Pollen Analysis.

Background, Laboratory Techniques and Identification

Abstract

The historical background of pollen analysis is given in a short review. Preparation methods used for different kinds of samples are listed and described. Preparation of slides and analytical work is briefly surveyed. Some of the parameters used at the identification of pollen and spores are explained.

Historical background of pollen analysis — a short review

Already in 1885 the Swiss geologist Früh described pollen and spores observed in peat samples. A Swedish zoologist Trybom, studied bottom samples from lakes in 1888 and concluded that pollen grains are very resistant to decay and can serve as index fossils in palaeontology (Erdtman, 1943). But the birth of pollen analysis can be dated to 1916, when a meeting of Scandinavian scientists was held in Kristiania (Oslo), Norway. The Swedish geologist Lennart von Post presented his paper on tree pollen in bogs (fossil pollen identified in peat) (von Post, 1918).

The method to count pollen grains in samples taken at different layers in a stratigraphy was elaborated by Gustav Lagerheim, von Post's teacher. However, von Post transformed the analysis method into a refined dating instrument for Quaternary geologists. Although Lagerheim can be considered the spiritual father of pollen analysis he never published his results.

Von Post was the first one to construct pollen diagrams giving a visual picture of the vegetational development as reflected by changing pollen spectra at different levels in a stratigraphy (von Post, 1918). He soon realized that the first occurrence of e.g. pollen of spruce, one of the latest immigrating tree species in Sweden, could be used as a dating level in pollen diagrams. The same horizon was traced in other bogs and the stratigraphy could be dated by means of pollen analysis.

Palynology is the study of pollen grains and spores, describing their construction, function and medical properties. Pollen analysis has later passed many stages and developed into the excellent instrument for palaeoecological research, which it is today.

Some of the most important scientists of palynology and pollen analysis may be mentioned: During more than 30 years of palynological research, Gunnar Erdtman built up the Palynological laboratory in Stockholm, Sweden, with an extensive reference slide collection containing modern pollen and spores of plants from all over the world. In the 50ies, 60ies and 70ies many palynological research laboratories were initiated around the world, inspired by Erdtman's work. Erdtman published several basic text books on pollen analysis and palynology. Pioneer investigations with different applications of pollen analysis for example in archaeology have been carried out by Knut Faegri, Norway, Johs Iversen and Jørgen Troels-Smith, Denmark.

Preparation methods

References used are: Faegri and Iversen, 1975; Påsse, 1976; Moore and Webb, 1978; Björck *et al.*, 1978; van der Kaars and Smit, 1985; Berglund and Ralska-Jasiewiczowa, 1986.

Preparation of soil samples is carried out in order to concentrate pollen and spores, and facilitate analysis. The purposes are: deflocculation/disintegration and removal of «humic acids» (= organic colloids), removal of extra organic and minerogenic matter, (staining and) mounting in fluid or solid medium.

Depending of sample composition, different preparation methods or combination of methods are used for peat, sediments (gyttja, clay), rock samples or modern reference material. (Preparations of recent reference material and rock samples are not discussed in this paper).

Method: author	suitable for:
Potassium hydroxide, von Post's field method	organic samples rich in pollen: peat, gyttja
Acetolysis, Erdtman	»
Sieving field method, van der Kaars and Smit	coal, lignite, sand, silt, clay rich organic matter
Hydrofluoric acid (HF), Assarson and Granlund	minerogenic material: gyttjaclay, clay
Sedimentation-separation, Påsse	minerogenic samples poor in pollen: clay, silt, till

The main steps to concentrate pollen and spores are: (Fig. 1)

1. Adding exotic pollen or spores (markers) for concentration calculations (Stockmarr, 1971);
2. Eliminate calcium carbonates — HCl;
3. Deflocculation/dispersion, removal of « humic acids » — KOH/NaOH;
4. Remove coarse organic debris — sieving;
5. Remove mineral grains — HF/sedimentation — separation;
6. Eliminate extra organic matter (cellulose) — acetolysis;
7. Staining (can be excluded);
8. Mounting (in glycerol or silicone oil).

It can be pointed out that many laboratories have elaborated their own small variations of the separate steps in the preparation schemes.

von Post's field method

a. 2-3 mm^3 of the sample is put on a slide;
b. 5-10 % KOH is added;
c. heat over a spirit flame, add water during boiling;
d. some drops of glycerine are added and the concentrated sample is covered.

van der Kaars and Smit's field method

(Requires more equipment brought into the field, than the above described method).

a. crush the sample if necessary (coal, lignite) and sieve through a tea-strainer;
b. ca 20cc of crushed sample is suspended in 10 % sodium pyrophosphate;
c. stir sample and let it settle for about 30 min.;
d. sieve through 7 microns screen (repeat if necessary);
e. wash with distilled water and then with alcohol, centrifuge;
f. stain and mount.

Erdtman's acetolysis method

Used on organogenic deposits in which coarse plant remains (root-lets, mosses, cellulose) should be removed.

a. deflocculation (KOH), and removal of coarse plant remains by sieving;
b. deshydration with glacial acetic acid;
c. heating in acetolysis mixture consisting of 9 parts anhydric acetic acid and one part conc. sulphur acid;
d. washing with glacial acetic acid;
e. washing with distilled water;
f. staining and mounting.

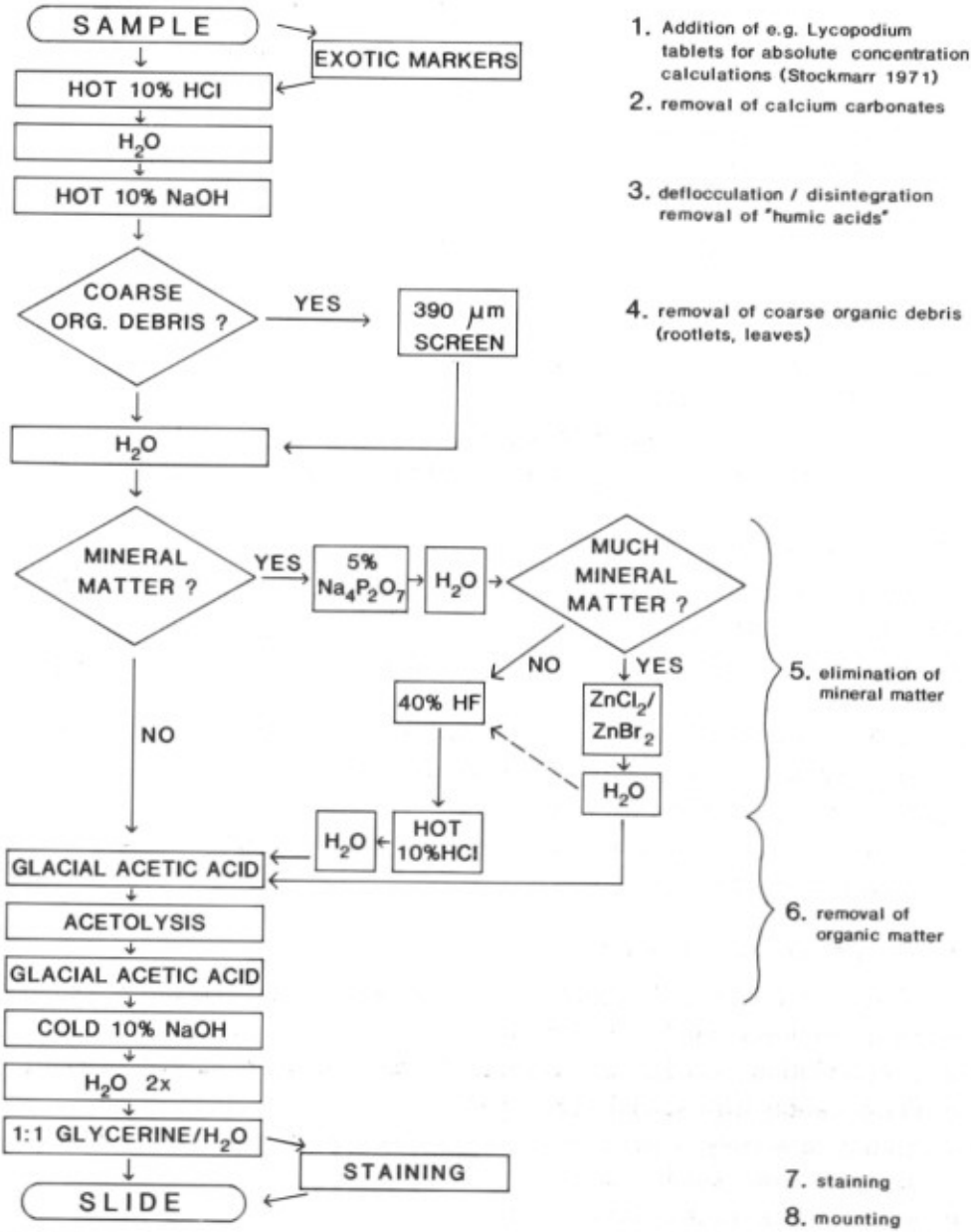

Fig. 1. Laboratory techniques for pollen preparation (modified after Berglund and Ralska-Jasiewiczowa, 1986).

Assarson and Granlund's hydrofluoric acid (HF) method

Used on minerogenic samples like clay, gyttja clay, silt to remove mineral particles before the acetolysis is used (see above). Coarse mineral grains (e.g. sand particles in drift peat) are removed by suspension in water and decanting.

a. removal of calcium carbonates — HCl;
b. deflocculation — KOH/NaOH;
c. hydrofluoric acid is added. The samples can either be heated in a water bath or left standing in cold HF for 2 days to remove mineral particles e.g. silica;
d. after the HF-treatment, acetolysis is carried out.

Sedimentation-separation method

Minerogenic samples like clays, boulder clays (tills) expected to contain low or very low pollen concentrations may alternatively to the HF-method be treated according to a *sedimentation-separation method* (Påsse, 1976; Björck *et al.*, 1978).

a. the clay particles are removed by suspension in 5 % sodium pyrophosphate, left for two hours, when pollen and spores sink to the bottom of the cylinder;
b. the clay particles in the suspension are decanted;
c. repeat suspension and decanting;
d. separation of coarse mineral grains with a heavy liquid (bromoform, zinc bromide, zinc chloride) of density 2.0-2.3 (mineral grains have a density over 2.6, pollen grains 1.3-1.7);
e. treatment according to the acetolysis method.

The advantages of the sedimentation-separation method are:

1. highly concentrated clean samples, which facilitates identifications;
2. no treatment with HF is necessary;
3. silicous microfossils such as diatoms, sponge spicules, cysts are not dissolved and can be noted in the slides together with pollen and spores.

Oxidation

Only used if there is lignine present in the samples, and carried out after the acetolysis method has been applied.

Oxidizing agents are chloric oxids, nitric acid or hydrogen peroxide. Oxidation clears microfossils which have darkened.

Preparation of slides and microscopical work

To facilitate identification of pollen and spores, they may be stained by adding fuchsin or safranine. Mounting media used can be either solid or

fluid, or both, e.g. solid: glycerine jelly; liquid and solid: silicon oil; liquid: glycerine.

The use of a solid medium like glycerine jelly has two disadvantages: the pollen grains will swell after some time, and the pollen grains are fixed in their positions and cannot be moved for identification. The advantage is that the slides can be stored for a long time.

Silicone oil is used preferably when size measurements on pollen grains are going to be carried out, because they do not swell in this embedding medium. The most common method is to mount the material in glycerine, where the pollen grains are kept in a liquid medium and can be turned around for identification.

The analytical work is carried out in the following way:

a. the slide is preliminarily checked if pollen is present and if the amount of material (concentration) is convenient for analysis. The pollen concentration should not be to high as small pollen grains tend to cluster along the edges of the cover glass, and big pollen grains (saccate pollen) will remain in the centre of the slide. If only a few traverses are crossed at the analysis there may be statistical errors;
b. a magnification of x 300-500 is recommended. By closer examination x 1000-1200 oil immersion lenses and phase contrast equipment should be used;
c. depending of the aim of the study, 500-2000 pollen grains of terrestrial plants are counted at the different levels in the sampled sequence;
d. the pollen and spore types observed as well as other organic remains, such as coal particles and algae, are noted in a form, where also name of the site, type of material, sample number, sample depth, preparation method used and analyser should be recorded.

Pollen and spores — morphology, taxonomy and identification

In the descriptions below the terminology used principally follows Faegri and Iversen. Their *Textbook of Pollen Analysis* (Faegri and Iversen, 1950, 1964, 1975) together with *An Illustrated Guide to Pollen Analysis* (Moore and Webb, 1978) are recommended as handbooks. The terms used in palynology have been compiled and explained in special publications (Iversen and Troels-Smith, 1950; Kremp, 1965).

Palynology (palynos = Greek word for fine flour) is the science of pollen and spores. The pollen grains are formed in the anther, where each pollen mother cell gives rise to four pollen grains. When the grains are liberated, they separate, but in some species they remain fixed together as

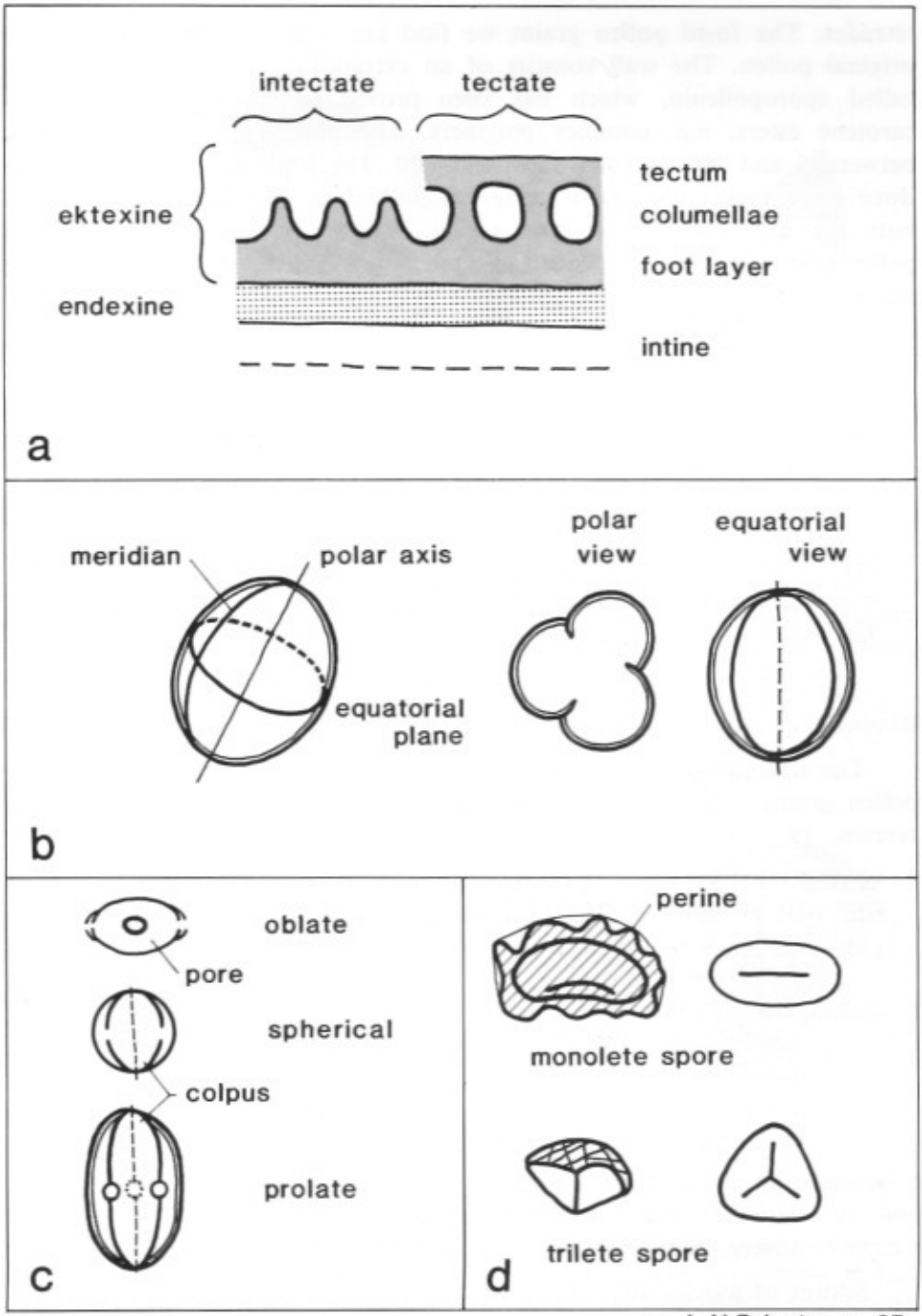

Fig. 2. Descriptional terms used in palynology (after Faegri and Iversen, 1975). a: Terminology of the pollen grain exine. *b: positional designations. c: The three main shapes of pollen grains in equatorial view. d: Spore types.*

tetrades. The fossil pollen grains we find are only the outer walls of the original pollen. The wall consists of an extraordinarily resistant substance called sporopollenin, which has been proved to contain carotenes and carotene esters, e.g. complex polymers. The pollen grains range in size between 5 and 200 microns, mostly 10-100. The fresh pollen grain includes three main parts: innermost living cell and inner wall layer, *intine*, which both are not fossilized, and outer wall layer *exine*, comprising the fossil pollen grain. The *exine* is divided into two layers called *endexine* and *ektexine* according to Faegri and Iversen.

The *ektexine* has a three-layered structure. As seen in Fig. 2a there is a lowermost foot-layer covered by a more or less dense carpet of small granules called *columellae*. The *columellae* are in some cases fused together as a roof or *tectum*. The pollen grains can according to their possession of a roof or not be *tectate*, *intectate* or *semitectate*.

The positions of different features on the pollen grain are described with terms of a sphere or ellipsoid e.g. poles, polar axis, polar view, equator, equatorial plane, equatorial view, meridian and so on (Fig. 2b). The outer shape of the grains varies, the main types being spherical, compressed — oblate, and long, drawnout — prolate (Fig. 2c).

Two types of apertures exist, through which the pollen tube emerges. Round apertures are called *pores*, and furrows are named *colpi*.

The sculpturing of the *exine* is very important at the identification of the pollen grains. Three main groups can be distinguished: (after Faegri and Iversen, 1975, Table 1, see also Fig. 3).

a. sculpturing elements are very small and do not appear clearly, the surface seems to be smooth:
   psilate (Fig. 3b, d, f)
   perforate, foveolate;

b. sculpturing elements of equal breadth and height:
   scabrate (Fig. 3g, j)
   verrucate (Fig. 3e)
   echinate (Fig. 3i)
   gemmate, baculate, clavate;

c. sculpturing elements elongated:
   reticulate (Fig. 3a, h)
   striate, rugulate.

Spores of mosses and ferns have a different wall construction compared to the pollen grains. The spore wall has no layer containing *columellae*. Ferns (bracken) have an outer layer called *perine*, which is easily lost, and thus often missing on fossil spores. The aperture is either *monolete* — a straight furrow, or *trilete* — a three-parted furrow (Fig. 2d).

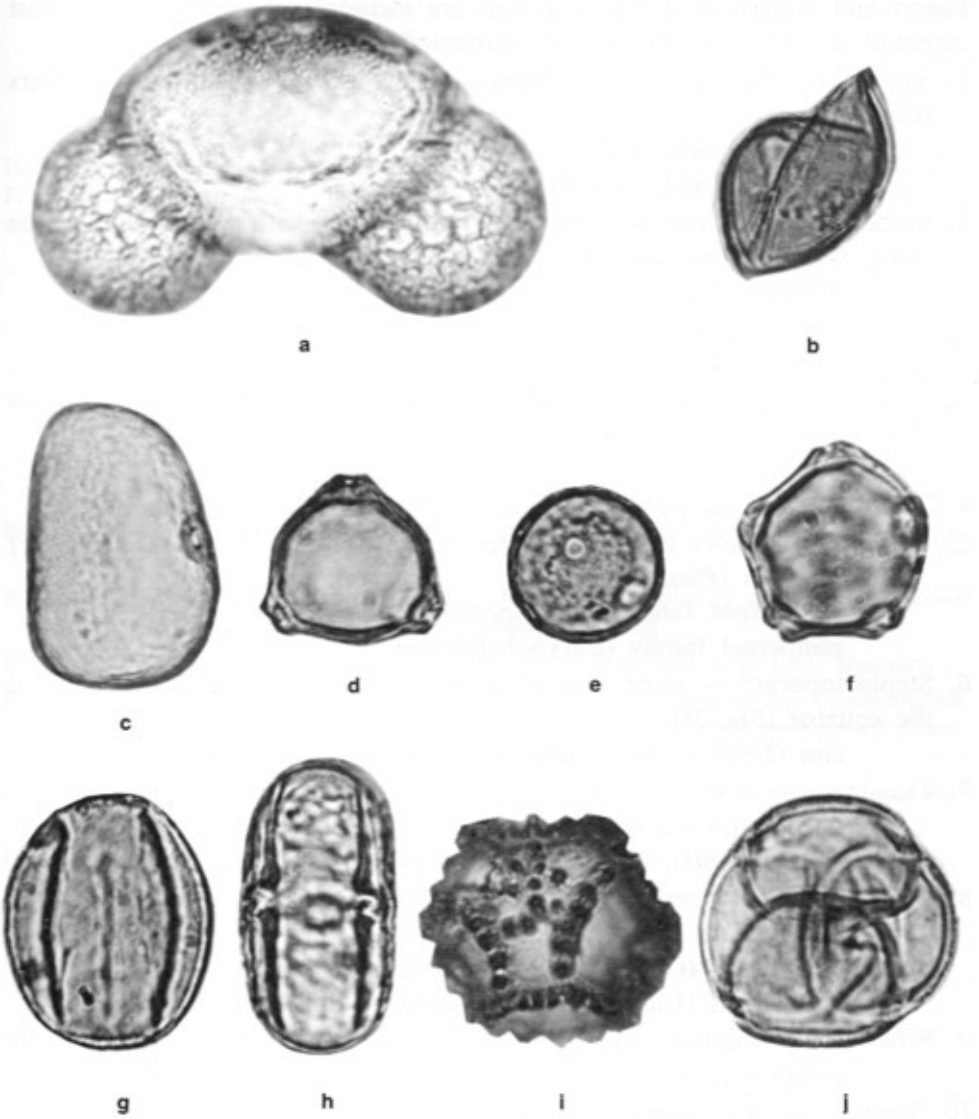

Fig. 3. Pollen types representing ten different groups in the identification key by Faegri and Iversen (1975). (a) vesiculate, saccate — Pinus *(pine); (b) inaperturate* — Juniperus *(juniper); (c) monoporate* — Secale cereale *(rye); (d) triporate* — Betula *(birch); (e) periporate* — Plantago *(plantain); (f) stephanoporate* — Alnus *(alder); (g) tricolpate* Quercus *(oak); (h) tricolporate — Leguminosae (leguminose); (i) fenestrate — Compositae liguliflorae (composite); (j) tetrad — Ericaceae (heather). All micrographs in light microscope at x 1000.*

The pollen keys used for identification are based on the number of apertures and their position on the surface of the grain. In the pollen key of Faegri and Iversen 24 different groups are included, of which the ten most common are described below and illustrated in Fig. 3.

1. Saccate or vesiculate pollen grains — a central body with two bladders (Fig. 3a)
   conifers, pine (*Pinus*),
   spruce (*Picea*), fir (*Abies*)
2. Inaperturate — no obvious openings but provided with a weak zone of the *exine*, where the pollen grain splits or breaks (Fig. 3b)
   Juniper (*Juniperus*), yewtree (*Taxus*)
   sedges (Cyperaceae, on which false apertures *lacunae* occur)
3. Monoporate — one pore, surrounded by a thickening, called *annulus* (Fig. 3c)
   grasses (Gramineae)
4. Triporate — three pores, birch (*Betula*), hazel (*Corylus*) (Fig. 3d).
5. Periporate — more than three pores distributed over the surface (Fig. 3e)
   plantain (*Plantago*),
   goose foot family (Chenopodiaceae),
   pimpernel family (Caryophyllaceae)
6. Stephanoporate — more than three pores (usually 4-6) arranged around the equator (Fig. 3f)
   elm (*Ulmus*), alder (*Alnus*), hornbeam (*Carpinus*)
7. Tricolpate — with three colpi (Fig. 3g)
   oak (*Quercus*), ash (*Fraxinus*),
   willow (*Salix*), buttercup family (Ranunculaceae)
8. Tricolporate — three colpi and three pores (Fig. 3h)
   lime (*Tilia*), beech (*Fagus*),
   composites (Compositae tubuliflorae),
   umbellifers (Umbelliferae), leguminose family (Leguminosae)
9. Fenestrate — windows arranged in a geometrical pattern, echinate (Fig. 3i)
   composites (Compositae liguliflorae)
10. Tetrads — four pollen grains sticked together, each grain (Fig. 3j)
    tricolporate or monoporate, heather (Ericaceae),
    reed mace (*Typha latifolia*)

Finally it should be stressed that the use of identification keys must be supplemented with studies of reference slides to achieve good and reliable results. Illustrated pollen floras can also be studied as a complement, e.g. Beug, 1961; Erdtman *et al.*, 1961, 1963; Florin, 1969; Moore and Webb, 1978; Ciampolini and Cresti, 1981; Nilsson (*ed.*), 1973-1987 and many others.

Periodicals containing palynological studies are above all *Pollen et Spores, Grana* and *Review of Palaeobotany and Palynology.*

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