CHAPTER II
MATERIALS AND METHODS

The polliniferous materials were obtained from mature but unopened flowers of fresh, fixed and dried specimens. Care was taken to minimize the variations due to the position of anthers in flower or flowers on the inflorescence so as to get fully developed grains. Pollen grains were then preserved in vials containing glacial acetic acid. Sampling, whenever found possible, has covered the available spectra of ecological habitats.

The preparation of pollen samples for the study was made by the acetolysis method (Erdtman, 1952). After acetolysing the grains for 3 minutes, the mixture was heated to 100°C in a water bath for one minute and was washed 4–5 times in distilled water.

For light microscopic (LM) analysis acetolysed grains were embedded in unstained glycerine jelly and slides were sealed with paraffin. Pollen samples examined in scanning electron microscope (SEM) were initially acetolysed and then transferred
from water onto the cover glasses mounted on specimen stubs. After allowing them to dry, they were vacuum-coated with carbon, followed by gold-palladium (Walker, 1974a). Olympus System Microscope - model BHA - was used for light microscopic analysis and all the measurements were made under oil immersion (100 x) using an ocular (12.5 x) and an ocular micrometer of which 1 division, after calibration, amounted to 1 μ.

Photomicrographs were taken on Carl-Zeiss photomicroscope I using ORWO NP55, 35 mm film. Scanning electron microscopy was carried out on Cambridge Stereoscan S4 using ORWO Np22, 120 mm film at Physical Research Laboratory, Ahmedabad. LM negatives were printed on Agfa hard glossy single weight bromide paper, keeping the magnification (1000 x) constant unless otherwise stated and SEM negatives on Agfa normal glossy single weight bromide paper.

In general, terminology of Reitsma (1970) is largely followed for describing the pollen and to a less degree that of Nair (1974a) and Walker and Doyle (1975), the latters' especially for size and shape classes. Pollen grains are described in the following order: nature of occurrence, shape classes, polar axes (p), equatorial axes (e), second equatorial (in bilateral grains, see Nair, 1961), axes (e1), size classes, polar outline, dimensions across the polar view, equatorial outline, apertures, mesocolpia, apocolpia, exine, sexine, ectoexine (Ec/s), endosexine (En/s), nexine, ectonexine (Ec/n), endonexine (En/n) and sculpturing. For describing the shape of apertures and echinae or similar sculptural outline at optical section, the terminology of simple symmetrical plane shapes suggested by Systematic Association Committee and taken from Exell (1962) was adopted.

Subtypes given alongwith the description of pollen grains of taxon represent a minor deviation from the typical pollen of a given taxon but subtypes are not recognised when variation in pollen grains of a given taxon is constant, for instance number
of pori in a polypantoporate pollen; further, wherever pollen
dimorphism occurs and grains are almost in equal proportion
in the assemblages they are described under type I and type
II and so on. Each pollen type recognized in the chapter on
discussion embodies a given set of arbitrarily recognized
palynological characters. A concise palynological review has
been provided in the form of critical notes for each family
referring to the relevant literature.

Each dimension cited in the text represents the mean of
at least 15 measurements except for shape classes for which
25-30 grains were measured; the mean measurement is given in
parenthesis between the range observed. Palynological terms
thought necessary to use in the present work are given in
glossary at the end of the work.

Pollen slides upon which the light microscopic part of the
study was based have been deposited in palynological collections
in the laboratory of Bioscience Department, Sardar Patel
University. Authentically identified plant materials and
herbarium specimens from which the collection of anthers were
made have been voucheded and deposited in the University
Herbarium, Vallabh Vidyanagar and the author's own collection
in the Herbarium of Botanical Survey of India, Western Circle
(BSI), Pune.

Artificial, bracketed keys based on pollen data are given
wherever found possible to delineate genera or species in a
family and also for all the pollen types recognized in the
chapter on discussion. Stress is laid in keys on number, position
and other details of apertures, sculpturing, size and shape of
grains in order of preference. Any slight constriction, bridge,
rupture or jagged appearance at the equatorial region of the
colpus of a grain has been taken to represent a porus (see
Moore and Webb, 1978) and thus a colporoidate condition.
As the pollen morphology, with some exceptions, is consistent with the level of relative advancement and relationships postulated in the phylogenetic system of angiosperm classification (see Cronquist, 1981; Takhtajan, 1980) an attempt is made to arrange the families studied within the circumscription of Takhtajan's system of classification of flowering plants (1980).