APPLICATION OF TRANSMISSION ELECTRON MICROSCOPE ANALYSIS TO THE RECONSTRUCTION OF FORMER VEGETATION

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SUMMARY

With the aid of a modified single-stage replica method the taxonomic identification of pollen from Chenopodiaceae and Amaranthaceae to the generic level appears to be possible. The implications of these identifications for vegetation reconstruction and the interpretation of pollen diagrams are discussed.

1. INTRODUCTION

Conventional light-microscopic methods used in pollen analysis are not sufficiently discerning for the identification of certain pollen types further than the family or even family group level (compare, *e.g.*, "Cheno-Amaranthaceae"). In a number of cases this hampers the reconstruction of former vegetation types.

In a diagram of a section from Eastern Macedonia, Greece, *cf. fig. 1*, published

Fig. 1. Macedonia with the Tenagi Philippon

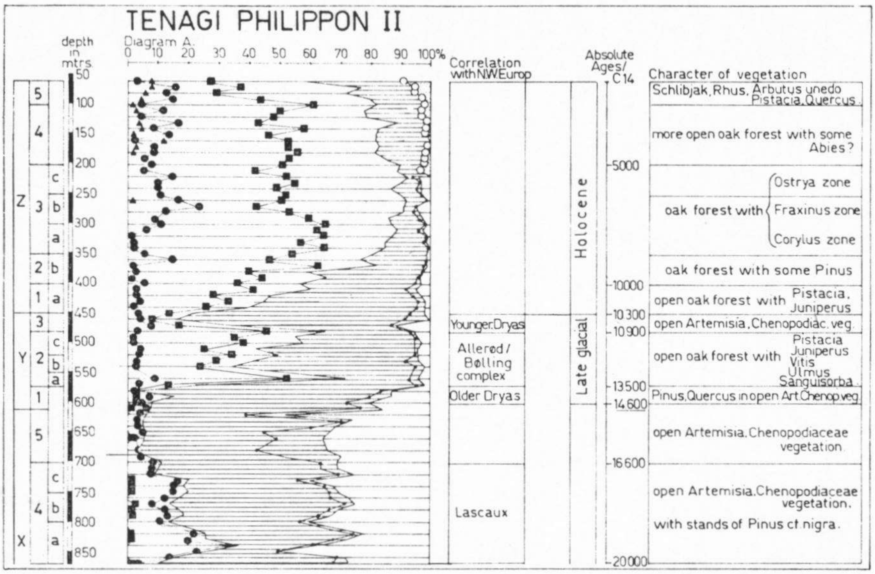


Fig. 2. Pollen diagram

by WIJMSTRA (1969), some pollen zones characterized by relatively high percentages of Chenopodiaceae and *Artemisia* occurred (*fig. 2*). These higher percentages could be ascribed to the presence, at the time of deposition, of a vegetation type indicative of a steppe-like landscape, but conceivably also to the occurrence, at that time, of border zone vegetation types surrounding lakes with a moderately high salinity.

With the intent of solving this problem, replicas of recent chenopodiaceous pollen grains were made which were examined in the electron microscope and compared with pollen replicas from the fossil material. This comparison was facilitated by the fact that the characters shown by transmission electron-microscopy have been proved to be easily quantifiable (TSUKADA 1967). An attempt was made to avoid the introduction of new terms for the description of the nannomorphological features of the replica surface. The addition of the prefix "micro-" to the current palynological terms seems more practicable than the introduction of an entirely new nomenclature.

2. METHODS

2.1. Preparation

A technique for the preparation of single-stage surface replicas has been described by ROWLEY & FLYNN (1966):

A drop of 50% acetone containing pollen grains is placed on a piece of cleft mica of about 1 cm^2 and allowed to dry at room temperature. Subsequently carbon is evaporated until a layer of 35 mμ thickness is built up, under constant rotation of the mica support (15 rev./min.). The carbon film with the pollen is separated from the mica on the surface of distilled water, placed in 2-amino-ethanol, and heated at a temperature of 145–155 °C for 10 min to 3 hours. The replicas are then washed in distilled water at 90 °C and are ready for observation after having been picked up on electron microscope grids.

A similar method was used by LIEM & VAN ANDEL (1968) and was also applied by the present authors, but with the following modifications:

a. A suspension of acetolysed (recent or fossil) pollen grains in distilled water (instead of acetone) was used;

b. instead of the mica carrier a smooth thin plate of $CaSO_4$ was used, prepared by mixing pure $CaSO_4$ powder with water to a paste of which approximately 0.5 cm^3 was pressed between two glass plates and placed in an oven for 20 min. at a temperature of 65 °C. After the glass plates have been removed the $CaSO_4$ surface is ready for use;

c. carbon is evaporated on the rotating carrier with the pollen from a distance of 12–15 cm from 3 different angles until the surface of an adjacently attached gold foil has re-assumed its original (gold) colour after having passed through the colours orange, red, blue, green and silver-green, as follows:
At an angle of 30° with the horizontal plane until the interference colour is orange-red;
at an angle of 60° with the horizontal plane until the interference colour turns blue; and
at an angle of 90° with the horizontal plane until the original gold colour has returned.
In this way a continuous carbon layer of fairly even thickness forms over the pollen grains and the supporting $CaSO_4$ substrate;

d. after the marked pollen-containing areas have been divided into 2×2 mm^2 squares, the $CaSO_4$ plate with the carbon film is immersed in a concentrated solution of NH_4Cl or NH_4NO_3. After a few hours the squares become separated from the $CaSO_4$ and float at the surface. They are picked up, washed once in distilled water, and placed in 2-amino-ethanol, to render the still adhering pollen grains soluble in water.

The use of $CaSO_4$ instead of mica has, in the authors' opinion, the following advantages:

a. the pollen suspension does not spread so far over the surface as sometimes happens on mica (which may be troublesome when the material to be studied does not contain much pollen);

b. the pollen grains are better visible against the white $CaSO_4$ background, almost to the naked eye;

c. the carbon film invariably becomes detached from the carrier even if the pollen suspension contains some contamination such as clay or plant fragments. When the fine sculpture of fossil pollen grains is to be investigated, the pollen can be studied in the same state as used for the light-microscopic examination, with the restriction that the sample must be suspended in water. This has the advantage that other pollen grains present in the sample are replicated at the same time, whilst the time-consuming use of a micro-manipulator can be avoided;

d. the carbon layer also replicates the crystalline structure of the $CaSO_4$, which makes the film stronger and lessens its tendency to curl up. In case the film nevertheless does curl up, the somewhat rougher surface still permits the penetration of the 2-amino-ethanol. When the curled-up carbon square is subsequently transferred from the 2-amino-ethanol to water of 90°C, a simple up-and-down agitation usually suffices to flatten the square again.

A minor disadvantage is that the complete separation of the carbon film from the $CaSO_4$ carrier requires several hours, but this is more than compensated for by the reliability of this modification. As already stated by ROWLEY & FLYNN (1966), this method can be used for small objects other than pollen. Good results were obtained with some algae (*Pediastrum*, *Scenedesmus*) and with fungal spores.

2.2. Micromorphological criteria for the taxonomic identification of Chenopodiaceae and Amaranthaceae pollen

Electron microscopic examination of the replica surface of pollen of Chenopodiaceae and Amaranthaceae shows the following new features that can be used for identification (TSUKADA 1967):

a. spinules or micro-echinae;

b. holes, hollows or pits (= micro-foveae)

In the present study these features were used for identification combined with light-microscopic criteria as given by MCANDREWS & SWANSON (1967). As a result our identification of the pollen grains under discussion is based on the following characteristics:

A. the diameter of the pollen grain;

B. the ratio of pore distance to diameter of the pollen grain, measured as indicated by MCANDREWS & SWANSON (1967);

C. the pit/spinule ratio;

D. the number of spinules in the area between two pores and their common tangents (see *plate 2*).

3. RESULTS

Table 1 shows the results of the combined light and electron microscopic observations. From the values in this table can be concluded that the characteristic features of the fossil pollen Type I correspond with those of the recent genus *Krascheninnikovia* (=*Eurotia*). Type I probably represents the pollen of *K. ceratoides*. Fossil Type II, which is less abundant, has all the characteristics of *Kochia* pollen and may indeed be the pollen of a species of *Kochia*. Both fossil pollen types were found in samples from a depth of 6.80 m and 6.46 m, shown in the diagram as the part of the Upper-pleniglacial lying between the Older Dryas and the Lascaux interstadial.

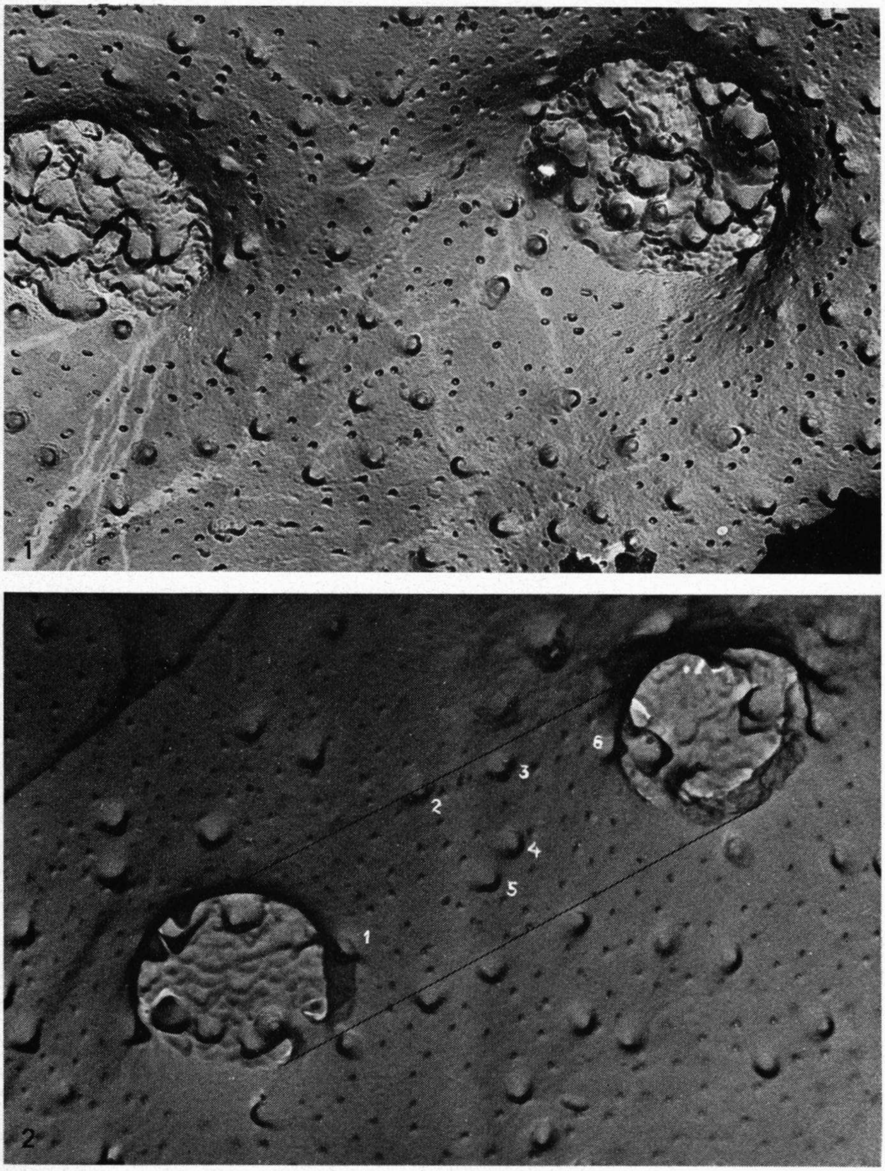


Plate 1

1. *Beta vulgaris* L., surface replica of pollen grain × 19.000 (recent).
2. Illustration of estimation of value D.
   Number of micro-echinae (spinules) in the area enclosed by 2 pores and their common tangents, on a pollen grain of *Chenopodium album*, × 19.000 (recent).

Table 1. Four quantifiable characters of pollen from Chenopodiaceae and Amaranthaceae are tabulated, *viz.*: A. ratio of pits to spinules. B. number of spinules between two adjacent pores and their common tangents. C. ratio of distance of two adjacent pores to the diameter of the pollen grain. D. diameter of pollen grain in μ.
10–20 observations obtained from four electron micrographs provided the data in columns A and B
30–100 measurements obtained by light-microscopic observation provided the data in columns C and D.

Species	A	B	C	D
Recent species:				
Halimione portulacoides (L.) Aellen	4.0±21.	10–15	0.173–0.204	27.6±2.4
Chenopodium album L.	9.8±1.2	4–8	0.186–0.222	27.2±2.0
Kochia laniflora (S.G.Gmel.) Borbás	3.8±0.9	6–11	0.160–0.183	20.2±2.4
Atriplex hastata L.	6.4±1.2	11–13	0.167–0.209	21.2±1.1
Beta vulgaris L. subsp. maritima (L.) Arcangeli	4.6±1.2	8–14	0.214–0.292	14.6±0.9
Bassia hirsuta (L.) Aschers.	3.5±1.4	4. 11	0.141–0.166	22.1±1.4
Arthrocnemum glaucum (Delile) Ung.-Sternb.	3.9±1.0	40	0.225–0.278	24.7±1.7
Amaranthus lividus L.	1.6±0.4	33–42	0.240–0.300	17.2±1.3
Krascheninnikovia ceratoides (L.) Gueldenst.	1.3±0.3	20–32	.0.207–0.241	18.9±1.9
Krascheninnikovia lanata (Pursh) Meeuse & Smit (ined.)	1.5±0.4	45–55	0.197–0.295	22.5±2.3
Fossil pollen:				
Type I	1.1±0.2	22	0.195–0.230	17.8±2.2
Type II	3.5	9	0.181–0.196	22.3±1.0

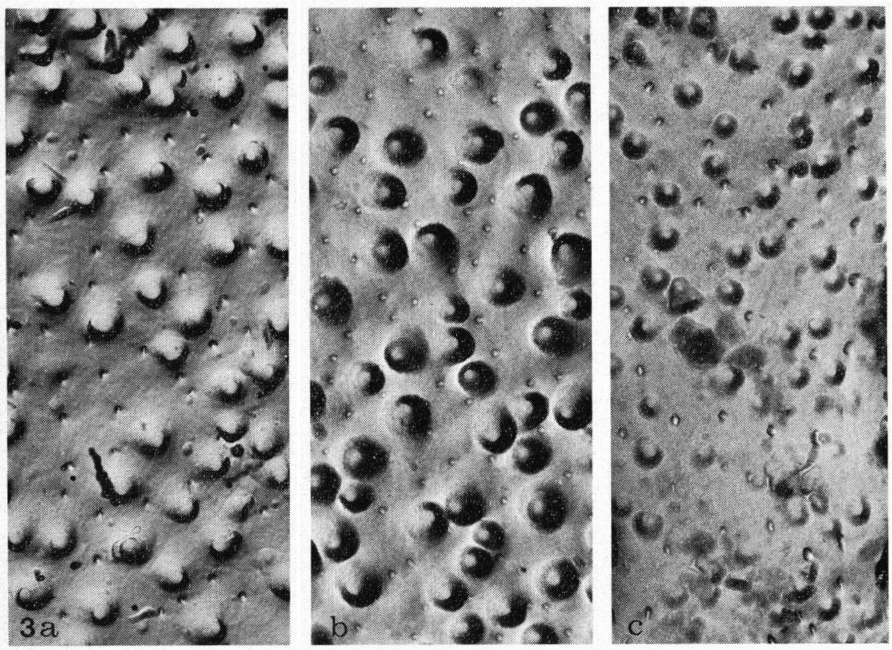
4. DISCUSSION

4.1. Pollen identification

The preparative technique employed yielded pollen surface replicas showing all the nannomorphological details required for taxonomic identifications of Chenopodiaceous pollen grains mentioned by TSUKADA (1967). The pit/spinule ratio and the distribution of the micro-sculptural elements of pollen grains of the genus *Krascheninnikovia* prove to be somewhat different from those of other genera. As a matter of fact the pit/spinule ratio of *K. ceratoides* is even lower than the value of 1.5 considered to be critical for the distinction between chenopodiaceous and amaranthaceous grains. This necessitated the additional study of the only amaranthaceous taxon which may have occurred in former plant communities in Europe, *viz., Amaranthus lividus*. Surprisingly, this species appears to have a higher pit/spinule ratio than has hitherto been recorded in the family Amaranthaceae, *viz.*, 1.6.

From these new data the following conclusions can be drawn:

1. The value 1.5 of the pit/spinule ratio may still be used for the distinction between pollen of Chenopodiaceae and Amaranthaceae, but there are a few exceptions, so that it does not provide an absolute taxonomic criterion and should rather be considered to be only a very useful indication.



3. a. *Krascheninnikovia ceratoides*, recent pollen, part of surface, × 34.000.
b. *Krascheninnikovia lanata*, recent pollen, part of surface × 34.000.
c. fossil pollen, part of surface, probably *Krascheninnikovia* spec., × 34.000.

2. The deviating pit/spinule ratio of the pollen grains of the genus *Krascheninnikovia*, which corresponds with that of certain fossil pollen grains, very strongly suggests that one of the fossil pollen types recovered from sediments belongs to this genus.

4.2. Palaeo-ecological implications

For a long time the probable occurrence in the Mediterranean area of certain Weichselian plant communities, which must have had a preference for an arid environment, has been much debated (see *e.g.* MENENDEZ AMOR & FLORSCHÜTZ 1963; MONSONAN 1964). The discussion concerns the regular presence of *Artemisia* and "Cheno-Amaranthaceous" pollen grains and of some macrofossil remains in Weichselian sections (FRENZEL 1967). Since *Artemisia* and certain Chenopodiaceae and Amaranthaceae are not only frequent inhabitants of halophytic environments but are often also indicators of aridity, it follows that reliable specific or genetic identification of the chenopodiaceous and amaranthaceous taxa is required before a more conclusive interpretation of paleoecological conditions can be given. The strikingly high representation of pollen of Chenopodiaceae and of *Artemisia* during certain Weichselian intervals in the diagram of Tenagi Philippon, Macedonia, Greece (WIJMSTRA 1969) confronted us again with aspects of the above-mentioned discussion. The

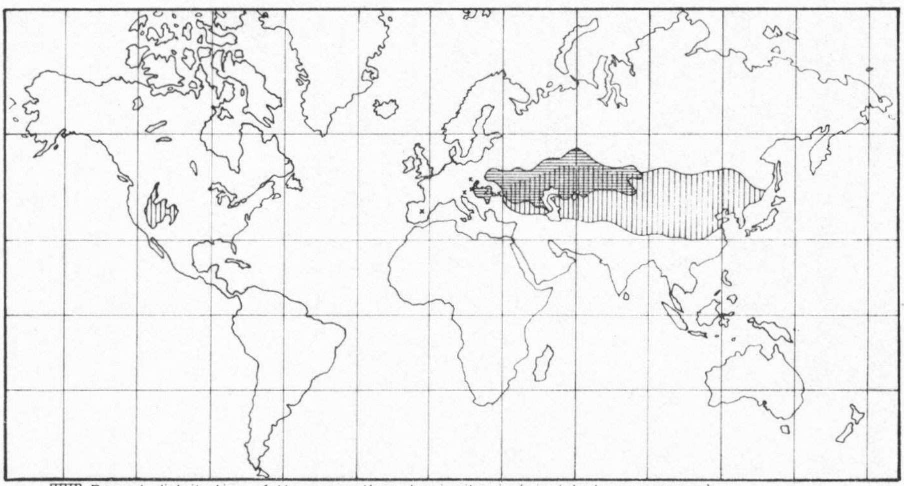


Fig. 3. Distribution of the genus *Krascheninnikovia* and of *Kochia laniflora*

results of the replica studies, augmented with some light-microscopic observations, clearly established the presence of pollen grains of at least one species of *Krascheninnikovia* (= *Eurotia*) and render the occurrence of a species of the genus *Kochia* highly probable.

The present range of distribution of these genera (see *fig. 3*) leaves but little doubt concerning the preference of these taxa for the arid steppe milieu. The phytosociological survey of high mountainous areas of Central Asia made by WALTER (1968) also clearly emphasises the preference of these components for extremely arid and cold sites, such as the Pamir and the Tschangtang.

Climatological data provided by Walter for the Pamir indicate an annual rainfall of 102.6 mm, the rains mainly falling in the period May-August. The average annual temperature registered by the Pamir station varies between - 1.0 °C and + 2.8 °C, but during the summer the air temperature, measured 2 m above the soil surface, may attain values between 20 °C and 22 °C, closer to the soil 28 °C, and at the soil surface even 52 °C. At night the temperature may drop below zero with the exception of only 10 to 30 nights per year. A permafrost layer is, however, only present in some small local areas.

From the above-mentioned evidence we may deduce that in Greece during those phases of the Weichselian in which *Krascheninnikovia* was recorded in the Macedonian section an extremely dry climate prevailed with an average temperature appreciably lower than to-day but possibly with extreme fluctuations.

In other Mediterranean diagrams also high percentages of *Artemisia* and Chenopodiaceae have been found (BONATTI 1966; BOTTEMA 1967; BEUG 1967; FRANK 1969, FLORSCHÜTZ, MENENDEZ-AMOR & WIJMSTRA, in the press).

It seems permissible to assume that in the areas concerned at one time the same arid steppe vegetations were present as in the Macedonian area during the

Weichselian. It appears also from the Mediterranean diagrams that the A.P. percentages vary with the elevation of the site of the section. The A.P. percentages reach a maximum at a moderate elevation of 700 m to 800 m as far as pollen of *Fagus*, *Quercus*, and *Pinus* is concerned. This phenomenon leads to the conclusion that in zones lying at an altitude of 700–800 m the aridity was somewhat less extreme, so that refugia for trees may have existed (see also VAN DER HAMMEN, WIJMSTRA & ZAGWIJN, in the press).

We may also conclude that in the Mediterranean area pluvial conditions did not occur in the Pleniglacial (WIJMSTRA 1969). The new evidence must be taken into account if an attempt at reconstruction of atmospheric circulation patterns during glacial times is made.

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