed by fusion of PAS-positive spheres in the pollen tube near the FA. In other species, as *Petunia*, the pollen tube collapses or is squeezed by the surrounding tissue.

In *Petunia* the high amount of released tube cytoplasm causes the bursting of the synergid and penetration of its cytoplasm in between the egg cell, central cell, and persistent synergid. However, in no case could tube cytoplasm be observed inside other cells than the penetrated synergid, and the neighboring cells were not affected by the close contact with this degenerated synergid cytoplasm. Since in *Petunia* walls are absent in the chalazal part of the egg apparatus, and the cells are surrounded only by plasma membranes, one might ask in what way the egg cell and central cell are protected, considering the dramatic cytoplasmic changes that occur in the penetrated synergid.

It is obvious that the penetrated synergid is involved in the transfer of the male gamete material to the egg cell and central cell. The first question to consider is in what condition the male material is released from the porn tube: as naked nuclei or as complete cells?

6 Fusion of Gametes

There is abundant evidence that the male gametes of angiosperms are cellular and complete descriptions of their ultrastructure are available. However, most studies focused their attention on sperm cells in mature trinucleate pollen grains or pollen tubes. The sperm cells are believed to be surrounded by a plasma membrane only, in contrast to generative cells which mostly show a cell wall. Their cytoplasm contains mitochondria, ER, ribosomes, dictyosomes, and microtubules. Plastids are frequently reported to be absent. Unfortunately, there are only a few reports dealing with the sperm material inside the synergid. Attempts at observing and recording the initial stages of the fertilization process in living material were not very successful. The major problem is the low transparency of the penetrated synergid in optical microscopy.

There is, however, some evidence that the male gametes are deposited in the embryo sac as

complete cells. With phase-contrast microscopy Cass and Jensen observed sperm nuclei surrounded by a distinct clear zone in the penetrated synergid in barley. This clear zone closely resembles the zone observed between the nucleus and boundary of the sperm cell in the pollen grain, which represents the male cytoplasm. Cass and Jensen also, rarely, observed penetration and discharge of a pollen tube into the persistent synergid. In these cases the discharge of complete sperm cells could be established. The movement or transport of the sperm cells after their discharge into the synergid remains an unresolved question.

According to Steffen, isolated sperm cells show autonomous, amoeboid movement, which could be sufficient to reach the female egg cell and nucleus. However, Cass showed that isolated living sperm cells of barley did not exhibit any directional motility. Ultrastructurally, the presence of microtubules in these sperm cells could be established. The position of the tubules and the shape changes observed indicate that their chief role is regulation of cell shape. The suggestion of a microtubular role in regulation of cell shape is in agreement with the findings of Sanger and Jackson. They induced generative cell-shape changes by treating pollen with isopropyl N-phenyl carbamate or colchicine; both compounds destroy cytoplasmic microtubules.

6.1 Fusion of Nuclei

Next in the process of double fertilization is the fusion of male and female nuclei. Based on light microscopic observations, various modes of transfer of genetic material and nuclear fusion have been described. Three types of karyogamy are recognized, depending on differences in the condition of the sexual nuclei at the moment of fertilization: Premitotic, Postmitotic, and Intermediate. In the first type fusion is completed before the zygotic mitosis. This type is usually common in Gramineae and Compositae. In the second type the fusion is completed after the initiation of the zygotic mitosis during prophase- metaphase. It occurs in a number of Liliaceae (Fig. 7.3). The third type is characterized by an incomplete mixing of the sexual nuclei. The chromosomes remain apart for varying lengths of time and can still be seen in the prophase of the zygotic nucleus. Similar observations have been described by Hu and Zhu. In the classical light microscopic literature it is usually stated that the male nucleus sinks into the female nucleus or becomes immersed into it. According to Luxova, in barley the membrane of the egg nucleus appears locally inter- rupted temporarily to allow the entry of the sperm nucleus. Also, in *Caltha palustris* and *Ranunculus acris* the disappearance of the membrane of the egg nucleus prior to nuclear fusion has been observed.

However; ultrastructural studies have shown that no interruptions or breakdown of nuclear membranes are involved in the nuclear fusion. The observed mechanism is shown in Figs. 7.4 A-E. The nuclear fusion starts with local contact and fusion of the outer membranes of the two nuclear envelopes which then become continuous. In these process elements of the neighboring and attached ER can be involved (Fig. 7.4 A). Next sequence in the fusion process is the contact and fusion of the inner membranes of the nuclear envelopes. In this way bridges are formed between the two nuclei and their nucleoplasm become continuous (Fig. 7.4B). Subsequently, the nuclear bridges enlarge and coalesce until no separating membranes are left

between the two nuclei (Fig. 7.4 C-E). During the nuclear fusion process, cytoplasmic portions containing organelles become trapped between the nuclei, but this cytoplasm is squeezed out by the enlarging nuclear bridges. The observed sequence indicates that the membranes of both nuclei contribute to the envelope of the zygote nucleus, Soon after fertilization is achieved, division of the primary endosperm nucleus occurs, leading to the formation of endosperm. The zygote remains undivided for some time, although considerable cellular and cytoplasmic changes can take place.

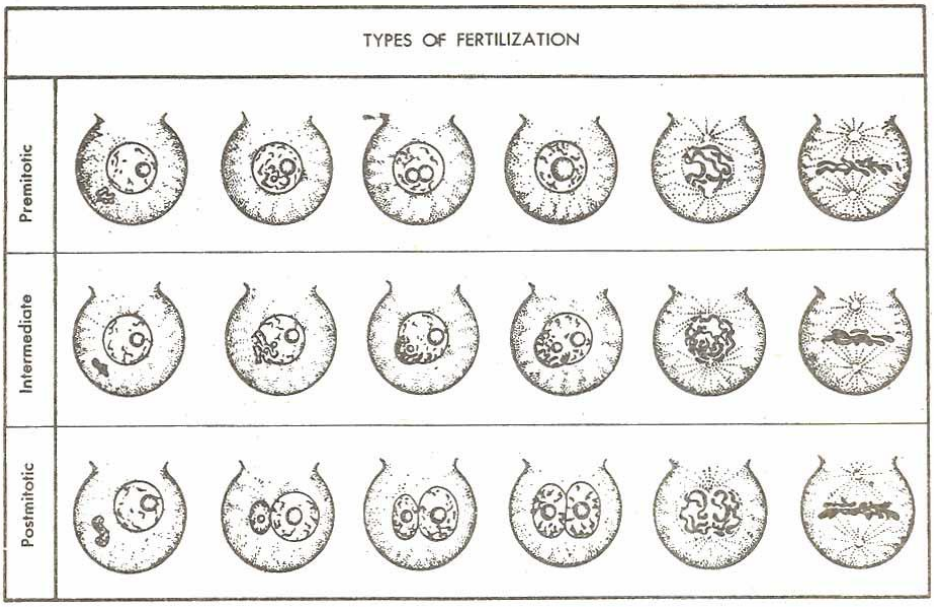

Fig. 7.3 Types of nuclear fusion in angiosperms, as based on the moment of first nuclear contact and completion of mixing of chromatin. In the "premitotic" type the nuclear fusion is completed before the zygotic division. In the "intermediate" type the chromatin of male and female nuclei remain partly separated until the first mitotic division. In the "postmitotic" type the chromatin of male and female nuclei remain separated completely until the mitotic division of zygote.

In cotton and *Hibiscus* the zygote shows drastic shrinkage after its formation. Shrinkage is accompanied by increasing density of the cytoplasm and loss of vacuolar volume. In cotton zygote starch is formed, the amount of ER increases, and the cytoplasm contains abundant RNA. In both *Epidendrum* marked changes in the number and aggregation of the ribosomes, leading to the formation of large helical polysomes, have been observed. In some species, as *Capsella, Epidendrum, Hordeurn,* and *Quercus*, the zygote does not shrink after its formation. Usually, a reorganization of the cytoplasm takes place as in *Hordeum* where a peripheral localization of vacuoles is achieved. In Capsella, directly after fertilization, the dictyosomes are activated in relation to the formation of zygote cell wall. It is noticeable that the zygote wall lacks plasmodesmata. The zygote divides after a period of varying length following fertilization.

In the fertilized central cell the nucleus divides very rapidly following fertilization. In cotton,

initially, little or no cytoplasmic reorganization has been observed. The early free-nuclear endosperm is a highly active, rapidly growing tissue. As in the zygote, large helical polysomes are formed. In Petunia, however, marked cytoplasmic changes occur in the central cell after fertilization and prior to the first nuclear division. There is formation of extensive RER and, simultaneously, with an increasing density of the cytoplasm, there is a decrease in the starch content of the plast.

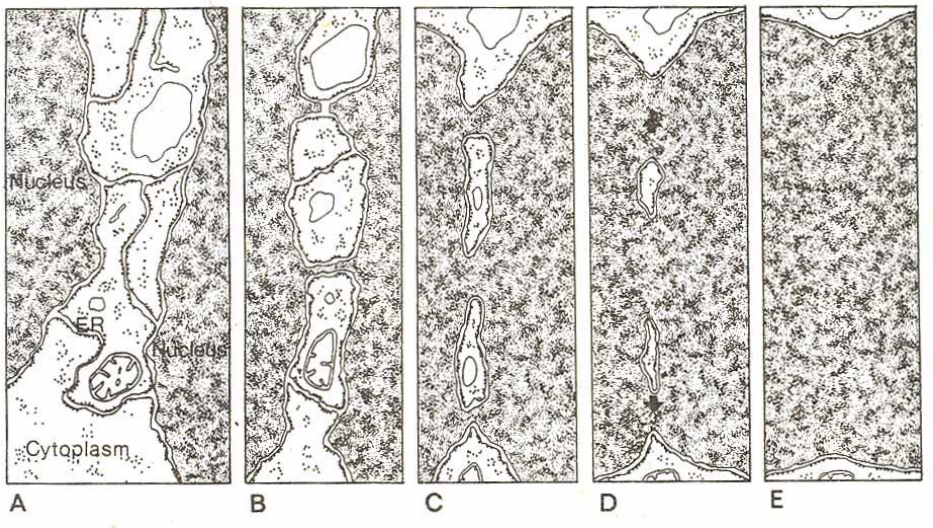

Fig. 7.4 Diagrammatic presentation of the cytoplasmic events leading to the complete fusion of gametic nuclei in plants. A Contact of *ER.* B Fusion of outer nuclear membrane. C Formation of nuc1ear bridge. D Enlargement and coalescence of bridges. E Fusion completed

6.7 The Progamic Phase and Fertilization

Summarizing the previous notes, it becomes clear that the whole process from pollination till fertilization is a very complex interaction between gametophyte and sporophyte, and also between the two gametophytes with their gametes.

This complexity involves also the relation to animals as pollen vectors or visitors to flowers which results in pollination. The position and morphology of the stigma is clearly related to the mode of pollen transport. Most of these characters are all built up by the sporophyte, and result in the interaction of pollen and stigma. Thus, some factors in pollination, as sticking, exchange, the moment of pollen transport, and the intensity of the visit or duration of flowering, are pre- requisites for a successful landing of the pollen.

Physical and chemical forces are involved in sticking of the pollen to the stigma. The pollen and stigma surface coatings, genetically determined, function in the first steps of recognition or rejection. In this context the preexisting sporophytic or gametophytic nature is important. The recognition signals may function on electric, membrane-bound or on molecular level, but are still not clear. Also, the recognition of interspecific pollen resulting in incongruency is unknown. The stage of pollen development and its vitality determine the moment of

germination too. In this process of interaction, comparable with host-parasite relations, many steps are discerned. The sequence of these steps is not yet all known and need further investigation.

The pathway of the pollen tube passes through different zones. It penetrates tissues, or runs along wet phases, or gas-filled areas. Besides, the tube growth is a directed process and needs nutrition. The influence of the different tissues on each other, and the direction of growth, includes a mechanism of control and at- traction or regulation. The stigma, style, and ovary are all involved in relation to the pollen tube, growth. The study of the ontogeny of these organs, on structural and physiological level including hormonal control, would help in a further elaboration of this interaction process.

The factors for selective degeneration in the egg apparatus, and acceptance of the pollen tube have already been under study for a long time. Probably these phenomena depend not only on the normal developmental process, but are influenced by pollination too. The most intriguing pathway of the sperm cells in the pollen tube, the partition and directed movement in the egg apparatus still await a detailed explanation.

In summary, the progamic phase is a developmental process, expressed in the growth and function of the stamen and the germination of pollen. It includes a genetic programmed process sequence, influenced by the surrounding tissues which are involved in nutrition and can act on the direction of the growth. This complicated mechanism, partly to be compared with the fertilization in lower plants and lower tracheophytes, needs a structural and functional approach with a good genetically defined plant material under controlled conditions. The progamic phase and fertilization will be followed by seed formation. It is, therefore, necessary to include in these preceding phases even the preparation to subsequent seed development.

Chapter 8 The Embryo

1 Zygote

Although examples of other cells endowed with embryogenic potential are not unknown, the fertilized egg or zygote usually represents the forerunner of the embryo in almost all spermatophytes. The zygote is a unicellular system which, through a programmed sequence of events, gives rise to a multicellular embryo with well-differentiated organs and, as such, embodies within itself the multifarious properties of an adult plant. In angiosperms the zygote is located at the micro- pylar pole of the embryo sac with its basal portion attached to the embryo sac wall, while the apical portion projects into the central cell. Even under the light microscope, the cell presents a characteristic polarized appearance with the micropylar pole vacuolate, while the chalazal pole contains the nucleus and most of its cytoplasm. A good deal of new information has come to light about the structure of zygote with the application of electron microscopy. Before attempting to understand the process of embryogenesis in angiosperms, it is essential to look into the organization and environment of this unique cell.

1.1 Structure and Composition

In the unfertilized egg of *Papaver nudicaule* the nucleus is sited towards the micropylar pole with a large vacuole towards the chalazal pole (cf. synergids). However, at fertilization there is a reversal of cytoplasmic polarity; the nucleus shifts to the chalazal pole and the micropylar pole is occupied by a vacuole. A post-fertilization shift of nucleus and cytoplasmic contents towards the chalazal pole has also been observed in *Zea mays*. Such conspicuous organelle relocation does not characterize the fertilized eggs of other species such as *Capsella*, *Epidendrum, Petunia,* and *Linum* where these organelles are so oriented in the unfertilized egg itself. However, the ER becomes more elaborate, and the ribosome density increases. Whereas, in most species, the ribosomes in the unfertilized egg occur singly, either in the cytoplasm or in association with ER, they now show a distinct tendency to aggregate into polysomes (cotton, *Capsella* and *Epidendrum*). In cotton, particularly, the bulk of the ribosomes forms helices of up to 20 subunits, and much of these represents a new population initiated after fertilization. In *Lycopersicum* the ribosomal system of the egg undergoes dissolution, and a new population is formed at a distance from the zygote nucleus; this region contains a considerable number of ER cisternae and resembles ergastoplasm. A large number of mitochondria and dictyosomes also accumulate in this area. During this period the cytoplasm shows a sharp increase in Azure B staining. These features could be interpreted as indication of mRNA synthesis in the zygote.

Other evidences suggestive of intense metabolic activity include internal differentiation of mitochondria and plastids, as well as increase in their number. While the increase in number is presumably due to a multiplication of the organelles present in the original egg cytoplasm, (at least) in some instances it is attributable to a contribution from the sperm cell. In the majority of cases, uniparental maternal inheritance is the rule for plastids as they are selectively

excluded from the generative cell during pollen mitosis and, hence, the sperms are apoplastidic. However, evidence from genetic studies involving mutant plastids points to a biparental inheritance in some taxa. The generative cell in these, e.g. *Castilleja, Lobelia, Oenothera,* and *Pelargonium* has one or several plastids. Plastids of sperm origin have been traced within the zygote of *Oenothera* and *Plumbago.* The sperm organelles could be identified by their smaller size and different shape. It is important to realize that mere transmission of the male organelles into the egg during fertilization is not of much consequence, unless such organelles are capable of survival and multiplication in the zygotic environment, as well as during subsequent embryonic stages of development. That this is possible is indicated by cytological studies in *Oenothera,* and breeding experiments in *Pelargonium zonale*. The fate of the male organelles in *Plumbago*, however, still awaits investigation.

Several investigators have experienced difficulty in detecting DNA in the unfertilized egg by tinctorial methods, particularly the Feulgen. However, soon after fertilization, the zygotic nucleus returns to a Feulgen-positive state. While some explanation has been offered to account for the aberrant response of the unfertilized egg, it may imply that the imposed restraints to staining, if any, are either removed or lost after fertilization. Other cytological changes apparent after fertilization include the accumulation of starch, lipids, and protein in the zygotic cytoplasm. A distinct oil droplet occurs in the basal part of the zygote in *Jasione montana*, and this droplet disappears after embryogeny has advanced.

2.2 Size Adjustments

The fertilized egg, in several species, undergoes changes in its size before embarking on division. This is particularly striking in *Gossypium* where the volume of the zygote decreases to half that of the egg in 8-10 h after fertilization. Similar shrinkage in volume is also characteristic of the zygote of *Hibiscus*. A less drastic reduction in volume has been observed in *Nicotiana tabacum*, the decrease being up to 27% in 40-50 h following fertilization. However, as the zygote embarks on division 7 days after fertilization, the possibility of further shrinkage in this species cannot be ruled out.

On the other side of the scale are taxa such as *Datura stramonium, Cypripedium insigne,* and *Jasione montana* in which the zygote enlarges during the pre-division phase. Elongation of the zygote before it divides is regularly seen in members of Lentibulariaceae, Orobanchaceae, Scrophulariaceae, and others. In Capsella bursa-pastoris there is an immediate but temporary decrease, but soon the original volume is restored.

1.3 Polarity

One of the striking features associated with the zygote is the polarized appearance of the cell, both under light and electron microscopes. The nucleus and most of the cytoplasm gather at the chalazal end, and a large vacuole occupies the micropylar end of the cell. At the ensuing mitosis the two daughter cells are, therefore, destined to inherit differentially distributed cytoplasmic elements and, therefore, the course of embryogenesis is already foreshadowed in the zygote itself: the chalazal daughter cell (apical cell) forms the embryo proper, while the

basal cell forms the suspensor.

The timing, as well as the mechanism, of fixation of polar axis in the zygote is of great interest to a student of morphogenesis. Our knowledge is very inadequate not only in respect of the zygote of angiosperms but all the vascular plants as well. The zygote in Fucales - particularly *Fucus* - has been extensively used to probe the cytological, biochemical, and biophysical basis of determination of polar axis, and several reviews have appeared. That the pattern of polar development during embryogenesis in *Fucus* bears some resemblance to that in angiosperms has been recognized for some time.

It is necessary to discuss the stages which characterize the development of a polar axis in the zygote of *Fucus*; concerning angiosperms Raghavan has already provided an excellent account. The spherical fucoid zygote has initially an apolar homogeneous appearance. The first sign of polarity is the localized accumulation of subcellular components in the cytoplasm, and the formation of a protuberance within 8-14 h following fertilization. An asymmetric division ensues resulting in a smaller rhizoidal cell, and a larger thallus cell. The events leading to the polarized reorganization of cytoplasmic elements has been explained by Jaffe on the basis of self-electrophoresis which includes: (1) decreased permeability of the plasma membrane on one side of the cell brought about by a variety of external agents, such as light, gradients of pH in the medium or in the concentration of substances, such as hormones; (2) amplification of the initial membrane difference through the accumulation of cations at the region of de- creased permeability, followed by the establishment of a polarized electrophoretic gradient across the cell; (3) movement of electrophoretically polarized cytoplasmic elements (such as protein molecules) across the gradient, and often a movement of the nucleus.

As of now there is no evidence to suggest that such a mechanism exists in higher plants. Obviously, this is an area that requires much attention. Besides, one has to keep in mind other factors while discussing the problem of polarity in angiosperm zygote. Whereas in the fucoid zygote the structural and biochemical polarity occurs in a previously homogeneous egg cytoplasm, the egg is itself polarized in angiosperms. It may be more fruitful, therefore, to study the mechanism of cytoplasmic redistribution during the formation and maturation of the egg itself. Moreover, the fucoid eggs are released by the parent plant into the adjoining sea water, and fertilization is effected externally. By contrast, in angiosperms the egg is anchored to the embryo sac wall at the micropylar pole.

2 Early Embryogenesis

2.1 Cell Patterns

In most investigated angiosperms, the zygote divides by a transverse wall resulting in two superposed cells, the apical and basal cells. These cells as well as their derivatives have been assigned specific abbreviations, originally by Soueges and the French School, and are now conventionally employed in embryological literature. The apical cell is designated ca and the basal cell cb (Fig. 8.1, see also Fig. 8.4).

Rarely, as in Balanophoraceae, Dipsacaceae (*Scabiosa*), Loranthaceae, and Piperaceae, the division of the zygote is by a vertical or oblique wall which is so oriented as to be almost longitudinal.

The transverse division of the zygote and the respective contributions of the two daughter cells to the subsequent formation of the embryo and its suspensor are considered as the basis for the recognition of embryogenic types.

The division of the zygote in some taxa is such that the apical cell ca may be smaller than the basal cell cb. However, in a statistically significant number of angiosperms, the basal cell may be approximately equal to or appreciably smaller than the apical cell. Any discussion on the nature of factors that control the specific size relationship between the apical and basal cell in a two-celled proembryo must, at the moment, remain largely conjectural for want of critical information concerning zygotic division in higher plants. It may involve shape and polarity of zygote itself, qualitative and/or quantitative biochemical differences in the microenvironment of the embryo sac adjoining the micropylar and chalazal end of zygote, the morphogenetic destination of the two cells, or some other factor.

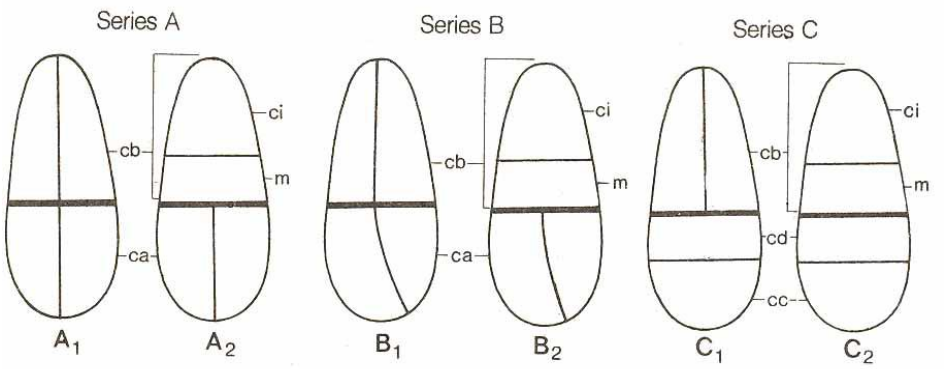

Fig. 8.1 Proembryonal tetrads; classification based primarily on the plane of division in apical cell: vertical in *Series A,* oblique in *Series E,* and transverse in *Series* C. Further subdivision takes into account the mode of division of basal cell which may be either vertical(*A1, B1, C1*) 01; transverse (*A 2, B2 , C2*)

2.2 Tetrad, Quadrant, and Octant Proembryos

The division of each of the two cells of the proembryo results in a four-celled tetrad arranged in specific patterns. The major patterns, or Series, are designated A, B, and C (Fig. 8.1). The partition wall in the apical cell is vertical in Series A, oblique in Series B, and transverse in Series C. In each Series two variations are possible, depending upon whether the basal cell initially undergoes a vertical or a transverse division and, accordingly, these tetrads are designated *Al* and *A2*, *B1* and *B2*, and *C1* and *C2* (Fig. 8.1). When the basal cell undergoes a transverse division (as in Series *A2*, *B2*, and *C2*) the more basally placed daughter cell is designated *ci* and the intermediate daughter cell m (Fig. 8.1).

In a tetrad of Series C, when the apical cell divides transversely, two superposed cells *cc* and *cd* (Fig. 8.1) are formed. These cells may again divide and produce a tetrad of the second

order (Fig. 8.2). Here the cell cc behaves like *ca*, and the cell *cd* as *cb*. The tetrad of the second order may conform to Series A (Fig. 8.2), B or C. Tetrads of the third and fourth order are also possible (Fig. 8.2) if the transverse divisions continue at the apical pole of the proembryo. The derivatives of *cc* are designated *ce* and *cf*(Fig. 8.2), and that of *ce* as *cg* and *ch* (Fig. 8.2). It should be emphasized that, in all these instances, the potential embryonal region is progressively shifted to the distal pole of the proembryo (Fig. 8.2); the basal part does not contribute to the embryo proper. Soueges attached considerable importance to the constitution of the tetrads, and their contribution to the embryo proper.

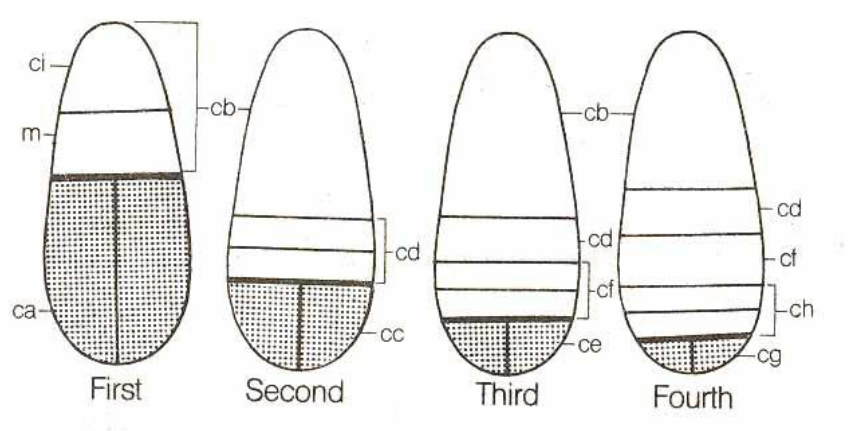

Fig.8.2 Proembryonal tetrads of *Series* C. showing tetrads of firstm, second, third. and fourth order. respcctivcly. The potential embryonal pole (*shaded*) shifts progressively to the extreme micropylar end

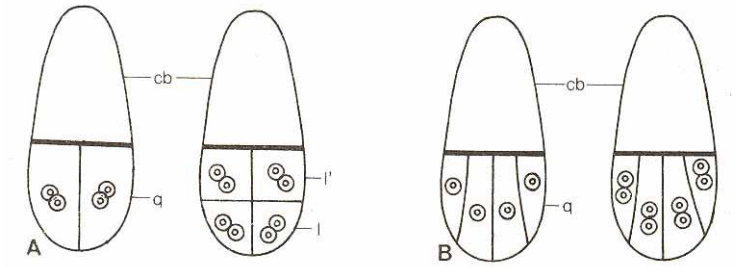

Fig. 8.3 Quadrants (four cells at distal end of proembryo) produce octants (eight cells) either in two tiers(A) or one tier (B). When in two tiers, the apical tier is designated/and the basal tier *l′*.

The tetrad stage is followed by another vertical division in a plane usually at right angles to the first vertical division in the distal cell *ca, cc, ce, or cg*. These four juxtaposed cells constitute the quadrant *q* (Fig. 8.3) which through another division gives rise to an octant. The concept of quadrant and octant relates to the formation of four and eight cells at the distal end of the proembryo. The octant may be in two tiers *l* and *l′* (Fig. 8.3 A), or one tier (Fig. 8.3 B). The quadrant and octant are of much significance as the progenitors of the cotyledonary and epicotylary *loci* of the shoot pole and, in addition, partially or fully contribute to the hypocotyledonary portion of the embryo.

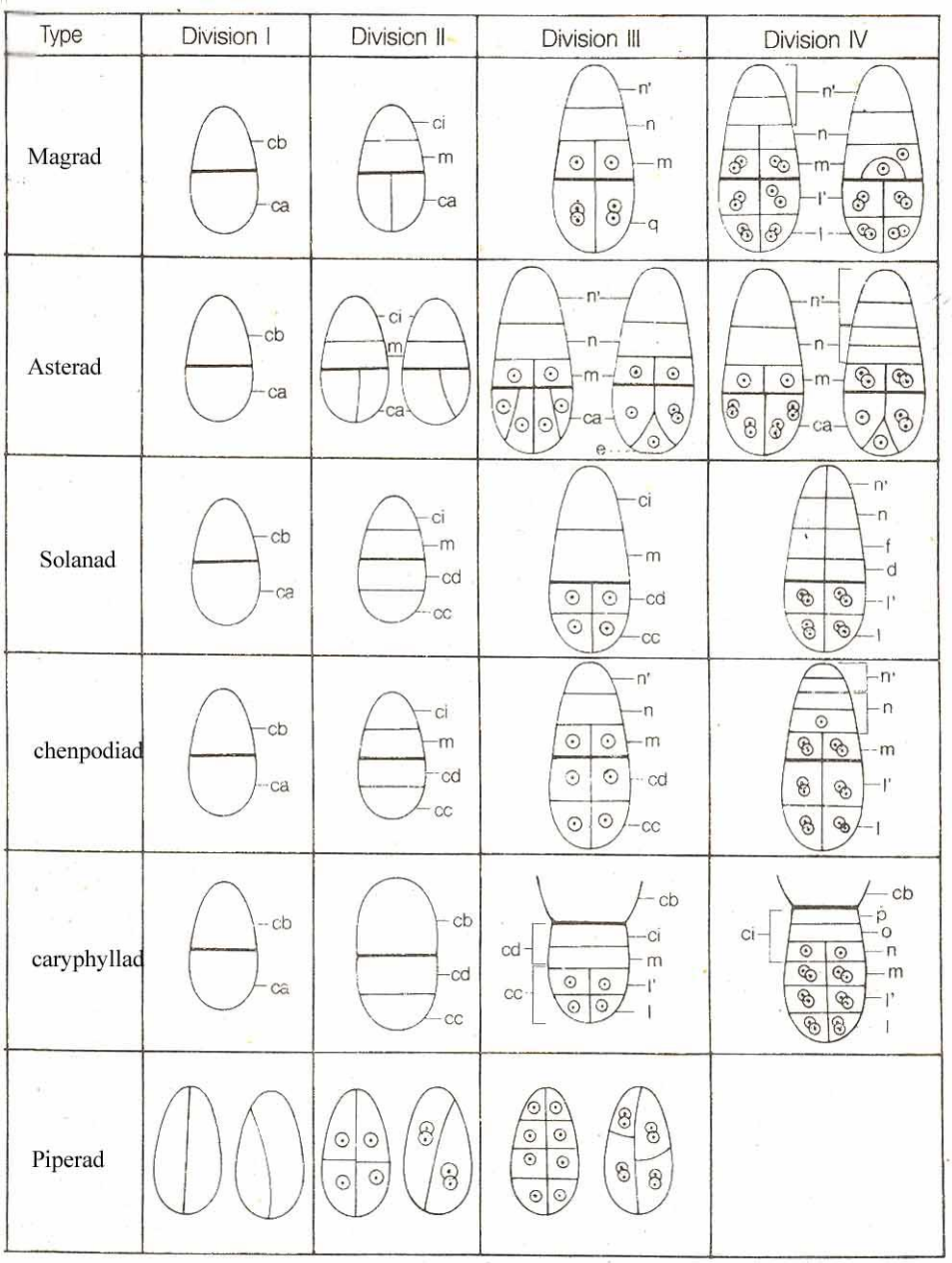

Fig. 8.4 Schematic representation of main types of embryogeny.

The derivatives of the basal cell, immediately next to embryonal mass, contribute to the hypophyseal region. Now the embryo is complete. The remaining derivatives of the basal cell [including the cells corresponding to the derivatives of the basal cell in tetrad of the second, third, and fourth order (see Fig. 8.2)] constitute the suspensor. It is noteworthy that the contribution of zygote to the embryo proper, volumewise, may range between 100% as in *Penaea* (where the suspensor does not differentiate and the apical and basal cell contribute equally to the embryo proper), to just about 6% as in some species of *Corydalis.*

The foregoing account outlines the general course of development of the embryo in angiosperms. There is considerable variation in the sequence and chronology of the early divisions in various tiers of the proembryo, the orientation of the partition walls, and the contribution of the tiers to the further development of embryo and suspensor. A particular course of development may be specific to individual species, or genus, or even entire family, but variation may occur even within the same species(Fig. 8.4).

2.3 Stages Leading to Mature Embryo

Further development from the octant stage following numerous cell divisions in various planes causes the proembryo to assume a globular configuration. The globular proembryo passes through a phase before the cotyledons *pco,* and epicotyl *pvt* become outwardly evident at specified *loci*, and there is a transition from radial to bilateral symmetry. The embryo is now heart-shaped, so characteristic of the dicotyledons.

In the monocotyledons, however, the early course of development is no doubt similar to that in dicotyledons, but there are fundamental departures at the time ofdifferentiation in the globular proembryo.

During early embryogenesis two characteristic cells, or groups of cells, become evident. These are generally referred to as hypophysis *h*, and epiphysis *e*. The intermediate cell *m*, between the embryonal mass and suspensor, cuts off a discoidal cell designated "hypophysis" by Hanstein. Correspondingly, at the opposite end, i.e. at the apex of the embryonal region, a wedge-shaped cell may be cut off. This has been designated epiphysis by Soueges. According to Soueges, these cells are so characteristic of the dicotyledonous embryos that they are considered to represent two polar morphogenetic units, the root pole and the shoot pole. The hypophysis may be a single cell, or a group of cells. The initials of the root cortex *iec* and the root cap *co* are derived from the hypophysis. The hypophysis is pushed into the embryonal mass in the course of development. There is considerable variation in the origin and organization of hypophysis.

The epiphysis originates in the terminal tier of dicotyledonous embryos, and gives rise to the shoot tip. The epiphysis may be a single cell or a group of cells, and its organization also varies. The epiphysis may not be easy to recognize in several dicotyledons, and it remains to be seen whether it is differentiated during embryogenesis of all dicotyledons.

Irrespective of the variations in the sequence and disposition of cells in the tiers of the proembryo, ultimately there is hardly any difference in the organization of the embryo.

3 Differentiation in Embryo

The three-dimensional growth of the embryo is initiated when vertical walls are laid in the terminal cell of the linear proembryo. In due course, through divisions in various planes, the globular stage is attained. Differentiation sets in with the establishment of the cotyledonary and epicotylary *loci* in the shoot pole; the hypocotyledonary region and the root pole with the hypophysis can be distinguished. The organization of the *loci*, however, is not strictly correlated with the cell lineage, or the tier systems. In the globular proembryo the primary tissue systems, through differential vacuolation in the homogeneous mass of cells, become

marked out. The cotyledonary, epicotylarly, and hypocotyledonary regions along with the hypophyseal region become identifiable through further cell divisions, growth, and development in the respective organs. The hypocotyledonary region, through cell increase and elongation, contributes to the main axial sys- tern of the later embryo. This differential growth phase of the embryo leads to the organization and activity of the respective meristems through the accentuation of the root-shoot polar differences. Swamy and Padmanabhan, while discussing this phase of development of the embryo at the organizational level, point out that the existing systems of classification, based on the sequence of cell divisions in the proembryo, do not reflect the importance of underlying factors of growth, differentiation, and organization as applied to the dicotyledons as a whole. There are too many variants to give them any real status either from the morphogenetic or the phylogenetic point of view.

4 Dicot and Monocot Embryo

Hanstein (1870), on the basis of his study of Alisma plantago, claimed that it represented the monocotyledonous type of development. When a large number of taxa had been investigated, particularly by Soueges and his collaborators, the essential similarity in the sequence of divisions in the young embryo in monocots and dicots was noted. Therefore, both mono- and dicoty- ledonous species figured in embryogenic classification. For example, Schnarf and Johansen included monocotyledonous species such as *Muscari Liliurn, Jutneus* and others along with dicotyledonous species in the Crucifer (Onagrad) type, Soueges, likewise, included several monocots in Period I though he designated a separate Megarchetype for them on the erroneous assumption that in all monocotyledons the stem tip was lateral, originating from the middle tier and, that, only the cotyledon was terminal. During early embryo- genesis, quadrant and octant stages, and up to the globular proembryo, there is a similarity of lineage and configuration of the cells in monocots and dicots. At the time of differentiation in globular proembryo, fundamental differences arise. Swamy ad Krishnamurthy point out that in the organization of embryonic shoot apex, the dicotyledons and monocotyledons follow entirely diverse morphogenetic patterns. In the dicotyledons the axial cells which constitute the epiphysis have a retarded growth in contrast to, the faster rate of growth of circumaxial cells, In the monocots (Fig. 8.5 A-K), on the other hand, one-half of the terminal cell and its derivatives have the characteristic of the epiphysis with retarded growth, whereas the other half with a rapid rate of growth forms the cotyledonary locus. The apparent lateral position of the stem tip in later stages is due to the more rapid growth of the cotyledon. Recent studies on a number of monocotyledons by Haccius, Swamy, Lakshmanan and others have clearly demonstrated that the epicotyl and cotyledon arise from one and the same terminal tier. These two loci are differentiated by the first vertical wall in the terminal tier. At the quadrant stage in the terminal region, the differences become quite apparent. Lakshmanan studied a series of transections of the terminal tier to explain the major difference between the dicotyledons and monocotyledons (Fig. 8.6 A-E). In the dicotyledons the two oposite cells of the terminal quartet engender the pair of cotyledons (Fig. 8.6 B); in the

monocoty- ledons the number of cells of the quartet that gives rise to the cotyledon varies, with practically all the four cells (except a few derivatives of one of them) giving rise to the cotyledon as in Philydraceae (Fig. 8.6 C); three cells of the quadrant as in Pontederiaceae, Sparganiaceae, Iridaceae (Fig. 8.6 D); only the adjacent two cells as in Hydrocharitaceae, Potamogetonaceae, and Amaryllidaceae (Fig. 8.6E). Campbell observed that in *Zannichellia* both the shoot apex and cotyledon arise from the terminal segment.

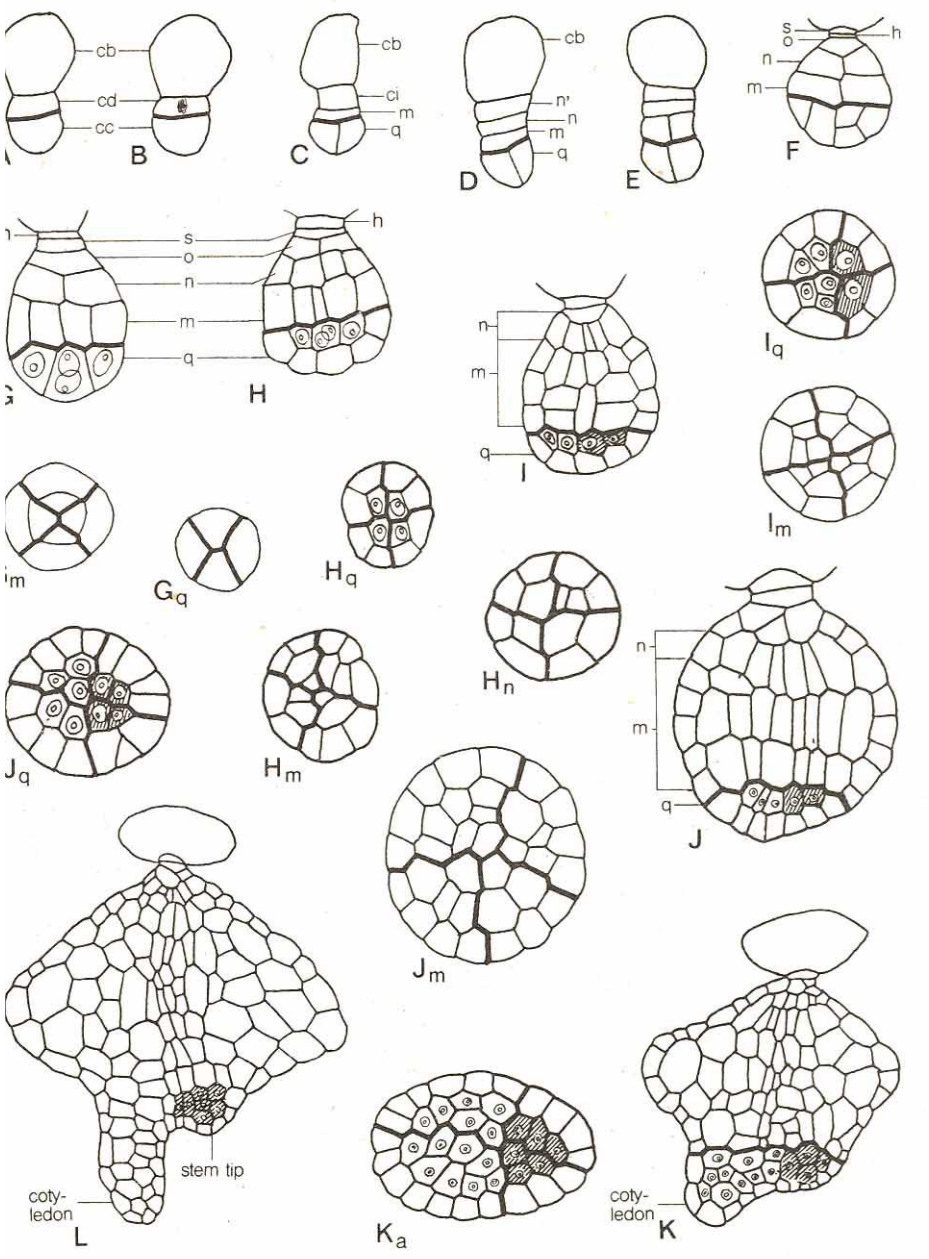

Fig. 8.5 *Halophila ovata,* stages in the development of embryo. In monocotyledons one-half of the apical cell exhibits retarded growth *(shaded regions* in I-L) and gives rise to the stem tip, whereas the other half grows rapidly by cell divisions and forms the cotyledon. Hence. the shoot tip is not lateral in origin.

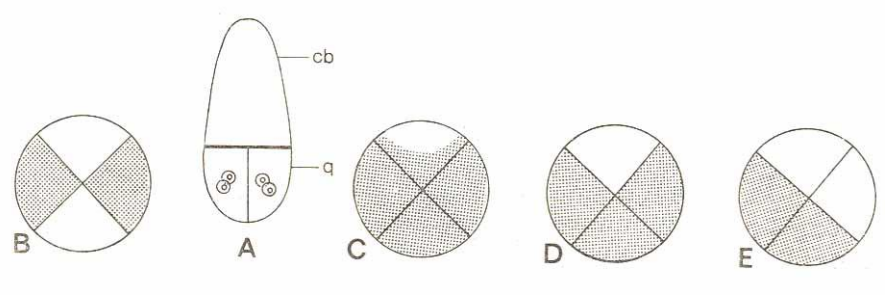

Fig. 8.6 Derivation of cotyledons in monocotyledons and dicotyledons. A Quadrant proembryo. B-E Transections of terminal tier of A; *stippled portion* represents progenitors or cotyledon. B Development in dicotyledons. C-E Development in various monocotyledonous taxa.

5 The Grass Embryo

Among the monocotyledons, the grasses present some remarkable features in their embryogeny as well as the structure of mature embryo and, therefore, deserve special attention. Within the caryopsis, the mature embryo is placed towards the base on the dorsal side and is relatively small in relation to the endosperm (Fig. 8.7 A, C). The scutellum is a long, shield-like vascularized structure laterally attached to the axis when seen in a sagittal longitudinal section (Fig. 8. 7 B,D). The scutellar surface in contact with the endosperm bears a secretory epidermis. The point of attachment of the scutellum to the axis, known as the scutellar node, demarcates the axis into two regions. The upper region is the epicotyl which comprises a shoot apex and leaf primordia. The epicotyl is sur- rounded by a hollow sheath called the coleoptile (Fig. 8.7 B, C). During germination the coleoptile often becomes green and photosynthetic and the first foliage leaf breaks through it. In some grasses, for example *Triticum* and *Arena* (Fig. 8.7 B), there is an erect flap-like outgrowth devoid of vasculature on the antiscutellar side of the axis and is designated the epiblast; in others such as *Zea* (Fig. 8.7 D) the epiblast is not present. The area between the base of the coleoptile and the point of attachment of the scutellum has been referred to, by some authors, as the mesocotyl. Below the scutellar node the axis terminates in a radicle with a root cap, and is ensheathed at the root pole by a structure called coleorhiza (Fig. 8.7 B, D). The root emerges through the coleorhiza during germination.

The grass embryo is different from most other monocotyledonous embryos in that the Origin: of the first root primordium is endogenous. Hence, the coleorhiza has been interpreted as the suppressed primary root, the seminal root it encloses being adventitious. Interestingly, in cultured barley embryos, the scutellum and coleoptile developed leaf hairs showing green pigmentation, whereas the coleorhiza developed root hairs.

It has been suggested that the epiblast denotes the remnant of a second cotyledon; no doubt because it is always positioned opposite the scutellum. Many regard it as an outgrowth of the coleorhiza.

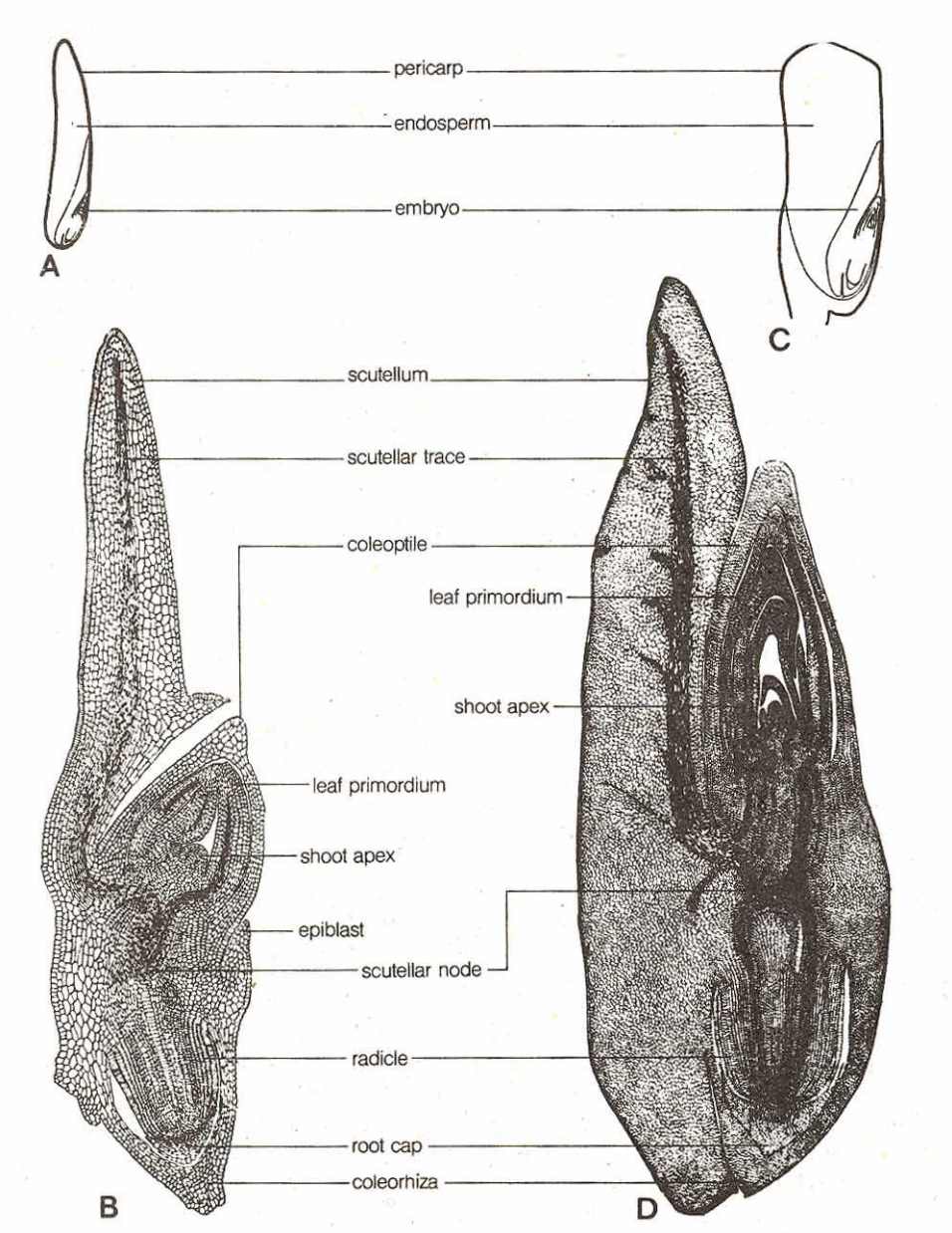

Fig. 8.7 The grass embryo. A, B *Avena sativa;* C, D *Zea mays.* A and C are longisections of caryopsis indicating the position of the embryo; B and D are tongisections of the embryo.

As in most other monocotyledons, the shoot apex of the grass embryo also appears to be lateral. It was pointed out in the previous section that in the light of recent work on monocotyledons, both the stem tip and cotyledon arise from the terminal proembryonic tier, but due to overgrowth of the cotyledon, the stem tip comes to occupy a lateral position. The asymmetry of the embryonic apex in grasses arises from the very beginning through partitioning of the proembryo by oblique walls.

The mature embryo of grasses with its special structures has proved to be of systematic value.

6 Ultrastructural and Cytochemical Aspects

The apical cell and the basal cell resulting from the division of the zygote are cytologically as well as biochemically distinct from one another, and exhibit an amplification of the reorganization and polarity that characterized the egg following fertilization. The two cells exhibit a marked difference in their ultrastructure. The apical cell is generally more electron-dense than the basal cell, and probably contains more ribosomes per unit volume. Vacuoles are smaller and fewer in the apical cell but are larger in the basal cell in *Gossypium, Capsella , Linum, Quercus*, and *Petunia*. In cotton, the plastids are perinuclear in the apical cell but their distribution is casual in the basal cell. Plastids are almost completely absent in the zygote of *Quercus garnbelii*, but they make a marked appearance and gradually increase in number through the 6- to 12-celled stage. Although typically the zygote has plasmodesmatal connections with the synergids and the central cell, these connections are completely severed in the two-celled proembryo signalling, no doubt, a change in the nature of physiological interactions with the immediate milieu. However, plasmodesmata can be observed on the common wall separating the two cells of the proembryo. In cotton, the tube-containing ER present in the zygote gets sequestered into the basal cell rather than the terminal cell.

In Capsella, the basal cell divides transversely, and the daughter cells again undergo transverse divisions forming a long, filamentous suspensor. The cell nearest to the micropyle becomes large and vesicular, and is referred to as the basal cell of suspensor. This cell becomes highly vacuolate with a large, somewhat lobed nucleus. The apical cell, in the meanwhile, undergoes a vertical division and, after the quadrant and octant stages, produces a globular proembryo.

During early stages of development the daughter cells become successively smaller in size because the embryo, as a whole, either increases slowly in size, may not increase at all, or actually decreases in size. In the embryo of cotton, Pollock and Jensen have shown that cell-size in the embryo shows a progressive decrease until, at the 100-cell stage, average cell size is about one-twentieth that of the zygote; thereafter cell size remains constant until the 1,000-cell stage when there is a further reduction by one-half. This feature appears to be characteristic of other species such as *Capsella and Quercus*.

In a young proembryo the cells of the embryo proper contain few dictyosomes and scanty ER, but a large amount of polysomes. Mitochondria and plastids are common and the proembryo stains intensely for protein and nucleic acid. In *Capsella*, and in Diplotaxis, even when the octant cells form a protoderm, there is insignificant ultrastructural difference between the different cells of the embryo proper. However, a sharp contrast can be observed between cells of the embryo proper and those of' the suspensor.

Ultrastructural differences within the embryo become evident at the heart-shaped stage. The cells of the procambium and ground meristem are more vacuolate than those of protoderm, but

the density of ribosomes is more or less comparable in all the regions. Plastids of protoderm and ground meristem show differentiation of lamellae, while those of procambial cells remain relatively undifferentiated.

During further growth the hypocotyl and the cotyledons elongate; the apical meristem of the epicotyl is organized. The root apical meristem is also formed at the radicular end. In several taxa the plastids in the embryonal cells differentiate into chloroplasts so that the embryo becomes green.

7 Suspensor: Structure and Function

There is a great deal of variation in the organization of the suspensor. In some taxa as *Lycopsis, Penaea, Tilia,* and *Viola* there is no suspensor at all. A reduced suspensor characterizes *Bryonia. Euphorbia, Myosotis,* and *Ruta*, among others. Usually, in taxa in which the endosperm produces micropylar haustoria, the suspensor is not very prominent and is usually short-lived. A long filamentous suspensor is characteristic of the crucifers; *Pedicularis* of Scrophulariaceae also has a long suspensor of about 20 cells. In Crassulaceae, Fumariaceae, Podostemaceae, Rubiaceae, Trapaceae, and Tropaeolaceae the suspensor becomes massive and haustorial. The family Fabaceae exhibits a wide variation in the organization of the suspensor--from its virtual absence to very massive structures which may be filamentous, or clustered like a bunch of grapes. Orchidaceae are also characterized by a variable structure of the suspensor.

The growth rate of the suspensor is usually faster during earlier stages of embryogeny. In *Diplotaxis,* for instance, the suspensor already has its maximal cell number between four and seven when the proembryo is still an octant. The growth is very rapid between the globular and the heart-shaped stages of development. Thereafter, further increase in length ceases, and during later stages the suspensor begins to degenerate. The average length of the suspensor in this taxon is 200 gm, while that of the embryo is 40 gm. The faster rate of growth during early development may be accompanied by an increase in fresh weight as well. In *Phaseolus coccineus* the suspensor is heavier than the embryo for the first 6 days of development, but the rate of weight increase is slower in the suspensor. There is negligible increase in weight after the 11th day. After the 6th day the embryo weighs more than the suspensor. The suspensor in *P. coccineus* comprises nearly 200 cells which are all present by the 8th day. The maximum length attained by the suspensor is 1,500 gm while the mature embryo measures 2,200 um.

Ultrastructrally, the suspensor cells are usually filled with small vacuoles, are less electron-dense, and contain fewer ribosomes than the cells of the embryo groper. The ceil walls separating individual suspensor cells are traversed by numerous plasmodesmhta in *Capsella*, *Helianthus, Stellaria,* and *Diplotaxts*, but there is no protoplasmic continuity between the suspensor and the central cell. The suspensor cells, in several plants including *Phaseolus* and *Ipomoea purpurea,* exhibit an abundance of stacked agranular endoplasmic reticulum and free ribosomes, but in *Helianthus* and *Diplotaxis* the ER is rough.

A karyological phenomenon of frequent occurrence in suspensor cells is endopolyploidy,

mainly by endoreduplication.

A further feature of interest refers to the presence of cell-wall ingrowths in the suspensor cells of several species. A number of organelles, particularly ER, mitochondria and dictyosomes have been observed adjacent to the wall embayments lined by plasma membrane. The ultrastructure of this "wall-membrane apparatus" is characteristic of the so-called transfer cells which are supposed to be involved in short-distance translocation of metabolites by enhancing the surface-to-volume ratio of cells. Taken together, these features have been interpreted as indicative of a role in the uptake or exchange of nutrients necessary for the growth of the embryo.

During late stages of embryo development, the suspensor undergoes degeneration and is apparently resorbed by the embryo. Previously it was assumed that the suspensor "becomes crushed and obliterated", but it has recently been demonstrated in *Tropaeolum majus* that the death of suspensor cells is actuated by a basipetal wave of autolytic and autophagic process, and is characterized by the rupture of tonoplast and the disorganization of protoplast.

Currently, the suspensor is considered to function in two ways: (1) as an organ that absorbs nutrients from surrounding somatic tissues of the seed for onward transmission to the developing embryo, and (2) as a source of supply of important nutrients and growth regulators to the developing embryo.

It has been pointed out earlier that in several families of angiosperms the sus- pensor produces haustorial extensions that are supposedly involved in absorbing nutritive material from the endosperm, nucellus, integument(s), placenta, and other tissues. This idea appears quite attractive in the light of ultrastructural studies on the suspensor of several taxa such as *Capsella, Diplotaxis, Phaseolus, Pisum,* and *Stellaria*. The suspensor cells have highly convoluted labyrinthine wall projections characteristic of transfer cells involved in short-distance translocation. Further, plasmodesmata occur on the walls separating individual suspensor cells and, probably, between suspensor and the embryo proper.

Several investigators have implicated the suspensor in a secretory role. Satina and Rietsema observed that the basal cell of the proembryo in *Datura* had oil globules. Since oil was first detected in the basal cell and only later in other cells of the proembryo, the authors concluded that the suspensor acts as a source of oil to the developing embryo. Pritchard stated that the primary suspensor cell in *Steuaria* was responsible for the elaboration of proteinaceous material.

There is evidence to indicate that the suspensor contains several growth regulators. Corsi cultured intact and suspensor-deprived embryos of *Eruca* in vitro at three stages of development, and noted that the presence of suspensor is greatly beneficial to the growth of the embryo proper.

It is very clear from the above findings that there is overwhelming evidence to suggest that the suspensor acts as a stage-specific supplier of growth regulators to the embryo proper, during development.

In conclusion, the suspensor appears to play a very dynamic role not only in nourishing the

embryo proper at specific developmental stages, but also in exercising control on its proper growth by supplying several important phytohormones.

Chapter 9 The Endosperm

1 Introduction

Historically, the fate of the second male gamete involved in triple fusion was traced initially in Liliurn martagon and Fritillaria tenella. Following this discovery, double fertilization was reported in a large number of angiosperms. The fusion product of one sperm with the secondary nucleus results in the formation of the primary endosperm nucleus. The nuclei or cells derived through mitoses of this nucleus and daughter nuclei constitute the endosperm. The families which lack endosperm are: Orchidaceae, Podostemaceae, and Trapaceae.

The endosperm is essentially a triploid tissue, but can be diploid as in *Butomopsis* and even pentaploid as in Fritillaria. The division of the primary endosperm nucleus is followed by repeated divisions of the daughter nuclei. On the basis whether a wall is formed, or not, after the division of the primary endosperm nucleus, the endosperm is classified as of' the Cellular or Nuclear type. There is also the intermediary Helobial type.

The endosperm tissue is responsible for the growth and germination of the embryo. The classical role of supplying nutrition to the developing embryo needs a reassessment. Recent studies, however, indicate that the endosperm does not have a significant role in nourishing the proembryo during early embryogenesis when the endosperm is in an actively-dividing stage. In seeds the persistent endosperm acts as the repository of reserve food materials, and in cereals it constitutes the edible portion.

2 Nuclear Endosperm

In Nuclear type the primary endosperm nucleus and the daughter nuclei undergo free-nuclear divisions (Fig. 9.1A-E). These nuclei remain embedded in a cytoplasmic sheath around the central vacuole. The number of free-nuclear divisions, before wall formation, varies in different taxa. In the majority the endosperm, eventually, becomes cellular (Fig. 9.1F). In *Limnanthes* and *Oxyspora paniculata*, however, free-nuclear condition persists until the endosperm is almost completely consumed by the developing embryo. In *Scleria foliosa*, at the two-nucleate stage, a tubular extension is formed at the micropylar end of the embryo sac. It remains enucleate with scanty cytoplasm, and acts as a haustorium (Fig. 9.2A). Centripetal wall formation is restricted only to the upper region of endosperm (Fig. 9.2A). The chalazal portion remains coenocytic, and functions as a haustorium (Fig. 9.2 B, C).

2.1 Wall Formation

Wall formation is usually centripetal; it is initiated around the proembryo and gradually extends towards the chalazal region of the embryo sac. In most plants, wall formation once initiated continues until there are no more free nuclei. The cells divide until the maturity of the seed, and get filled with reserve food.

In a few families, *Cruciferea, Cucurbitaceae, Leguminosae,* and *Proteaceae,* wall formation

is no doubt initiated but does not proceed beyond the central region. The chalazal region, thus, remains free nuclear (Fig. 9.3). The free-nuclear zone not only shows much variation but also endopolyploid nuclei.

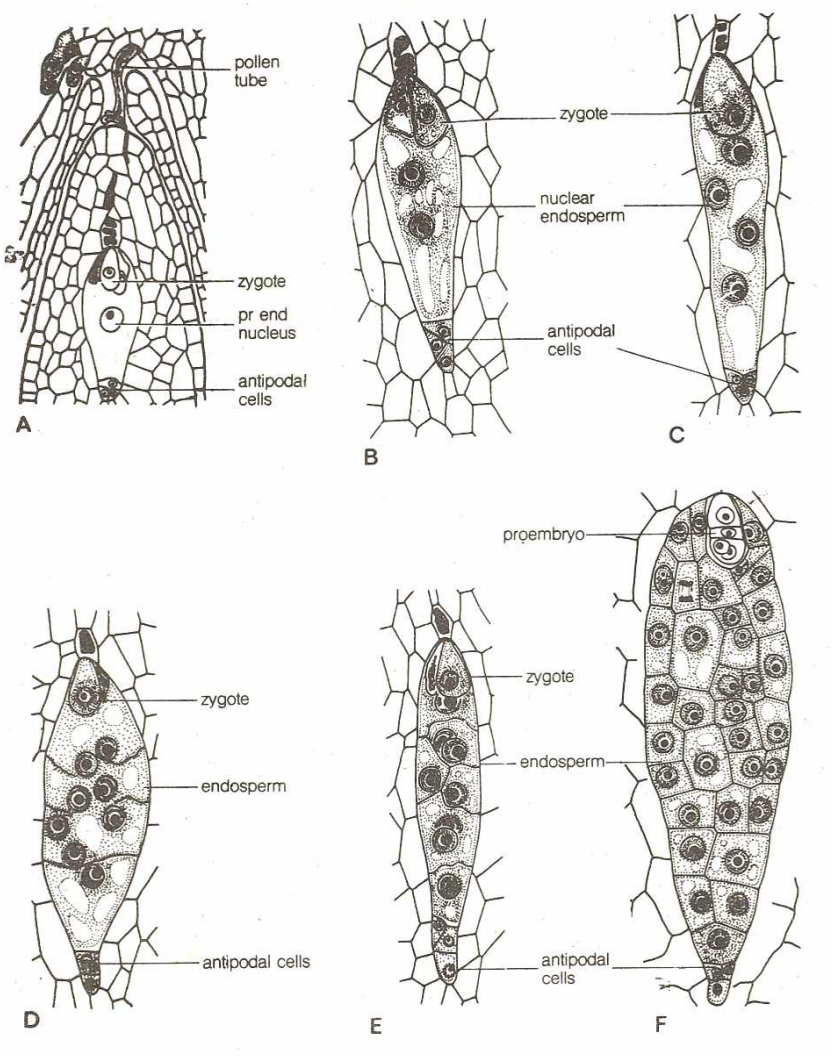

Fig. 9.1 *Diplacrum caricinum,* endosperm development. A Longisection of micropylar portion of ovule showing pollen tube, zygote, and primary endosperm nucleus. B, C Zygote, two- and four-nucleate endosperm. D Zygote and eight-nucleate endosperm; centripetal wall formation occurs al this stage. E, F Zygote (E) and proembryo (F) , and cellular endosperm. Note persistent degenerated megaspores in A-E, and antipodal cells in A-F.

2.2 Cucurbitaceae

Dissections and microtome sections have revealed many interesting types of chalazal haustoria in Cucurbitaceae (Fig. 9.3). The micropylar broader portion, which becomes cellular, is referred to as endosperm proper, thus demarcating it from the free-nuclear chalazal zone. Occasionally, the peripheral cells in the lower region of endosperm proper bulge out during

later stages of the development of seed. *In Luffa aegyptica* and *Melothria maderaspatana* such bulges are usually referred to as "secondary" haustoria.

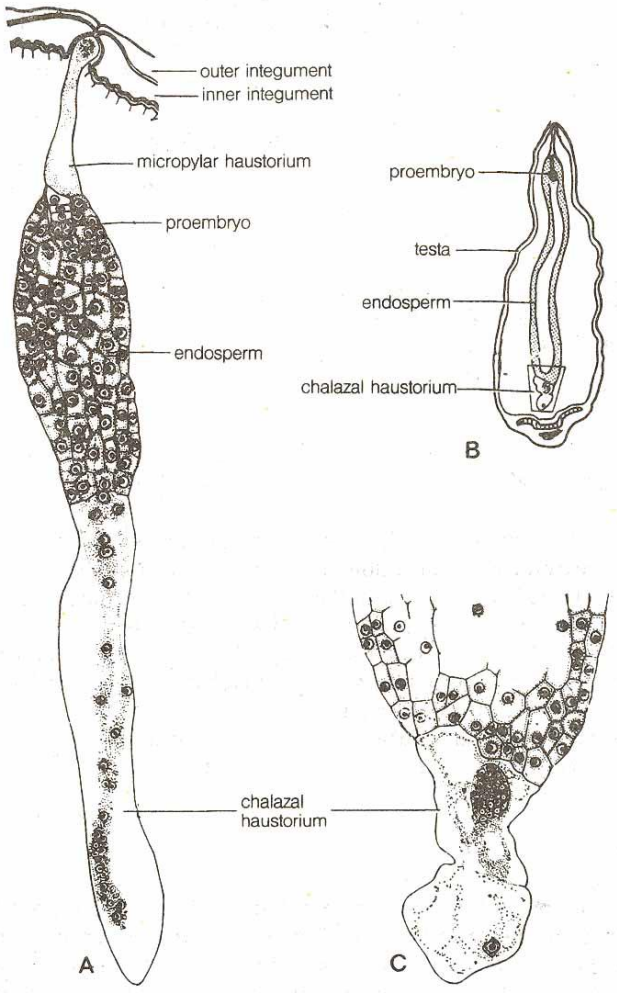

Fig. 9.2 *Scleriafoliosa.* A Endosperm with micropylar and chalazal haustoria. B Longisection of seed showing a central cavity in the endosperm. C Portion of chalazal haustorium from B.

The lower coenocytic portion elongates gradually and forms a tubular process. This region contains dense cytoplasm with numerous nuclei of various sizes, shapes, and ploidy levels. The tip of the haustorium in *Cucurbita pepo* and *Cucumis sativus* becomes swollen. The development of the endosperm, with particular reference to the structure of the haustorium, has been studied in *Benincasa cerifera, Cucumis melo, Luffa aegyptica,* and *Melothria heterophylla.* In *Cucumis* the length of the haustorium varies from 1,170 to 3,006 pm; in *Benincasa* 270 to 1,170 um; and in *Luffa* 360 to 990 um. *Melothria* shows only a rudimentary haustorium which is represented by a short protuberance consisting of small cells. There is a correlation between the length of seed, length of haustorium, and haustorial activity (Fig. 9.3).

The haustorium presumably transports metabolites from the circumjacent tissue to the developing embryo. Occasionally, in some taxa, the coenocytic haustorium cuts off multinucleate chambers which later segment into uninucleate cells. This aspect requires further study, both at the light microscopy and the ultrastructural level.

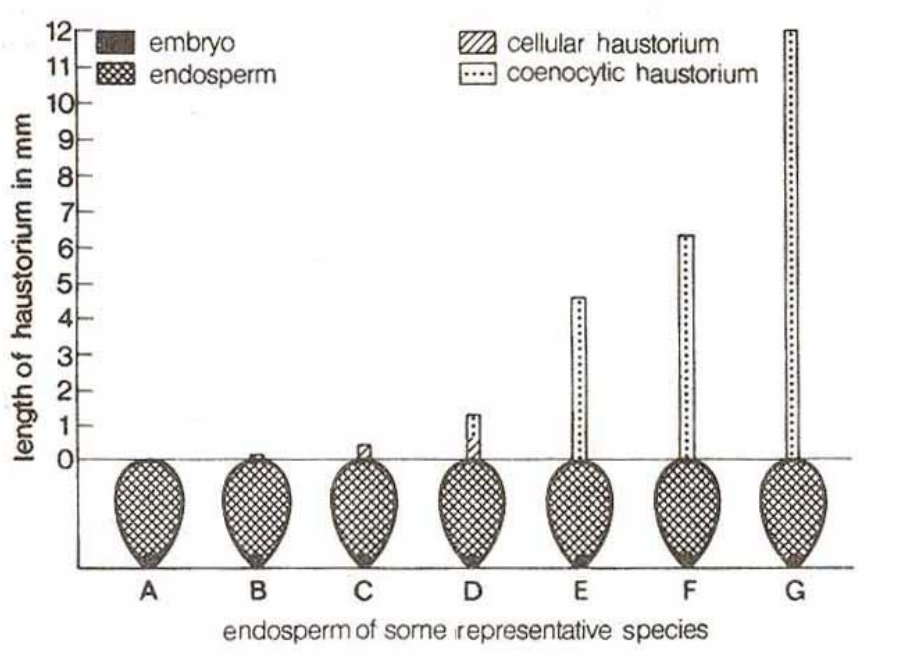

Fig. 9.3 Cucurbitaceae; endosperm at globular proembryo to show cellular portion and coenocytic haustorium. A. *Blaslania garcini* B *Melothira heterophylla,* C *Citrulus fistulosus,* D *Benincasa cerifera,* E *Melothira maderaspalana.* F *Trichosanthes anguina.* G *Cucurbita ficifolia.*

2.3 Leguminosae

In many genera of the Phaseolae, after the free-nuclear phase, wall formation usually occurs in the vicinity of the embryo. The lower half of the endosperm remains free-nuclear, assumes vesicular contour, contains hypertrophied nuclei, and functions as a haustorium. The shape and size of the coenocytic haustorium varies in many taxa, and is clearly demarcated from the cellular region. In *Cassia* it is bulbous, in *Mimosa pudica* constricted, and in *Calliandra* coiled. *Cyamopsis* and *Desmodium* are exceptions, and cellularization occurs in the coenocytic portion.

2.4 Palmae

The correlation between the size of fruit and activity of endosperm is exemplified in *Cocos nucifera.* In 50 mm long fruits the embryo sac is still filled with liquid endosperm which contains many free nuclei and cytoplasm, usually referred to as "liquid syncytium". The endosperm nuclei, at this stage, are active and undergo mitotic divisions. When the fruit reaches 100 mm, many endosperm vesicles appear in the liquid syncytium. Such vesicles arise due to coalescence of cytoplasmic material that surrounds the free nuclei, are 10-30 um in

diameter, and confain as many as 40 nuclei. Occasionally, a single nucleus enlarges without under- going any division, and fills the entire vesicle. These vesicles often coexist with enucleate endosperm nodules. Several interesting features are reported in about 150 mm long fruits. The Vesicles and nuclei migrate to the chalazal end and, finally, the vesicles disintegrate. The contents of vesicles mix with the existing free endosperm nuclei, and form a gelatinous syncytium. The nuclei in the syncytium elongate, and show amoeboid movement: Mitotic activity (in these nuclei) is rapid, and followed by wall formation. The cellular endosperm is referred to as the coconut meat which never fills the entire cavity, probably owing to the large size of the embryo sac.

The coconut milk, which is the liquid endosperm, contains growth hormones, protein granules, oil droplets, and many nuclei. Its use in tissue culture experiments dealing with growth and morphogenesis is well documented, and it also induces cell division in embryogenic pollen grains. Carrots and coconuts are made for each other, and their interdependence in experimental embryology is well known. The activity of the coconut milk at biochemical level, however, still remains an enigma.

Dissections of endosperm in *Grevillea robusta* revealed that the chalazal region remains free-nuclear, shows streaming of cytoplasm, becomes coiled, and is aptly designated "vermiform appendage". This appendage invades the chalazal region of the ovule and functions as an aggressive haustorium. In *Lomatia polymorpha*, in addition to the main haustorium, numerous single-celled, finger-like outgrowths arise all over the surface of the endosperm and, thus, increase the absorbtive surface.

3 Cellular Endosperm

In the Cellular type the initial and subsequent nuclear divisions are followed by cell-plate formation. During earlier development there is considerable variation in the method of cytokinesis in various families. Also, one or more cells at the micropylar, chalazal, or both ends become specialized to form a haustorium. The haustorial function is circumstantial, and presumed to be due to the hypertrophied nature of the nucleus and dense-staining of the cytoplasm.

4 Helobial Endosperm

The Helobial type of endosperm is characterized by the division of the primary endosperm nucleus, giving rise to two unequal chambers - the micropylar chamber usually larger than the chalazal. Free-nuclear divisions occur in the micropylar chamber before it undergoes cellularization. By contrast, the nucleus of the chalazal chamber either remains undivided, or divides once or twice. The chalazal chamber usually remains coenocytic but, in a few cases, it gets partitioned into cells.

4.1 Salient Features in Monocotyledons

The significant characteristics of Helobial endosperm in the monocots are: 1. The primary endosperm nucleus is situated in the chalazal region of the embryo sac adjacent to the

antipodal cells. 2. The division of the primary endosperm nucleus is followed by a wall which separates a large micropylar and a small chalazal chamber. The cytoplasm of the chalazal Chamber stains deeply. 3. Before undergoing division, the nucleus of the micropylar chamber always migrates to the upper part of the embryo sac. 4. The first two divisions in the micropylar chamber are flee-nuclear; cell-wall formation, if any, occurs during later stages. 5. The chalazal endosperm chamber, also called the "basal apparatus", has a haustorial function. The division of the nucleus in this chamber, if it occurs, is always free nuclear. If wall formation occurs, it is significantly belated as compared to the micropylar chamber. 6, The endosperm cells in the chalazal chamber lag behind in the rate of nuclear division as well as in number, as compared to the micropylar chamber.

5 Ruminate Endosperm

The ruminate endosperm in the mature seeds of angiosperms has attracted much attention. The word ruminate is derived from the Latin "ruminatus" meaning chewed. The studies indicate that there is no chewing up of the endosperm tissue, but the ruminate condition results owing to an irregular growth activity of the seed coat as well as the endosperm. The endosperm surface, thus, becomes uneven. The rumination is absent in the perichalazal region.

Seeds which reveal a network of lobes in sections in any plane are termed "lab- yrinth seeds". This structure may be either due to rumination of the endosperm, or lobing and folding of the cotyledons. In seeds thus, when lobing and folding of cotyledons occur together with rumination, the labyrinth structure is very prominent, In *Kingiodendron sp., Ervcihe griffithii* and *E. tomentosa* the seeds show excessive folding of cotyledons, followed by intrusion of the testa in between the folds and lobes, causing the labyrinth condition. In both taxa the mature seeds lack endosperm.

Ruminate endosperm could be an ancestral character still occurring in present-day seeds, belonging to both advanced and primitive taxa.

6 Central Cell

The central cell is the largest cell in the embryo sac, generally with two haploid nuclei but variations in the number of nuclei are well known. Morphologically, both the polar nuclei are similar: occasionally, the upper (micropylar) may be larger than the lower (chalazal). The polar nuclei are usually completely fused at the time of fertilization although, in some cases, they are partially fused. Serial sectioning of these nuclei in Petunia revealed that they are connected by several narrow bridges and that, in a number of other places, only their outer membranes are continuous. Both the nuclei evaginate and make contact at several points along their adjoining surfaces. This is followed by fusion of the outer nuclear membrane and, ultimately, the inner nuclear membrane. This is also true of cotton and *Capsella bursa-pastoris*. The formation of fusion nucleus is accompanied by fusion of the two nucleoli.

7 Wall Formation in Endosperm

In the Nuclear type of endosperm there is no switching back and forth of karyokinesis and cytokinesis; the changeover to wall formation occurs once. In the Cellular type, karyokinesis is always coupled with cytokinesis. In the Helobial type, karyokinesis and cytokinesis are coupled in the first division. Subsequently, uncoupling results in the formation of coenocytic cells that later become cellular as a result of recoupling of karyokinesis and cytokinesis. Irrespective of the type of endosperm development, the process of wall formation is common in the coenocytic endosperm of either the Nuclear or the Helobial type. What factors regulate the control of cellularization in Nuclear and Helobial endosperm is not clear. The advantage of the endosperm becoming compartmentalized is interpreted as one way of storing carbohydrates in an insoluble form, which might be used later during digestion of the endosperm.

In *Helianthus annuus* wall formation in the endosperm starts at the micropylar end and extends towards the chalazal end of the embryo sac. The wall formation is not associated with the mitotic spindle apparatus, and the walls grow freely as the wall material is deposited by the coalescence of vesicles arising from golgi near the growing tip of the wall. By contrast, the usual mode of wall formation is by cell plate, as reported in *Dianthus chinensis*. The presence of a large number of dictyosomes and their smooth vesicles in close proximity to the free ends of wall supports the growth of walls. In fertilized cotton embryo sacs, wall formation in endosperm occurs approximately 10 days after pollination, whereas in cultured unfertilized embryo sacs wall formation in endosperm occurs on the third day of culture after dozens of free nuclei have been formed. The pattern of growth of walls in fertilized and unfertilized endosperm is comparable. The walls are initiated at the embryo sac wall, and grow towards the large central vacuole of the embryo sac, by apparent coalescence of dictyosome vesicles and, possibly, involvement of RER.

8 Embryo-Endosperm Relationships

The endosperm has been classically assigned the function of nourishing the embryo. The intense metabolic activity of young endosperm may partially reflect the production of hormones and other substances which influence the early growth and morphogenesis of *Capsella* embryos in vitro. In *Capsella bursa-pastoris* the endosperm develops up to the heart-shaped stage of the embryo, and probably does not contribute significantly to its nutrition. During early embryogeny in *Helianthus annuus* the endosperm seems to be of limited nutritional value. The persistent synergid has cytoplasm similar to that present before fertilization, has complete cell wall, and shows increased lipid contents, and starch in plastids. Thus the persistent synergid is functional and contributes significantly to the growth and development of the embryo. The endosperm which is still developing, and has not reached a certain size, cannot provide nutrition to the embryo. Thus, the young endosperm, during early stages of development of the embryo, needs adequate nutrients for its own growth. During late embryogeny, however, the endosperm does have a large pool of reserve materials which is utilized for later development of the embryo. It is reasonable to postulate that the embryo is

not wholly dependent on the endosperm for its nutrition, but gradually consumes it. The high osmotic environment may be essential for the normal development of the embryo, and may also prevent it from precocious germination. The osmotic gradient in the endosperm tissue is, to some extent, determined by the concentration gradient of amino acids. Of the 23 free amino acids identified in the endosperm tissue, 12 or 13 are characterized by high concentration in the chalazal portion of the endosperm rather than in the micropylar portion, during different stages of development of the ovule. Ryczkowski further states that the differentiation of embryo is influenced not only by the concentration gradient of low molecular compounds, but also by proper osmotic value of these compounds.

Jensen proposed a hypothesis to explain shrinkage of zygote based on a presumed osmotic gradient by the initial rapid growth of the endosperm that would cause water to move from the vacuole of the zygote to the endosperm. Thus, proper osmo-regulation by endosperm is one of the important functions during early embryo- genesis.

Chapter 10 Polyembryony

1 Introduction

Angiosperms show the unique phenomenon of double fertilization. The egg, after fertilization, develops into an embryo. It is nourished in the younger stages by the suspensor, and in later stages by the endosperm - a post-fertilization tissue resulting from the fertilization of polar nuclei. Besides the egg, other constituent cells of the embryo sac、 with or without fertilization, produce embryos. Moreover, the activity of one or more cells of the ovule, outside the embryo sac, also leads to the development of accessory embryos. Such embryos are asexual. Thus, in addition to the zygotic embryo, sexual and/or asexual non-zygotic embryo(s) may also develop in the same seed. Such a condition is referred to as "polyembryony".

2 Classification

Polyembryony can be broadly classified into "simple" and "multiple" depending upon the presence of one or more embryo sacs in the same ovule.

2.1 Simple Polyembryony

By its nature, that is whether it develops with or without fertilization, simple polyembryony can be sexual or asexual. The examples of sexual polyembryony are the embryos originating from the egg cell and synergid; supernumerary edilrryos also arise from"the proembryo and suspensor. Asexual embryos develop rithin the embryo sac without fertilization. If the embryos develop from the nucellar and integumentary cells without the interpolation of the gametophytic phase, the polyembryony is said to be "adventive" or "sporophytic".

The failure of meiosis in megaspore mother cell, or the development of embryo sac in sporophytic tissue (nucellar or integumentary), result in asexual diploid gametophyte; then the egg (and synergid) can produce diploid embryos. Haploid embryos develop parthenogenetically from the synergid, or egg, of a reduced female gametophyte.

2.2 Multiple Polyembryony

In multiple polyembryony accessory embryos are produced from two or· more embryo sacs in the same ovule. The development of embryo in each embryo sac conforms to the method described for simple polyembryony. Multiple gametophytes in an ovule may develop if, during ontogeny, two or more ovules (one embryo sac in each ovule), due to spatial demand, before or after the differentiation of integuments, fuse and function as a single ovule; fusion or division of two or more nucelli with common integuments, and one embryo sac in each nucellus; multiple archesporium leading to the formation of several embryo sacs. In seaisland cotton (*Gossypium barbadense*), Harland (1936) described the development of a haploid-diploid twin which resulted from multiple embryo sacs in an ovule. The fertilization of egg cell in one embryo sac induced the parthogenetic development of the egg cell in the

other embryo sac.

In multiple embryo sacs within an ovule, whereas the egg cell (diploid) of the unreduced embryo sac may develop parthenogenetically, the egg cell (haploid) of the reduced embryo sac may develop after fertilization forming a diploid-diploid twin. If the unreduced egg is fertilized and the reduced egg develops parthenogenetically, the twin would be triploid-haploid.

Asker (1979) refers to four possible modes of reproduction in facultative apomicts: (1) the unreduced egg cells, by parthenogenesis, produce uniform maternal offsprings; (2) the unreduced egg cells, by fertilization, give rise to autotriploids (U hybrids); (3) the reduced egg cells, by parthenogenesis, give rise to (poly) haploids, and (4) the reduced egg cells, through fertilization, produce variable sexual offspring (R hybrids).

3 Nucellar Polyembryony

Nucellar (adventive) polyembryony is reported in 16 out of 172 crassinucellate families of angiosperms (Rangaswamy 1981). The embryos develop from the nucellar cells with and without the interpolation of the gametophytic phase.

The nucellar cells destined to develop into embryos contain dense cytoplasm and starch. They divide actively, develop into small embryonic masses, and push their way into the embryo sac. Some of the embryonic masses take the lead, grow further, and differentiate into embryos. Those towards the micropylar region are better organized than those situated farther down laterally. These embryos exhibit various stages of development, as well as irregular organization. They usually lack a suspensor. Nevertheless, a well-defined suspensor has been observed in Citrus microcarpa. The cotyledons are mostly unequal. Some embryos may show tricotyly, and fasciation at the radicular end, so that the seedlings have multiple shoots but a single root.

The origin of the embryo may be autonomous, sporadic, induced, or stimulated. When the embryos develop autonomously, the entry of the pollen tube, or stimulation due to degenerated aposporous megagametophytes, accelerates the induction and development of nucellar embryos as in Ochna serrulata. Plural embryos can be induced in the nucellar cells by distant hybridization, application of physical or chemical stimuli, or pollination of flowers with abortive or foreign pollen. Stimulation by pollination in Epilobium hirsutum with the pollen of E. dodonei, E. montanum, and E. angustifolium induces nucellar embryos. This requires reinvestigation.

The successful development of nucellar embryos depends upon (1) fertilization of egg, and formation of endosperm in the sexual embryo sac, (2) development of normal endosperm but without the fertilization of egg, and (3) rarely, even in the absence of fertilization of the polars or the egg. There is considerable information en nucellar einbryos in fruit trees like *Citrus, Eugenia, Mangifera*, and some others.

4 Integumentary Polyembryony

The development of embryos from the integumentary cells is not dependent on the fertilization of the egg cell, and there is no interpolation of the gametophytic phase. In the asexual individuals of *Spiranthes cernua* the inner integument exhibits an unusual phenomenon. The terminal cell, as well as the lower adjoining cells, enlarge and become richly cytoplasmic (Fig. 10.1A-C). By the time the sexual embryo sac shows four degenerated nuclei (Fig. 10.1D), the richly cytoplasmic integumentary cells divide and produce two to six proembryos in the micropylar region of the ovule (Fig. 10.1 E, F). Such embryos remain arrested and do not grow very much, nor do they differentiate.

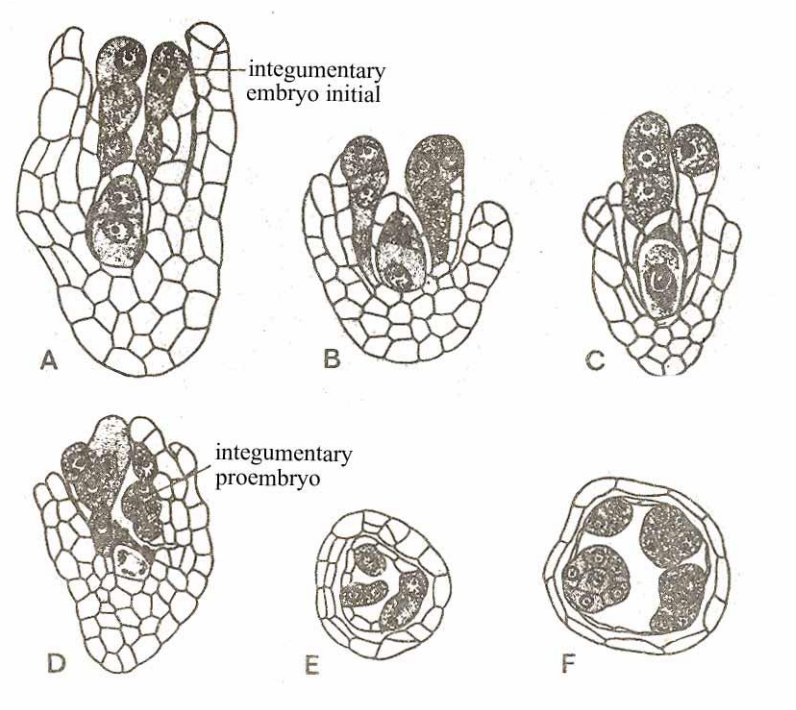

Fig. 10.1 *Spiranthes cernua.* A-D Various stages of adventive integumentary proembryos in micropylar region of a bitegmic ovule; longisections. A Dyad. B T-shaped tetrad. C Functional megaspore D Degenerated nucellus, and degenerated four-nucleate embryo sac. E, F Transections of ovules, in micropylar region, to show adventive proembryos.

When the innermost layer of the integument becomes distinct by its morphological and cytological features, it is referred to as "endothelium", and is a common feature in unitegmic, tenuinucellate ovules. In *Melampodium divaricatum*, in a number of ovules the cells of the innermost layer of the integliment become richly cytoplasmic, and undergo repeated divisions. Thus, one finds several developmental stages of the proembryo; some may simulate the earlier stages of a zygotic embryo. None of the proembryos develop far enough to differentiate. In *Carthamus tinctorius* supernumerary embryos develop from the endothelial cells during post-fertilization stages. They also do not show any differentiation, and do not reach maturity.

In *Vincetoxicum nigrum*, the development of additional embryos due to the cleavage of the

group of embryonic cells formed by the zygote. In *V. officinale* the supernumerary embryos develop from the inner cells of the integument. This may also be true of *V. nigrum*, and Guignard may not have carefully followed the ontogeny of the adventive embryo. A reinvestigation may prove rewarding.

It is questionable whether such multicelled and globular structures should at all be considered as proembryos? So far, not a single instance of a polyembryonic seed due to differentiated integumentary or endothelial embryos are known. It would be interesting to induce, in vitro, embryos from integumentary cells.

5 Zygotic and Suspensor Polyembryony

5.1 Zygotic Polyembryony

In *Erythronium americanum* the development of supernumerary embryos results from the cleavage of the apical cells of the embryogenic mass of cells produced by the zygote. A similar condition occurs in *Tulipa gesneriana*, *E. dens-canis, Cocos nucifera, Primula auricula*, and *Corydalis cava.*

In *Cymbidium bicolor* the zygote divides vertically or obliquely, the daughter cells get somewhat separated, and divide further to form two independent proembryos. As is common in the Orchidaceae, the proembryos develop only up to the globular stage in mature seed. The frequency of polyembryony is 2%, and the monozygotic twin is unattached.

Like *Erythronium,* in *Eulophia epidendraea, Habenaria platyphylla,* and *Godorum densiflorum* also the zygote produces an irregular mass of cells, and some cells at the apical end develop simultaneously into multiple proembryos (Fig. 10.2A). The additional embryo may also arise due to budding of the proembryo (Fig. 10.2B).

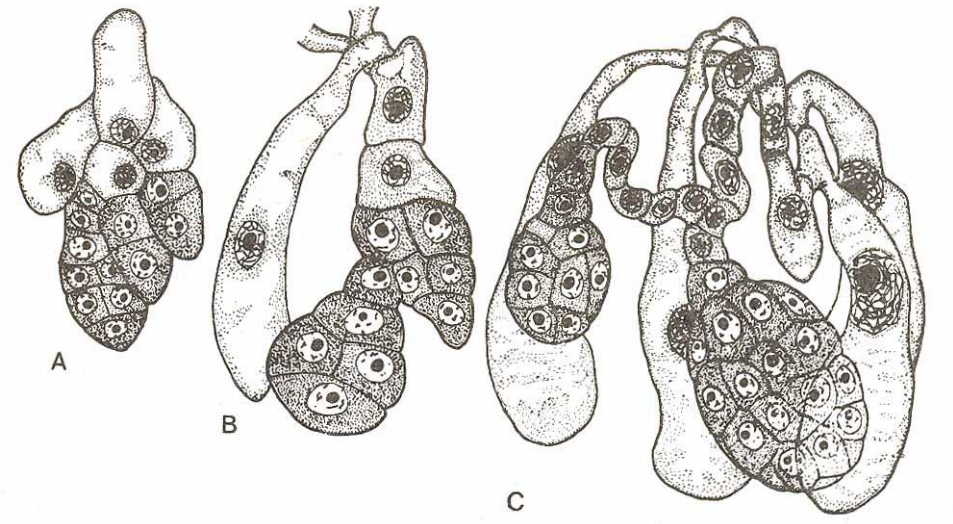

Fig. 10.2 Zygotic and suspensor polyembryony in *Eulophia epidendraea* (whole mounts). A The zygote has produced a group of cells, and three proembryos have developed therefrom. B Budding of an embryo from globular proembryo. C Twin proembryos presumed to have arisen by splitting of a single proembryo. Note the haustorial suspensor cells in A-C.

In Eulophia the filamentous proemtryo becomes branched and an embryo may be formed at the tip of each branch" (Fig. 10.2 C). In one case as many as three proembryos developed.

Due to incomplete cleavage of the zygotic proembryo, in *Stachyurus chinensis*, two proembryos develop side by side. *Sedum spectabile* is an unusual instance, where the additional embryo is produced by cleavage of the apical cells of the three-celled proembryo. This should be reinvestigated.

Of the 49,903 maize seeds examined, observed 32 pairs of twin embryos. The authors state that this condition was either due to cleavage of the proembryo, or a result of the fertilization of two reduced female cells in the embryo sac by two sperms. Besides these diploid-diploid twins, 15 were haploid-haploid, and 2 haploid-diploid.

5.2 Suspensor Polyembryony

The cells of the uniseriate suspensor develop buds in *Zygophyllum fabago*. Suspensor polyembryony is of common occurrence in Acanthaceae: *Dipteracanthus patulus, Acanthus ilicifolius,* and *Sericocalyx scaber.*

The plural embryos, which develop from the proembryonal mass, or a suspensor cell, have the diploid number of chromosomes.

6 Synergid Polyembryony

The embryos from the synergids develop frequently, after fertilization. When the synergid and egg cell are fertilized by two sperm cells of the same pollen tube, there is no possibility for the secondary nucleus to be fertilized. The embryos collapse in the absence of endosperm. When more than one pollen tube is involved in fertilization, the development of the embryos from the egg and synergid is accompanied by well-organized endosperm. The synergid and egg cell are both fertilized in *Najas majvr*, the embryos develop equally, and resemble each other. Such diploid gametophytic polyembryony has been observed in several other members. Entry of additional pollen tubes has been observed in *Cuscuta rflexa*, *Tamarix ericoides,* and *Pennisetum squamutatum*. In the latter two taxa the diploid synergid embryo develops along with the zygotic embryo.

In *Argemone mexicana*, the synergids are ephemeral but, sometimes, one synergid persists after fertilization. It is smaller than the zygotic proembryo; both have independent suspensors, and the proembryos lie juxtaposed. Sachar confirmed the twin comprises a diploid zygotic and a haploid synergid proembryo. The zygotic embryo alone develops further so that the seed is monoembryonate.

In *Dioscorea composite* the zygotic and synergid embryos show comparable development until a late stage, they follow a uniform pattern of development up to organogenesis,in Nechhamandra alternifolia.

The development of haploid embryo from the synergid, along with the diploid zygotic embryo, is reported in several taxa: *Lilium martagon, Arabis lyallii, Erythraea centaurium,* and *Aristolochia bracteata*. The suspensor of the zygotic as well as of the synergid embryo of *Arabis lyallii* elongates into similar filamentous structures with a small embryonal mass at the

apical end. Both the embryos have a reduced suspensor in *Erythraea centaurium,* and develop up to the heart-shaped stage. The zygotic and the synergid embryos are simultaneously nourished by the endosperm.

In *Pennisetum squamulatum* the diploid synergid embryo develops along with the zygotic embryo. The suspensor of the zygotic embryo is typically multicellular as in the Gramineae, and that of the synergid is partly uniseriate and partly biseriate.

At times, one or both the synergids develop into haploid embryo(s) as in *Azadirachta indica*. In *Carthamus tinctorius* triplets develop due to fertilization of the egg and both the synergids.

It is difficult to explain the mechanism of stimulus that induces the unfertilized synergid to develop into an embryo. Such embryo complexes occur frequently either in distant crosses, or as a result of a special pretreatment with pollen (e.g. X-ray treatment), or exposing the ovaries, just after pollination, to varying temperature. To begin with the zygotic and synergid proembryos are morphologically similar. However, the haploid proembryo remains arrested since the diploid pro embryo (perhaps being more compatible) utilizes most of the ‘'endosperm, starving the weaker one.

6.1 Twins and Triplets

The multiple embryos inside a seed can be of varied origin and with different ploidy. But the frequency of polyembryony is extremely low and, therefore, it becomes difficult to assess the origin of additional embryos.

6.1.1 Twins

1. Haploid-Haploid: In nature the occurrence of haploid-haploid twins is rare. The twin may develop from an unfertilized synergid and an unfertilized egg cell (of the same embryo sac) as in *Orchis maculata*. Haploid twins are also reported in *Gossypium barbadense* and *Sesbania aculeata*; it was not ascertained whether these twins originated from the parthenogenetic development of the egg and synergid, or both synergids. A reinvestigation is desirable.

2. Haploid-Diploid: The haploid-diploid twins are of common occurrence. The haploid embryo develops from an unfertilized synergid, while the diploid originates from the fertilized egg: *Plantago lanceolata, Linum usitatissimum, Solanum tuberosum* and *Phleum prdtense, Orchis maculatus. Listera ovata,* and *Platanthera chlorantha.*

3. Haploid-Triploid: In *Phlellm* the unfertilized synergid gives rise to the haploid embryo while the triploid embryo arises due to the fertilization of a diploid (unreduced) egg of aposporous embryo sac, or fertilization of the haploid egg cell by two haploid male gametes,or one diploid (unreduced) male gamete.

4. Diploid-Diploid: Conjoined and unattached monozygotic twin seedlings, developing from the cleavage of the proembryo, are reported in *Asparagus officinalis*.

The diploid-diploid twin can also arise by fertilization of a synergid and egg cell as in *Geum rivale*. If the two male gametes from the same pollen tube fertilize the haploid egg and haploid synergid, there is a possibility of the formation of identical twins as in some orchids (Hagerup 1947). Diploid twins are also reported in *Nicotiana tabacum* and *Medicago sativa.*

Two more pathways are likely for the development of diploid-diploid twins. There is a

possibility of the formation of more than two male gametes in the same pollen tube, so that the supernumerary male gametes can fertilize the egg, synergid(s), as well as effect triple fusion, producing a diploid twin or triplet and normal endosperm.

Secondly, a diploid-diploid twin can also result due to the endoduplication of chromosomes of a haploid synergid, and fertilization of the haploid egg.

5. Diploid-Triploid: Such twins are reported in *Triticum*. The diploid embryo develops from the zygote, while the triploid embryo is of endospermic origin. However, the following possibilities are worthy of consideration: (1) In an unreduced embryo sac the diploid synergid may develop parthenogenetically, while the diploid egg is fertilized by one haploid sperm. (2) In a reduced embryo sac, the haploid synergid is fertilized by a haploid male gamete, and the haploid egg by two haploid male gametes.

6.1.2 Triplets

In *Sagittaria graminea* double fertilization is a normal feature, and one of the synergids collapses during the process. In a few cases the synergids simulate the egg cell with the vacuole in the upper part and nucleus in the basal part, and two pollen tubes enter the embryo sac. One embryo sac showed three twocelled proembryos, and remnants of two pollen tubes at the micropylar end. The author concludes that the two additional proembryos developed from the two synergids, both fertilized by the male gametes from the second pollen tube. Thus, there is a diploid-diploid-diploid triplet.

A diploid-triploid-diploid triplet can arise by the fertilization of an unreduced diploid egg by a haploid male gamete, and parthenogenetic development of the diploid synergids.

Chapter 11 Gametophytic Apomixis

1 Introduction

"Apomixis" in angiosperms means asexual (agamic) reproduction by see, i.e., "agamospermy" ("seed apomixis"). The normal counterpart of this widespread anomaly of reproduction is "amphimixis", i.e., sexual reproduction. Orginally, Winkler (1908) defined apomixis in a wider sense, including vivipary, pseudovivipary, and other forms of vegetative propagation such as bulbils, runners, rhizomes, etc. This broader definition is now rather rarely used.

Apomixis leads to maternal (metromorphous, incorrectly: matromorphous) offspring which normally are genetically exact copies of the mother plant.

Agamospermy covers two fundamentally different modes of reproduction:

1. "Adventitious embryony" in which the embryo, i.e., the new sporophyte, arises directly from a somatic cell of the ovule, usually from the nucellus ("nucellar embryony"). Only the endosperm is produced in the embryo sac. This purely sporophytic form of agamospermy often causes polyembryony, and is dealt within Chapter 10 "Polyembryony".

2. "Gametophytic apomixis" in which the embryo sac, i.e., the female gametophyte, arises from an unreduced embryo sac initial. A distinction is made between: a) "diplospory" in which the unreduced embryo sac originates from a generative cell (female archesporial cell, megaspore mother cell), either directly by mitosis (Antennaria type), or indirectly by modified meiosis, resulting in an unreduced restitution nucleus (Taraxacum type, Ixeris type), and b) "apospory" in'which unreduced embryo sacs originate from somatic cells of the ovule (usually from the nucellus). "Apomeiosis" is a concept which covers both diplospory and apospory.

Diplospory and apospory circumvent meiosis, but just as important are the mechanisms that prevent fertilization, in other words the capability of the unreduced egg cells to develop asexually by parthenogenesis. These two main components·of gametophytic apomixis - diplosporyjapospory (apomeiosis), and parthenogenesis - allow the maintainance of the alternation of generations, but without an alternation of nuclear phases. Therefore, gametophyte and sporophyte are both of the same level of ploidy.

A third factor in apomixis influences the formation of endosperm: the relation of polar nuclei to fertilization. There are two possibilities:

1. The polar nuclei must be fertilized so that only the egg cell is capable of parthenogenetic development; the endosperm formation is pseudogamous (unreduced "pseudogamy").

2. Both egg cell and polar nuclei are independent of fertilization; both embryo and endosperm formation are parthenogenetic; i.e., autonomous ("autonomous apomixis" = "diploid, unreduced parthenogenesis").

Maternal offspring are, to summarize, the result of a cooperation of different, independent processes which can be conceived as "basic components of gametophytic apomixis". The two

most important of these components are diplosporousjaposporous formation of embryo sac, and parthenogenetic formation of embryo. If 100% of the offspring were maternal, then "obligate apomixis" would be the case. However, most apomicts retain traces of sexual reproduction in that one or both of these basic components are not always completely effective so that, rarely, aberrant offspring occur; in extreme cases so rarely that,t hey are hardly detectable. These "facultative apomicts" can produce, besides maternal offspring, aberrants in three different ways:

1. Fertilization of unreduced egg cells results in "B_{III}hybrids" = V-hybrids, usually formed as "2n+n hybrids" and, rarely, as "2n + 2n hybrids".

2. Fertilization of reduced egg cells, i.e., normal, sexual reproduction, results in "B_{II} hybrids" = R-hybrids, formed as "n+n hybrids" or, exceptionally, as "n + 2n hybrids".

3. Parthenogenetic development of reduced egg cells ("haploid parthenogenesis" or "haploid pseudogamy") results in "polyhaploids" (n + 0), e.g., in dihaploids from tetraploid mother plants, or in (mono) haploids from diploid mother plants. "Haploid parthenogenesis" is the most important case of "nonrecurrent apomixis", counterpart of "recurrent apomixis" = "stable apomixis" which includes "diploid parthenogenesis" and results in maternal offspring (2n +0). Another instance is "androgenesis".

2 Embryology of Gametophytic Apomicts

2.1 Development of Unreduced Embryo Sacs

The different types of unreduced embryo sacs are defined on the basis of their origin: diplosporous embryo sacs originate from generative cells (female archesporium, megaspore mother cells), aposporous ones from somatic cells. If the origin of unreduced embryo sacs cannot be established, apomeiosis in its original definition should be used as a covering term.

2.1.1 Diplospory (Earlier Synonym: "Generative Apospory")

The simplest and most frequent means of development of unreduced embryo sacs from generative cells in *Antennaria alpina* and is, therefore, called "Antennaria type".

Antennaria Type (=Mitotic Diplospory). The megaspore mother cell does not enter into meiosis but after a long interphase, growth, and pronounced vacuolation, characteristic of functional megaspores, proceeds directly to the first mitosis. The female meiosis is omitted, and the megaspore mother cell functions as an unreduced ("diploid") megaspore. Two further mitoses lead to the mature, eight-nucleate embryo sac. The Antennaria type is widely distributed, and some of the important genera comprising apomictic species are: *Antennaria* (not all the apomictic species), *Burmannia coelestis*, *Erigeron and Eupatorium*, *Hieracium, Poa alpina and P. nervosa, Parthenium, Calamagrostis, Zephyranthes, Cooperia, Nardus stricta.* In *Elatostema eurhynchum* a parietal cell is formed as in sexual relatives.

Taraxacum Type. In this type, discovered in *Taraxacum,* the megaspore mother cell enters into the meiotic prophase but, because of asynapsis, there is mostly no pairing; the univa1ents remain scattered over the whole spindle of metaphase I and, therefore, are not shared between the poles. The first meiotic "division" results in a meiotic restitution nucleus enclosing the

complete somatic chromosome complement. Therefore, the second meiotic division continues with the unreduced chromosome number and results, after cytokinesis (cell wall formation), in an unreduced dyad instead of a reduced tetrad. Usually, the chalazal dyad cell undergoes three mitoses leading to an eight-nucleate embryo sac. The restitution nucleus formation in the Taraxacum type applies, primarily, only to the female meiosis. The important representatives of the Taraxacum type are among the Compositae: *Taraxacum, Erigeron, Chondrilla,* certain strains of *Antennaria carpatica.* Examples from other families are scarce: *Arabis Holboellii, Agropyron scabrum, Paspalum* (only certain species; most are aposporous).

Other Types of Diplospory. Both the Antenl1aria type and the Taraxacum type are met with in such diplosporous apomicts the sexual relatives of which show monosporic embryo sac development (Polygonum type). Only a few diplosporous apomicts belong to groups in which sexual plants show tetrasporic development (Drusa type, Fritillaria type). As in the Taraxacum type, an asyndetic meiotic prophase leads to a restitution nucleus which undergoes a division corresponding to the second meiotic division, but this nuclear division is not followed by a cell division. Therefore, the resulting coenomegaspore contains two unreduced instead of four reduced nuclei. Two further mitoses lead to an eight-nucleate embryo sac. This so-called Ixeris type has been reported not only in *Ixeris dentala,* but also. (although sometimes with slight deviations) in a few other tetrasporic Compositae such as *Rudbeckia,* and *Erigeron*, and in Plumbaginaceae in *Statice oleaefolia.*

Only two diplosporous species are known in groups with disporic embryo sac development (Allium type): *Allium nutans* and *A. odorum.* In these species the chromosome number is doubled by a premeiotic endomitosis and, therefore, a normal female meiosis (with pairing of identical chromosomes forming "autobivalents") leads to a tetrad of unreduced nuclei. As in the Allium type, there is no cell wall formation' in the chalazal-dyad so that only two further mitoses are necessary to achieve maturity. This "Allium ilutans type" as well as the Ixeris type show strikingly how apomicts conserve the embryological mechanisms of their sexual relatives as best as possible. Premeiotic chromosome doubling plays an important role in apomictic ferns. Postmeiotic chromosome doubling by endomitoses and reduplications are rarely reported, e.g., in *Potentilla collina, Saccharum,* and *Ranunculus auricomus.*

2.1.2 Female meiosis in diplosporous apomicts

Asyndesis can result either in a disturbed meiosis with irregular distribution of chromosomes which leads to aneuploidy, or in a meiotic restitution nucleus which leads to an unreduced dyad. Finally, meiosis can be entirely lacking.

2.1.3 Apospory (Earlier Designation: "Somatic Apospory")

The unreduced embryo sacs develop from somatic (vegetative) cells lying, as a rule, m the center of the nucellus, often adjoining the chalazal pole of the megaspore mother cell, dyad, or megaspore. Or, rarely, deeper in the chalaza, or even in the inner integument. Often there is only a single aposporous initial per ovule. Even if there are more, usually only one of them matures into an aposporous embryo sac.

Aposporous initials are first recognizable by their growth and the enlargement of their

nucleus, especially the nucleolus, and by vacuolation causing compression and resorption of adjacent generative as well as vegetative cells. The darker staining of certain nucellar cells before vacuolation, especially with iron-hematoxylin, has turned out to be a very unreliable criterion for potential aposporous initials. Polarization and orientation of the spindle of the first mitosis are usually parallel to the nucellus, as in reduced embryo sacs.

This bipolar aposporous type is called the "Hieracium type", after the first aposporous plant analyzed embryologically. It is widely distributed, but is rather rare in Compositae: *Hieracium subg Pilosellae* (Euhieracium follows the Antennaria type)*, Crepis* (some American species), and some Australian Compositae.

A peculiar form of apospory occurs in Panicoideae (Gramineae). There is no initial polarization in the embryo sac, and the vacuole develops at the chalazal end. The spindle of the first mitosis lies crosswise at the micropylar end. A second mitosis leads to the mature, four-nucleate embryo sac. Thus, this monopolar "Panicum type" of apospory contains the three-celled egg apparatus and single polar nucleus, while antipodals are absent. Such four-nucleate, monopolar embryo sacs (not to be confused with the Oenothera type) are always unreduced; if reduced embryo sacs also occur in the same plant, they are always eight-nucleate and bipolar.

The Panicum type, discovered in *Panicum maximum*, is known only in Panicoideae and Andropogoneae, but is very widespread. Important genera comprising apomictic species are: *Bothriochloa-Dichanthium-Capillipedium, Cenchrus, Chloris, Digitaia, Eriochloa, Heteropogon, Hyparrhenia, Panicum, Paspalum, Pennisetum, Sorghum, Themeda, Urochtoa,* etc. Occasionally, four-nucleate embryo sacs have two polar nuclei and one synergid, or may arise by diplospor.

In Panicoideae the unreduced embryo sacs are usually four-nucleate, and the reduced ones eight-nucleate. However, exceptions have been reported. In certain species unreduced, aposporous embryo sacs can also be bipolar and, therefore, eight-nucleate. Vice versa, in certain cases, reduced embryo sacs can also give the impression of being four-nucleate due to fusion of the two polar nuclei and early degeneration of the antipodal nuclei, which is atypical for grasses. The genus *Paspatum* is especially instructive. Besides usual apomictic species there are intermediate species with unreduced embryo sacs sometimes four-nucleate, sometimes eight-nucleate, or with a variable number of nuclei. The other extreme is demonstrated by a few species of *Paspatum* in which also the unreduced embryo sacs are always bipolar and eight-nucleate. There are even reports of diplosporous species in *Paspatum*.

In most aposporous apomicts (Hieracium type and Panicum type) asynchronous development of unreduced embryo sacs in the individual ovules of the same bud is typical, contrary to the conditions in most sexual plants.

2.1.4 The Female Meiosis in Aposporous Apomicts

The meiosis is independent of the aposporous embryo sac development, a fact largely. verifled for various apomicts. In principle both these processes can occur simultaneously in

the same ovule but, usually, in wild apomicts the megaspore mother cell, or the products of its meiotic divisions, are sooner or later eliminated so that only unreduced, aposporous embryo sacs persist. This elimination can take place in various ways. The simplest and most effective, but surprisingly rare, is the interruption in development and the degeneration of the megaspore mother cell during meiotic prophase. More often, in aposporous apomicts, the female meiosis can, in principle, be completed, but mostly the megaspores degenerate. However, further development is still possible, at least in some ovules. The meiotic development has to compete with aposporous embryo sacs which are usually more or less advanced in development, not having been delayed by meiosis.

The timing of the induction of aposp6ry is decisive. The sooner an aposporous initial is induced, the fewer are the chances of development of the megaspore. *P. adscharica* with very early induction of apospory is totally aposporous; *P. recta* with delayed induction produces meiotic embryo sacs in about 3 % of the ovules. Similar observations have been reported for various other apomicts, e.g., *Ranunculus auricomus* where apomictic wild forms show early induction of apospory, in certain ovules even before meiotic prophase. Crossing with sexual *R. cassubicifolius* and each backcrossing increasingly delays the induction of apospory until, in certain hybrids, apospory can only be determined in older buds in which the female meiosis has been completed.

Effects similar to those, caused by such variations in timing can arise from variations in the number of developing aposporous initials in each ovule, owing to an enlargement of the induced nucellar region. However, even in these cases of "multicellular apospory" usually only one aposporous embryo sac completes development.

2.1.5 Apomicts with Multicellular Female Archesporium

These occur especially in Rosaceae. Some are purely aposporous, such as certain species of *Potentilla, Sorbus, Malus, Aphanes,* and, probably, *Alchemilla*. *Potentilla verna* is an example for diplospory. In other *Potentilla*, and especially in *Rubus*, diplospory occurs besides apospory. This often makes the interpretation of embryological preparations difficult because, exactly as in sexual plants, it is not always easy to delimit the generative region since there are often transitions between ordinary megaspore mother cells, potential ones, and adjoining somatic nucellar cells. Many cases are indeed clear. In others, e.g., *Potentilla* and *Rubus* it is sometimes hardly possible to distinguish between diplospqry and apospory. Therefore, a term which covers both of these pathways of producing unreduced embryo sacs is indispensable. Such a term is apomeiosis as originally defined, embryo sac formation without reduction of the number of chromosomes.

2.1.6 Mature Unreduced Embryo Sacs

Diplosporous as well as aposporous (apart from the four-nucleate Panicum type) unreduced mature embryo sacs are in principle of the same pattern as the reduced embryo sacs typical for the related sexual species, even in such details as secondary division of antipodals. In apomictic *Crepis* these divisions are fewer than in sexual relatives. However, structure and organization of unreduced, especially aposporous embryo sacs, show more or less

characteristic variability, chiefly in the number of mitoses of certain embryo sac nuclei. In particular, unreduced egg cells and polar nuclei can deviate from the expected number. Variations can also occur in the fournucleate Panicum type. In the autonomous apomict *Burmannia coelestis,* where the synergids have no function, egg apparatuses with one or even two additional egg cells instead of synergids.

Supernumerary egg cells easily explain the occurrence of twin embryos from one and the same embryo sac. According to their position in the embryo sac such additional embryos are sometimes interpreted as "synergid embryos", i.e., as cases of apogamy. However,this is hardly justified as in angiosperms the extremely reduced megagametophytes have no vegetative prothallial cells but only highly specialized - synergid and antipodal cells; their dedifferentiation is difficult to imagine.

2.2 Formation of Endosperm and Embryo

Maternal offspring originate only when the unreduced egg cells, containing the complete maternal genetic information, are capable of parthenogenetic development, as a zygote would lead to Bill offspring with increased polyploidy. The differentiation of a normal embryo presupposes a normal differentiation of the endosperm. For this, pollination and fertilization of the polar nuclei are necessary only in "pseudogamous apomicts", while in "autonomous apomicts" the endosperm develops autonomously, i.e., both the egg cell and polar nuclei develop parthenogenetically.

2.2.1 Autonomous Endosperm Development

Plants showing autonomous apomixis (synonym: diploid parthenogenesis) do not depend on pollination for maternal reproduction because neither the unreduced egg cell nor the polar nuclei require fertilization; nevertheless, they are only rarely completely male-sterile. A very instructive example for the independence from pollination is the dioecious *Antennwia alpina* s.l. in which hundreds of taxa are known only as female plants. Most apomictic Compositae show autonomous endosperm development; in other families autonomous apomicts occur only sporadically, e.g., *Burmannia coelestis, Elatostema*, *Cafqmagrostis, Cortaderia jubata, Alchemilla.*

Cytological analyses of endosperm in autonomous apomicts, although not very numerous, show that the degrees of polyploidy are rather variable, due mainly to the variability in number and extent of fusion of polar nuclei. Moreover, there are, as in sexual plants, endomitotic duplications. The fusion of polar nuclei is not a prerequisite for mitotic activity in endosperm. In apomictic *Hieracium* both the synergids remain intact, whereas in sexual species one synergid is always destroyed. In *Taraxacum scanicum*, even in autonomous apomicts occasional fertilization of polar nuclei is still possible. Most embryo sacs show, as expected, intact synergids; only rarely one of them is destroyed (obviously, in such cases fertilization of polar nuclei has taken place). It is remarkable that synergids, as well as pollen grains, remain latently functional, at least in certain 'autonomous apomicts, although they are not needed to ensure fertility.

2.2.2 Pseudogamous Endosperm Development (Pseudogamy)

The initiation of endosperm development requires fertilization of polar nuclei. Possibly, an exception could be *Arahis Holboellii* where the stimulus of pollination is said to be sufficient for endosperm development; this should, however, be reinvestigated.

The most reliable evidehce that fertilization of the polar nuclei is necessary is provided by cytological analyses of metaphases in Nuclear endosperm, or in early stages of Cellular endosperm. Moreover, fertilization has been confirmed by direct observation:

1. of the triple fusion,

2. of the penetration of one male gamete into fused or free polar nuclei, whereas the other male gamete "is arrested at the boundary of the egg cell without penetrating into the egg cell cytoplasm",

3. of the synergids, one of which is destroyed, and

4. of the growth of pollen tube into the embryo sac and its penetration and discharge into the degenerating synergid.

Electrophoresis of proteins shows that the endosperm in different ovules of pseudogamous apomicts as genetically not identical and, therefore, must have resulted after fertilization, as only the male gametes are reduced and, consequently, show recombination.

Pseudogamy is typical for most apomictic Rosaceae and apomictic Gramineae. In apomictic Compositae, on the other hand, pseudogamy occurs only exceptionally as in *Parthenium*. Important examples from other families are *Zephyranthes texana, Hypericum perforatum, Arabis Holboellii, Cooperia pedunculata,* and *Ranunculus auricomus.*

Generally, all apomictic species within the same genus show either autonomous or pseudogamous endosperm development. Genera comprising both alternatives are so rare that there is no reason to suppose that pseudogamy is a precursor of autonomous apomixis (in the sense of a complete breakdown of double fertilization). Such exceptions are known in *Poa* (all apomicts except P. nervosa are pseudogamous), and *Malus*. This is important in the cultivation of *Malus,* since only autonomous species can set fruit without pollination.

Facultative pseudogamy has been noted in a male sterile strain of *Nardus stricta*: when pollination occurs, polar nuclei are fertilized; but when pollen is not available endosperm can develop autonomously.

Male-sterile strains of pseudogamous apomicts depend on the pollen supply from neighboring plants.

The degree of polyploidy of the endosperm of pseudogamous apomicts is even more variable than that of autonomous apomicts. Certain levels of polyploidy usually predominate, but not always those expected from the embryological data.

The causes of this variability of the levels of polyploidy are, on the one hand, the number of unreduced polar nuclei and their extent of fusion; on the other, the number of fertilizing male gametes. It is possible that polar nuclei (fused or free) can be fertilized by two male gametes. Development without fertilization seems to be exceptional, in the cultivation of *Malus*, since only autonomous species can set fruit without pollination.

The seed-setting in pseudogamous apomicts is often poor and can furthermore depend on

the male parent, chiefly on its level of ploidy. Pseudogamous apomicts can also show a kind of seed incompatibility (endosperm incompatibility) after crossing between plants of different levels of ploidy, although the sterility (viability) barriers are not as effective as in sexual plants. Similarly, a misproportion in the genetic composition of the endosperm can lead to its degeneration and, as a consequence, also of the embryo. As yet, the results are far too fragmentary to give a clear picture. Seed incompatibility, at least in pseudogamous *Ranunculus auricomus*, is an effective mechanism to isolate apomicts from their sexual relatives.

Therefore, correlated with the change to apomictic reproduction, an adaptation in the optimal endosperm ratio must have taken place, in order to avoid seed incompatibility. Pseudogamous plants have different mechanisms to ensure a genomic endosperm ratio similar to that typical for sexual Plants:

1. Fertilization of one unreduced polar nucleus by one reduced male gamete, occurring in the four-nucleate, nionopolar Panicum type.
2. Fertilization of two free unreduced polar nuclei, each by one reduced male gamete.
3. Fertilization of two fused unreduced polar nuclei by one unreduced male gamete.
4. Double fertilization of fused unreduced polar nuclei by two reduced male gametes. This feature is genetically fixed and not connected with apospory.

To what extent such mechanisms are a prerequisite for normal development of endosperm and, therefore, influence seed-setting and fertility, should be investigated more closely.

"Pseudogamous heterosis" i.e., the peculiar phenomenon that vigor of maternal offspring can depend on the male parent, has been observed in certain apomictic strains of *Rubus* and *Malus*. In pseudogamous Panicum maximum seed dormancy and the rates of germination can depend on the male parent. Such influences can only originate from the endosperm, as it is the only genetically variable tissue in the seeds of pseudogamous plants.

2.2.3 Embryo Development

In many pseudogamous apomicts the embryo starts to develop, in most ovules, only after the initiation of the endosperm, as in sexual plants: e.g., *Rubus, Hypericum perforatum, Ranunculus auricomus, R. cassubicus, Hierochloe,* and many aposporous Panicoideae such as *Panicum maximum, Pennisetum ciliare, P. dubium.*

Other pscudogamous apomicts (possibly the majority), while still in the bud stage, before anthesis, show precocious embryo development in most ovules ("precocious embryony"). The embryo precedes the endosperm which develops only after fertilization of polar nuclei. Examples for this derived feature are *Poa, Parthenium, Ttipsacum dactyloides,* and others.

Transitions between these two features are known. Certain genera contain species with both features, e.g., *Potentilla* and *Paspalum*. There are also species in which only single strains deviate, e.g., *Bothriochloa ischaemum* and *Potentilla tabernaemontani.*

In autonomous apomicts both features are known, but precocious embryony prevails. Of course, here also the endosperm can be initiated before anthesis. In others, such as *Crepis* and *Cortaderia jubata,* the division of the egg cell takes place only after endosperm formation has

begun.

The importance of precocious embryony is obvious. The division of egg cell before anthesis makes fertilization impossible, and prevents formation of Bill hybrids. They, however, can still be produced from occasional ovules with delayed development containing persistent undivided egg cells even after anthesis.

In "hemigamy" (a linguistically wrong synonym: "semigamy") the nucleus of the second male gamete enters the cytoplasm of the egg cell but does not fuse with its nucleus, and degenerates sooner or later.

In "androgenesis" (male parthenogenesis) the embryo originates from the nucleus of the male gamete, whereas the egg cell nucleus in the two-nucleate (one male and one ♀) "zygote" degenerates. Androgenetic offspring contain, therefore, only the paternal chromosome complement. In nature, androgenesis is just another additional feature occurring in certain apomicts, as well as in certain sexual plants. The importance of androgenesis lies in its application in plant breeding.

2.3 Male Meiosis and the Male Gametophyte

Diplospory as well as apospory are phenomena concerning only the female reproductive structures. The male meiosis is not primarily affected but is often more or less normally concluded, similar to the female meiosis in apospory. Irregularities are secondary. It is noteworthy that even in the Antennaria type the male meiosis can be more or less normal.

Unreduced pollen from restitution nuclei is relatively rare, even in diplosporous apomicts of the Taraxacum type. The formation of female restitution nuclei is caused by a special factor which only affects the female, but not necessarily also the male sporogenesis.

Autonomous apomicts, which do not need viable pollen for endosperm initiation, show the whole range from completely normal male meiosis (with only bivalent formation, e.g., Mendel's *Hieracium aurantiacum*) to complete male sterility. A male-sterile dioecious apomict is *Cortaderia jubata.* Certain aposporous specie of *Cortaderia* and *Lamprothyrsus* are known from female plants only. Apparently, all possible disturbances, up to complete degeneratfon of the male meiosis, can accumulate (e.g., in Taraxacum).

Pseudogamous apomicts, which depend on pollen for till fertilization of polar nuclei, have, on an average, pollen of a much better quality; but even here com-.

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As stated earlier, neither can diplospory/apospory totally exclude meiosis, nor can the capacity of parthenogenesis totally exclude fertilization. In apomictic wild types both these components of sexuality must be suppressed by auxiliary mechanisms such as failure of stages of megasporogenesis or gametogenesis, aposporous competition, and precocious embryony. In spite of this, exceptionally, maturation of reduced embryo sacs, or fertilization of reduced or unreduced egg cells can occur later, during embryogenesis, and especially during endosperm development, decisive hindrances have to be overcome before an aberrant offspring can develop. Unfortunately, however, these stages are difficult to approach methodically and,

therefore, have hardly been investigated. Even during germination, and afterward, aberrant embryos often show signs of weakness compared to the vigorous maternal ones and, eventually, some of them die. This applies not only to polyhaploid but also to B_{II} and B_{III} hybrid embryos and seedlings.

In principle, therefore, gametophytic apomicts still preserve the possibility of producing aberrant offspring, although this is exceptional. Thus, it becomes clear that apomixis and sexuality are not alternatives, as was formerly assumed, but independent modes of reproduction which can occur side by side. Apomixis and sexuality are in a state of balance which, during the course of development between megasporogenesis and seed germination, or even later, shifts more and more in favor of apomixis. The quantitative expressions of this state of balance between apomixis and sexuality are:

1. the "degree of diplospory/apospory" (common designation: "degree of apomeiosis"), i.e., the percentage of ovules with unreduced embryo sac initials or stages, and

2. the "udegree of apomixis", i.e., the percentage of maternal offspring (offspring or apomictic origin). From a theoretical point of view, the degree of apomixis (as well as the degree of pseudogamy) should also include polyhaploid offspring.

The degree of diplosporyjapospory (apomeiosis) reaches values which are, as a rule, lower when determined by embryological analyses, higher when calculated from cytological embryo or endosperm analysis, and still higher when calculated from cytogenetical offspring analyses. A rise to degrees of apomixis approximating 100% is the quantitative indication of the suppression of sexuality in the course of development. In populations with an extremely high degree of apomixis, traces of sexuality are often difficult to prove.

3.2 Possibilities of Influencing the Degree of ApomixiS

The degree of diplospory japospory, the degree of apomixis, and the extent of suppression of sexuality are genetically determined but can often, to a certain extent, be influenced quantitatively by factors beyond the mother plant.

3.2.1 Male Parent

The male parent may influence whether a (reduced or unreduced) egg cell develops parthenogenetically or after fertilization. Such influences by the male parent may often take place via the fertilized endosperm, but it is not known how. However, the level of ploidy of the male parent is not always decisive, as shown, for example, by recent pollination experiments in *Ranunculus auricomus*. Obviously, certain plants produce pollen with a greater tendency to fertilize egg cells inclined by nature to parthenogenetic development.

3.2.2 Environmental Factors

Environmental factors influence the degree of apomixis in many cases. In *Calamagrostis purpurea*, instead of mitotic embryo sacs (Antennaria type, typical for this species), under certain circum: meiotic embryo sacs in some of the inflorescences (which, because of genetic self-incompatibility, remain sterile after selfing).

3.2.3 Other Factors

X-ray and other ionizing radiation experiments (some including mutagenic agent such as

ethyl methane sulfonate) did not affect the state of balance between apomixis and sexuality in *Taraxacum vulgare, Paspalum dilatatum, Potentilla,* and *Pennisetum pedicellatum.* Only in two predominantly apomictic varieties of *Poa pratensis* X rays caused a slight increase in the number of sexually formed aberrant offspring which, however, reverted again rather rapidly to apomixis. Instances of mutagenic breakdown of apomixes, or reversal to sexuality, are not known. A case of mutagenic induction of a tendency toward apospory leading to facultative apomixis in diploid *Pennisetum typhoides* was reported. Further research on mutagenesis concerning genes involved in reproduction would be highly desirable.

Chromosome doubling by means of colchicine has been carried out on many apomicts, but only in a few cases was any influence on apomixis detected.

4 Causes and Consequences of Apomixis

4.1 Heterozygosity and Variability

Maternal reproduction means genetic fixation of any heterozygous gene combination and leads, in spite of hybridity, to uniform offspring, to populations of genetically exact copies of the mother plant. An extreme heterozygosity is almost characteristic of all apomicts investigated genetically as, after crossing with sexual mother plants, manifold segregations occur. Crossings are possible as nearly all apomicts produce a certain amount of viable pollen. Therefore, in spite of apomixis there is, in principle, still the possibility of a limited gene recombination and variability and, consequently, a restricted adaptability.

There are also some asexual sources of variability, besides traces of sexuality, which both increase heterozygosity. Mutation is one of them; however, its importance should not be overestimated, above all that of recessive mutations, as apomicts are in general polyploid. Autosegregation, i.e., changes or rearrangements of the genotype during egg cell formation in plants with parthenogenesis, is probably more important in diplosporous apomicts which form restitution nuclei, such as the Taraxacum type. There are two possibilities: (1) In "subsexual" reproduction a partially asyndetic meiotic prophase with a few bivalents leads to a restitution nucleus with very limited crossing-over. (2)Individual chromosomes can be lost during the formation of restitution nuclei. Other anomalies such as occasional gains or losses of individual chromosomes through nondisjunction occur in apomicts as well as in sexual plants, and will not be discussed here. The synergids do not always discharge the sperm nuclei, thus preventing, sometimes, double fertilization. If the reduced egg cell develops parthenogenetically, the spindle may lie crosswise to the axis of the embryo sac so that the daughter nuclei "are not placed in the gradient of polarity in the egg cell plasm" and may fuse ("automixis"). Nevertheless, total homozygosity does not result, as the original, tetraploid strains are highly heterozygous.

The effects of heterosis in sexual crop plants show strikingly significant advantages of heterozygosity. The fixation of a pronounced heterozygosity is, however, not compatible with sexual reproduction - apart from complex heterozygotes. No doubt, the well-known superiority of many apomictic strains is a further example of the positive effects of

heterozygosity. Another parallel to heterosis is that hybrid offspring of apomicts, if any, are almost always inferior.

4.2 Causes of Gametophytic Apomixis; Components of Apomixis in Sexual Plants

In sexual plants meiosis and gametophyte formation are bound together so that unreduced gametophytes result, at most, from meiotic restitution nuclei. In apomictic plants unreduced embryo sacs are formed from meiotic restitution nuclei only in the Taraxacum and Ixeds types, which are remarkably rare, except in Compositae. In the majority of apomicts, on the contrary, the bonds between female meiosis and embryo sac formation are broken up. This applies to both mitotic diplospory (Antennaria type) and apospory, but it hardly ever occurs in sexual plants. Even in aposporous Panicum maximum the sexual hybrids formed unreduced embryo sacs, if at all, not by apospory but through meiotic restitution nuclei.

The hypothesis that the bonds one has to imagine linking female meiosis and embryo sac formation in normal sexual plants must be opened somehow in most gametophytic apomicts.

4.3 A pomixis and Polyploid

4.3.1 Polyploid

Allopolyploidy is just as typical for gametophytic apomicts as hybridity and heterozygosity. Triploidy is relatively rare and, in most cases, restricted to the Taraxacum and Ixeris types of diplospory. More common are higher levels of polyploidy up to dodecaploidy, and, sometimes, even higher. However, tetraploids are the most frequent. Very often different levels of polyploidy are realized within the same species. Some apomicts, especially with high levels of polyploidy, show a tendency to revert by haploid parthenogenesis.

4.3.2 Diploid Apomicts

Diploid apomicts are very rare in nature. The apospory cannot be transmitted by haploid (monoploid) gametes but only by diploid (reduced or unreduced) or polyploid ones. Diploid apomicts can, therefore, be formed only as dihaploids but not as hybrids at least in *R. auricomus.*

4.3.3 Dihaploids

Dihaploids, formed by parthenogenetic development of reduced (i.e., diploid) egg cells of partially apomictic tetraploids, are known from many investigations. Most dihaploids are sexual, very often rather weak and more or less sterile, but not invariably. Some of these apomictic dihaploid *R. auricomus* plants are no doubt vigorous enough to survive in natural competition. May be one or another of the known diploid apomictic wild strains mentioned above have resulted from tetraploid apomicts by haploid parthenogenesis?

5 Apomixis and Breeding

Gametophytic apomixis is the result of a delicately balanced interaction of different, genetically independent components. Although many details are known, there remain nonetheless important gaps in the theoretical knowledge of apomixis. The most serious gaps concern the physiological background of its components, particularly with respect to the formation of megagametophyte (diplosporyjapospory), sporophyte (parthenogenesis), and

endosperm.

Any breeding program stands or falls with the availability of hybrids. In highly apomictic plants one can try different possibilities of influencing the degree of apomixes to increase the rate of hybridization. Different breeding research programs in course for *Poa* will serve as an illustration. If the suppression of the mechanisms of sexual reproduction is so effective that "obligate apomicts" result, their pollen call be used to pollinate sexual relatives which, however, are usually diploid. If no sexual relatives are available, facultative apomicts of the same level of polyploidy may do as female parent. Every successful hybridization involving apomicts causes abundant variability in offspring, due to the heterozygosity typical of apomicts - not least for reproductive mechanisms, but highly apomictic new types can often be selected only in succeeding generations following hybridization. In certain especially favorable cases such as *Pennisetum ciliare* or *Panicum maximum* obligate apomicts occur. It is not always easy to identify aberrants in apomictic populations and to define the stability of each line without genetic markers. Subjective visual assessments made in the field can be supported by chemical or statistical methods. Special problems on the breeding of apomictic plants, on the search for related sexual plants and complications due to polyploidy, on breeding schedules, on selection and improvement of new varieties, etc., cannot be discussed here.

The reasons for the practical interest in apomixis, and for the great efforts in making its manipulation possible, are obvious. Apomictic reproduction would allow one to fix any desired heterozygous gene combination and to maintain new, heterozygous "varieties" with valuable agronomical properties, genes for resistancy to diseases, etc. Most alluring is the prospect of being able to perpetuate heterosis effects by apomixis and to produce seeds of "hybrid varieties" (F_1 hybrids) without constant crossings of the. parent lines. Moreover, the manipulation of gametophytic apomixis would profit by the fact that different virus infections can be prevented by seed reproduction, thanks to the double barrier of meristem and megagametophyte - in contrast to vegetative propagation. This might enable one to invigorate and rejuvenate old varieties.

The hitherto rather modest successes in introducing apomixis into sexual plants, jn spite of the great efforts undertaken, are at least partly due to the fact that there is as yet no other practicable way than that of proceeding empirically. A real advance can only- be expected from a thorough theoretical knowledge concerning the causes and the genetics of gametophytic apomixis and the exact functioning of the genes involved and, above all, the physiological background of the components of gametophytic apomixis. Extensive mutation research would seem to me to be particularly promising. It is to be hoped that an increasing practical need will give new impetus to fundamental and systematic research, as this most important and widespread anomaly of reproduction in angiosperms is still not really understood.