An early application of pollen. Relief carving from the Palace of Syrian King Ashuir-nasir-pal II. 883–859 B.C., discovered at Nimrud, the modern Calah, now in the Metropolitan Museum of Art, New York.

The standing figure, a human body with outspread wings, is fructifying the tree, which has the form of a palm. The flowers of the tree are sprinkled with water from the vessel in the left hand. The male palm flowers held in the right hand are used to transfer pollen to the female flowers.

R. G. Stanley H. F. Linskens

POLLEN

Biology Biochemistry Management

With 64 Figures and 66 Tables

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Preface

Pollen transmits the male genetic material in sexual reproduction of all higher plants. This same pollen is also well suited as a research tool for studying many patterns of plant and animal metabolism. In addition, an increased knowledge of pollen may help plant breeders accelerate efforts to improve the world's food and fiber supply.

This volume focuses upon pollen biology and chemistry; it attempts to integrate these facts with management practices involved in pollen applications.

People have long been involved with pollen. Pollen applications are recorded in the rites of ancient civilizations (see Frontispiece). From the earliest times many benefits have been attributed to the inclusion of pollen in man's diet; also, since the mid-19th century air-borne pollen has been recognized as detrimental to many people's health.

Disciplines concerned with man's cultural history and the earth's changing ecology find pollen a particularly useful and accessible tool. Identifiable parts of pollen have survived over 100 million years. But most books dealing with pollen are generally concerned with the identification of the plant source, an aspect of the science of palynology; other books emphasize the natural vectors transmitting pollen, the pollination mechanisms. Very few works include the biochemistry or biology of pollen. Yet extensive studies by physicians, as well as plant breeders and apiculturists, have contributed a sizeable body of research relating to pollen.

We have endeavored to review these many historical and recent studies and to indicate some areas of pollen biology and biochemistry where critical knowledge is still lacking. These deficiencies in our knowledge, and their relation to improved management practices, present significant research challenges for the future.

The main details of pollen germination and growth to fertilization will be covered in a related volume now in preparation. A second volume will include such topics as incompatibility reactions, stigma responses, population effects, tropism metabolic and cytological changes during growth, and the influence of different chemicals and treatments on *in vitro* and *in vivo* growth. Hopefully, this and the successor volume will encourage increased applications of pollen in research leading to an improved understanding of many basic cell processes, and provide insights to help improve the yields of desired crops.

We would like to express our appreciation for the encouragement and assistance provided by many colleagues during the years this volume was in preparation. Colleagues who have kindly reviewed parts of this volume include: G. Barendse, Charles A. Hollis, III, W. Jorde, Edward G. Kirby, III, Marianne Kroh, F. Lukoschus, James L. Nation, Joop K. Peter, Frank A. Robinson, Wal-

TER G. ROSEN and INDRA VASIL. J. BRAD MURPHY patiently reviewed the total manuscript and creatively aided in its correction and improvement.

Great devotion and care in preparing this manuscript was provided by Miss BONITA CARSON assisted by Miss ANN MCLOCKLIN of the School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences at the University of Florida. PATRICIA STANLEY shared or assumed much of the burden of proof-reading drafts of the manuscript.

All these many meaningful contributions to our effort are gratefully appreciated and sincerely acknowledged.

ROBERT G. STANLEY
HANS F. LINSKENS

Postscript

R. G. STANLEY †, April 15, 1974

A few weeks after delivering the manuscript to press BOB STANLEY died in a tragic way. This book will therefore be his last work, his ultimate contribution to a field of his special interest, to which he contributed so much.

With melancholy and gratitude I recall the twenty years of our friendship and close scientific cooperation, which were brought to an abrupt end by Bob's untimely death.

H. F. LINSKENS

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I. Biology

Chapter 1. Development

Our understanding of the elements and patterns of pollen development represent the outgrowth of extensive light microscopic studies of many 19th century plant anatomists. These insights have been refined, classified and extended by studies with the transmission electron microscope, and most recently by observations with the scanning electron microscope. Some overlapping vocabulary and uncertainty in the meaning of names and labels attached by different workers to various pollen elements has occurred. To help avoid misunderstandings in this volume, the descriptive vocabulary will be clarified in these initial pages. Examples will be given to illustrate a few of the most common patterns of pollen differentiation.

Terminology

A flowering plant, a sporophyte, produces spores. Similar to the reproductive cycle in higher animals, some cells in diploid plants undergo meiotic division, resulting in cell clusters with haploid numbers of chromosomes. In angiosperms the organs which form male spores, the *microspores*, are called *anthers*. Female spores, *megaspores*, are formed in the *ovary* at the base of the *pistil*. In gymnosperms the clusters of male cells are formed on *microsporophylls*; the megaspores are borne on *megasporophylls*. The sporophylls, evolutionarily modified leaves, are usually grouped together in a cone structure, the *strobilus*.

The anther normally consists of two lobes, each with two elongated *microsporangia*, the *pollen sacs*, in which pollen development takes place (Fig. 1-1). The anther lobes, or *thecae*, are fused together by connective tissue which consists of vegetative cells, with a small, central vascular bundle. The *filament*, a stalk containing a single vascular bundle, is the connector which supports and attaches the anthers to the *receptacle* in the flower. The anther plus the filament are called the *stamen*.

Initially, a microspore is not ready to continue the life cycle. After formation of the microspore, one, two, or three mitotic divisions occur, followed by a resting period varying from a few hours to many months. After mitosis, the mature microspore is referred to as a *pollen grain*.

The term microspore should be limited to the uninucleate structures released from tetrads after meiosis. The term pollen grain describes the above structure after mitosis of the microspore nucleus and containing the developed *vegetative* (tube) and *generative cells* or *male cells* (Vasil, 1967).

The pollen grain, ready to germinate and grow, is correctly considered the multicellular *male gametophyte*. *Male gametes*, or sperm cells, are formed in the pollen grain or the *pollen tube*, which forms on germination of the pollen grain. By

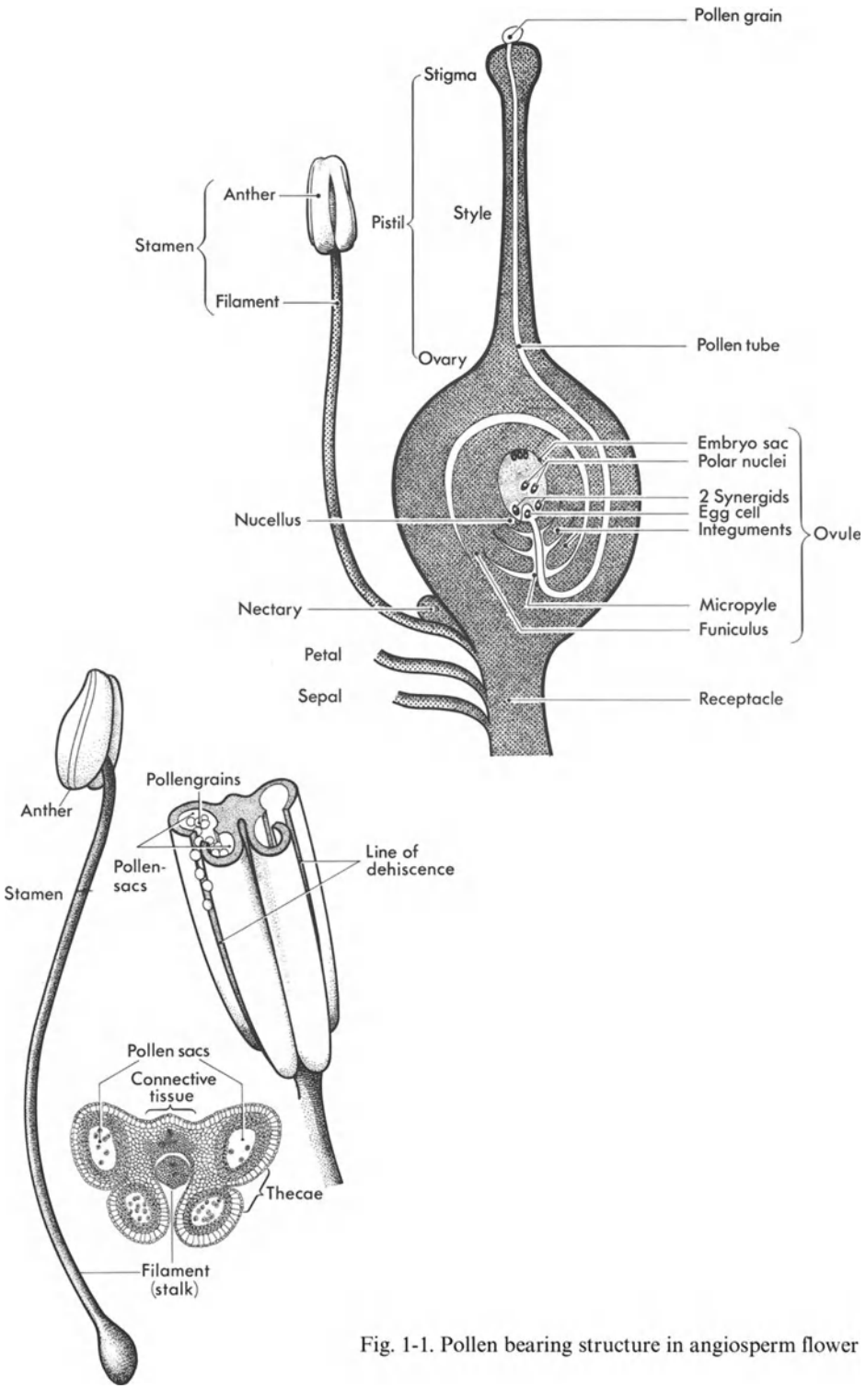

Fig. 1-1. Pollen bearing structure in angiosperm flower

convention, the thecae of the anthers are considered microsporangia and the *pollen mother cells* contained within the microsporangia are termed *microsporocytes* or *meiocytes.*

Young anthers contain *archesporial cells* which differentiate to form the *parietal cell* layer and *sporogenous* tissue. The parietal cells produce the outer wall of the anther, and an innen layer, the *tapetum.* Cells of the sporogenous tissue give rise to many *pollen mother cells* (PMCs) which divide to yield the microspores which mature and are shed as pollen grains.

The multicellular pollen grain transfers the male genome to the female organ by *pollination.* During formation and development, the male gametophyte depends on the parental sporophytic tissue for nutrition. In contrast, the female egg cell in the embryo sac is never independent of the sporophyte. The structure of the pollen grain and growth of the pollen tube are related to the role of conveying the male cells to the egg cell, i.e. the fertilization process, and secondarily, to stimulating development of the fruit or seed which encloses the mature embryo.

In angiosperms, the fused megasporophylls, the *pistil,* has a receptive surface for pollen, the *stigma.* The stigma is connected by the stylar column to the *ovary,* an enlarged basal portion of the pistil. The ovary contains the *ovules,* each with an embryo sac containing several cells. One of these cells is the egg cell with which a male cell must fuse. The male cells, conveyed down the style via the pollen tube, enter the ovary through the *micropyle.*

In pollination of gymnosperms, pollen is transferred directly to the *micropyle* of the ovule. In comparison, angiosperm pollen is transferred to the stigma and must grow through stigma and stylar tissues before entering the micropyle.

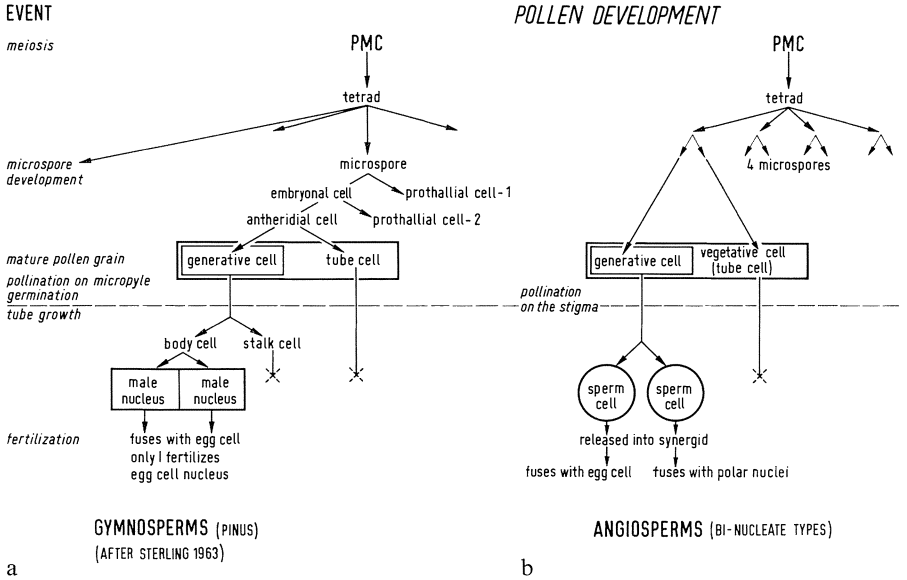

Fig. 1-2a and b. Comparison of pattern of pollen development in (a) gymnosperm and (b) angiosperm

Development Pattern

As already indicated pollen development differs in gymnosperms and angiosperms (Fig. 1-2).

Gymnosperms

Microspores are formed in the microstrobili which generally develop in the axils of scale leaves near the tips of branches. Each microstrobilus usually bears many microsporophylls in a spiral arrangement around the central axis, each with two or more microsporangia on the lower side.

Microspore mother cells undergo meiosis giving rise to the spore tetrad, each yielding 4 microspores, ultimately the pollen grains. Each pollen grain is sealed in a double layered wall. The outer pollen wall in many species forms two conspicuous wings or sacs which contain air. Such structures reduce the free fall velocity of these wind-dispersed, *anemophilous*, pollen. Gymnosperm pollens show the complete gametophytic development pattern; the nuclei divide several times (Fig. 1-2) and resulting mature pollen grains, in *Pinus* for example, contain the following cells: two nonfunctional prothallial cells, a central vegetative or tube cell and the generative cell, the latter two originating from the *antheridial* initial. With formation of these cells, gymnosperm pollen grains are shed from the microsporangia.

Angiosperms

Angiosperm pollen development can be separated into three major types (WULFF, 1939):

The Normal Type. Most commonly in angiosperms, the microspores begin to enlarge and exine formation is initiated immediately after meiosis. At division of the nucleus the microspore has already reached a definitive size, vacuoles are present and account for most of the microspore's volume. Because of the vacuoles, the cytoplasm is confined to a peripherial layer and the nucleus is in an acentric position. A chemical or bioelectric potential gradient has also been suggested as the sources of nuclear displacement (VAZART, 1958). Prior to the first mitosis the amount of deoxyribonucleic acid (DNA) in the microspore nucleus increases (BRYAN, 1951). The *first* mitotic division is generally not synchronous among the developing pollen grains in all the stamen within a flower, or even within the same anther (KOLLER, 1943). In some species where the walls separating microspores are very thin, mitotic divisions may be synchronous (MAHESHWARI and NARAYANASWAMI, 1952). The normal type of angiosperm pollen development is found in the majority of the monocotyledons and dicotyledons, even in species with bi- or trinucleate pollen.

The Juncus Type. This type of pollen development differs from the normal one in that division of the primary microspore nucleus takes place before growth of the pollen; vacuolization starts before formation of the exine is initiated. Also,

after the division of the generative nucleus, further growth of the grain can occur. This type of development is found in the Cyperaceae and the Juncaceae.

The Triglochin Type. The third common type of pollen development is somewhat intermediate. The young microspores, after separation, grow slightly and form a thin exine. But the main growth period, including formation of vacuoles and definitive sculpturing of the exine, starts after formation of the generative cell. The ripe pollen grain is binucleate. This third type of pollen development is found in *Najas*, *Ceratophyllum*, *Ruppia*, *Apomogeton*, *Triglochin* and others.

The above described types of pollen development are mostly descriptive, but they may be of taxonomic value. Mature pollens, as well as fossil types, can be divided into many classes based on development and wall pattern. This is the subject of comparative palynology and is reviewed in the famous books of ERDTMAN(1952, 1969), WODEHOUSE (1935), KREMP (1965) and others. In this book we are concerned with the biochemical aspects of pollen, and the sources of variation in the maturing pollen grain.

Synthesis and Division

Pollen grains develop through a series of regulated events which occur in a definite time sequence.

Induction of Meiosis

Meiotic division has 3 important features:

1. *Transformation of the chromosomes* by crossing-over processes;
2. *Rearrangement of the genomes* by random distribution of the homologous chromosomes, and
3. *Reduction of the chromosome number* from 2n (diploid) to n (haploid). Up to the present time many textbooks emphasize the third event. If this were the most important aspect of meiotic division, nature could renounce the complex processes of meiosis as a compensation of fertilization and, therefore, sexuality. However (1) and (2) are decisive because these events make recombination possible, which is probably the most important contribution of sexuality to evolution.

DNA Synthesis

The diploid PMC is genetically distinguished at a very early stage of premeiotic division. DNA synthesis takes place during premeiotic interphase (TAYLOR and MCMASTER, 1954). A second period of DNA synthesis takes place during prophase I in late zygonema and early pachynema. A distinct type of DNA is synthesized during this period (HOTTA et al., 1966). This DNA synthesis is a consequence of the break-repair mechanisms and may represent delayed replication of part of the chromosomes. If this interpretation is correct, the major determinant in differentiating meiotic from mitotic cells is the regulation mechanism which delays the

reproduction of some critical, essential DNA component. It may be speculated that, functionally, this component is an axial sequence of nucleotides. Crossing over during meiosis may be closely associated with the delayed reproduction of this element. The DNA replicase is inhibited at this division. A special histone (SHERIDAN and STERN, 1967) appears to be involved in meiotic induction. The histone pattern in developing pollen is opposite to that during ribonucleic acid (RNA) and protein synthesis. Low or no nucleohistone staining indicates a high rate of RNA and protein synthesis; high nucleohistone staining corresponds with reduced RNA and protein synthesis.

Histones

Histones apparently play a role in DNA-dependent RNA and protein synthesis in microspores and two-celled pollen grains (SAUTER and MARQUARDT, 1967; SAUTER, 1971). Acidic peptides as well as basic histone proteins may function as derepressor molecules. While the exact mechanism of induction is not known, other chemical changes are recognized. These changes include modified protein and enzyme patterns (LINSKENS, 1966) and periodic changes in sulfhydryl groups (LINSKENS and SCHRAUWEN, 1964).

RNA Synthesis

RNA synthesis is most active just before division (G-2), after the first mitosis and after microspore nuclear division (STEFFENSEN, 1966; LINSKENS and SCHRAUWEN, 1968b). A changing ribosomal pattern accompanies pollen development. Transition from the diploid sporophytic stage to the haploid gametophytic stage is concommitant with formation of new polysomal fractions (LINSKENS and SCHRAUWEN, 1968a). Changes in RNA or histones may also influence or reflect changes accompanying nuclear differentiation in pollen development (GEORGIEV, 1969).

In the transition to meiosis during pollen development it is possible to distinguish (a) transition to the incipient phase of meiosis due to synthesis of a special informational molecule following a signal in the transcription process; and (b) biochemical events which direct the course of meiosis. The latter includes two distinct events: chromosome pairing and crossing over between homologous chromosomes. Both events are dependent on metabolic signals to form enzymes at the right time, in proper amount and sequence, and on the nutrient pool which must deliver energy-rich compounds and chemical building moieties for the synthetic processes.

Induction of Polarity

Generally, no polarity is observed in pollen mother cells. The second meiotic division takes place at an angle of 90° to the first division. Polar differentiation during meiosis and pollen development was investigated in *Tradescantia* (SAX and

EDMONDS, 1933; SCHMITT and JOHNSON, 1938; LACOUR, 1949; BRYAN, 1951). Protein granules in the microspores disintegrate during prophase, generating vacuoles and pressing the nuclei against the ventral wall. Unequal distribution of protein synthesized just prior to mitosis occurs during anaphase. The mass of RNA and protein is shifted to the spindle pole where the vegetative nucleus will be formed. This asymmetric division and cytoplasmic gradient results in polarity. The generative nucleus, in which chromosomes are arranged in the compact, spindle-like, DNA-rich resting stage, is present in a smaller cell with a small portion of its cytoplasm lacking RNA. The vegetative nucleus remains more or less spherical but increases in size and shows a distinct nucleolus as protein synthesis starts.

As will be discussed subsequently, normal pollen development can be strongly influenced by genetic and environmental factors, resulting in abnormally small or large pollen grains (VON WETTSTEIN, 1965). Elevated temperatures, or introduced factors such as X-rays, γ-irradiation or chemicals can disturb polarity and result in male sterility. Pollen grains with nondisjunction of translocated chromosomes can form normal tetrads, but deficiencies in protein metabolism can yield uninucleate grains, with a high degree of sterility (SAX, 1942). Another disorder of polarity reported at asynapsis in *Picea* pollen grains results in formation of one, ring-like, air-filled wing (ANDERSSON, 1947).

In some cases of sterile pollen it is difficult to determine the source of abnormality. Among the 39 different species studied by ZIELINSKI and THOMPSON (1966) the five species of *Pyrus* producing sterile pollen are a good example of the difficulty in recognizing the origins of pollen degeneration. All five species had two normally appearing meiotic divisions. The breakdown in development occurred somewhere during the maturation process after liberation of the microspores from the tetrads and before anthesis. This post-meiotic phase is one in which tapetal activity is particularly critical. External factors may influence the tapetal cells and if tapetal cell metabolism is drastically upset, microspores do not develop normally.

Differentiation of Pollen Grain Nuclei

After formation of the microspores, a resting period generally occurs before the first division of the nucleus in the microspore. The length of this dormant stage varies from a few hours to several months (DAHLGREN, 1915; FINN, 1937). During this period, pollen is particularly sensitive to temperature (KOLLER, 1943). The mitosis which follows results in two distinct cells: a vegetative cell (tube cell) with the vegetative nucleus, and a generative cell with the generative nucleus. Differentiation seems to depend on unequal division of basic protein of the pre-mitotic nuclei (MARTIN, 1960). The problem of heteropolarity associated with the pollen mitosis is still under discussion (STEFFEN, 1963).

The products of the division of the microspore nucleus, the vegetative and the generative cells, are qualitatively different. The generative cell is smaller, hyaline and contains less RNA; its associated cytoplasm is separated from the vegetative cell cytoplasm by a plasma membrane. This has been demonstrated by light

(HOFMEISTER, 1956) and electron microscopy (BOPP-HASSENKAMP, 1959; BAL and DE, 1961; SASSEN, 1964; LARSON, 1963, 1965). Thus, cytoplasmic dimorphism exists within the cells of the pollen grain. Differentiation also can be shown by X-ray absorption (DAHL et al., 1957). In the vegetative nucleus, nucleoli are usually larger than those in the generative nucleus; decreased staining ability of the vegetative cell is believed to be due to the lower DNA content of the nucleus (LACOUR, 1949). The protein content of the vegetative nucleus is about twice that of the generative nucleus (STEFFEN, 1963), and the proteins seem to be more acid (BRYAN, 1951). The histone staining pattern also differs in the vegetative nucleus and generative cell nucleus. In the vegetative nucleus of *Lilium candidum* pollen, RNA was detected bound to the histone, a condition not observed in the generative cell nucleus (JALOUZOT, 1969).

Two hypotheses have been proposed to explain the *mechanism for the morphological and physiological differences* between the genetically alike cells of the pollen grain. Theory I assumes that difference in the DNA content are the cause of differentiation. The generative nucleus DNA increases over that of the vegetative nucleus (TAYLOR and MCMASTER, 1954). That would mean that all other differences in assembly of subcellular organelles, e.g. plastids, mitochondria, and spherosomes, result from nuclear differentiation (STEFFEN and LANDMANN, 1958; RICHTER-LANDMANN, 1959). Theory II accounts for differentiation by the fact that after division the two nuclei are in different cytoplasmic environments. One explanation for these differences in cytoplasm is based on the quantity (GEITLER, 1935), the other on the quality (LACOUR, 1949). Qualitative differences would also result from a quantitative difference in RNA content of the surrounding cytoplasm or from difference in hydration of the nucleus and viscosity of the cytoplasm (PINTO-LOPES, 1948). One reason for concentrating research on the pattern of the spindle in pollen development is because asymmetry of the spindle mechanism may be a key factor in establishing nuclear differences. The mechanism and time of differentiation of the two nuclei is a complex and intriguing problem.

Origin of the Sperm Cells

In angiosperm pollen development, the nucleus in the generative cell subsequently divides once more, forming two sperm nuclei. This division may take place (a) in the anthers, so that the ripe pollen contains three functional cells (COOPER, 1935), (b) in the mature pollen grain after pollination but before germination (CAPOOR, 1937), (c) just after germination on the surface of the stigma, which is the most common case in angiosperms, or (d) at the time the pollen tube reaches the embryo sac (D'AMATO, 1947).

BREWBAKER (1957) advanced a theory relating the nature of the pollen grain nuclei at maturity to the mechanism of incompatibility. He suggested that two-celled pollen grains occur in homomorphic plants which express pollen incompatibility reactions in the style and are generally linked to gametophytic incompatibility. In pollen which is three-celled at maturity, the stigmatic incompatibility reaction is most commonly observed, representing the sporophytic incompatibility. When pollen is shed in the three-celled stage, the incompatibility reaction is

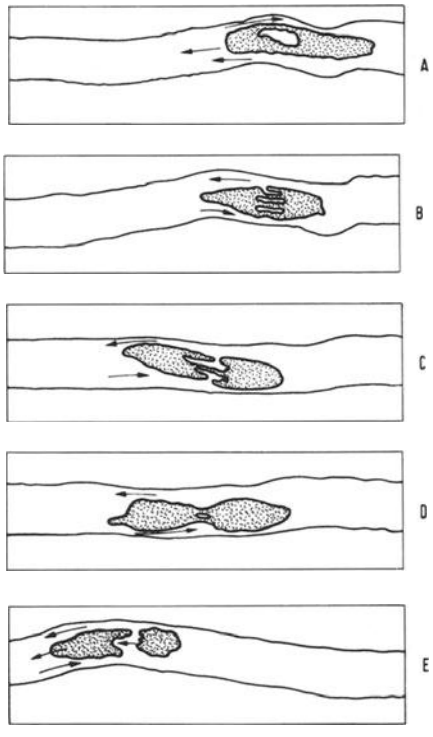

Fig. 1-3 A-E. Generative cell division in the pollen tube. Two male cells separate (E) but are retained in generative cell cytoplasm. Arrows indicate direction of cytoplasmic streaming and nuclear movement

stronger and occurs earlier. Three-celled pollen is viable for much shorter periods after dehiscence than pollen shed in the two-celled stage.

In the development of gymnosperm pollen, the antheridial initial cell gives rise to the tube cell and generative cell as it does in the angiosperms. However, in gymnosperms the generative cell divides to produce a *spermatogenous cell* (or *body cell*) and a *sterile cell* (Fig. 1-2). The latter, called the "stalk cell" by STRASBURGER (1892) and the pollen "wall cell" by GOEBEL (1905), is a terminal cell which disintegrates in the mature pollen grain. The two male cells in gymnosperm pollen arise from the spermatogenous cell. A pollen grain in most extant species of gymnosperms and angiosperms produces just two male gametes. However, in cycad pollen, particularly *Microcycas*, as many as 24 male cells may be produced (FAVRE-DUCHARTRE, 1963).

Factors contributing to formation of sperm cells in the pollen grains while still in the anthers are not known. Division of the generative cell can be experimentally induced by changing the water availability (GEITLER, 1942; PODDUBNAYA-ARNOLDI, 1936). BREWBAKER (1957) speculates that during the second mitosis, the generative cells deplete the sugars and other energy-supplying metabolites in giving rise to sperm cells. Yet corn and other trinucleate grass pollens are among pollens with the highest known percent endogenous carbohydrates at maturity (Table 9-1).

The two sperm cells produced during germination generally assume a spindle-like or lappet shape (Fig. 1-3). Each sperm nucleus is surrounded by cytoplasm

containing cell organelles, including mitochondria, ribosomes, and small but autonomous plastids, all contained within the plasmalemma (RENNER, 1934; KOSTRIUKOWA, 1939; KAIENBURG, 1950; SASSEN, 1964).

When formation of the sperm cells takes place in the pollen tube, division of the generative nucleus presents a special spacial problem. While in many species the spindle fibers are visible in a phase contrast microscope, in some cases a normal spindle cannot be observed. In such cases chromosomes often appear arranged in a row or in a tandem arrangement. Cytoplasmic microtubules, usually found in plant cells, appear as spindle fibers attached to the chromatids during anaphase. Although they are more easily observed in developing microspores (ROWLEY, 1967; ROSEN, 1971) they are also present in pollen tube cytoplasm (FRANKE et al., 1972). Fixation technique can easily destroy or obscure microtubules in prepared sections.

The nuclei of the sperm cells complete their division in the pollen tube relatively slowly; they remain in delayed telophase with strong chromatization and general absence of nucleoli. Finally, the two male sperm cells separate within the generative cell membrane (Fig. 1-3 E) and ultimately become separate sperm cells. Occasionally, abnormal nuclear divisions may occur increasing the number of sperm cells (MAHESHWARI, 1949).

A complete male gametophyte, ready to fulfill its function in fertilization, consists of three fully organized cells, each with cytoplasm and organelles which can undergo independent mutation (RENNER, 1922). The sperm cells participate in double fertilization in angiosperms, one forming the 2n zygote, the other usually giving rise to the 3n endosperm. In gymnosperms one sperm cell generally disintegrates, the other produces a 2n zygote. Figure 1-2 presents a diagramatic comparison of the development of the male gametophyte in the angiosperms and gymnosperms.