7. MOLECULAR STRUCTURES OF THE PARTIALLY DISSOLVED FOOT LAYER AND ENDEXINE OF PINUS GRIFFITHII MCCLELL POLLEN GRAINS

M. KEDVES, A. TÓTH, Á. KÁROSSY and A. VARGA

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany of the J. A. University, H-6701, P. O. Box 993, Szeged, Hungary

Abstract

Pollen grains of *Pinus griffithii* MCCLELL were dissolved partially with pyrrolidine. The molecular systems of the foot layer and the endexine were investigated on highly magnified pictures. The new observations are as follows: 1. Different kinds of molecular structures were observed. 2. Two major types were observed: Chain molecules and microtubular structures. 3. The distances between the centre of the shadows of the atoms are about 1.5–1.6 Å. 4. The bordering between the foot layer and the endexine and the dark and light endexine lamellae are not distinct on molecular level. 5. There are no characteristic molecular differences between the different layers investigated. 6. A common molecular structure is also represented by chain-molecules at the ontogenetically different layers (ectexine and endexine).

Key words: Palynology, recent, *gymnosperm*, molecular system.

Introduction

The sporoderm has several peculiar characteristic features from different points of view. Investigations on this subject were carried out on several levels and methods. One of them is the study of the biopolymer system of the partially degraded wall structures. Taking into consideration the different results more or less well delimited fields of researchs can be distinguished. In our point of view the sporopollenin biopolymer system is in the first place of our interest. The different levels of the organization were summarized in 1989a (KEDVES). In our laboratory several investigations were carried out about the structure and symmetry of the biopolymer system of the plant cell wall. Regarding the so-called sporopollenin type plant cell walls, two major components have been established within the biopolymer system in angstroem dimension: the quasi-crystalloid skeleton and the stabilizing system. The quasi-crystalloid skeleton was investigated with several methods: 1. Two dimensional modelling and symmetry operations, based on the TEM pictures of partially degraded exines (cf. KEDVES 1988, 1989b, 1990, 1991a, KEDVES and FARKAS, 1991, KEDVES, FARKAS, MÉSZÁROS, TÓTH and VÉR, 1991, etc.) 2. Three dimensional modelling (cf. KEDVES, 1991b, 1992). 3. Computer modelling (KEDVES, M. and KEDVES, L., 1994, 1996). The quasi-crystalloid skeleton of the spore-pollen wall was

designed by COLLINSON, HEMSLEY and TAYLOR, W. A. (1993) as mycelles within the colloidal-crystal biopolymer system. Regarding the stabilizing biopolymer structures of the metastable quasi-crystalloid skeleton till this time we have few data (cf. KEDVES and TÓTH, 1994).

The subject of this contribution was planned into the stabilizing biopolymer system investigation research program, based on our previous hypothesis of the molecular symmetry of the organic solvents (GÉVAY and KEDVES, 1989).

The aim of this paper is the evaluation on molecular level the similarities and the differences between the foot layer and endexine, and between the dark and light lamellae of the endexine.

Material and Methods

In our preliminary report (KEDVES and PÁRDUTZ, 1992) we published some data of the results of several experiments. It seems to be useful to cite in this place the previously published methods again which correspond to the present contribution; p. 39: "Experiment No 669: 20 mg air dried pollen material + 5 ml pyrrolidine. Temperature: +5 -5 °C, length of time: 25 days." P. 35: "We have the opportunity to take picture of 400.000 x resolution 2-3.5 Å with the new TEM instrument of the Biological Research Center of the Hungarian Academy of Science (OPTON 902)."

The evaluation of the molecular systems was made on high magnified pictures (5 Million).

Results

The general survey TEM picture (Plate 7.1., fig. 2) well illustrates that the electron density of the foot layer is much more stronger than that of the endexine lamellae. Beneath the foot layer there is a light endexine lamella, "a". Altogether five lamellae are represented in our TEM picture (Plate 7.1., fig. 2). The microphotographs on the magnification of 1 Million well represents the pecularities of the ectexine/endexine bordering on both sides (Plate 7.1., figs. 1,3). It is well illus-

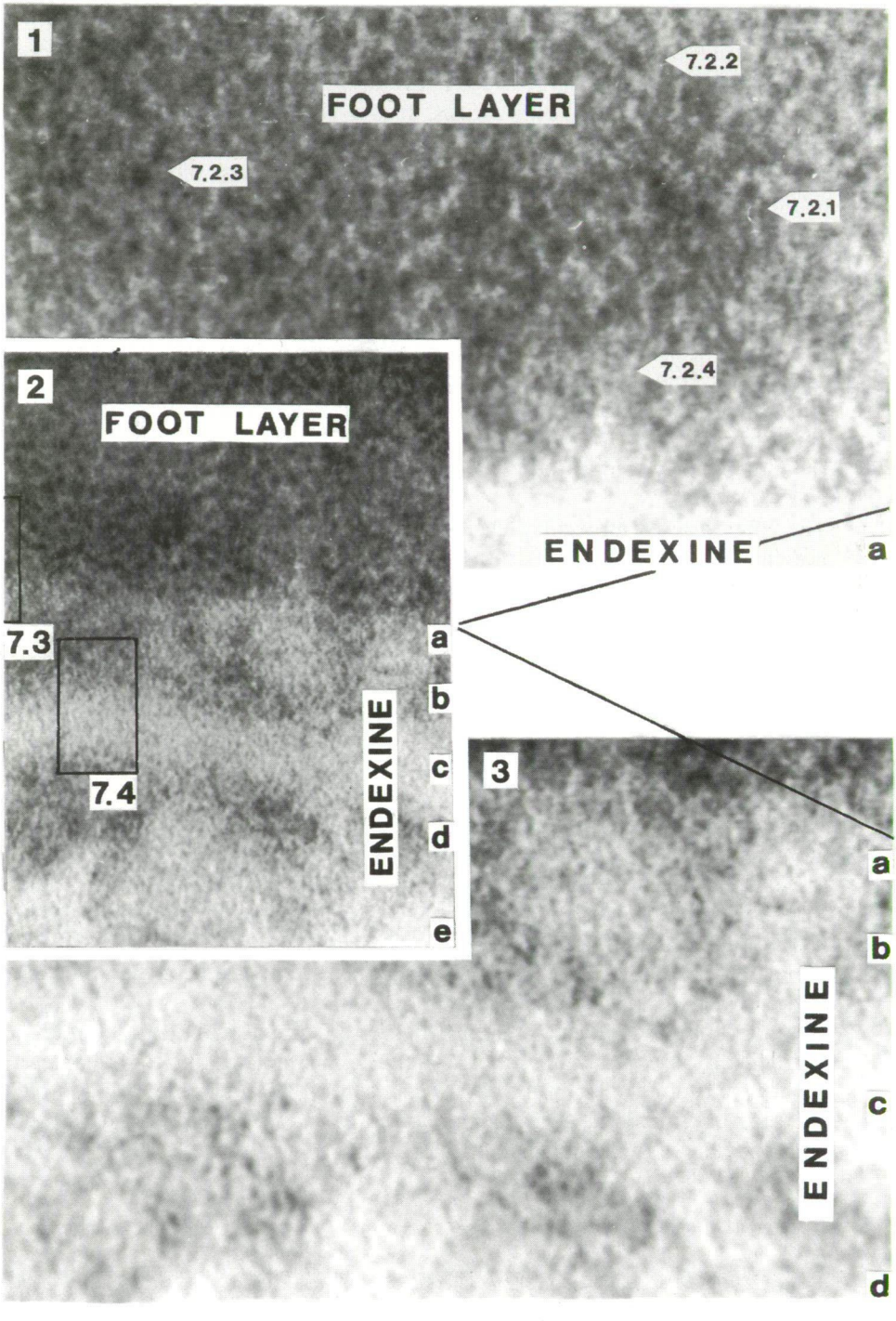
Plate 7.1. ►

1-3. *Pinus griffithii* MCCLELL, Recent. Experiment No: 669, negative no: 435. TEM pictures of the partially dissolved exine.

1. Inner part of the foot layer and the first, outermost (a) endexine lamella. On the foot layer four biopolymer structures are marked with arrows. The numbers of the arrows indicate the highly magnified pictures of these molecular systems; for example 7.2.3. = picture 3, in plate 2, of the present paper, no 7. 1,000.000x.

2. General survey picture of the partially dissolved foot layer and endexine. Five lamellae of the endexine (a – e) are illustrated in this picture. The position of the highly magnified pictures illustrated in the plates 3 and 4 are marked with frames. 500.000x.

3. General survey picture of the bordering part of the foot layer/endexine on biopolymer level. The lowest part of the foot layer, and four endexine lamellae are illustrated. As it is well shown in picture no 1 also, the line of the bordering is not definitely remarkable after partial dissolution. 1,000.000x.


1
FOOT LAYER
7.2.2
7.2.3
7.2.1
7.2.4
ENDEXINE
a
2
FOOT LAYER
7.3
7.4
ENDEXINE
a
b
c
d
e
3
a
b
ENDEXINE
c
d

trated, that at the bordering zone it is not so easy to mark strictly the dividing line. The different kinds of biopolymer structures are also well illustrated in the pictures of Plate 7.1. In the foot layer there are are several microtubular molecular systems, e.g. fig. 1, Plate 7.1., marked with arrows. It is a central unit of 2.5-3.5 Å, surrounded by about 6-8 further units. The total diameter of the microtubular systems is 7-10 Å. The highly magnified pictures of these microtubular molecular systems are illustrated by the magnification of 5 Million in the Plate 7.2., figs. 1-4. As of the molecular structures of the foot layer/endexine bordering, the following can be established; (Plate 7.3.):

1. Several molecular chains were observed oriented in the radial direction of the wall.
2. These chains can also be divided and are continuous from the endexine, and penetrate into the ectexine (foot layer).
3. In this way, one part of the molecular system of the ontogenetically different two layers is common.

To the molecular system of the two kinds of endexine lamellae we can point out as follows (Plate 7.4.):

1. The differences on molecular level between the dark and the light lamellae are not characteristic. Moreover the dividing line between two kinds of lamellae is very obscure. Cf. lamellae b – c – d.
2. Based on our present day knowledges two major molecular components were discovered with this kind of dissolution within the endexine.

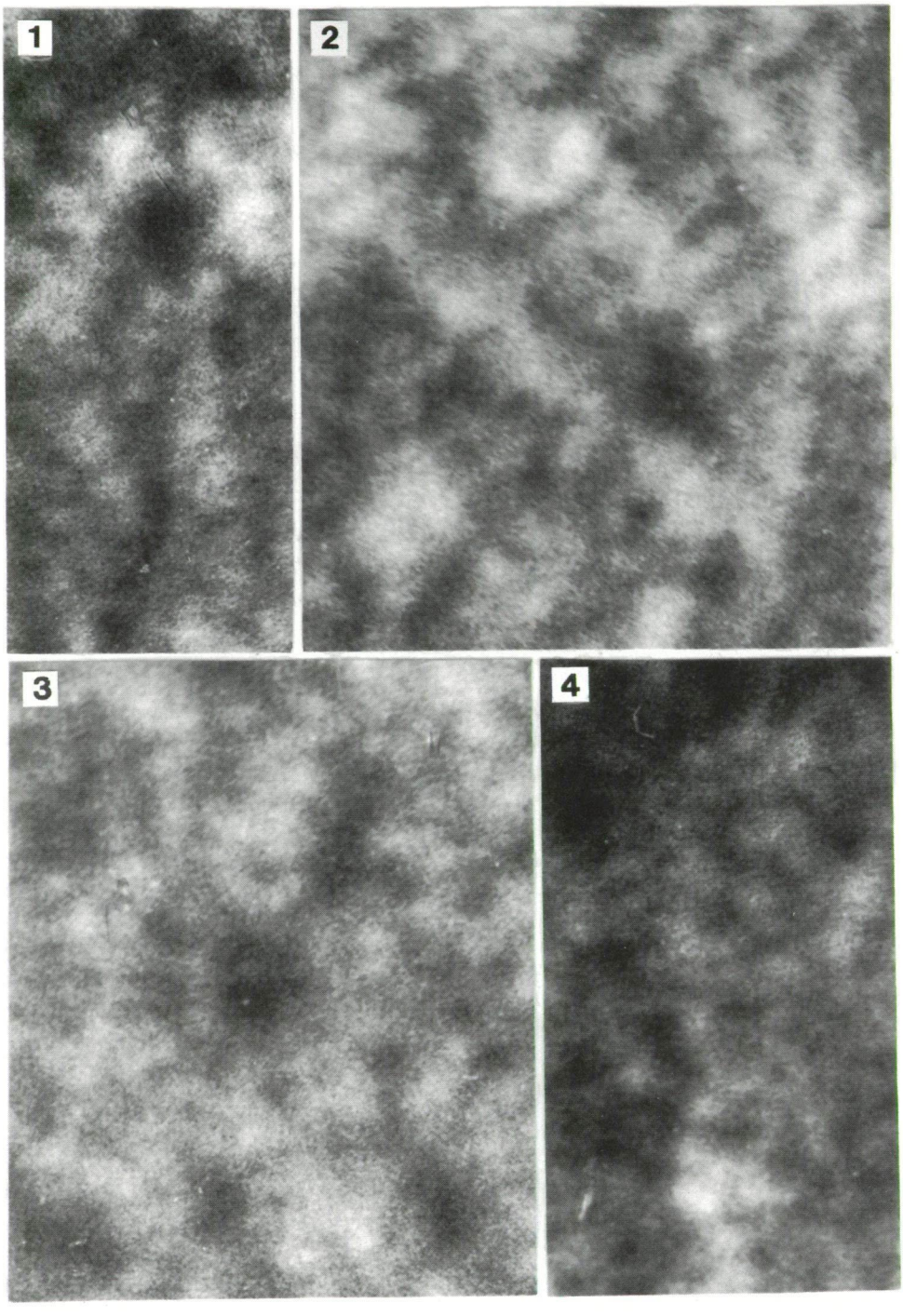
2.1. The radially oriented chain molecules, discussed previously. There are light molecular chains also in all probability these are holes of the dissolved molecules.

2.2. The second component is a strong, electron dense molecular system and fills up the holes between the less electron dense chains-molecules. The molecular symmetry of this component is not yet well known; pro parte presumably globular.

3. It seems that the molecular chain system is more loose within the dark lamellae. In this way the probably globular units with strong electron density are the factors of the dark lamellae. In contrast to this, the more compact chain-molecular system of the light lamella does not contain such molecular structures of strong electron density.
4. The microtubular molecular system which is very common in the foot layer was sporadically observed only in the dark endexine lamellae. The total diameter of the microtubules is about 8–10 Å. The central microchannel is 4 Å large. Till this time this kind of molecular structure had not been observed at the light lamellae of the endexine.

Plate 7.2. ►

1-4. Pinus griffithii McCLELL, Recent. Experiment No: 669, negative no: 435. TEM pictures of the partially dissolved foot layer. The highly magnified pictures of the molecular units marked in fig. 1, plate 7.1. 5,000.000x.


1
2
3
4

Discussion and Conclusions

Based on the molecular structure observed on the highly magnified TEM pictures of the partially degraded foot layer and endexine of the pollen grains of *Pinus griffithii* McClell, the following can be emphasized:

At this experiment in all probability pyrrolidine dissolved the quasi-crystalloid skeleton.

The characteristic stabilizing units described first from the intine of *Pinus griffithii* dissolved partially with diethyl aether were not observed. It may be presumed that pyrrolidine dissolved the whole Penrose system of quasi-crystalloid skeleton and the holes (frustration sensu NELSON, 1986) filling biopolymers.

The more or less radially oriented chain-molecular system is interesting and peculiar.

Concerning the microtubular system the following is worth of mentioning: A similar system was observed during our investigations of the interaction on molecular level of the pollen grains of *Thalictrum flavum* and input the microscopic *Fungi, Gliocladium roseum.* But it is necessary to note the microtubules of *Gliocladium roseum* and *Thalictrum flavum* are smaller (total diameter 2–4 Å) than at the exine of *Pinus griffithii.* This phenomenon seems to be very common on different levels of the molecular systems of the plant cell walls. For example regular pentagonal units were observed in molecular dimensions (sensu strictu), on biopolymer (angstroem dimensions) and finally in nanometer dimension. At the 1st and 2nd cases the quasi-periodic symmetry was demonstrated by KEDVES, FARKAS and TÓTH (1993).

Acknowledgements

This work was financially supported by the Grant OTKA 1/7, T 014692.

Plate 7.3. ►

Pinus griffithii MCCLELL, Recent. Experiment No: 669, negative no: 435. Highly magnified picture of the molecular system of the foot layer/endexine bordering. The position of the illustrated part is marked in picture 2, plate 7.1. 5,000.000x.

ENDEXINE
FOOT LAYER
a

Bibliography

COLLINSON, M. E., HEMSLEY, A. R. and TAYLOR, W. A. (1993): Sporopollenin exhibiting colloidal organization in spore walls. – Grana Suppl. *1,* 31-39.

GÉVAY, G. and KEDVES, M. (1989): A structural model of the sporopollenin based on dodecahedrane units. – Acta Biol. Szeged. *35,* 53-57.

KEDVES, M. (1988): Quasi-crystalloid basic molecular structure of the sporoderm. – 7 Internat. Palynol. Congr. Brisbane, Abstracts, 82.

KEDVES, M. (1989a): Quasi-crystalloid biopolymer structures and its highly organized degrees. – Acta Biol. Szeged. *35,* 59-70.

KEDVES, M. (1989b): Méthode d'étude des biopolymères de la paroi pollinique à structure quasi-cristalloïde. A method of investigation of the quasi-crystalloid structure of pollen wall biopolymers. – Rev. de Micropaléontologie *32,* 226-234.

KEDVES, M. (1990): Quasi-crystalloid basic molecular structure of the sporoderm. – Rev. Palaeobot. Palynol. *64,* 181-186.

KEDVES, M. (1991a): TICOS polyhedra, as a model in the pentasporan organization. – Plant Cell Biology and Development (Szeged) *2,* 43–48.

KEDVES, M. (1991b): Three dimensional modelling of the biopolymer structure of the plant cell wall I. – Plant Cell Biology and Development (Szeged) *2,* 63–74.

KEDVES, M. (1992): Three dimensional modelling of the biopolymer structure of the plant cell wall II. – Plant Cell Biology and Development (Szeged) *3,* 67-87.

KEDVES, M. and FARKAS E. (1991): Basis of the tertiary rotation and TICOS modelling of the quasi-crystalloid biopolymer skeleton of the plant cell. – Plant Cell Biology and Development (Szeged) *2,* 36-42.

KEDVES, M., FARKAS, E., MÉSZÁROS, K., TÓTH, A. and VÉR, A. (1991): Investigations of the basic biopolymer structure of the ectexine of *Alnus glutinosa* (L.) GAERTN. – Plant Cell Biology and Development. (Szeged) *2,* 49-58.

KEDVES, M. and KEDVES, L. (1994): Computer modelling of the quasi-crystalloid biopolymer structure I. – Plant Cell Biology and Development (Szeged) *6,* 68–77.

KEDVES, M. and KEDVES, L. (1996): Computer modelling of the quasi-crystalloid biopolymer structure II. – Plant Cell Biology and Development (Szeged) *7,* 82–88.

KEDVES, M. and PÁRDUTZ, Á. (1992): Transmission electron microscopy of partially dissolved exine of different disaccate *gymnosperm* pollen grains. – Plant Cell Biology and Development (Szeged) *3,* 38–66.

KEDVES, M., PÁRDUTZ, Á. and VARGA, A. (1996): Ultrastructural study on the pollen-microbial interactions. – Taiwania, *41,* 43–52.

KEDVES, M. and TÓTH, A. (1994): Premiers résultats du système de biopolymère stabilisateur du squelette quasi-cristalloïde de l'exine. – Plant Cell Biology and Development (Szeged) *5,* 79–86.

NELSON, D. R. (1986): Quasicrystals. – Sci. Amer. *254,* 43–51.

Plate 7.4. ▸

Pinus griffithii MCCLELL, Recent. Experiment No: 669, negative no: 435. Highly magnified picture of the molecular system of the endexine lamellae b, c and d. The position of the illustrated part is marked in picture 2, plate 7.1. 5,000.000x.

ENDEXINE
b
c
d