## Chenopod and Amaranth Pollen: Electron-Microscopic Identification

Abstract. *Electron-microscopic criteria for distinguishing chenopod and amaranth pollen have been found in the number of minute holes and spinules, and especially in the ratio between them, which is* $< 1.5$ *in amaranths and* $> 1.6$ *in chenopods. Sixty-eight percent of total fossil pollen from a Classic Maya level (carbon-14 age 1380* $\pm$ *120 B.P.) in a lake-sediment core from El Salvador belongs to wild amaranths, which presumably invaded corn fields. Fossil chenopodiaceous pollen from a depth of 3.85 meters (about 4000 years old) at Tinte, the Netherlands, is mostly* Atriplex littoralis, *which was evidently very common on coastal marshes in the middle sub-Boreal period.*

Chenopods are not only halophytes, common on salt marshes and elsewhere in coastal regions (*Atriplex* and *Salicornia* species), but also are indicators of aridity (*1*) and invaders of waste lands and of root crop fields (*Chenopodium, Salsola, Kochia,* and others) (*2*). Some chenopods are cultivated as vegetables (*Spinacia oleracea* and *C. album*) and as edible grains (*C. quinoa* and *C. canahua*). A few amaranth species (*Amaranthus caudatus, A. cruentus,* and *A. hypochondriacus*) are also cultivated as grains, especially in Central and South America (*3*) and in Nepal and northern India (*3, 4*), whereas others (*A. hybridus, A. quitensis, A. powellii,* and others) grow in waste and cultivated grounds. Paleoecologists have long regretted that, whereas both chenopods and amaranths grow in various distinctive habitats, chenopod pollen (except for *Sarocobatus vermiculatus*) cannot be distinguished certainly from amaranth pollen by optical means (*5*). Customarily, it is shown as "cheno-am" on pollen diagrams (*6–8*), although 24 chenopod species were reported from the upper Pleistocene periglacial zone of the Russian Plain (*9*). Specific or generic identification of cheno-am pollen is badly needed for more conclusive interpretation of paleoecologic conditions.

Electron microscopy now provides a method for reliable identification. The technique for examining modern nonacetolyzed pollen grains by electron microscopy has been well established (*10*) but has rarely been employed for fossil grains (*11, 12*). The technique employed in this study is an adaptation of the polymethyl-methacrylate heat-pressure method (*13*). The suspension of pollen and other organic matter, treated by 10 percent KOH, is first filtered through No. 12 bolting silk (if necessary, a combination of two or three different bolting silks can be used for concentration of fossil pollen), and acetolyzed. The residue, suspended in absolute alcohol, is evenly spread on the surface of a polymerized methylmethacrylate plate (about 1.0 by 1.5 cm). The plate is then sandwiched between two clean plates of glass, held tightly by two clamps, and heated to about 110°C for about 30 minutes. The residue is completely dried so that the acetolyzed flat pollen is sufficiently replicated in the plastic film, which is then shadowed with carbon and chromium. The electron microscope is a Hitachi HS-7S. Figure 1 presents some examples of electron micrographs of modern and fossil cheno-am pollen obtained by this technique.

Table 1 shows five measurable characters in six amaranth species, 16 chenopod species, and two fossil cheno-am pollen assemblages. The data for grain size and pore number, obtained by ordinary microscopy, are based on measurements of 50 and 10 grains, respectively. For measurement of spinule and minute-hole densities, five different portions were chosen in each of four electron micrographs (of four different grains), so the data represent 20 observations per sample. For fossils, each grain micrographed was examined in five different areas.

Cheno-am pollen grains are periporate. The electron micrographs show that the exine surface (between the large conspicuous pores) is surmounted by spinules 0.25 to 0.4 $\mu$ in height and perforated by minute holes 40 to 100 m$\mu$ in diameter. That both spinules and holes differ in density between the two families is clearly shown in Fig. 1, where the upper four micrographs are of chenopods, and the lower two are amaranths. The smallest number of spinules per 4 $\mu^2$ observed in amaranths is 23.7 $\pm$ 2.7 S.E. (*Amaranthus powellii*) and the largest counted in chenopods is 18.7 $\pm$ 1.4 S.E. (*Allenrolfea occidentalis*), suggesting a division at about 20 spinules per 4 $\mu^2$. The

Table 1. Five measurable characters of pollen in the goosefoot and amaranth families: (A) pollen diameter in microns; (B) total number of pores per grain; (C) spinule density per 4 $\mu^2$; (D) minute-hole density per 4 $\mu^2$; (E) ratio of minute-hole to spinule densities. Uncertainties are standard deviations. In (A) there are 50 observations; in (B), there are 10 observations that were obtained with a light microscope; in (C), (D), and (E) there are 20 observations, composed of four electron micrographs from four different grains in each species.

| Species | Measure | | | | |
|---|---|---|---|---|---|
| | (A) | (B) | (C) | (D) | (E) |
| Modern amaranths | | | | | |
| *Amaranthus caudatus* | 24.0 ± 2.6 | 25–35 | 30.2 ± 19.2 | 44.9 ± 11.2 | 1.47 ± 0.44 |
| *A. cruentus* | 30.6 ± 6.2 | 33–46 | 29.4 ± 15.7 | 44.4 ± 12.2 | 1.51 ± 0.35 |
| *A. hybridus* | 23.7 ± 1.4 | 28–40 | 30.8 ± 8.6 | 41.5 ± 12.4 | 1.37 ± 0.28 |
| *A. hypochondriacus* | 23.4 ± 2.7 | 30–48 | 30.3 ± 8.1 | 45.5 ± 12.7 | 1.51 ± 0.44 |
| *A. quitensis* | 21.1 ± 5.0 | 38–45 | 41.1 ± 11.3 | 47.7 ± 12.9 | 1.18 ± 0.22 |
| *A. powellii* | 23.3 ± 1.7 | 30–48 | 23.7 ± 11.9 | 31.9 ± 18.7 | 1.36 ± 0.45 |
| Modern chenopods | | | | | |
| *Allenrolfea occidentalis* | 22.4 ± 2.6 | 36–47 | 18.7 ± 6.0 | 54.7 ± 16.5 | 3.08 ± 1.19 |
| *Atriplex littoralis* | 19.6 ± 3.0 | 38–48 | 8.4 ± 2.8 | 51.3 ± 12.2 | 6.35 ± 2.30 |
| *A. portulacoides* | 25.1 ± 4.5 | 50–65 | 10.7 ± 2.9 | 67.3 ± 18.1 | 6.45 ± 2.00 |
| *A. wrightii* | 25.0 ± 1.0 | 54–60 | 13.8 ± 4.4 | 70.7 ± 17.9 | 5.24 ± 1.71 |
| *Beta vulgaris* | 19.5 ± 0.9 | 32–40 | 12.8 ± 4.2 | 58.9 ± 17.6 | 4.71 ± 1.46 |
| *Chenopodium album* | 27.2 ± 2.0 | 65–75 | 6.2 ± 2.0 | 67.6 ± 16.6 | 11.60 ± 3.52 |
| *C. ambrosioides* | 24.1 ± 7.2 | 85–107 | 9.3 ± 3.2 | 15.7 ± 2.0 | 1.75 ± 0.53 |
| *C. capitatum* | 21.4 ± 2.0 | 30–36 | 5.0 ± 3.1 | 38.0 ± 8.4 | 7.93 ± 0.14 |
| *C. hybridum* | 21.3 ± 2.1 | 40–62 | 6.3 ± 2.0 | 33.2 ± 9.4 | 5.37 ± 1.72 |
| *C. murale* | 20.7 ± 6.0 | 35–40 | 7.3 ± 2.5 | 38.4 ± 9.9 | 5.58 ± 1.96 |
| *Kochia scoparia* | 31.1 ± 2.6 | 72–85 | 8.1 ± 2.3 | 23.0 ± 19.0 | 2.90 ± 0.97 |
| *Salicornia ambigua* | 27.1 ± 2.0 | 52–65 | 12.7 ± 4.4 | 57.9 ± 22.8 | 4.74 ± 1.93 |
| *Salsola pestifer* | 24.2 ± 1.0 | 38–54 | 7.3 ± 2.0 | 60.1 ± 15.7 | 8.33 ± 2.45 |
| *Sarcobatus verniculatus* | 27.5 ± 2.5 | 14–17 | 16.8 ± 7.8 | 64.0 ± 17.4 | 3.89 ± 0.69 |
| *Spinacia oleracea* | 34.1 ± 1.0 | 60–75 | 3.4 ± 1.3 | 26.2 ± 8.0 | 8.18 ± 2.90 |
| *Suaeda salsa* | 21.1 ± 1.9 | 49–66 | 7.3 ± 2.3 | 55.4 ± 17.8 | 8.04 ± 2.50 |
| Fossils | | | | | |
| Cuscachapa (10-m level) | 24.3 ± 2.2 | 30–54 | | | |
| *Amaranthus* wild | | | 32.7 ± 2.7 | 38.0 ± 8.4 | 1.20 ± 0.20 |
| *Amaranthus* cereal | | | 36.6 ± 18.4 | 55.2 ± 27.9 | 1.51 ± 0.76 |
| *Chenopodium* | | | 7.6 ± 3.4 | 15.0 ± 6.0 | 2.58 ± 1.64 |
| Tinte (3.85-m level) | | | | | |
| *Atriplex littoralis* | 22.1 ± 2.0 | 48–68 | 12.2 ± 3.0 | 67.6 ± 14.9 | 5.70 ± 1.50 |

minute holes are less satisfactory, their density ranging in amaranths from 32.0 ± 4.2 to 47.7 ± 2.9 S.E. per 4 $\mu^2$, and in chenopods from 23.0 ± 1.5 to 70.7 ± 4.0 S.E. per 4 $\mu^2$; the amaranth range is within the chenopod range. Presumably, even in a single species, the absolute number of spinules and minute holes can be affected by changes in pollen size such as may be induced by acetolysis or the replication procedure. In maize pollen, increase of size was found to decrease the number of spinules per unit area (*12*). However, the ratio of minute holes to spinules per unit area should not be altered by changes of pollen size; this ratio is less than 1.5 in amaranths (about 1.5 in the cultigen and < 1.4 in the wild progenitor) and more than 1.6 in chenopods, showing various values from 1.75 ± 0.64 S.E. (*Chenopodium ambrosioides*) to 11.6 ± 0.74 S.E. (*Chenopodium album*).

Other usable electron-microscopic criteria, not listed in Table 1, are under study; they include the distance between neighboring pores and the number of spines on the pore membrane. The latter character, even if valid, cannot always be used for fossil pollen identification, since the operculum (pore membrane) may come off the grains, leaving a flat impression on the plastic plate.

Two samples of fossil pollen have been tested by the new criterion of spinule-hole ratio, the lambda test (*14*) being used to validate comparisons between and within genera. The first sample is from a core in Lake Cuscachapa, El Salvador, at the southern fringe of the area of Maya culture. In this core, the major vegetational-disturbance zone, confirmed by the dominance of cheno-am pollen (about 80 percent) and by maximal occurrence of maize pollen, was in pollen zone Ch-2 (*8*), the top of which was dated by carbon-14 at 1380 ± 120 B.P. (before the present), or Classic (*15*). Twenty fossil cheno-am pollen types from the 10-m level in this zone were randomly examined by electron microscopy. Of these, 17 are almost certainly wild amaranths, and are statistically indistinguishable from *Amaranthus powellii* (one is shown in Fig. 1f). One is most like the cultivated *A. hypochondriacus*, though it could also be *A. powellii*. The remaining two are chenopods. Small as the sample is, it is reasonable to infer that at least 68 percent of the total pollen is from wild amaranths, perhaps 4 percent is from cereal amaranths, and 8 percent is from chenopods, suggesting some cultivation of grain amaranths, but that most were wild types growing in maize fields.

That generic and perhaps specific identification of chenopods is possible is shown by the second test, from the 3.85-m level at Tinte, western Netherlands (the corresponding level at Lodderland in Voorne was dated 3840 ± 75 B.P.) (*16*). The level is located in pollen zone IVa, the middle sub-Boreal period (*16*), at the top of the lower peat, which is overlain by tidal lagoon deposits. In this level, cheno-am pollen constitutes about 85 percent of the tree pollen. A priori, one would suspect a halophytic chenopod, as in any sample of *Phragmites* peat in the Dutch coastal region. Of the ten chenopodiaceous grains electron-micrographed and statistically examined, all are clearly *Atriplex* (compare Figs. 1c, modern, and 1d, fossil). Moreover, all ten are statistically closer to *Atriplex littoralis* than to *A. portulacoides*; the possibility of *A. wrightii* is rejected ($P < .05$) for only four of the ten, but this American species is unlikely to be represented in the Netherlands. In the tidal clay containing molluscs, immediately above this peat, cheno-am pollen suddenly decreases to about 10 percent, perhaps as a consequence of the rise of sea level, but this sample has not been

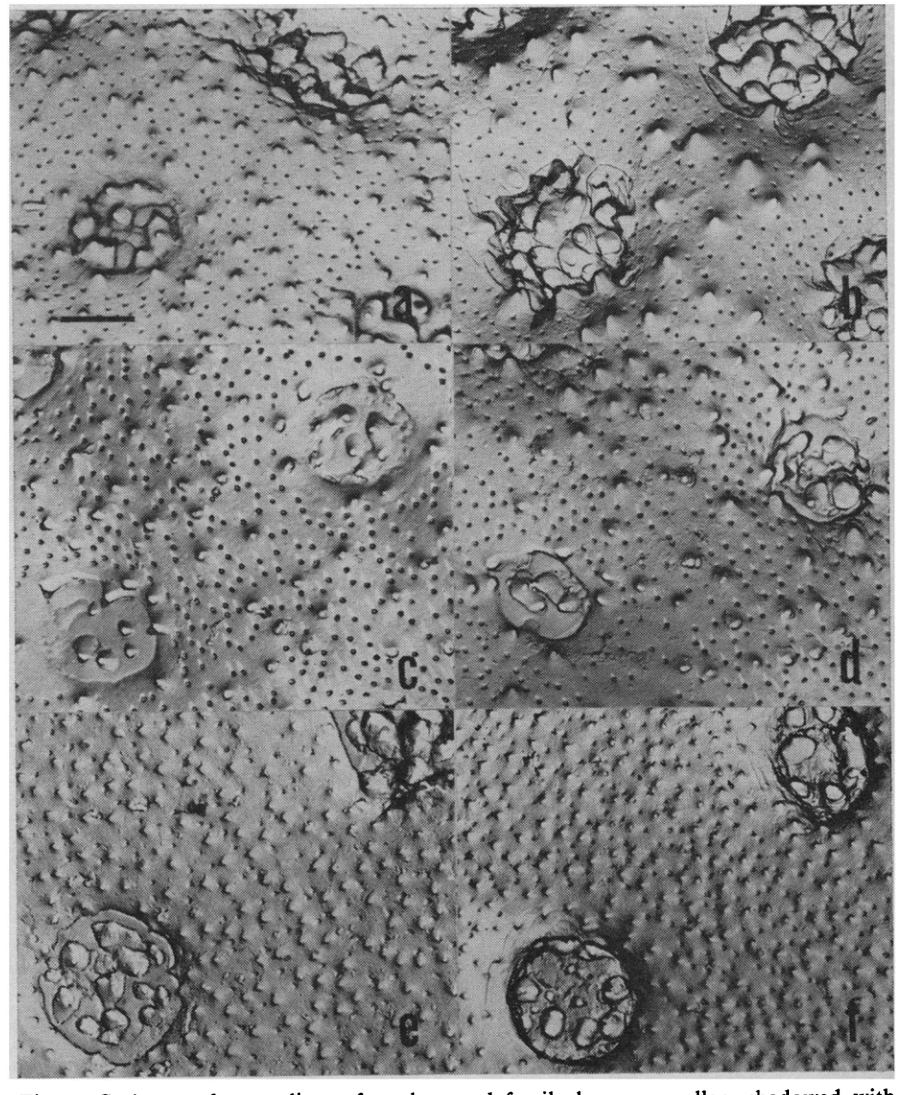


Fig. 1. Carbon surface replicas of modern and fossil cheno-am pollen, shadowed with chromium; (a) modern *Kochia scoparia*; (b) modern *Salsola pestifer*; (c) modern *Atriplex portulacoides*; (d) *fossil Atriplex littoralis* (?), taken from a depth of 3.85 m (dated about 4000 B.P., middle sub-Boreal period) at Tinte near the western Dutch coast; (e) modern *Amaranthus hypochondriacus*; and (f) fossil *Amaranthus* sp. from a depth of 10 m (dated about 1400 B.P., Mayan Classic period) from Lake Cuscachapa core, El Salvador. All figures ×10,000. Black line in (a) equals 1 $\mu$.

examined with an electron microscope.

Fossil pollen grains previously studied by electron microscopy were picked out of the sediments by capillary tube (*11*) or by needle (*12*). The present development of the technique makes it possible to work directly from pollen residues regularly prepared from sediments. By this method over ten known or unknown fossil pollen replicas are encountered in each 200-mesh grid, and all pollen from 2.0 $cm^3$ of clay-gyttja sediment from the Cuscachapa core or of peat from the Tinte core can be accommodated on about 45 grids. Electron-microscopic pollen analysis is thus technically feasible and promises to solve many serious identification problems in paleoecology.

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## Enzymatic Identification of Fish Products

Abstract. *Comparative enzymological techniques were used to distinguish between the muscle lactate dehydrogenases of 26 fish species. Intergeneric differences in enzymatic properties were frequently encountered. The techniques revealed, in addition, that some commercial samples of frozen fish fillets, labeled "haddock," contained cod lactate dehydrogenase.*

During a comparative study of lactate dehydrogenases (*1, 2*), we examined certain properties of these enzymes in fishes of the family Gadidae. This group of fishes includes haddock (*Melanogrammus aeglefinus*) and cod (*Gadus callarias*) and others commonly used for food. At one point in the study we used haddock muscle from a package of frozen fillets labeled "haddock." A cell-free extract prepared from this commercial sample was subjected to starch-gel electrophoresis (*3*). The gel was treated with a stain specific for lactate dehydrogenase (*4*). Surprisingly, the LDH (*5*) of this sample was electrophoretically identical with LDH from a sample of authentic cod muscle,

Table 1. Electrophoretic mobility and thermostability of muscle LDH from various fish species.

| Species | Distance moved * (cm) | Inactivation temperature † (°C) |
|---|---|---|
| *Teleosts* | | |
| Gadidae | | |
| Atlantic cod, *Gadus callarias* | 5.3 | 52 |
| Haddock, *Melanogrammus aeglefinus* | 1.7‡ | 43 |
| Pleuronectidae | | |
| Pacific halibut, *Hippoglossus stenolepis* | 0.0 | 56 |
| Petrale sole, *Eopsetta jordani* | 2.0 | 50 |
| Scombridae | | |
| Atlantic bluefin tuna, *Thunnus thynnus* | −1.5 | 48 |
| Pacific yellowfin tuna, *Thunnus albacares* | −1.5 | |
| Mackerel, *Scomber scombrus* | 0.3 | 56 |
| Scorpaenidae | | |
| Hawaiian scorpionfish, *Scorpaenopsis gibbosa* | 2.5 | 47 |
| Ocean perch, *Sebastes marinus* | 3.0 | 48 |
| Anoplopomatidae | | |
| Sablefish, *Anoplopoma fimbria* | 2.2 | 55 |
| Sparidae | | |
| Scup, *Stenotomus chrysops* | 1.0 | 48 |
| Cyprinidae | | |
| Carp, *Cyprinus carpio* | 2.6 | 67 |
| Clupeidae | | |
| Herring, *Clupea harengus* | 0.2 | 39 |
| Albulidae | | |
| Bonefish, *Albula vulpes* | .2 | 54 |
| Elopidae | | |
| Tenpounder, *Elops saurus* | .1 | 54 |
| Chanidae | | |
| Milkfish, *Chanos chanos* | .8 | 55 |
| Salmonidae | | |
| Brook trout, *Salvelinus fontinalis* | .3‡ | 51 |
| Osmeridae | | |
| Smelt, *Osmerus mordax* | 5.0 | 48 |
| Esocidae | | |
| Chain pickerel, *Esox niger* | 0.5 | 50 |
| *Nonteleosts* | | |
| Garpike, *Lepisosteus spatula* | 3.5 | § |
| Sturgeon, *Acipenser transmontanus* | 0.6‡ | 49 |
| African lungfish, *Protopterus* species | 1.7 | 50 |
| Atlantic spiny dogfish, *Squalus acanthias* | −2.0 | 61 |
| Pacific spiny dogfish, *Squalus acanthias* | −2.0 | 61 |
| Seven-gill shark, *Notorhynchus maculatum* | 0.6 | 49 |
| Lamprey, *Petromyzon marinus* | 0.4 | 51 |
| Pacific hagfish, *Eptatretus stouti* | −0.9 | 64 |

* Distance moved toward the anode by the major form of LDH present in a muscle extract. Extracts were made by grinding a 1-g portion of white (epaxial) muscle in 5 ml of cold 0.25*M* sucrose in a glass homogenizer. The extracts were clarified by centrifugation and stored at −10°C. Electrophoresis was carried out with 0.02-ml samples of extract under standard conditions (*3*); phosphate-citrate buffer at *p*H 7.0 was used. The gel was then treated with a nitroblue tetrazolium staining mixture specific for lactate dehydrogenase (*3, 4*). A sample of chicken $H_4$ LDH was used as a standard on each gel; under these conditions, this enzyme moved 2.2 cm. † Temperature required for 50 percent inactivation in 20 minutes (see *1*). Muscle extracts were diluted tenfold or or 100-fold in a buffer, *p*H 7.5, containing 0.14*M* NaCl, 0.0005*M* $MgSO_4$, 0.00015*M* $CaCl_2$, 0.01*M* tris, and 0.1 percent bovine serum albumin, and then heated at various temperatures for 20 minutes. The heated samples were quickly cooled to 0°C and later assayed at 23°C for LDH activity (*3*). ‡ Five closely spaced spots of LDH activity were observed in these extracts. The mobility value given is for the central spot, which was also the most intensely staining spot (*9*). § The muscle LHD of this species was unstable at 0°C when diluted tenfold or 100-fold in the standard buffer.