

The Institute of Biology's
Studies in Biology no. 107

Pollen and Allergy

R. Bruce Knox



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Professor of Botany
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General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date the Institute of Biology has sponsored this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

The features of the series include the attention given to methods, the selected list of books for further reading and, wherever possible, suggestions for practical work.

Readers' comments will be welcomed by the Education Officer of the Institute.

1979

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Preface

Pollen grains, the structures housing the male gametes of plants, have been associated with man throughout his history. This little book provides an introduction to the life of the pollen grain and, in particular, deals with its dual role in plant fertilization and human allergic disease.

In the last decade, our understanding of the function of the intricately-sculptured outer walls of pollen grains has greatly advanced. They contain informational molecules that act as recognition factors. These determine, on the one hand, acceptance of an appropriate mate by the female stigma for seed-setting, and on the other hand, the onset of seasonal asthma and hay fever in susceptible humans. The mechanisms behind these responses are presented together with an account of pollen dispersal by air currents in city atmospheres and in the pollen load of honey bees.

I thank Professor J. Heslop-Harrison, F.R.S., for first stimulating my interest in pollen, my colleagues, especially Dr A. Clarke and Mrs S. Ducker, for helpful appraisal of the manuscript, Anne Pottage for assistance with Fig. 2-5 and Marj Robertson for secretarial assistance.

Melbourne, 1978

R. B. K.

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1 The Pollen Grain

The pollen grain is the specialized structure which houses the sperm or male gametes of flowering plants. The word pollen was introduced by the great Swedish botanist, Linnaeus, in 1760 and is derived from the Latin root, fine flour, referring to its dry granular nature. It comprises two or three cells combined as a unit, and typically contains approximately 20% protein, 37% carbohydrate, 4% lipid and 3% mineral composition.

1.1 Pollen in history

Man has been aware of pollen throughout recorded history. The earliest records are contained in prehistoric artworks, stone carvings and bricks from the palaces of the Assyrian kings in the Hammurabi period (800 B.C.) and demonstrate early recognition of the sexes in plants. They illustrate mythological giant winged creatures apparently pollinating the female inflorescences of the date palm by shaking the male inflorescences over them. Herodotus, during his travels in Assyria, recognized the dioecious nature of the date palm, but this was later dismissed by Aristotle on the grounds that plants were not motile and had no need for separation of the sexes. His pupil, Theophrastus, had no such delusions, stating that 'the fruit of the female date palm does not perfect itself unless the blossom of the male with its dust is shaken over it'. He concluded that this process of fertilization was not unique to the date, and was likely to occur in all plants.

Theophrastus noted the parallels between this and another ancient practice, the caprifcation of figs. The cultivated fig, *Ficus carica*, has hollow fruits or syconia containing female flowers. For fruit-setting, branches of the wild goat fig (caprifig) are tied onto the trees at flowering. The fruits of the goat fig contain fertile anthers at the top and sterile female flowers below which are hosts for the gall wasp larvae. Pollination is carried out by the gall wasps which emerge from the female flowers within the goat fig fruits and, in order to escape, have to push through the dehiscing anthers which surround the exit pore. They carry the pollen on their body surface to the receptive female fruits of the cultivated figs. Theophrastus' report indicates some understanding of the role of insects in pollination.

Man's interest in pollen at that time, and in the succeeding Dark Ages, was largely for its pharmacological properties. In the sixteenth century, Turner, in his herbal, repeated the Greek Dioscorides' note that the 'flore' (pollen) that is found in the centre of a rose 'is good against the reume or flowing of the gummess'. Little progress in understanding the

real nature of pollen was made until the seventeenth century with the advent of modern scientific inquiry. The sexual nature of the flower was foreshadowed by the English plant anatomist, Nehemiah Grew. Grew aptly described dehiscence of the anther: 'At these clefts, it is that they disburse their powders; which as they start out, and stand between the two lips of each cleft have some resemblance to the common sculpture of a pomegranate with the seeds looking out at the cleft of its rind.' Those who have examined a ripe pomegranate will appreciate his analogy. The presence of furrows on the surface of lily pollen was noted by the Italian biologist, Malpighi, who considered pollen as a mere secretion prior to maturation of the ovary.

The breakthrough in recognizing pollen as the male element in plant life was made by Rudolf Camerarius, a professor at Tubingen. He experimented with anther manipulation and emasculation in species such as dog's mercury, *Mercurialis*, and castor bean, *Ricinus*, and concluded in his book published in 1694: 'in plants, no production of seeds takes place unless the anthers have prepared the young plant in the seed. It is thus justifiable to regard the anthers as male, while the ovary with its style represents the female part . . . the stamens are the male sexual organs in which that powder which is the most subtle part of the plant is secreted and collected.'

This pioneering work inspired others to carry out experiments on pollination. James Logan, governor of Pennsylvania in 1739, set up trials of corn, *Zea mays*, demonstrating that pollen from the tassels travelled in air currents to the cobs. The role of insects in pollination was demonstrated by Joseph Koelreuter, director of the grand-ducal botanic garden at Karlsruhe, in experiments published between 1761 and 1766. He showed that insects may be attracted to flowers by their nectar, which bees used to make honey. Later, Christian Sprengel, rector of Spandau, became so involved in pollination experiments that he was accused of neglecting his parish. He established the role of floral nectaries in producing nectar as an attractant for insect pollination, and noted that nectar-guides, coloured markings on the flower petals, are placed to indicate the sites of nectaries. He made remarkable observations on flowers which imprison and even destroy the insects that pollinate them.

Despite these advances in our understanding of pollination, little progress was made in coming to grips with the mechanism of fertilization. The concepts were rather vague, perhaps because of the limitations in the scientific instruments of the day. Pollen was said to burst on the stigma, releasing its fertilizing granules which found their way through stylar channels to the ovary (Needham, 1740). Koelreuter was more perceptive. He reported that the oily fluid secreted from the surface of pollen mixed with stigma secretion to form a new substance which flowed through the style to the ovule where he believed fertilization occurred.

All these concepts were to change at the turn of the nineteenth century, with the availability of new and more powerful microscopes, which gave

both higher magnification (up to 500 times) and higher resolution. The French artist and botanist, Turpin and an Italian microscopist, Amici, discovered independently in the 1820s the existence of the pollen tube. Amici followed this work with the discovery of the nutritional dependence of the pollen tube on the pistil, in 1846. Its role as the carrier of the sperm to the ovule was demonstrated later by the French botanist Brogniart.

At this time, great progress was being made in elucidating the structure of the pollen grain. Koelreuter, in the mid-eighteenth century, had considered that the pollen grain had a cellular core covered by two coats, the outer being tough and elastic. This was confirmed by Mirbel, who noted the presence of germinal apertures. Fine details of pollen wall structure were obtained by Hugo von Mohl, who introduced sectioning procedures, which superseded the former tearing or macerating methods. He developed a classification of pollen based on the geometrical configuration of the apertures. Working at St Petersburg in 1833, Julius Fritsche produced the first natural classification of pollen based on both morphological and chemical studies of the pollen walls. He named the outer patterned wall *exine*; and the inner smooth wall *intine*, and demonstrated their differential solubility in sulphuric acid. Later, Fischer showed a progression in thickening and complexity of exine structure, and increased prominence and number of germinal apertures in pollen of more highly evolved species.

In the final quarter of the nineteenth century, the last hurdle to understanding the nature of pollen was overcome: the existence of the sperm cells and their origin through the alternation of generations was established. As early as 1842, the apothecary Wilhelm Hofmeister had demonstrated spermatozoa in several lower plants, even before their discovery in animal systems; but it was not until the work of Celakovsky in 1874 and, later, Strasburger that proof of the alternation of generations in flowering plants was obtained. Controversy surrounded the cellular nature of the sperm, which was not resolved until 1965, with the advent of the transmission electron microscope. The remaining landmarks of the twentieth century form the basis for this book and will be considered in the chapters that follow.

1.2 Form of pollen grains

Pollen occurs in a number of shapes, mostly variations of spheres, and in sizes, ranging up to about 300 μm (Figs 1-2, 1-3 and 1-4). Their geometry is determined to some extent by the number and position of the germinal apertures: round to spherical grains having 0-many apertures, long grains having two apertures, triangular (3-sided) grains having three apertures and quadrate (4-sided) grains having four apertures.

The apertures are a major feature of grain morphology. Apertures may be long furrows, pores or a combination of the two structures (Fig. 1-1).

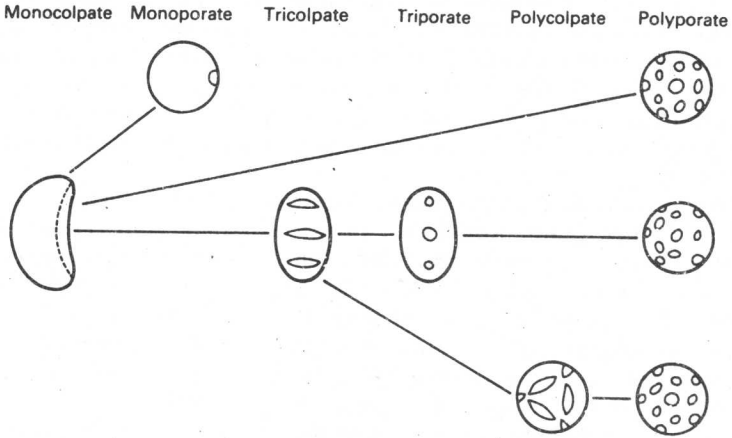


Fig. 1-1 Evolution of pollen shape and type in angiosperms. (Adapted from TAKHTAJAN, A. (1959). *Die Evolution der Angiospermen*. Jena.)

Pores are circular structures, while furrows are defined as having their long axes more than twice their width. Pollen grains show a distinct polarity in organization, with the furrows running longitudinally down through the equator of the grain from the poles at either end. Thus the polar view (from the top or bottom of the grain) gives a distinctly different impression of morphology from the equatorial view (from the sides). In polar view, as determined by the convergence of the furrows towards the pole, the equator is essentially the outline of the grain.

Monocotyledons generally have pollens with a single aperture, usually a broad furrow termed a colpus. In dicotyledons, three, four or five

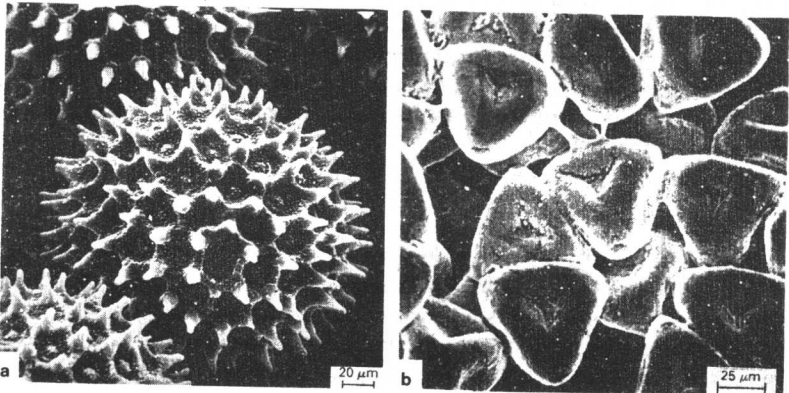


Fig. 1-2 Pollen grain shapes. (a) Morning glory, *Ipomaea*, pollen grain, showing germinal apertures surrounded by spines. (b) *Eucalyptus* pollen grain showing germinal apertures at corners of triangles. Grains are covered with pollenkitt.

apertures are commonly found, and may be pores or a combination of pores with furrows, described as *colporate* (Figs 1-2, 1-3 and 1-4). These are usually radially symmetrical about the equator. Other dicotyledons have many apertures, and in these cases, they are usually pores. Pores are often covered by a lid or operculum of sporopollenin, which is shed at germination. The scheme suggested by A. Takhtajan in 1959 for the evolution of pollen aperture types is shown in Fig. 1-1.

Another dimension of form appears when individual pollen grains adhere together to form polyads or pollen masses of various kinds. Tetrad or polyad grains result when the tetrad of microspores remain in contact after the dissolution of the callose special wall that surrounds them at the end of meiosis. Tetrads of grains are typical of certain

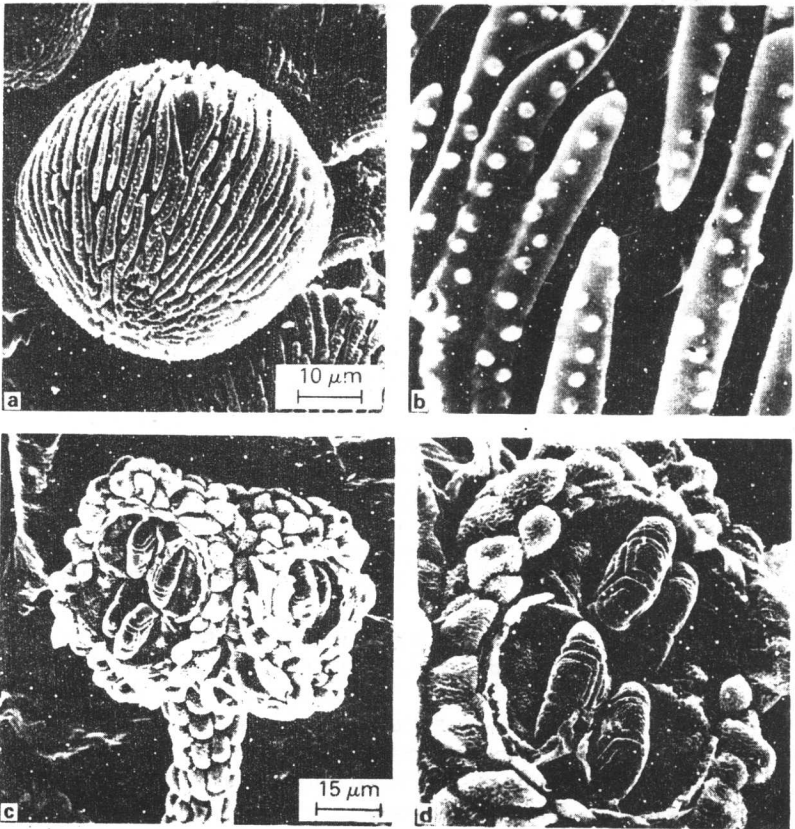


Fig. 1-3 Form of pollen grains. (a-b) Trumpet lily, *Datura*, pollen grain. (a) Shows striate exine surface patterning. One of the three germinal apertures is visible. (b) Detail of exine surface showing porous nexine. (c-d) Anther of *Acacia subulata*, an Australian wattle, showing dehiscence of polyads, each of 16 grains.

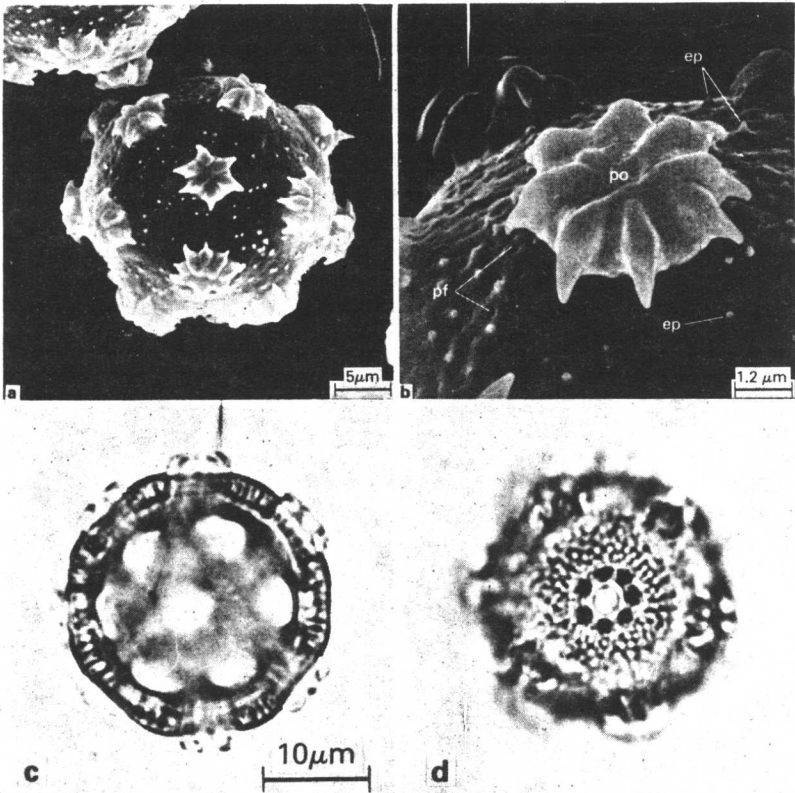


Fig. 1-4 Unusual sculptured pollen grains of *Pupalia lappacea*, Amaranthaceae. The spherical grains have ornamented germinal apertures (po). The exine is ornamented with small spines (ep) and perforated by micropores (pf). (c) and (d) show a surface and equatorial view of the same grains in the light microscope using phase contrast optics after they have been cleaned by acid treatment (acetolysis). (From ZANDONELLA, P. and LECOCQ, M. (1977). *Pollen et Spores*, 19, 119.)

families, for example the heath families, Epacridaceae, Ericaceae, the sundews, Droseraceae, and rushes, Juncaceae, and of certain genera within the Australian families Goodeniaceae and the acacias, Mimosaceae. It is not known why the grains adhere, but in tetrads of *Lechenaultia* (Goodeniaceae) the rods of the exine connect the grains, suggesting that close contact may be established early in development. Monads, dyads, triads and nullads can occur when one, two, three or all the grains are sterile. In the mimosa, *Acacia*, 4, 8, 16, 32 or 64 grains regularly associate in polyads (Fig. 1-3), depending on the number of meiotic tetrads held within segments of the anther.

Pollen grains associate in masses in orchids (Orchidaceae) and

milkworts (Asclepiadaceae). In orchids, an array of pollen associations is found from free individual grains in apostasioid orchids, adhesive masses in the lady's slippers, cypripediloid orchids, and tetrads loosely united by elastic viscin threads of tapetal origin in the orchidoid and neottioid orchids (Fig. 1-5). The orchidoids possess pollen sacs organized into many interconnected packets or massulae. In the neottioids, all the pollen within a sac is associated together into large structures called pollinia which can be easily separated into mealy or powdery masses. In the higher orchids, such as the Ophrydeae, pollinia may be hard masses, each of which is a separate dispersal unit.

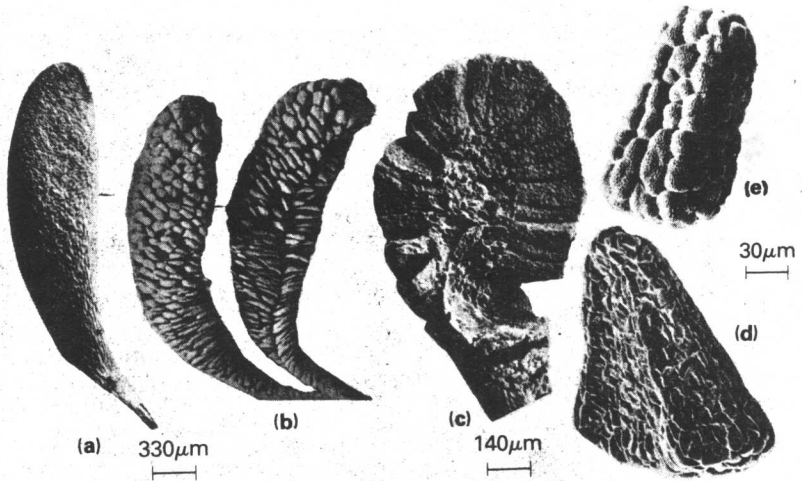


Fig. 1-5 Structure of pollinia and massulae of orchids as seen by scanning electron microscopy in (a) *Spiranthes*, (b) *Haemaria*, and (c) *Ophrys* which all have pollen sacs arranged into discrete pollinia, (d) *Orchis* and (e) *Gymnadenia* where the pollen sacs are aggregated into massulae. (From SCHILL, R. and PFEIFFER, W. (1977). *Pollen et Spores*, 19, 5-118.)

1.3 Pollen-wall structure

The pattern of the exine is determined by the kind of structural elements of which it is composed. There are two basic types of exine structure (Fig. 1-6). In *pilate* types, the rods are surmounted by a prominent knob which may be fused together to form intricate patterns, for example, the net-like exine of lily and crucifers. In *tectate* types, the rods are covered over by a roof which is often ornamented by spines, knobs and other features (Fig. 1-5). The outer covering is perforated by micropores, often concentrated around the base of spines giving access

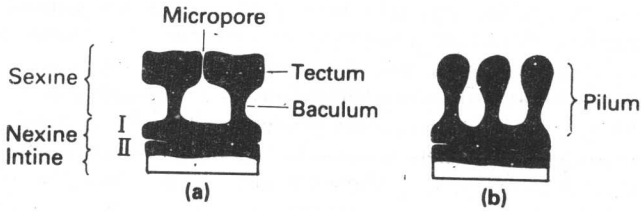


Fig. 1-6 Diagrams of the stratification of pollen walls; (a) shows a wall with a tectate exine and (b) with a pilate exine. (Modified from HESLOP-HARRISON, J. (1968). *Science*, N.Y., 161, 230.

to chambers within the outer exine layer. The chambers within are crypt-like, the tectum being supported by rods sited on a floor layer. In pollen of *Hibiscus* in the family Malvaceae, the floor layer is enormously thickened.

The exine is made of *sporopollenin* which is remarkable for its resistance to both physical and enzymic degradation, and is believed to be produced by the oxidative polymerization of carotenoid pigments and carotenoid esters. The exine covers the pollen grain, except at the germinal apertures where it is usually absent or much reduced. It is present in most terrestrial plants but may be reduced to a thin membranous structure or even absent in some aquatic monocotyledons.

The inner wall layer, the intine, is smooth and does not contribute to pollen patterning but is exposed to the surface at the germinal apertures where it may be thickened and more complex in structure. Like the primary cell wall of somatic cells, it consists of cellulose microfibrils and a matrix of hemicelluloses, pectic polymers and proteins. It is present in all pollens and is often enormously thickened. The intine is developmentally quite distinct from the exine: it is laid down much later – at the early vacuolate period (see § 2.3).

1.4 Pollen analysis and the history of vegetation

The exine layer is endowed with all the patterning attributes remarkable to pollen which may be specific to family, tribe, genus or species. It is possible to relate pollen fossils preserved in geological deposits to living plant pollens (Fig. 1-6), providing valuable clues to the history of plant life in past geological eras. Fossil pollen deposits are dated by use of standard palaeontological procedures. In the case of pollen preserved in the relatively recent Quaternary peat deposits, radiocarbon dating is used. Measurements can be related to age within the past 30 000 years with some accuracy. Sediments which are geologically much older, are deposits of materials that originated elsewhere but have been deposited at the sampling site. Pollen grains within the sample can be extracted, identified and counted. The data, when assembled into pollen

diagrams, gives an impression of the relative frequency of the genera or groups in the flora of the period, subject to obvious limitations that only the airborne pollen of wet environments, such as swamps, bogs and lake beds, tends to be preserved in large numbers in the fossil record. Pollen is preserved in such environments because they provide anaerobic conditions restricting oxidation of the sporopollenin. Not only do the results provide information about the vegetation of a particular area, but also about climatic changes that have occurred. An excellent review of the history of the British vegetation has been given by GODWIN (1975) and for central Europe by STRAKA (1975).

The principles used to identify fossil pollens are particularly interesting. Palynologists depend upon matching unknown fossils with present day reference pollens, making the important assumption that there have been no evolutionary changes in exine structure. The older Tertiary fossils present problems, since some are of extinct types, while others can still be related to a specific lineage (Fig. 1-6). For purposes of classification, fossil grains are given their own nomenclature, with their own generic and specific epithets. The antarctic beech, *Nothofagus*, becomes in fossil form *Nothofagidites*. The mimosa, *Acacia* becomes *Polyadopollenites*, named after its polyad pollen structure. In Australia, fossil eucalypt-like pollen is called *Myrtaceidites eucalyptoides* and has proved to be almost indistinguishable from other modern genera of Myrtaceae such as the apple myrtle, *Angophora*, the turpentine tree, *Syncarpia*, and the New Zealand pohutukawa or rata vine, *Metrosideros*.

The time ranges for various pollen grains in the fossil record are shown for the island continent of Australia in Fig. 1-7. Angiosperms first appear in the Lower Cretaceous, but it is not until the Upper Cretaceous that the first angiosperm pollen grains can be confidently identified. Many types of pollen make their debut in the Paleocene and Eocene, and fewer in the Oligocene and Miocene. The daisy family, or Compositae, makes its first appearance in the Miocene in Australia and elsewhere in the world. This huge family has worldwide distribution in nearly every ecological niche and has used almost every life form, yet it was apparently one of the last to evolve (Fig. 1-7). In Australia, one of the earliest pollen types to appear is that of the fossil *Nothofagidites*, which is recorded at low levels in the Paleocene and mid-Eocene, and in abundance in late Eocene and Miocene records. The late Miocene and Pliocene are notable for a fall in *Nothofagidites*, and are dominated by Myrtaceae. Later, in the Pleistocene, there is a rise in herbaceous plants such as grasses and Compositae.

These changes in vegetation can be related to what is now known of the history of continental geography - the movements of the continents over the earth's surface. Australia was connected to Antarctica until the Mid-Eocene time, which may account for the abundance of antarctic beech, *Nothofagus*, in the pollen assemblages until this period. Subsequently, Australia came into collision with the South-East Asian plate in mid-Tertiary, and changes are reflected in the fossil record, which provide

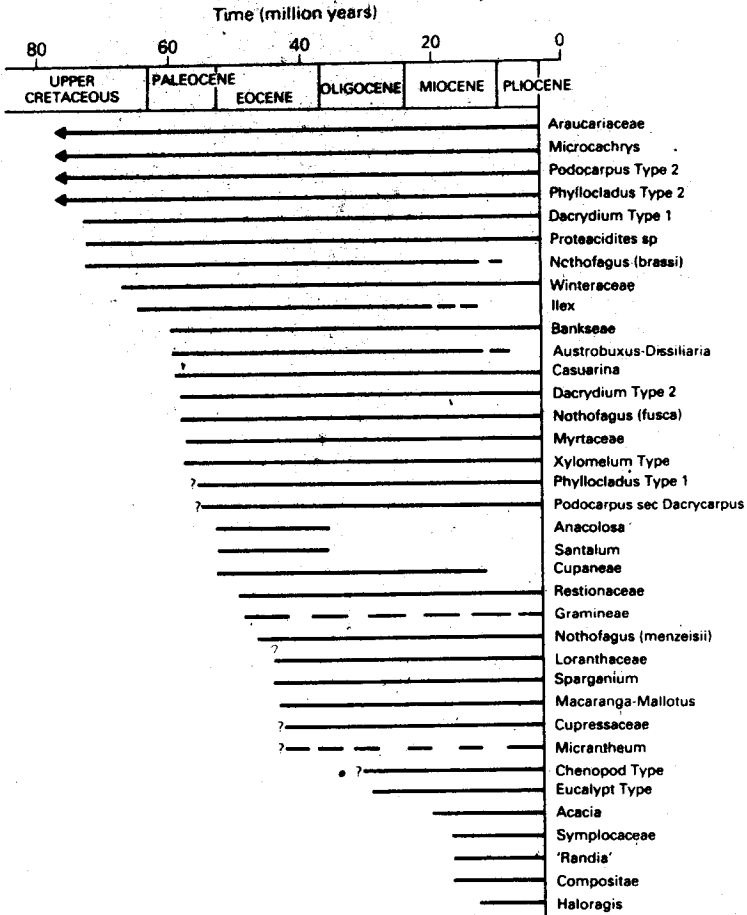


Fig. 1-7 Appearance of fossil angiosperm pollen during the Pleistocene period in Australia. (Adapted from MARTIN, H. (1978). *Alcheringa*, in press.)

important evidence for transcontinental migration of vegetation. H. Martin in Sydney recently considered that two forms of migration have occurred: (i) dispersal under favourable conditions; and (ii) climatically-controlled migration. In Australia, there is palynological evidence for a withdrawal from the central region, now desert, to the more climatically-favourable east coast; and also for a northward dispersal or withdrawal towards Queensland of present-day subtropical plants; and finally a withdrawal southwards to Tasmania seen, for example, in the present occurrence of the Antarctic beech, *Nothofagus gunii*. Fossil pollen data provides important evidence for such migration and the climatic control of plant evolution.

2 Formation of Pollen

Pollen is an integral part of the life cycle of flowering plants which is founded upon an alternation of two generations, as in lower plants. The dominant generation is the diploid vegetative plant body – the sporophyte. It bears haploid spores, single cells that produce either male or female gametes within structures called gametophytes. The pollen grain is the male gametophyte. At maturity, it may contain two, or in some cases three, cells. In about two thirds of flowering plant families, the pollen grains are shed from the anther in the two-celled condition (Fig. 2-1). One is destined to divide after germination to produce the two sperm cells, and the other to regulate pollen function. In other families, the sperm cell division occurs in the maturing pollen grain so that the pollen grains are three-celled (Fig. 2-1).

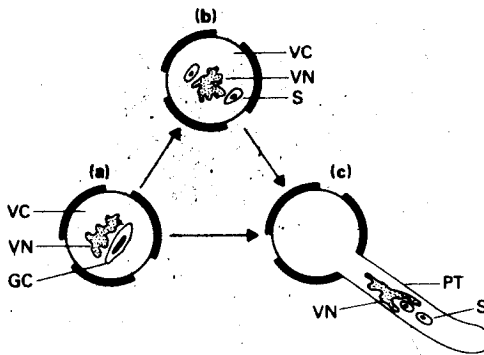


Fig. 2-1 Diagram showing mature binucleate (a) and trinucleate (b) pollen grains. The binucleate grains become trinucleate during germination (c). VC, vegetative cell; GC, generative cell; VN, vegetative nucleus, S, sperm cells. (Adapted from DEXHEIMER, J. (1970). *Rev. Cytol. et Biol. vég.*, 33, 169-234.)

Pollen is formed within the anther, which shows considerable variation in form but typically is an elongate structure containing four pollen sacs or loculi. The anther wall contains four layers (from outside): the epidermis, middle layer, tapetum and sporogenous cells (Fig. 2-2). The epidermis and middle layer are important for protection of the developing anther. The middle layer usually differentiates into the endothecium, with massive bar-like wall thickenings at maturity which are concerned with anther dehiscence. The tapetum is a nurse tissue surrounding the sporogenous cells, and there are two characteristic

forms: parietal or secretory types in which the cells remain in position around the anther cavity; and amoeboid or plasmodial types where the cells become invasive, their cell walls breaking down so that the cytoplasm and nuclei make intimate contact with the developing microspores.

2.1 Meiosis and its consequences

Meiosis comprises two periods of cell division, which have important genetic consequences.

(i) Reduction of chromosome number by half, allowing for subsequent fusion of gametes to restore the original number.

(ii) Genetic recombination resulting from crossing over of chromosome segments ensuring that every microspore is different from its sibling, and that new variation may be expressed in the progeny.

The cytology and genetics of meiosis are well established (KEMP, 1970) and it is the cell biology of the events that is new and exciting. Much of the pioneering work in biochemical and electron microscopic aspects has been carried out by J. Heslop-Harrison, now at the Welsh Plant Breeding Station, Aberystwyth, and H. G. Dickinson at the University of Reading, England. They consider that meiosis 'is correlated in time with one of the most dramatic developmental changes known in plants, the transition from the sporophytic to the gametophytic phase of the life cycle. The sporophyte is the more elaborate of the two generations in morphology and biochemical capacities, and no doubt we see in it the expression of the greater part of the potentialities of the genome. The male and female gametophytes possess an independent metabolism, but they are highly reduced in morphology and range of functions, suggesting that a large part of the genome is repressed during this phase of the cycle. This repression must be imposed by some event – or sequence of events – occurring in the meiocytes or in the early life of the spores they produce. Furthermore, diplophase information which might interfere with the development of gametophytic functions must presumably be expunged during the same period before the cytoplasm becomes host for the gametophytic nuclei after the quadripartition of the meiocyte.' This is achieved by changes in the cellular organelles, reflecting a reprogramming or reorganization within the gametophytic cells; and by changes in structure and permeability of the cell walls during the sporophyte-gametophyte transition that initially enhance then inhibit communication between them.

The changes begin during early prophase of meiosis I, where in the anthers of lily there is a fall of up to 50% in total cellular RNA by pachytene, the period when chromosome pairing and crossing over becomes cytologically detectable. The adenine/guanine base ratio of the RNA alters, indicating a change from ribosomal RNA to a residual RNA associated with the chromosomes. Electron microscope studies of the pollen mother cells have revealed the elimination of a major part of the