



HOKKAIDO UNIVERSITY

Title	Ontogenetic development of pollen tetrads in <i>Vaccinium smallii</i> A. Gray (Ericaceae)
Author(s)	Sarwar, A.K.M. Golam; Ito, Toshiaki; Takahashi, Hideki
Citation	日本花粉学会会誌, 58(2), 61-71
Issue Date	2012-12-31
Doc URL	https://hdl.handle.net/2115/55508
Type	journal article
File Information	Sarwar et al.pdf



1) Date of submission of revised manuscript: September, 2012

2) Type of manuscript: Research Article

3) Title: Ontogenetic development of pollen tetrads in *Vaccinium smallii* A. Gray (Ericaceae)

Name(s) of author(s): A. K. M. Golam Sarwar¹⁾, Toshiaki Ito²⁾ and Hideki Takahashi³⁾

Address(s): ¹⁾ Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202,
Bangladesh

²⁾ Research Faculty of Agriculture, Hokkaido University, N8 W8, Sapporo 060-8589,
Japan

³⁾ The Hokkaido University Museum, N10 W8, Sapporo 060-0810, Japan

4) Running title: Pollen tetrad development in *Vaccinium*

5) Total pages for text: 12

6) Total number of table(s): 0

7) Total number of figure(s): 29 (in 4 plates)

8) Total page(s) for captions for Figures: 3

9) Corresponding Author: Professor Dr. Hideki Takahashi

The Hokkaido University Museum

Kita 10, Nishi 8, Sapporo 060-0810, Japan.

Tel. & Fax: +81-11-706-4508

E-mail: hide@museum.hokudai.ac.jp

Ontogenetic development of pollen tetrads in *Vaccinium smallii* A. Gray (Ericaceae)

The ontogenetic development of pollen tetrads in *Vaccinium smallii* was investigated by light and transmission electron microscopy with special attention to the mode of exine deposition. The deposition of the primexine matrix represented by a fibrillar surface coat started during the cytokinesis of the microspore mother cell within a thick callose wall. The deposition of dark lipoidal globules of sporopollenin precursor substances was observed within the primexine matrix. After the disappearance of these globules, radially directed pro-columellae appeared within the primexine matrix, and the primexine gives rise to the tectum, columellae and foot layer after the callose dissolution. The position of future apertures was observed at a thick zone and/or a thin layer of the primexine matrix. In both regions, the pro-columellae, protectum and foot layer were not formed. Major binding mechanism of the permanent mature pollen tetrads in *V. smallii* is attributed to 1) the early deposition of the primexine matrix on the microspore mother cell undergoing cytokinesis, and 2) thin and tenuous callose depositions, 3) the common primexine matrix, and 4) the cytoplasmic channels between the adjoining microspores of the meiotic tetrad within a thick callose wall.

Key words: binding mechanism, ontogeny, pollen tetrads, primexine, *Vaccinium*

Introduction

The cosmopolitan family Ericaceae contains 4084 species and 121 genera ⁽¹⁾, which comprises 8 subfamilies: Enkianthoideae, Monotropeae (including Pyrolaceae), Arbutoideae, Cassiopoideae, Ericoideae (including Empetraceae), Harrimanelloideae, Styphelioideae (including Epacridaceae) and Vaccinioideae ⁽²⁾. Members of this family are highly diverse not only in life forms, leaf morphology and inflorescence characteristics, but also in palynological features. Pollen grains of Ericaceae are dispersed either as monads (mutually isolated grains; e.g., in Enkianthoideae) or compound grains. The latter can be further categorized into pseudomonads, reduced tetrads with one or more aborting cells (e.g., in Styphelieae of Styphelioideae), normal tetrads (commonly without viscin threads but occasionally with them such as in Rhodoreae of Ericoideae), and polyads of indefinite number of tetrads (e.g., in *Chimaphila* of Monotropeae). Because of the large size of the family Ericaceae, our knowledge on its pollen ultrastructure and development is still poor and therefore further studies are required.

Transmission electron microscopic (TEM) images of mature pollen exine in Ericaceae have been published only for several genera ^(3–18). Available information on pollen wall development at the TEM

level is even more scarce and is restricted to three subfamilies of Ericaceae: Styphelioideae (*Styphelia* ⁽¹⁹⁾), Monotropoideae (*Chimaphila*, *Orthilia*, and *Pyrola* ^(20–22)), and Ericoideae (*Calluna* ⁽²³⁾).

Vaccinium L. of the subfamily Vaccinioideae, with about 500 species, is the third largest genus in the Ericaceae ⁽¹⁾. Although pollen morphology of this genus has been studied widely, the majority of researchers used light microscopy (LM). Although scanning electron microscopy (SEM) has also been used to study the mature pollen, a single study employed TEM (⁽¹³⁾ and references therein). Our previous study ⁽¹³⁾ of *Vaccinium* gave an outline of pollen morphology and the details of its taxonomic significance in the sectional classification. However, information on the pollen tetrad development in *Vaccinium*, especially on the exine development, is still lacking. Published works on embryology (⁽²⁴⁾ and references therein) do not provide much information in this regard. Hence the objective of this paper is to fill the existing knowledge gap through a detailed description of the main processes of the pollen tetrad development of *V. smallii*, and to compare the results obtained with the already existing information from the other genera of Ericaceae and angiosperm taxa.

Materials and methods

Flowers of *Vaccinium smallii* A. Gray were collected at different developmental stages from the cultivated plants at the Botanic Garden of Hokkaido University, Sapporo, Japan. The anthers were fixed in 2.5% glutaraldehyde and 1% para-formaldehyde in 50 mM phosphate buffer (pH 7.4), and post-fixed in 1% osmium tetroxide in the same buffer. Fixed materials were dehydrated in an ethanol series and embedded in Epon 812 epoxy resin. Sections were cut on a Reichert-Jung Ultracut N ultramicrotome using glass or diamond knives. In order to confirm the developmental stages thick sections (ca. 1 µm) were collected on glass slides, stained with toluidine blue, and observed with a Zeiss compound microscope (Axiophot, Germany) using bright-field optics. Thin sections (ca. 0.1 µm) were collected and dried on formvar-coated copper slot grids, and double-stained with saturated 2% uranyl acetate (20 min) and lead acetate (3 min) solution, and observed and photographed with a Hitachi H-800 transmission electron microscope at 75 KV. Descriptive terminology used in this paper follows Takahashi and Sohma ⁽²⁵⁾, Punt et al. ⁽²⁶⁾, El-Ghazaly et al. ⁽²⁷⁾, and Hesse et al. ⁽²⁸⁾.

Results

Cytokinesis of microspore mother cell within the callose

The tetrasporangiate anther wall consisted of epidermis, two layers of hypodermal cells, and one, two, or rarely three layers of tapetal cells (Fig. 1). The tapetal cells had electron-dense cytoplasm denser than that of the sporogenous cells. Following the meiotic nuclear division, cytokinesis started with the

formation of cell plates between four meiotic nuclei of each microspore mother cell (MMC), enveloped in a thick callose wall. Initially, the MMC had an elongated triangular or tetragonal shape (Figs. 1 and 2). The primexine matrix represented by a fibrillar surface coat was deposited between the callose wall and undulating plasmalemma of MMC forming four meiotic microspores (Fig. 3). Its thickness was not uniform (Figs. 4 – 6). In some cases, dark lipoidal globules were observed within the fibrillar surface coat on the plasmalemma of MMC (Figs. 7 and 8).

Microspore tetrad within the callose

Soon after the primexine development had begun, the radially directed pro-columellae traversed the primexine matrix (Figs. 9 – 12). The cytoplasm was rich in ribosomes and contained dilated cisternae of primarily rough endoplasmic reticulum (RER). Structure of the primexine matrix coating the microspore surface appeared similar on distal (apocolpial) and proximal (intersporal) surfaces. Each tip of the cylindrical cytoplasmic microprojections of the plasmalemma was situated at the bottom of the respective pro-columella (Figs. 10 – 12). The pro-columellae (probacula) developed simultaneously all around the microspore except for the future apertural regions.

The adjoining microspores were connected by cytoplasmic channels (cytoplasmic bridges) across thin and tenuous callose layer in the intersporal space between microspores (Fig. 13). The adjoining cells in each meiotic tetrad were also connected to each other by the common primexine matrix (Figs. 13 – 15). Cytoplasmic channels occurred less frequently compared to the common primexine matrix.

Protectum was faintly observed at the outer surface of the primexine matrix (Figs. 12, 15 and 17). At the probable future apertural regions, the primexine matrix was either completely absent (Fig. 16) or quite thick (Fig. 17), and no pro-columellae, protectum and foot layer were observed in both cases. The tapetal cytoplasm became more electron-dense and contained numerous RER. Lipoidal electron-dense granules were found on the surface of the tapetal cells (Figs. 14 and 16).

Free microspore tetrad

After the dissolution of callose wall, the four microspores of the tetrad were connected and surrounded by thick tectum, and the discontinuous common tectum was formed between the neighboring four microspores (Fig. 18). There were many starch grains and lipid globules in the microspore cytoplasm. The outer pollen wall was composed of tectum, columellae, foot layer, endexine, and intine. The outer wall was thicker than or as thick as the inner wall (Fig. 18). At this stage, the endexine showed more electron-density than the ectexine. Thick intine was deposited near the endoaperture (os) regions (Fig. 18). Exine of the ectoaperture (colpus) region was characterized by the lack of tectum and columellae, very thin foot layer, and thick endexine (Fig. 19). The inner exine (septal exine) was composed of a common tectum, columellae, foot layer and thin endexine (Fig. 20). A transparent space was sometimes observed

at the central part of the common tectum between the microspores (Fig. 20). These spaces appear to correspond to those occupied by thin and tenuous callose layer between four microspore cells within the callose wall.

Microspore mitosis producing pollen tetrad

The microspore nucleus first divided to form a large central vegetative nucleus and a smaller generative nucleus pressed against the outer pollen wall (Fig. 21). Subsequently, the cell wall was formed producing two distinct cells; a larger vegetative cell and a smaller generative cell. All generative cells observed were situated at the distal pole of the grains constituting the permanent tetrad and the partition wall between vegetative and generative cells was connected with intine of distal pole (Fig. 22). The first mitosis of whole grains in the anther sac might have taken place synchronously. At this stage, aperture (colpus) region was characterized by the lack of ectexine (tectum, columellae and foot layer), and by thick endexine and intine (Fig. 23).

Mature pollen tetrad

The cytoplasm of mature pollen grains was filled with many starch grains and lipid globules, which made nuclei difficult to see (Fig. 24). Distal pollen wall of the mature grains was composed of thick ectexine (thick tectum, thin columellae, and thick foot layer), thin endexine with more electron-density, and intine (Fig. 25). The electron density of the ectexine decreased, the differentiation became much more distinct between the ectexine and endexine. The exine was about 1 μm thick at the outer distal regions. The common tectum between the neighboring grains within a mature tetrad were found to be fragmentary, and thin columellae connected the thick foot layers of adjoining grains (Fig. 26). Total thickness of inner common exine was only about 0.5 μm . The aperture (colpus) region was characterized by the lack of ectexine, thick endexine, and thick intine with the membranous granular layer (Fig. 27).

In SEM, mature pollen grains were observed as united forming a compact tetrahedral tetrad, 3-colpor(oid)ate, with an average of 43.9 μm in tetrad diameter (D) and 35.0 μm in grain diameter (d), and the exine sculpture being coarsely rugulate to coarsely rugulate-psilate with secondary sculptures (Figs. 28 and 29).

Discussion

Ontogenetic timing

Among the members of the family Ericaceae, the meiosis in the MMC occurs during two different periods: the flowering season, e.g. in *V. smallii* and the summer of the year prior to flowering,

e.g. in *Rhododendron schlippenbachii* (Sarwar and Takahashi, unpublished data). These results support and confirm the previous finding⁽²⁴⁾.

Two major patterns of microsporogenesis, simultaneous and successive cytokinesis, have been recognized, and the tetrad geometry was found to reflect which of these patterns actually occurs⁽²⁹⁾. The result of present investigation supports and confirms that the simultaneous type of microsporogenesis occurs in the genus *Vaccinium* as well as in the family Ericaceae^(4, 19 – 24), Sarwar and Takahashi, unpublished data). Eudicots are characterized by simultaneous microsporogenesis⁽²⁹⁾.

Exine development

General phenomena at the initial stages of exine development in *V. smallii*, i.e. while the microspores are still within the callose wall, are similar to those described in many angiosperms⁽³⁰⁾. Moreover, new features in the exine development are the initiation of the primexine matrix represented by a fibrillar surface coat (Figs. 1 – 8) during the cytokinesis stage of MMC following meiotic nuclear division.

In the similar stage of development, the dark lipoidal globules were also observed within the fibrillar coat between the plasmalemma of MMC and inner surface of callose wall (Figs. 7 and 8). Such an occurrence of dark lipoidal globules during cytokinesis may be a peculiar phenomenon in higher angiosperms. But, this feature is common in the primitive angiosperms and Cycadales⁽³¹⁾ and references therein). Gabarayeva and Grigorjeva⁽³¹⁾ suggested these dark lipoidal globules to be sporopollenin precursor substances. Being abundant at the cytokinesis stage, they almost disappear by the early microspore tetrad stage. It is clear that the disappearance of the globular form of this substance means either its transformation to other form, not so easily recognized with TEM, or its consumption. Another possibility is the dissociation these globule substances to microglobules as in *Stangeria eriopus*⁽³¹⁾. These globules may be related with the early and rapid deposition of primary exine in *V. smallii*, but their position is different from the protectal knobs in *Calluna*⁽²³⁾.

The columellae are the first pollen wall components to form, and these remain distinct throughout ontogeny in early tetrad stage, followed by the tectum and foot layer development (Figs. 9 and 18). The appearance of radially oriented pro-columellae in *V. smallii* is similar in *Pyrola*⁽²²⁾ than in *Calluna*⁽²³⁾.

Recently, Gabarayeva⁽³²⁾ reviewed and synthesized the ideas about the formation of sporoderm structure and discussed the probable role of ER and Golgi apparatus in the process of exine development. Even so each tip of cylindrical cytoplasmic micro-projection is always situated at the bottom of each pro-columella (Figs. 10 – 12 and 15). This phenomenon means some possible relations between cytoplasmic activity and the deposition of pro-columellae.

While the meiotic tetrads are still enclosed in the callose wall, the pro-columellae spread tangentially and eventually the tectum becomes faintly apparent on the outer surface of primexine matrix

(Figs. 12 and 15). During this stage, white lines are observed within foot layer in many plant species and this white line seems to be the “common mode of sporopollenin deposition”^(33 – 34). The white line between the foot layer and endexine may have some function in the morphological separation of the ectexine from the endexine⁽³⁵⁾. However, we did not observe any white line during the stage of *Vaccinium* pollen development partly due to the lack of the timely developmental stages. These might be also due to a packing and/or masking as a result of rapid deposition of the sporopollenin in *V. smallii* as argued by Takahashi and Sohma⁽²²⁾.

After the callose dissolution, the chemical reaction of sporopollenin undergoes transformation. At the beginning, accumulation of sporopollenin precursors results in an increase of the electron-density of the exine elements (Figs. 18 – 23). This is then followed by the maturation of sporopollenin causing decrease in the electron-density especially of ectexine elements (Figs. 24 – 27). Similar observations were reported for the pollen ontogeny of *Tarrena gracilipes*⁽³⁶⁾ and taken together they corroborate the exine substructure model described by Abadie et al.⁽³⁷⁾.

The endexine generally differs from the ectexine in the staining capability. Soon after the callose dissolution, the endexine is formed and it becomes more electron-dense than the ectexine. This is especially true for mature grains (Fig. 24).

The outer layer of intine shows more fibrillar and darker staining with debris of cytoplasmic canals, but the inner layer exhibits less staining and homogenous nonfibrillar nature (Fig. 25). Similar bi-layered intine has been reported for *Asimina triloba*⁽³⁸⁾ and some other taxa. The bi-layered intine is not common in dicots, but it may play some role in pollen germination⁽³⁹⁾. Recently, Rowley and Skvarla⁽⁴⁰⁾ reported and concluded that some nutrients and some other growth supporting materials and construction substances may pass not only through the apertures but also through the pollen wall in living microspores and pollen grains. Cytoplasmic canals may play an important role in this transport process through the intine.

Aperture ontogeny

The position of future apertures becomes apparent early in the primexine development while the meiotic tetrads are enclosed in the callose wall. The processes of aperture formation were reviewed by Rowley⁽⁴¹⁾ and different types of individual apertural induction processes have been described. A new type of aperture development was found in *Calluna*⁽²³⁾. The future apertural region was characterized by a thicker zone of fibrils (primexine matrix) than elsewhere. After the formation of nexine, apertures become prominent as endexine is interbedded with oncus material and no endexine component is present in the apertures of mature pollen⁽²³⁾. Ressayre et al.⁽⁴²⁾ reported that the additional callose deposits are the best indicators to predict the future aperture sites in some species.

The process of aperture formation in *V. smallii* does not completely follow in a manner described by the workers mentioned above. Although features of aperture development almost similar to those in *V. smallii* has been reported for *Pyrola*⁽²²⁾, a few details differ enough to merit comment. In *V. smallii*, either thin or completely absent primexine matrix (Fig. 16) and a thick zone of primexine matrix (Fig. 17) may form in the regions which are destined to be apertures in the mature grains. Both regions lack the pro-columellae, and later protectum and foot layer. Thick endexine and thick intine are observed in the apertures (colpus) of mature pollen (Fig. 27). The future apertural region gets induced by thick deposition of the primexine matrix without the formation of pro-columellae in *Pyrola*⁽²²⁾ and *Calluna*⁽²³⁾, and the same mechanism is found in *V. smallii* in this study. So the apertural region destined by thick primexine matrix deposition may be a common feature of the Ericaceae.

Hitherto, many research works point to the occurrence of cytoplasmic inclusions within the thick layer of the intine, especially near the aperture. In *V. smallii* also, the cytoplasmic inclusions of what appear to be cytoplasmic canals are found at the apertural region, especially at the oral region (Fig. 27). Although the role of these structures is not yet clear, one view is that they may include the enzymes that are important in the germination of the pollen grains⁽⁴³⁾.

Binding mechanism of pollen tetrad

Cytoplasmic channels between the adjoining microspores within the meiotic tetrad form the first physical basis of the tetrad cohesion. Similar to the study of the pollen development in *Pyrola*⁽²²⁾, we also observed the common primexine matrix on the plasmalemma surface of cytoplasmic channels between microspores (Fig. 13). The prolonged sharing of the cytomictic channels between adjoining MMCs might provide a good ground for the occurrence of the pollen grains united in groups more than four in number in *Chimaphila japonica*⁽²⁰⁾.

The deposition of a fibrillar surface coat, the primexine matrix at very early stage, when the cytokinesis starts between four meiotic nuclei of each MMC (Figs. 5 – 8), might be another important feature for the binding mechanism of pollen tetrads in *V. smallii*. The mechanisms of mature tetrad formation are discussed in detail by Takahashi and Sohma^(22, 25).

Microspores in the tetrads after the callose dissolution are sometimes interconnected with each other by the cytoplasmic channels (e.g. *Calluna*⁽²³⁾, *Hedycarya*⁽⁴⁴⁾). This sharing of common cytoplasm is also believed to play an important role in maintaining a close synchrony during the mitotic division of nucleus within the tetrad. In *V. smallii*, we observed also the synchronous mitotic division within the tetrad. In this material, however, the cytoplasmic channels disappear by the time of the onset of mitotic division. The synchronization of mitotic events may not always be explained by the presence of the cytoplasmic channels between the neighboring microspores in the tetrad. Furthermore, in the materials

considered, mitosis of nucleus takes place synchronously in all of the tetrads produced within the anther sac. Similar observation was also reported for *Pyrola* ⁽²²⁾.

Acknowledgements

We wish to express our sincere thanks to the Director and Curator of the Botanic Garden of Hokkaido University for allowing us to collect the experimental materials. We are also grateful to Dr. Gautam Pitambar for his suggestions to improve the English text. The first author (A.K.M.G.S.) acknowledges the receipt of MEXT (Japanese Ministry of Education, Culture, Sports, Science and Technology) Scholarship during the period of this study.

References

- (1) Kron, K.A., and J.L. Luteyn: Origin and biogeographic patterns in Ericaceae: new insight from recent phylogenetic analyses. *In*: Friis, Ib., and H. Balslev (eds.), *Plant Diversity and Complexity Pattern – Local, Regional and Global Dimensions*. Biol. Skrifter 55. The Roy. Aca. Sci. Lett., Copenhagen. pp. 479–500 (2005).
- (2) Kron, K.A., W.S. Judd, P.F. Stevens, D.M. Crayn, A.A. Anderberg, P.A. Gadek, C.J. Quinn, and J.L. Luteyn: Phylogenetic classification of Ericaceae: molecular and morphological evidence. *Bot. Rev.* **68**, 335–423 (2002).
- (3) Kim, K.H., S. Nilsson, and J. Pragłowski: A note on the pollen morphology of the Empetraceae. *Grana* **27**, 283–290 (1988).
- (4) Lutz, R.W., and R.D. Sjolund: Development of the generative cell wall in *Monotropa uniflora* L. *Plant Physiol.* **52**, 498–500 (1973).
- (5) McGlone, M.S.: Pollen wall structure of the New Zealand species of *Epacris* (Epacridaceae). *New Zealand J. Bot.* **16**, 83–89 (1978a).
- (6) McGlone, M.S.: Pollen structure of the New Zealand members of the Styphelieae (Epacridaceae). *New Zealand J. Bot.* **16**, 91–101 (1978b).
- (7) Pragłowski, J., and E. Grafström: The genus *Carpodetus* (Escalloniaceae): a pollenmorphological enigma. *Grana* **24**, 11–21 (1985).
- (8) Rowley, J.R.: Why the endexine and ectexine differ in resistance to oxidation: *Calluna* as a model system. *Grana* **40**, 159–162 (2001).
- (9) Ridgway, J.E.: Pollen germination in *Monotropa*. *Ann. Missouri Bot. Gard.* **57**, 384–385 (1970).
- (10) Sarwar, A.K.M. Golam: Pollen morphology of Ericaceae and its systematic significance. LAP Lambert Academic Press, Germany. 302 pp (2011).
- (11) Sarwar, A.K.M. Golam, and H. Takahashi: Pollen morphology and systematics in two subfamilies of Ericaceae: Cassiopoideae and Harrimanelloideae. *Bangladesh J. Plant Taxon.* **16**, 37–46 (2009).
- (12) Sarwar, A.K.M. Golam, T. Ito, and H. Takahashi: Pollenkitt ropes of *Notopora schomburgkii* Hook. f. (Ericaceae, Vaccinieae). *Jpn. J. Palynol.* **51**, 65–68 (2005).
- (13) Sarwar, A.K.M. Golam, T. Ito, and H. Takahashi: An overview of pollen morphology and its relevance to the sectional classification of *Vaccinium* L. (Ericaceae). *Jpn. J. Palynol.* **52**, 15–34 (2006).
- (14) Sarwar, A.K.M. Golam, T. Ito, and H. Takahashi: Pollen of *Ceratostema Jusseieu* (Ericaceae, Vaccinieae): Tetrads without septa. *J. Plant Res.* **119**, 685–689 (2006).
- (15) Sarwar, A.K.M. Golam, T. Ito, and H. Takahashi: An overview of pollen morphology in subfamily Arbutoideae (Ericaceae), and its systematic significance. *Jpn. J. Palynol.* **54**: 79-92 (2008).

- (16) Takahashi, H.: Pollen morphology of *Pyrola* and its taxonomic significance. *Bot. Mag., Tokyo* **99**, 137–154 (1986).
- (17) Takahashi, H.: Pollen morphology and its taxonomic significance of the Monotropeae (Ericaceae). *Bot. Mag., Tokyo* **100**, 385–405 (1987).
- (18) Waha, M.: Ultrastructure and function of pollen connecting threads in Ericaceae and other Angiosperm families. *Plant Syst. Evol.* **147**, 189–203 (1984). (in German with English abstract).
- (19) Ford, J.: Ultrastructure and chemical studies of pollen wall development in the Epacridaceae. In: Brooks, J. et al. (eds.), *Sporopollenin*. Acad. Press, pp. 130–173 (1971).
- (20) Takahashi, H.: Pollen development in *Chimaphila japonica* Miq. (Pyrolaceae). *Sci. Rep. Tôhoku Univ. Fourth Ser.* **37**, 263–272 (1979).
- (21) Takahashi, H.: Pollen morphology and development of *Orthilia secunda* (L.) House (Pyrolaceae). *J. Fac. Agr. Hokkaido Univ.* **63**, 145 – 153 with 4 plates (1987).
- (22) Takahashi, H., and K. Sohma: Pollen development in *Pyrola japonica* Klenze. *Sci. Rep. Tôhoku Univ. Fourth Ser.* **38**, 57–71 (1980).
- (23) Dahl, A.O., and J.R. Rowley: Microspore development in *Calluna* (Ericaceae) – exine formation. *Ann. Sci. Nat. Bot. (Paris), 13^e série* **11**, 155–176 (1991).
- (24) Hermann, P.M., and B.F. Palser: Stamen development in the Ericaceae. I. anther wall, microsporogenesis, inversion and appendages. *Amer. J. Bot.* **87**, 934–957 (2000).
- (25) Takahashi, H., and K. Sohma: Development of pollen tetrad in *Typha latifolia* L. *Pollen Spores* **46**, 5–18 (1984).
- (26) Punt, W., S. Blackmore, S. Nilsson, and A. Le Thomas: Glossary of Pollen and Spore Terminology. LPP Contrib. Ser. 1, LPP Found., Utrecht. 71 pp (1994).
- (27) El-Ghazaly, G., S. Huysmans, and E.T. Smets: Pollen development of *Rondeletia odorata* (Rubiaceae). *Amer. J. Bot.* **88**, 14–30 (2001).
- (28) Hesse, M., H. Halbritter, R. Zetter, M. Weber, R. Buchner, A. Frosch-Radivo, and S. Ulrich: Pollen Terminology – An Illustrated Handbook. Springer-Verlag, Wien. 261 pp (2009).
- (29) Furness, C.A., P.J. Rudall, and F.B. Sampson: Evolution of microsporogenesis in angiosperm. *Int. J. Plant Sci.* **163**, 235–260 (2002).
- (30) Stanley, R.G., and H.F. Linskens: Pollen: Biology, Biochemistry, and Management. Springer-Verlag, Berlin. 307 pp (1974).
- (31) Gabarayeva, N.I., and V.V. Grigorjeva: Exine development in *Stangeria eriopus* (Stangeriaceae): ultrastructure and substructure, sporopollenin accumulation, the equivocal character of the aperture, and stereology of microspore organells. *Rev. Palaeobot. Palynol.* **122**, 185–218 (2002).

- (32) Gabarayeva, N.I.: Principles and recurrent themes in sporoderm development. *In*: Harley, M.M., C.M. Morton, and S. Blackmore (eds.), *Pollen and Spores: Morphology and Biology*. Royal Botanic Garden, Kew. pp. 1–16 (2000).
- (33) Rowley, J.R., and D. Southworth: Deposition of sporopollenin of lamellae of unit membrane dimension. *Nature* (London) **213**, 703–704 (1967).
- (34) Dickison, H.G., and J. Heslop-Harrison: Common mode of deposition for the sporopollenin of sexine and nexine. *Nature* (London) **220**, 926–927 (1968).
- (35) Rowley, J.R., and J.J. Skvarla: Pollen development in *Epilobium* (Onagraceae): Late microspore stages (a review). *Rev. Palaeobot. Palynol.* **140**, 91–112 (2006).
- (36) Vinckier, S., and E. Smets: A histological study of morphogenesis in *Tarenna gracilipes* (Rubiaceae). *Grana* **44**, 30–44 (2005).
- (37) Abadie, M., M. Hideux, and J.R. Rowley: Ultrastructural cytology of anther. II. Proposal for a model of exine considering a dynamic connection between cytoskeleton, glycolemma and sporopollenin synthesis. *Ann. Sci. Nat. Bot.* (Paris) **13**, 1–16 (1986).
- (38) Waha, M.: Sporoderm development of pollen tetrads in *Asimina triloba* (Annonaceae). *Pollen Spores* **29**, 31–44 (1987).
- (39) Ubera, J.L., P.H. Fernández, M.G. Schlag, and M. Hesse: Pollen and tapetum development in male fertile *Rosmarinus officinalis* L. (Lamiaceae). *Grana* **34**, 305–316 (1996).
- (40) Rowley, J.R., and J.J. Skvarla: Pollen development in *Epilobium* (Onagraceae): from microspore mitosis to formation of the intine. *Grana* **46**, 130–139 (2007).
- (41) Rowley, J.R.: Germinal aperture formation in pollen. *Taxon* **24**, 17–25 (1975).
- (42) Ressayre, A., L. Dreyer, S. Triki-Teurtroy, A. Forchioni, and S. Nadot: Post-meiotic cytokinesis and pollen aperture pattern ontogeny: Comparison of development in four species differing in aperture pattern. *Amer. J. Bot.* **92**, 576–583 (2005).
- (43) Heslop-Harrison, J., Y. Heslop-Harrison, R.B. Knox, and B. Howlett: Pollen-wall proteins: “gametophytic” and “sporophytic” fractions in the pollen walls of Malvaceae. *Ann. Bot.* **37**, 403–412 (1973).
- (44) Sampson, F.B.: Pollen tetrads of *Hedycarya arborea* J.R. Forst. et G. Forst. (Monimiaceae). *Grana* **16**, 61–63 (1977).

Legends

- Fig. 1. The anther wall composed of thick epidermis (Ep), two layers of hypodermal cells, one, two, or rarely three layers of tapetal cells (T). Each microspore mother cell (MMC) during simultaneous cytokinesis is enveloped in a thick callose wall (C). Bar = 10 μm .
- Fig. 2. MMC during cytokinesis following meiotic nuclear divisions shows the cell plate formation (arrows). Each mother cell with four haploid nuclei (N; of which two nuclei are seen in this section) enveloped in a thick callose wall (C) is separated by the cell wall (W). Bar = 1 μm .
- Fig. 3. Stage similar to that of Fig. 2 showing the beginning of deposition of the primexine matrix (*) represented by the fibrillar surface coat on undulating plasmalemma of the MMC during cytokinesis. Also seen are Golgi apparatus (arrow), vesicles, and mitochondria (Mt) in the cytoplasm. Bar = 100 nm.
- Fig. 4. MMC during cytokinesis with the cell plate formation (arrow), enveloped in a thick callose wall (C). Nucleus (N). Bar = 1 μm .
- Fig. 5. MMC during cytokinesis following the meiotic nuclear divisions showing the cell plate formation (arrow). More continuous primexine matrix observed on the distal plasmalemma of the MMC. A nucleus (N) has a nucleolus. Bar = 1 μm .
- Fig. 6. Detail of Fig. 5 (rectangle). Surface of MMC showing the constant and somewhat thick fibrillar surface coat, primexine matrix (*). Callose (C) and cell wall (W). Bar = 1 μm .
- Fig. 7. MMC during cytokinesis with the cell plate formation (arrows). Electron-dense lipoidal globules observed within the fibrillar primexine matrix on the plasmalemma of MMC within the callose wall (C). Bar = 5 μm .
- Fig. 8. Detail of Fig. 7 (rectangle box). Surface of MMC during cytokinesis, showing electron-dense lipoidal globules (arrows) within the fibrillar primexine matrix. Bar = 1 μm .
- Fig. 9. A microspore (MS) tetrad within a thick callose wall (C). Each microspore contains a haploid nucleus (N) and many small vacuoles. Tapetal cell (T). Bar = 5 μm .
- Fig. 10. Early exine showing the primexine matrix (*) and pro-columellae (arrows) at the tip of radial cytoplasmic micro-projections. Bar = 0.5 μm .
- Fig. 11. Oblique section of developing exine showing the primexine matrix (*) and pro-columellae at the tip of radial cytoplasmic micro-projections (arrows) and/or vesicles (V). Mitochondria (Mt) and vacuoles (Va) in the cytoplasm. Bar = 0.5 μm .
- Fig. 12. Oblique section of developing exine showing the primexine matrix (*), pro-columellae (arrows) and protectum (arrowheads). Vesicles (V) and rough ER in the cytoplasm. Bar = 0.5 μm .
- Fig. 13. Adjoining microspores of the meiotic tetrad connected by both common primexine matrix (*) and cytoplasmic channels (arrows). Thin and discontinuous callose layer (arrowhead) between the adjoining primexine matrix. Many rough ER in the cytoplasm. Bar = 0.5 μm .

- Fig. 14. Adjoining microspores of the meiotic tetrad connected by common primexine matrix (*). Dark lipoidal granules (arrows) observed on inner surface of tapetum (T). Callose (C) and probacumellae, i.e., probacula (PB). Bar = 0.5 μ m.
- Fig. 15. The primexine matrix (*) connection between adjoining microspores. Vesicles (arrow) fusing undulating plasmalemma. Developing exine is composed of primexine matrix (*), probacula (PB), and faint protectum (PT). Callose (C). Bar = 0.5 μ m.
- Fig. 16. Possible future aperture region (arrow) destined by very thin or almost lack of primexine matrix. Lipoidal electron-dense granules on the surface of the tapetal cells (T). Bar = 1 μ m.
- Fig. 17. The regions of thick primexine matrix (arrows), probably initial parts of future apertural regions. Bar = 1 μ m.
- Fig. 18. A microspore tetrad after the dissolution of callose wall showing thick intine (I) near ora. Each microspore contains a nucleus (N) with a nucleolus. Bar = 10 μ m.
- Fig. 19. A colpus region in the same stage of Fig. 18, characterized by the lack of ectexine (T: tectum, Co: columellae, F: foot layer), thick endexine (En) (more electron-dense) and thick intine (I), Starch grains (S). Bar = 1 μ m.
- Fig. 20. Septal pollen wall in the same stage of Fig. 18. Adjoining tectum is connected (*). A transparent space (arrow) is observed at the central part of the common exine. Starch grains (S). Bar = 1 μ m.
- Fig. 21. The vegetative (VN) and generative cell nuclei (GN) separated after microspore mitosis, but cell plate formation not complete yet. Each nucleus has a large nucleolus. Pollenkitt-like substance (arrow) deposited on the outer surface of tectum. Starch grains (S). Bar = 5 μ m.
- Fig. 22. Generative cell (GC) formed at the distal region of the pollen grain. Partition wall connected with intine (arrow). Starch grain (S) included in generative cell as well as in vegetative cell (VC). Bar = 1 μ m.
- Fig. 23. Edge of aperture (colpus) region in the same stage of Fig. 22, characterized by very thin ectexine (T: tectum, Co: columellae, and F: foot layer), thick depressed endexine (En), and thick intine (I). Bar = 1 μ m.
- Fig. 24. A mature pollen tetrad showing many starch grains (S) and dark lipoidal globules (L) in the cytoplasm. Electron density of the ectexine decreased. Aperture (os) regions (arrows) characterized by the lack of ectexine, very thin endexine, and thick intine. Bar = 10 μ m.
- Fig. 25. A cross-section of exine at the distal area of mature pollen grains. Apocolpial pollen wall composed of thick tectum (T), thin columellae (Co), thick foot layer (F), thin endexine (En), and intine (I). Many starch grains (S) and lipoidal globules (L) in the cytoplasm. Bar = 1 μ m.
- Fig. 26. Tectum (arrows) becomes more fragmental in septal pollen wall between the adjoining three grains of the mature pollen tetrad. Bar = 1 μ m.

Fig. 27. Aperture (colpus) region of the mature pollen grain of the tetrad showing the lack of ectexine (T: tectum, Co: columellae, and F: foot layer), thick endexine (En), and thick intine (I). Bar = 1 μ m.

Fig. 28. A mature pollen tetrad showing an apocolpial region with three colporate apertures.

Fig. 29. A mature pollen tetrad showing the adjoining mesocolpial regions of three pollen grains. Three colporate apertures found.







