CHAPTER 4

POLLEN MORPHOLOGY
INTRODUCTION

The contemporary history of pollen morphology virtually started with the work of Prof. R.P. Wodehouse of USA, and as embodied in his book “pollen grains”, published in 1935. His work on pollen morphology has been a part of his aerobiological investigations, and thus he placed concentrated attention on the plants contributing to pollen flora of the air. However his concepts and observations fully reveals the depth of his thoughts and knowledge of pollen morphology. Wodehouse (1935) observed that the origin and development of the pollen morphoform are related to phyletic and other factors such as the external and internal environment. For example, the occurrence of pollen differences in closely related species of a genus is expressive of the phyletic phenomenon, the difference in the number and arrangement of furrows in related species are attributed to the internal factors such as the contact stimuli and the morphological differences in pollen are the direct consequence of the influence of external environment. However it has been pointed out that the various factors interact and function uniformly to give shape to the final morphological form of pollen.

Faegri and Iversen (1964) has categorized pollen grains into several morphological types on the basis of various characters, such as the ornamentation of exine, number and distribution of apertures, size and number of grains in the compound group etc. In general, there are five morphological categories in the pollen (spore) walls, resolved into three phylogentic groups namely (i) Primary (most conservative): Aperture ; (ii) Secondary (less conservative): Exine surface ornamentation; and (iii) Tertiary (least conservative): exine (wall) strata, shape and size.

A systematic combination of the various morphological characters make the pollen of a particular taxon entity by itself and that is how the pollen grains form a useful index in plant taxonomy & in analytical studies such as aerobiology (Nair et al, 1986).

Fernandes and Dahl (1952), demonstrated the importance of electron microscopy in gaining knowledge on the fine structure of pollen walls. An array of workers took
interest in the field, among them the name of Rowley (1959), Ehrlich (1958), Larson (1964), Erdtman & Dunbar (1966) may be mentioned. The results of these investigations have been reviewed by Bradley (1960), Heslop-Harrison (1963), and Van Campo et al (1966-67).

In the present chapter, morphology of the common pollen types recorded and identified from the Greater Silchar area are discussed on the basis of light microscopic and Scanning Electron microscopic observations.

MATERIALS AND METHODS

CHEMICALS REQUIRED:

1. Glycerene jelly (Erdtman, 1952):
   - Gelatin – 50g
   - Glycerine – 150ml
   - Phenol crystal – 7g
   - Distilled water – 175ml

2. Ethanol

PREPARATION OF REFERENCE POLLEN SLIDES:

The polliniferous materials from the dominant plant species were collected during their respective pollen seasons from southern part of Assam. To procure pure pollen, undehisced inflorescence/flower were collected and processed. The flowers after removal of extraneous parts were placed on a glazed brown paper kept in trays and dried at 35°C for 2-3 days in a vacuum oven. The dried inflorescence was crushed gently and the pollen grains thus released were sieved through 100, 200, 300 mesh/cm³ sieves. Pollen thus obtained was studied for their purity by light microscope. To assess the purity of pollen samples used for the examination of antigens, microscopic examination of all the pollen samples were carried out.
according to the method outlined by Cour & Loublier (1980). About 10 mg of pollen was placed in a centrifuge tube containing 2ml ethanol: glycerol solution in 9:1 ratio. After thorough shaking, the samples were centrifuged at 3000 rpm for 10 min. The supernatant was removed. The residue was then mixed thoroughly with a glass rod. A drop of suspension was obtained and was mounted on a micro slide with a drop of glycerene jelly. The slide was scanned under different microscopic fields for designed pollen, plant parts, contaminated pollen, dust particles and fungal spores. Percentage purity of pollen was calculated based on more than 1000 particle count. Pollen samples having purity greater than 95% only were processed for further experimentation.

Preparation of reference pollen slides were made following the method adopted by Wodehouse (1935). A small amount of above processed pure pollen was placed at the centre of a clean microscopic slide. One or two drops of alcohol (95%) was added over it, and then the oily and resinous substances were wiped off along with the excess alcohol with a tuft of twisted cotton. While the pollen grains still moist, sufficient amount of glycerin jelly (stained with safranin) was added. The pollen was stirred with a needle until evenly distributed on the slide. Then a slide cover was placed on the slide. Slides were labeled, allowed to dry, sealed with Canada balsam and kept flat in a slide cabinet for future use as reference. Identification was done with the help of the literature available (Faegri & Iversen, 1964 and Nair et al, 1986).

**METHOD FOR PREPARATION OF GLYCERINE JELLY:**
Glycerine jelly was prepared following the Erdtman’s method (1952). 150ml of glycerine and 175ml of distilled water were thoroughly mixed in a one litre beaker and heated in water bath for 2-3 hrs. While heating the glycerine solution, 50g of gelatine was added slowly with continuous stirring of the mixture with a clean glass rod to avoid clumping. After complete dissolution of the ingredients, 7g of phenol crystals were added as preservative. Then saturated solution of safranin was added drop by drop in the molten mixture till the mixture turned dark red. The fluid
was than filtered. The glycerine jelly thus prepared can be used as mountant after the preparation of permanent pollen slides.

METHOD FOR SEM PHOTOGRAPHY:
SEM photography was done in the Scientific Instrumentation Centre of Burdwan University, West Bengal.
A small amount of samples were placed on the stubs coated with double sided tape. The stubs were than placed in the IB2 Ion coater (used for gold coating) & the Ion coater was run for 5 min. 200 amston gold coating per sample has been found to be sufficient for SEM study. Then, the stubs were inserted inside the SEM chamber and the instrument was run for 15min to evacuate the air. After creating sufficient vacuum inside the chamber, the SEM photography was done. Photography was done at 500X to 8000X magnification.

KEY TO IDENTIFICATION OF POLLEN GRAINS
The following key is purely artificial and it helps only in differentiating the pollen grains on the basis of its aperture, exine structure, arrangement of spine, etc. Identification of all the important morphological characters of all the common pollen types of Greater Silchar area was done by using the reference pollen slides. The NPC system (Erdtman, 1969) was followed in characterizing the pollen type. Each pollen was identified upto its lowest taxonomic rank position.

Pollen grains in polyads
Exine granular

<table>
<thead>
<tr>
<th>Pollen grain (µm)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>36-37</td>
<td>Acacia auriculaeformis</td>
</tr>
<tr>
<td>65-70</td>
<td>Albizia lebbek</td>
</tr>
<tr>
<td>45 x 80</td>
<td>Pithecellobium saman</td>
</tr>
</tbody>
</table>
8 celled, exine psilate  

Pollen grains free

Aperturate

Aperturate simple

Colpate

1-Colpate

Exine psilate  

Exine granulate  

3-Colpate

Exine reticulate

Pollen 27\mu m in dia  

Pollen 23 X 30\mu m in dia  

Pollen 20-25\mu m in dia.

Exine psilate  

Exine verrucate

Pentacolpate

Exine psilate  

Exine reticulate

Mimosa pudica

Areca catechu

Cocos nucifera

Lantana camara

Argemone maxicana

Trewia nudiflora

Clerodendrum viscosum

Erythrina indica

Melastoma sp.

Liliaceae
Porate

1-porate

Exine granulate/psilate ...... Gramineae
Exine psilate ...... Cyperaceae

Pentaporate

Exine psilate ...... *Amaranthus spinosus*
Exine spinulate

Pollen 135μm in dia. ...... *Hibiscus rose-sinensis*
Spines longer with round end ...... *Ipomea carnea*
Exine retipilate ............... *Polygonum sp.*

Aperture composite

3-colporate

Exine psilate

Pollen 25μm in dia. ...... *Mangifera indica*
Pollen 60-70μm in dia. ...... *Delonix regia*
Exine spinulose

Pollen 30-35μm in dia. ...... *Helianthus annus*
Pollen 30μm in dia. ...... *Gynura nephalensis*
Exine granular

Pollen 30μm in dia. ...... *Cassia sophera*
Zonocolporate

3-zonocolporate

Exine reticulate

Pollen 30μm in dia. .... Moringa oleifera
Pollen 30 x 35 μm .... Cassia fistula
Pollen 20-25μm in dia. .... Cleome gynandra

Exine psilate ................. Cassia alata
Pollen 15 x 15 μm .... Terminalia arjuna

Exine granulate

Pollen grain 70 x 30μm.... Adathoda vasica
Pollen 30-35μm in dia. .... Ricinus communis
Pollen 15 x 20 μm .... Cannabis sativa

Exine regulate

Pollen 26 – 34 μm .... Tamarindus indica

Exine scarrate ............... Crotalaria sp.

Exine spinulate

Pollen 19 x 22 μm ....... Ageratum conyzoides
Pollen 16 x 20 μm ....... Chromolina odorata

Exine faveolate .............. Cassia siamea

4-zonocolporate

Exine psilate

30
Pollen 35 X 25µm in dia  ..... *Azadirachta indica*

Pollen 30 X 28 µm in dia. ..... *Melia azadrach*

5-zonocolporate
Exine reticulate

Pollen 18µm in dia. ..... *Emblica officinalis*

Oblate spheroidal, baculate or suboblate

3-colporate, exine granulate  ..... *Datura metal*

Bacculate, exine psilate  ..... *Peltophorum sp.*

Exine verrucate  ..... *Bougainvillea spp.*

Pollen grains observed under the microscope were categorized on the basis of their exine structure, aperture, size, etc. [Plate 3-7]. Most of the known/identified allergenic pollen grains were observed to be smaller in size and light in weight [Plate 3]. Pollen of the plants having entomophilous mode of pollination were also found to contribute to the aerobiology of the area under study [Plate 5].

The electron microscopic studies have helped a great deal in tracing the pattern of development of the pollen walls and also in getting a correct picture of the nature and number of stratified layers and its fine structure, while the electron photographs of the surface replica of exines provided the exact picture of the ornamentation patterns [Plate 8 & 9]. For proper identification of air-borne pollen materials, knowledge of their diagnostic morphological characteristics are essential prerequisite (Nair et al, 1986).
PLATE 3: Light microscopic photographs of some allergenic plant pollens collected from Greater Silchar area.

3.1: Surface view of *Argemone maxicana* pollen grain (X 40)

3.2: Polar view of *Helianthus annus* pollen grain (X 40)

3.3: Polar view of *Cleome gynandra* pollen grain showing pore structure (X40)

3.4: Polar view of *Bougainvillea spectabilis* pollen grain (X 40)

3.5: Surface view of *Amaranthus spinosus* pollen grain (X40)

3.6: Surface view of *Imperata cylindrica* pollen grain (X40)

3.7: Equatorial view of *Cassia fistula* pollen grain (X40)

3.8: Equatorial view of *Ageratum conyzoides* pollen grain showing spines on the surface (X40)
PLATE 4: Light microscopic photographs of some poaceae and cyperaceae pollen 
grains collected from Greater Silchar area.

4.1: *Oryza sativa* pollen grain showing monoporate structure (X100)

4.2: *Chrysopogon sp.* pollen grain showing monoporate structure (X40)

4.3: *Saccharum sp.* pollen grain showing pore structure (X40)

4.4: Cyperaceae pollen grain showing colpate structure (X40)

4.5: Cyperaceae pollen grain showing colpate structure (X40)

4.6: Cyperaceae pollen grain showing colpate structure (X40)
PLATE 5: Light microscopic photographs of some dominant pollen types collected from Greater Silchar area.

5.1: Surface view of *Albizia lebbeck* pollen grain (X100)

5.2: Surface view of *Clerodendrum viscosum* pollen grain (X100)

5.3: Equatorial view of *Cassia siamea* pollen grain (X100)

5.4: Surface view of *Zea mays* pollen grain (X100)

5.5: Surface view of *Crotalaria juncea* pollen grain showing pollen tube development (X100)

5.6: Equatorial view of *Adathoda vasica* pollen grain showing colpus and pore (X100)

5.7: Polar view of *Cassia sophera* pollen grain showing colpus (X100)

5.8: Surface view of *Areca catechu* pollen grain (X100)
PLATE 6: Light microscopic photographs of some dominant pollen types collected from Greater Silchar area.

6.1: Equatorial view of *Azadirachta indica* pollen grain (X40)

6.2: Surface view of *Melia azadarach* pollen grain (X40)

6.3: Polar view of *Gynura nepalensis* pollen grain (X40)

6.4: Polar view of *Delonix regia* pollen grain (X40)

6.5: Surface view of *Datura metal* pollen grain (X40)

6.6: Polar view of *Peltophorum ferugenium* pollen grain (X40)

6.7: Surface view of *Cassia occidentalis* pollen grain (X40)

6.8: Polar view of *Polygonum sp.* pollen grain (X40)
PLATE 7: Light microscopic photographs of some dominant pollen types collected from Greater Silchar area.

7.1: Surface view of *Lantana camara* pollen grain (X40)

7.2: Polar view of *Mimosa pudica* pollen grain showing colpate structure (X40)

7.3: Polar view of *Mikenia micrantha* pollen grain (X40)

7.4: Circular view of *Macaranga denticulata* pollen grain (X40)
PLATE 8: SEM photographs of some allergenic pollen types collected from Greater Silchar area.

8.1: Distal view of Cocos nucifera pollen grain showing colpus (X1500)

8.2: Surface view of Amaranthus spinosus pollen grain showing polyporate structure (X2500)

8.3: Polar view of Cassia alata pollen grain (X2500)

8.4: Polar view of Cleome gynandra pollen grain showing spine on the surface (X3000)
PLATE 9: Scanning Electron Microscopic photographs of some dominant pollen types collected from Greater Silchar area.

9.1: Surface view of *Polygonum* pollen grain (X1500)

9.2: Polar view of *Ipomaea carnea* pollen grain showing spinulose structure (X2500)

9.3: Surface view of *Erythrina indica* pollen grain (X2500)

9.4: Surface view of *Mimosa pudica* pollen grain showing colpate structure (X8000)

9.5: Polar view of *Cassia siamea* pollen grain showing spinulate structure (X1000)

9.6: Equatorial view of *Acacia auriculaeformis* pollen grain (X2000)

9.7: Polar view of *Clerodendrum viscosum* pollen grain showing tricolpate structure (X1500)

9.8 Surface view of *Bombax ceiba* pollen grain showing net like exine with pore (X2000)
Most of the atmospheric pollen grains recorded were monoporate (37.0%) which include the pollens of Graminae and Cyperaceae followed by the 3-zonocolporate (11.16%) which includes the pollen of *Aggratum conyzoides*, *Cassia spp.*, *Cleome sp.*, *Mangifera indica*, *Moringa sp.* and polyads (7.0%) which include the pollens like *Acacia*, *Albizia*, *Mimosa pudica*. etc. A morphological analysis of the Hawaiian pollen flora shows that the 3-colporate (of dicots) is dominant while the derived 1-porate forms (of monocots) and the specialized inaperturates are considerably less (Selling, 1947).