IV. Material and methods

IV.1 Sequence corings

Two types of sequence were cored in the Gietsenveentje: sequences for lithological descriptions and sequences for various types of analysis. The first type of sequence was described in the field, while the second type of sequence was sampled, packed in plastic and transported to the laboratory.

Some of the sequences used for lithological descriptions were cored by two employees of the Dutch Geological Survey, Mr De Vries and Mr Warkor. They used an Edelman sampler. The other sequences used for lithological descriptions were cored with a Russian sampler with a length of 50 cm and a diameter of 5 cm (AABY & DIGERFELDT 1986, 183; LANG 1994, 39).

In total, eleven sequences were sampled for various types of analysis. Three of these, viz. Gieten I, II and III, were cored with a Dachnowsky sampler with a length of 25 cm and a diameter of 3.6 cm (WEST 1968, 103; LANG 1994, 40). Another three sequences, viz. Gieten IV-P, IV-HR and IV-HL, were sampled from an open section, which was dug in the centre of the Gietsenveentje (see VI.1.4). Gieten IV-P and Gieten IV-HR were sampled with the help of rectangular sample tins, while Gieten IV-HL was sampled with a scoop: layers of 2.5-3 cm of sediment were cut out and put separately into plastic bags. Finally, four sequences, viz. Gieten V-A, V-B, V-C and V-D, were cored with two types of sampler. The largest part of each of these sequences was cored with a Russian sampler. The part near the Pleistocene subsoil, where the sediment is more compact and sandy, was sampled with a gouge (in German: *Holländischer Marschenlöffel*) with a length of 100 cm and a diameter of 3.5 cm (COUTEAUX 1962; AABY & DIGERFELDT 1986, 188; LANG 1994, 42).

Attemps were made also to sample cores with a large diameter for the analysis of macroscopic remains. A Dachnowsky sampler with a length of 40 cm and a diameter of 6 cm was used. Because of the presence of fibrous and loose peat sediment, it proved difficult to sample a complete core with this sampler; sampling was successful only at the location of sequence Gieten V-C.

IV.2 Sampling techniques

The samples to be used for various types of analysis were taken out of the sequences with the help of various techniques.

In sequences Gieten I and II, pollen samples with a thickness of 1 cm were taken. No volume measurements were made. In sequence Gieten III, pollen samples with a thickness of 0.5 to 0.7 cm were taken: they were sliced with the help of a cutting machine. Volume measurements of each pollen sample were conducted with the help of a cylindrical punch with a diameter of 1.05 cm.

In sequences Gieten IV-P, IV-HR and V-A to V-D, pollen samples with a thickness of 1 cm were taken, also with the help of the cylindrical punch. When a layer of sediment with a thickness of 1 cm was punched, the volume of the sediment in the punch measured 0.8659 cm^3.

In sequence Gieten IV-HL, large macroscopic samples with a thickness of 2.5-3 cm were taken from an open section. The macroscopic sample core of Gieten V-C, which has a diameter of 6 cm, was completely divided into pieces with a thickness of 5 cm.

The wood samples were collected from the pollen sequences. When relatively large pieces of wood were recognized, they were taken out of the core and stored separately.

The samples used for radiocarbon dating were collected from the pollen sequences. For conventional dating, relatively large samples are needed. In sequence Gieten I, a sample with a thickness of 10 cm was used for conventional dating. In sequence Gieten III, the sediment between 322 and 351 cm, which remained after the pollen samples had been taken, was divided into seven parts of 3.2-3.3 cm. These seven parts were dated conventionally. In sequence Gieten IV-P, two samples with a thickness of 2 cm were used for conventional dating. For AMS dating, only very small samples are needed (see IV.7). In sequences Gieten IV-P, IV-HR and V-A to V-D, samples with a thickness of 1 cm were used for AMS dating. These samples were taken out of the sediment with the help of the above-named cylindrical punch, at exactly the same level as the pollen samples.

The phosphorus samples were also collected from the pollen sequences. In sequences Gieten V-A, V- B and V-C, samples with a thickness of 1 cm were collected, again with the help of the cylindrical punch. The samples were always taken at a level not more than 1 cm from a pollen sample.

IV.3 Sample processing

All samples were processed in the laboratory of the Groningen Institute of Archaeology. The pollen samples were processed following the method described by Faegri & Iversen (1989). Two tablets with *Lycopodium* spores were added to each sample for the determination of pollen concentration and influx (see IV.8). The samples were mounted in silicone oil.

The macroscopic samples with volumes of 1 or 2.5 litres were put in water for a few weeks. By stirring, the samples were made to disintegrate. In the case of compact gyttja samples, this proved to be quite difficult. Subsequently, the samples were put through three sieves with meshes of 2, 0.5 and 0.2 mm respectively, so that three different fractions were obtained. These fractions were analyzed separately. In most cases, the relatively small 2 mm sieved sample was analyzed completely, while the larger 0.5 and 0.2 mm sieved samples were analyzed only partly. The results of the samples from the various sieves were combined and converted to a content of 1 litre.

The wood samples were used for making hand-cut sections with a razor blade along the transversal, radial and tangential planes. The sections were placed in water on an object slide and covered with a coverslip.

IV.4 Sample analysis

The microscopic slides with the pollen samples were analyzed with a light-transmitting Zeiss binocular microscope at 400x magnification. For difficult identifications, oil immersion was used at a magnification of 1000x. Several works were used for identifying pollen and spore types, including Faegri & Iversen (1989), Moore et al. (1991), Reille (1992) and Punt et al. (1976; 1980; 1981; 1984; 1988; 1991). Palynomorphs were identified with the help of publications of Van Geel (1978) and Van Geel et al. (1981; 1989). The reference collection at the Groningen Institute of Archaeology, containing modern pollen of thousands of species, was used to check the identifications. The nomenclature of the pollen types follows the standardized nomenclature of Birks (BIRKS 1973, 225; BERGLUND & RALSKA-JASIEWICZOWA 1986, 457):

Umbelliferae	Family identification certain, types or subgroups unidentified or unidentifiable.
Ericaceae indiff.	Family identification certain, one or more types are identified within the family, this is the "rest group".
Betula	Genus identification certain, types or subgroups unidentified or unidentifiable.
Jasione montana	Species identification certain.
Rumex acetosa/ acetosella	One fossil type present; only two taxa are considered likely candidates, but further distinctions are not possible on the basis of pollen or spore morphology alone.
Potentilla-type	One fossil type present, three or more taxa are possible candidates, but further distinctions are not possible on the basis of pollen or spore morphology alone.

The pollen morphology of *Rumex acetosa* and *Rumex acetosella* is discussed in chapter V.

The macroscopic samples were analyzed with a Wild M5 binocular microscope with incident light at magnifications of 6 to 50x. Several works were used to identify the macroscopic remains, including Beijerinck (1947) and Körber-Grohne (1964). The reference collection at the Groningen Institute of Archaeology, containing modern seeds of many species, in dry, charred and waterlogged conditions, was used to check the identifications.

The wood samples were analyzed with a Zeiss binocular microscope with coaxial illumination at magnifications of 40 to 400x. The reference work by Schweingruber (1978) was used to identify the wood remains. Also comparisons with recent material were made.

IV.5 The construction of the pollen diagrams

The principal part of the present study is formed by pollen analysis. The result of a pollen analysis is the pollen record, consisting of a very large

matrix of raw data. This matrix is much too unwieldy to be of direct use. Results are therefore presented in a simpler, graphic form: the pollen diagram. In a pollen diagram, the vertical axis represents depth and the horizontal axis the abundance of the pollen taxon in either proportional or absolute terms. The first type of diagram is a pollen percentage diagram, the second is a pollen influx diagram (discussed in IV.8). In the pollen percentage diagrams presented here, the curves are divided into five major groups (BERGLUND & RALSKA-JASIEWICZOWA 1986):

1. **Arboreal Pollen**: Ligneous terriphytic spermatophytes (trees, shrubs and climbing plants);
2. **Non-Arboreal Pollen**: Non-ligneous terriphytic spermatophytes (herbs) and pteridophytes (ferns);
3. **Local types**: Helophytes (plants of lake shores, fens and bogs) and limnophytes (aquatic plants);
4. **Van Geel palynomorphs**: Palynomorphs, predominantly bryophytes (mosses) and fungi, which have been assigned a type number by Van Geel (1978) and Van Geel et al. (1981; 1989).
5. **Charcoal particles**: Charcoal particles, subdivided into three size categories.

The Arboreal and Non-Arboreal Pollen together form the pollen sum (ΣP = AP + NAP). All pollen percentages, including the percentages of the last three groups, are calculated on the basis of the pollen sum. The **regional pollen** includes all pollen types included in the pollen sum; the **local pollen** includes all pollen types excluded from the pollen sum. According to Faegri & Iversen (1989), the pollen rain consists of three components:

(1) the gravity component: pollen falling straight down;

(2) the local pollen: that part of the pollen output which went into a diffusion cloud the centreline of which remained more or less parallel to the ground, increasing in diameter and being intercepted by the ground cover;

(3) the regional pollen: the truly airborne pollen, caught by updraughts and transported via greater altitudes than the tree-tops and the air currents created by the latter.

However, the terminology used by different authors is not consistent. As used by Janssen (1966), "local" corresponds to the gravity component of Faegri & Iversen and "extra-local" to the local pollen as defined by Faegri & Iversen. "Regional" and "extra-regional" both correspond to Faegri & Iversen's regional pollen. Because the definitions put forward by Faegri & Iversen are difficult to apply in actual practice and because the used terminology is not consistent, the best solution seems to be to use convenient, practical definitions of local and regional pollen. However, the problem of which pollen type belongs to local pollen and which to regional pollen, is a difficult one and each solution is subjective. In this study, the following definitions of regional and local pollen are used:

Regional pollen: pollen of plants generally growing outside the basin, at a fairly large distance from the core locations. Except for *Betula*, all common tree pollen types are included in the regional pollen. Because herbs and ferns that prefer more or less dry conditions are not thought to have occurred in the basin, and consequently in the neighbourhood of the core locations, they are included in the regional pollen.

Local pollen: pollen of plants growing within the basin, in the direct neighbourhood of the core locations. All pollen types of aquatic plants and of plants of lake shores, fens and bogs are considered to belong to this category.

All pollen percentage diagrams were drawn by the TILIA computer program, which was developed by Dr. E.C. Grimm of the Illinois State Museum, Springfield (USA).

IV.6 Processing of samples for the assessment of phosphorus content

The phosphorus samples were processed following the molybdenum blue method (MURPHY & RILEY 1962; BENGTSSON & ENELL 1986). In this procedure, a blue phosphomolybdate compound is developed in an aqueous solution. All phosphorus samples were processed at the Laboratory of Plant Ecology of the University of Groningen.

IV.7 Radiocarbon dating methods

The absolute age of Gietsenveentje samples was assessed at the Centre for Isotope Research of the University of Groningen with the help of two fundamentally different methods of radiocarbon dating:

1. the conventional method, which entails the measurement of the radioactivity of ^{14}C (MOOK & STREURMAN 1983);
2. the AMS method (Accelerator Mass Spectrometry), which entails the measurement of the $^{14}C/^{12}C$ ratio by mass spectrometry. Because the number of ^{14}C atoms can be measured directly, instead of only those atoms which decay radioactively (ELMORE & PHILLIPS 1987), it is possible considerably to reduce the amount of material needed for an analysis - roughly by a factor 1000. The AMS tandem in Groningen, which was used for the Gietsenveentje AMS dates, is of the new generation Tandetron (WIJMA & VAN DER PLICHT 1997).

IV.8 Assessing pollen concentration and pollen influx

In order to assess pollen concentration and pollen influx, tablets with a known number of a *tracer*, in this case *Lycopodium* spores, were added to each pollen sample with a known volume (STOCKMARR 1971). Each tablet contains ca. 12,542 spores (BERGLUND & PERSSON 1994). 2 Tablets were dissolved in warm water and added to each Gietsenveentje pollen sample (except for the samples of Gieten I and II). All Gietsenveentje pollen samples were taken with a punch with a volume of 0.8659 cm^3. The *Lycopodium* spores were counted in routine pollen analysis. With the help of the following formula, pollen concentration was calculated (BIRKS & BIRKS 1980, 207):

$$A = \frac{L_A \times P_C}{L_C \times V_S}$$

in which:
A = concentration in grains/cm^3
L_A = number of *tracer* units (*Lycopodium* spores) added to each sample
P_C = number of counted pollen grains
L_C = number of counted *tracer* units (*Lycopodium* spores)
V_S = sample volume in cm^3

Pollen influx was calculated as follows:

$$I = A \times v_S$$

in which:
I = influx in grains/cm^2/year
A = concentration in grains/cm^3
v_S = sedimentation rate in cm/year

The sedimentation rate was calculated as follows:

$$v_S = \frac{\text{date of depth B} - \text{date of depth A}}{\text{depth B} - \text{depth A}}$$

in which the dates are calibrated ^{14}C dates, expressed in cal BC; the depths are expressed in cm.

IV.9 Recording of recent vegetation plots and collection of surface samples

Vegetation plots were recorded following the (extended) method of Braun-Blanquet (1964). For practical reasons, the dimensions of the chosen examination areas were determined at 2 x 2 m (EVANS & MOORE 1985). A species list of each examined area was made. Subsequently, the contribution of individuals of each species to the vegetation cover was estimated on the basis of the combined estimation method, developed by Braun-Blanquet (1964) and extended by Barkman et al. (1964; see also SCHAMINÉE et al. 1995a, 72). This method implies that when a species covers an area less than 5%, its abundance, viz. the number of individuals, is estimated; when it covers an area of 5% or more, its cover area, viz. the percentage of the total area which is occupied by the species when the outline of its individuals is projected on the ground, is estimated.
Surface samples, which are a reflection of the modern pollen present in the air, were obtained by the sampling of moss polsters: in the moist and acid environment of a moss polster, pollen apparently is preserved very well (BOTTEMA 1995). As far as possible, only green moss stems and leaves were collected. Bradshaw (1981, 50) states that in this way, a pollen assemblage reflecting perhaps the last 5 years will be taken, the precise period depending on the growth rate of the moss. However, inevitably some remains of older moss organs and some soil parts will be collected together with the green moss parts, so that it cannot be excluded that pollen older than 5 years occurs in the surface samples.