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Title	POLLEN MORPHOLOGY AND DEVELOPMENT OF ORTHILIA SECUNDA(L.)HOUSE (PYROLACEAE)
Author(s)	TAKAHASHI, Hideki
Citation	Journal of the Faculty of Agriculture, Hokkaido University, 63(2), 145-153
Issue Date	1987-03
Doc URL	https://hdl.handle.net/2115/13054
Type	departmental bulletin paper
File Information	63(2)_p145-153.pdf

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POLLEN MORPHOLOGY AND DEVELOPMENT OF *ORTHILIA SECUNDA* (L.) HOUSE (PYROLACEAE)

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Received July 29, 1986

Introduction

The Pyrolaceae is a small family of evergreen perennials distributed in principally cool north temperate regions. The Pyrolaceae is related to the Ericaceae and often included within it as subfamily Pyroloideae[6,13,21]. In this study, I followed the treatment by Cronquist[2] who considered it as a separate family based on the small and undifferentiated embryo and its herbaceous or half-shrubby habit.

The four genera, *Orthilia* (1 sp.), *Pyrola* (c. 30 spp.), *Moneses* (1 sp.) and *Chimaphila* (7 spp.) included in the family Pyrolaceae, are clearly distinguished from each other by the palynological features: pollen unit and aperture condition[3,4]. In this family, palynological feature is one of the useful means for the clarification of the phylogenetic relationships.

Pollen development of *Chimaphila* polyads and *Pyrola* tetrads has been examined by transmission electron microscopy (TEM) by the author[14,17]. Although the scheme of the pollen development of *Orthilia* monad was listed in that study[17] for the comparison of the genera in this family, the presentation of TEM photographs has not been done until now. In this paper, the data on the pollen development of *Orthilia* is presented for the first time.

Pollen morphological variability within the genus *Chimaphila* and *Pyrola* have been revealed mainly in the scanning electron microscopy (SEM)[15,16]. Following these reports, the description of mature *Orthilia* pollen is given in SEM, TEM as well as in light microscopy (LM) in this study. Though several reports on the pollen morphology of *Orthilia secunda* in LM have been described, the descriptions are not identical. We need to make comparisons between the descriptions in LM and in EM.

Based on these pollen developmental and morphological data, the phylo-

genetic relationship between *Orthilia* and the related genus *Pyrola* of the Pyrolaceae is discussed.

Materials and Methods

For TEM observations on the pollen development, the samples were obtained from *Orthilia secunda* (L.) House naturally growing at the foot of Mt. Rausu in the Shiretoko Peninsula, Hokkaido. These were processed and examined as described in the previous studies[14,17,19].

For LM, SEM and TEM observations on mature pollen, the samples were obtained from the dried specimens deposited in the following herbaria: MO, SAPT and TI. Abbreviations of the herbarium names except for SAPT are according to the Index Herbariorum[7], and for SAPT see "News and notes" in Taxon **32**: 703 (1983). Pollen was processed in accordance with the methods outlined by the previous studies[15,16,18].

Specimens examined

U. S. A. Alaska: Northeastern Brooks Range, A. R. Batten 894, subsp. *obtusata* (TURCZ.) BÖCHER (MO 2344485)[345*]. MEXICO. Las Cruces, G. B. Hinton 1716 (MO 1207880)[346*]. SWEDEN. Södermanland: Svinaker, E. Asplund 1267 (SAPT)[72*]. U. S. S. R. C. Siberia, H. Hara s. n. (TI)[126*], Sakhalin: Sakaehama, B. YOSHIMURA & M. HARA s. n. (SAPT)[34*], Kurile Isl.: Etorofu, B. YOSHIMURA s. n. (SAPT)[33*]. JAPAN. Hokkaido: Isl. Rishiri, H. TAKAHASHI 2804 (SAPT)[86*]; Prov. Kitami, K. ITO s. n. (SAPT)[28*]; Mt. Rausu, H. TAKAHASHI 245 (SAPT)[2*]; Mt. Meakan, H. YOKOYAMA 4277 (SAPT)[31*]; Saru River, N. NISHIMURA s. n. (SAPT)[30*]; Mt. Soranuma, H. TAKAHASHI 1582 (SAPT)[1*]; Mt. Muine, H. KARIYA *et al.* s. n. (SAPT)[85*]; Mt. Tokushunbetsu, S. KAWANO 652 (SAPT)[29*], Nagano: Mt. Yatsugatake, K. ITO & M. TOHYAMA s. n. (SAPT)[32*]; Mt. Komagatake, H. HARA s. n. (TI)[127*]. (Asterisk indicates the reference number of the pollen slide collection deposited at the Botanic Garden, Faculty of Agriculture, Hokkaido University).

Results

Pollen development (Figs. 1-10)

Pollen mother cells (PMCs), each of which has a polyhedral shape, are separated from each other by a thin primary cell wall (Fig. 1). Adjoining cells are sometimes connected by cytomictic channels, ca. 0.5 μm in diameter, and an intercellular communication is shown by some organelles as seen in Fig. 2. At this moment, nuclear membrane shows a wavy contour, and the nucleolus and the synaptonemal complex are observed within the nucleus

(Fig. 3). These features mean that the developmental stage of Figs. 1-3 is around premeiotic or meiotic prophase. Subsequently, each PMC becomes spherical and progressively enveloped in a thick callose wall, which is deposited between the plasma membrane of PMC and the primary cell wall (Fig. 4). The nuclear membrane disappears and the chromosomes become arranged at the center of PMC (metaphase I). Fig. 5 shows PMC at telophase I when two daughter nuclei are under formation at the opposite poles. Many organelles are situated at the center of PMC, i. e., between two daughter nuclei. At this stage, some cytomictic channels are still found between PMCs. In meiotic division II, two daughter nuclei further divide into four nuclei which show a tetrahedral arrangement. The cytokinesis is accomplished by the callose deposition between the four nuclei (Fig. 6). At this stage, the cytomictic channels are no longer visible. Thereafter, four microspores are perfectly separated from each other by a thick callose layer. A thin primexine appears on the plasma membrane of each microspore still within the callose wall (Fig. 7). In Fig. 8, the primexine increases in thickness with three exinous sub-layers; tectum, probacula and foot layer which are well recognized in the primexine matrix (Fig. 9). The future aperture is recognized as the region where the primexine matrix is thick and where three sub-layers are less developed (Fig. 10).

Mature pollen (Figs. 11-21, Table 1)

Pollen grains in monads, small in size, (16-) 17-23 (-28) μm (P) × (15-) 17-20 (-22) μm (E), P/E = (0.9-) 1.0-1.2 (-1.3), prolate spheroidal to subprolate sometimes oblate spheroidal in equatorial view (Figs. 11-13), circular to semi-angular in polar view (Fig. 14). Apertures 3-colpor(oid)ate, rarely 2-colpor(oid)ate or 2-syncolpor(oid)ate (Fig. 15), colpi long, (13-) 15-18 (-20) μm, attaining over four-fifths of the polar axis, equatorially constricted. Lalongate slit-like, one or two ora (Figs. 16, 17) discernible at the equator of the colpi but frequently not clearly discernible. In SEM, the exine sculpture verrucate (Fig. 18), verrucae 0.2-0.5 μm in diameter, similar sculpturing all over the surface except for the aperture, that of the colpus membrane also verrucate (Fig. 19) with somewhat larger verrucae, 0.3-0.7 μm in diameter. In TEM, the exine similar thickness all over the surface except for the aperture (Fig. 20). The exine composed of a thick tectum, very thin infratectal bacular layer, thick foot layer and endexine (Fig. 21). Total thickness of the exine 0.6-0.7 μm.

Infraspecific variation: A somewhat larger pollen is observed in the specimen collected in Mexico (Table 1). In the specimen collected in Alaska, which is recognized as subsp. *obtusata* (TURCZ.) BÖCHER (MO 2344485), about 70% pollen grains have aberrant colpi; 2-colpor(oid)ate or 2-syncolpor(oid)ate.

TABLE 1. Pollen measurements in *Orthilia secunda*

Locality	Pollen slide number	Polar axis P (μm)	Equatorial axis E (μm)	P/E ratio	Figures
Alaska	345	17-(18)-23	17-(18)-22	0.9-(1.0)-1.1	15
Mexico	346	21-(23)-28	20-(20)-22	1.0-(1.1)-1.3	
Sweden	72	16-(17)-18	16-(18)-19	0.9-(1.0)-1.1	
C. Siberia	126	18-(19)-21	17-(18)-20	1.0-(1.1)-1.1	
Sakhalin	34	17-(18)-20	15-(17)-19	1.0-(1.1)-1.2	
Kurile Isl.	33	19-(20)-21	17-(18)-19	1.0-(1.1)-1.1	
Hokkaido	86	18-(20)-22	17-(18)-19	1.1-(1.1)-1.2	
Hokkaido	28	16-(18)-19	16-(18)-20	0.9-(1.0)-1.1	16, 17
Hokkaido	2	18-(19)-20	15-(17)-18	1.0-(1.1)-1.2	11-14, 19
Hokkaido	31	18-(20)-21	15-(18)-21	1.0-(1.1)-1.3	18
Hokkaido	30	17-(18)-21	16-(17)-19	1.0-(1.1)-1.2	
Hokkaido	1	17-(18)-19	15-(18)-19	0.9-(1.0)-1.2	20, 21
Hokkaido	85	18-(19)-21	17-(19)-20	0.9-(1.0)-1.1	
Hokkaido	29	18-(19)-20	17-(18)-20	1.0-(1.0)-1.1	
Honshu	32	18-(20)-21	15-(17)-19	1.0-(1.2)-1.3	
Honshu	127	18-(20)-21	16-(18)-20	1.0-(1.1)-1.3	

All pollen grains are acetolyzed and mounted in silicone oil (viscosity 3000 cs.). The measurements are as a rule based on 20 grains and the smallest-(mean)-largest values are indicated. In pollen slide number 345, normal pollen grains with 3-colporate aperture are selected for the measurements because pollen grains with 2-colpi occur in high percentage.

Discussion

Though the developmental stages after the meiotic tetrad were not observed in this study, the morphological events before the callose dissolution are basically in accordance with those which have been reported in many angiosperms having monad pollen until present[8]. Within the Pyrolaceae, however, the monad pollen in *Orthilia* is a remarkable feature clearly distinguished from the tetrad pollen in related genus *Pyrola*[3,4,5,6,9,11,12,20]. This palynological difference can be traced back to the stage when the four microspores are enveloped in the callose wall. In *Orthilia*, four microspores are perfectly separated from each other by a thick callose layer. On the other hand, in *Pyrola*, four microspores are imperfectly separated by a thin and discontinuous callose layer, in which the cytoplasmic channels and the connection of the primexine matrix are observed between the adjoining microspores[17]. The deposition and dissolution of the callose wall during the micro-

TABLE 2. Comparison between the representative descriptions in LM reported until now and in EM revealed in this study on the pollen morphology of *Orthilia secunda*

Palynological character	OVERBECK (1934)	OLDFIELD (1959)	ERDTMAN *et al.*, ERDTMAN (1961, 69)	NOWICKE (1966)	TAKAHASHI (1987)
Pollen unit	Monad	Monad	Monad	Monad	Monad
Grain diameter (μm)	15-20	19.5×17.8	19×17	c. 20×15.5	17-23×17-20
Exine sculpture	Almost smooth	An extremely fine granulation	Psilate or provided with very fine, low processes	Finely reticulated	Verrucate (verrucae 0.2-0.5 μm in diam.)
Exine wall	Slender and pale	c. 1-1.5 μm thick, endexine> ektexine	c. 1 μm thick, sexine≈nexine	c. 1 μm thick, sexine≈nexine	0.6-0.7 μm thick (tectum 0.2-0.3 μm, infratectal bacular layer 0.05 μm, foot layer 0.2-0.3 μm, endexine 0.1 μm)
Aperture	Three folds	3-colporate Furrow long with slightly irregular edge, constricted Pore small narrow slit across the furrow, sometimes not detected	3-colporate Colpal part c. 16 μm long, comparatively broad (contracted at the equator) with granulate membrane Ora small, indistinct	3-colporate Colpi c. 13 μm long Ora c. 1.3 μm in diam.	3-colpor(oid)ate rarely 2-colporate Colpi c. 15-18 μm equatorially constricted, colpus membrane with verrucae (0.3-0.7 μm in diam.) Ora lalongate one or two slits, or frequently not detected
Locality of the material examined	Europe	Europe	Europe	Canada	Old and New World

spore development of the angiosperms plays an important role in the separation of the four microspore cells derived from a PMC[19].

The developmental process of the exine wall of *Orthilia* is similar to that of many angiosperms. The primexine-template of the exine wall of the pollen grains, is detected on the plasma membrane of each microspore within the callose wall. The region of the future aperture is characterized by a thick primexine matrix, which seems to be a distinct feature from that in many angiosperms. The aperture of the pollen in many angiosperms is destined as the region lacking the primexine matrix or having a thin matrix (cf. TAKAHASHI & SOHMA[17]). The same mechanism for the determination of the aperture as in *Orthilia* has also been reported in the related genus *Pyrola* of the family Pyrolaceae[17].

Though there have been several reports on the morphology of *Orthilia secunda* pollen in LM, they are not necessarily identical. The comparison between the descriptions in LM and in EM revealed in this study is shown in Table 2. Sometimes the exine sculpture in LM observation appears differently from in SEM; the verrucate sculpture revealed in SEM has been erroneously described as "almost smooth" by OVERBECK[12] or "finely reticulated" by NOWICKE[9]. Furthermore, the exine thickness in LM observation is usually estimated to be thicker than in TEM observation; about 1-1.5 μm in LM against 0.6-0.7 μm in TEM.

The differences in the pollen of *Orthilia* and that of the related genus *Pyrola* are: in *Orthilia*, the pollen is monad, whereas in *Pyrola*, it is tetrad; in *Orthilia*, the colpus membrane has a verrucate sculpture, whereas in *Pyrola*, it has a psilate sculpture. From a quantitative point of view, the value of the grain size and the exine thickness in *Orthilia* (each E=17-20 μm, 0.6-0.7 μm) is smaller than in *Pyrola* (each d=21-30 μm, 1.0-1.5 μm). On the other hand, the pollen monad of *Orthilia* shows some similarities to the pollen tetrad of *P. minor* in the subgenus *Amelia* and/or *P. grandiflora* in section *Pyrola* of the subgenus *Pyrola* within the genus *Pyrola*, in having a small grain size and a verrucate exine sculpture (Table 3).

Orthilia has usually been recognized as a distinct genus from the genus *Pyrola* based on the character correlation including palynological data such as monad grain[6], but in recent Japanese flora[10], *Orthilia* is still included in the genus *Pyrola*. Although the pollen of *Orthilia* shows some resemblance to that of some members of *Pyrola*, the pollen developmental and morphological aspects revealed in this study indicate that *Orthilia* is clearly separated from *Pyrola* as a distinct genus.

An infraspecific variation in *Orthilia* pollen has been revealed in this

TABLE 3. Comparison of some palynological features between *Orthilia* and realted genus *Pyrola*

Genus	Orthilia	Pyrola			
Subg.		Amelia	Pyrola		
Sect.	(16/1)**	(9/1)	Pyrola (73/8)	Chlorantha* (36/4)	Scotophylla (16/2)
Pollen unit	Monad	Tetrad			
Sculpture of colpus membrane	V***	P			
Exine thickness (μm)	0.6–0.7	1.0–1.5			
Sculpture at distal pole	V	V	V, V–R R, R–P	R, R–P P	R–P, P
Grain size (μm)	17–20	21–25	21–29	23–27(30)	25–28

* *P. faurieana* H. ANDRES which has been included in section *Chlorantha* is excluded from it in this Table based on the author's opinion[15].

** (Examined specimens/examined species).

*** V: verrucate, R: rugulate, P: psilate sculpture. In grain size E value is adopted in *Orthilia* and d value (monad grain diameter) in *Pyrola* tetrad. The palynological features in *Pyrola* are according to TAKAHASHI[15].

study. Two colporate or two syncolporate aperture conditions are found in many pollen grains in slide number 345, which are obtained from the specimen recognized as subsp. *obtusata*. This abnormality in pollen morphology seems to be correlated with the irregularity in meiosis, because aberrant chromosomal behavior in meiosis of *Orthilia secunda* subsp. *obtusata* collected in Greenland have been reported by BÖCHER[1]. A larger grain size was detected in the specimen collected in Mexico (slide number 346). More specimens, however, must be examined in order to clarify the correlation between the grain size and their geographical distribution.

Summary

Pollen morphology and development in *Orthilia secunda* were described based on LM, SEM and TEM observations. Pollen morphological data revealed in this study were compared with those previously reported in LM. The pollen unit and the sculpture of the colpus membrane of *Orthilia* was different from that of the related genus *Pyrola*. The difference in the pollen unit can be traced back to the stage when the four microspores are enveloped in the callose wall. The palynological features revealed in this study support

a generic status of *Orthilia* distinct from the genus *Pyrola*. The pollen of *Orthilia*, however, shows some similarities to that of *P. minor* and *P. grandiflora* in having a small grain size and a verrucate exine sculpture. An infraspecific variation of pollen morphology was revealed. Frequent occurence of 2-(syn)colpor(oid)ate grains newly detected in subsp. *obtusata* may be correlated with the irregular meiosis which has been reported in this taxon.

Acknowledgements

For use of the electron microscopes, thanks are due to Prof. Emeritus T. UI, Dr. E. SHIKATA and Dr. K. ITO, Hokkaido University. Thanks are also due to Dr. T. TSUJII, director of Botanic Garden, Faculty of Agriculture, Hokkaido University, for his constant encouragement. I wish to express my sincere thanks to the directors and curators of the following herbaria: Missouri Botanical Garden, St. Louis, Missouri, U. S. A. (MO); Faculty of Agriculture, Hokkaido University, Sapporo, Japan (SAPT); Botanical Gardens Koishikawa, Tokyo University, Tokyo, Japan (TI), for allowing me to sample the polliniferous material. The pollen developmental aspects of this study were examined under the guidance of Dr. K. SOHMA, Tohoku University. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, No. 57740374.

Literature Cited

1. BÖCHER, T. W.: Studies in Pyrolaceae — two interesting wintergreens from west Greenland. *Bot. Tidsskr.* **57**: 28–37. 1961
2. CRONQUIST, A.: An Integrated System of Classification of Flowering Plants. Columbia University Press, New York, 1262 pp. 1981
3. ERDTMAN, G.: Pollen Morphology and Plant Taxonomy. I. Angiosperms. Almqvist & Wiksell, Stockholm, 539 pp. 1952
4. ERDTMAN, G.: Handbook of Palynology. Munksgaard, Copenhagen, 486 pp. 1969
5. ERDTMAN, G., BERGLUND, B. and J. PRAGLOWSKI: An introduction to a Scandinavian pollen flora. *Grana Palynologica* **2**(3): 3–92. 1961
6. HABER, E. and J. E. CRUISE: Generic limits in the Pyroloideae (Ericaceae). *Can. J. Bot.* **52**: 877–883. 1974
7. HOLMGREN, P. K., KEUKEN, W. and E. K. SCHOFIELD: Index Herbariorum. Pt. I. The Herbaria of the World. Regnum Vegetabile 106. Utrecht, 452 pp. 1981
8. MASCARENHAS, J. P.: The biochemistry of angiosperm pollen development. *Bot. Rev.* **41**: 259–314. 1975
9. NOWICKE, J. W.: Pollen morphology and classification of the Pyrolaceae and Monotropaceae. *Ann. Missouri Bot. Gard.* **53**: 213–219. 1966

10. OHWI, J.: New Flora of Japan (revised by M. KITAGAWA). Shibundo, Tokyo, 1716 pp. 1983
11. OLDFIELD, F.: The pollen morphology of some of the West European Ericales. *Pollen et Spores* **1**: 19–48. 1959
12. OVERBECK, F.: Zur Kenntnis der Pollen mittel- und nordeuropäischer Ericales. *Beih. Bot. Centralb.* **51**: 566–583. 1934
13. STEVENS, P. F.: A classification of the Ericaceae: subfamilies and tribes. *Bot. J. Linn. Soc.* **64**: 1–53. 1971
14. TAKAHASHI, H.: Pollen development in *Chimaphila japonica* Miq. (Pyrolaceae). *Sci. Rep. Tohoku Univ. Fourth Ser.* **37**: 263–272. 1979
15. TAKAHASHI, H.: Pollen morphology of *Pyrola* and its taxonomic significance. *Bot. Mag. Tokyo* **99**: 137–154. 1986
16. TAKAHASHI, H.: Pollen polyads and its variation in *Chimaphila* (Pyrolaceae). *Grana* **25**: 161–169. 1986
17. TAKAHASHI, H. and K. SOHMA: Pollen development in *Pyrola japonica* Klenze. *Sci. Rep. Tohoku Univ. Fourth Ser.* **38**: 57–71. 1980
18. TAKAHASHI, H. and K. SOHMA: Pollen morphology of the Droseraceae and its related taxa. *Sci. Rep. Tohoku Univ. Fourth Ser.* **38**: 81–156. 1982
19. TAKAHASHI, H. and K. SOHMA: Development of pollen tetrad in *Typha latifolia* L. *Pollen et Spores* **26**: 5–18. 1984
20. UENO, J.: Palynological notes on Ericaceae and Pyrolaceae from Japan and its neighbours. *Acta Phytotax. Geobot.* **20**: 101–111. 1962
21. WALLACE, G. D.: Interrelationships of the subfamilies of the Ericaceae and deviation of the Monotropoideae. *Bot. Notiser* **128**: 286–298. 1975

Explanation of Plates

Plate 1.

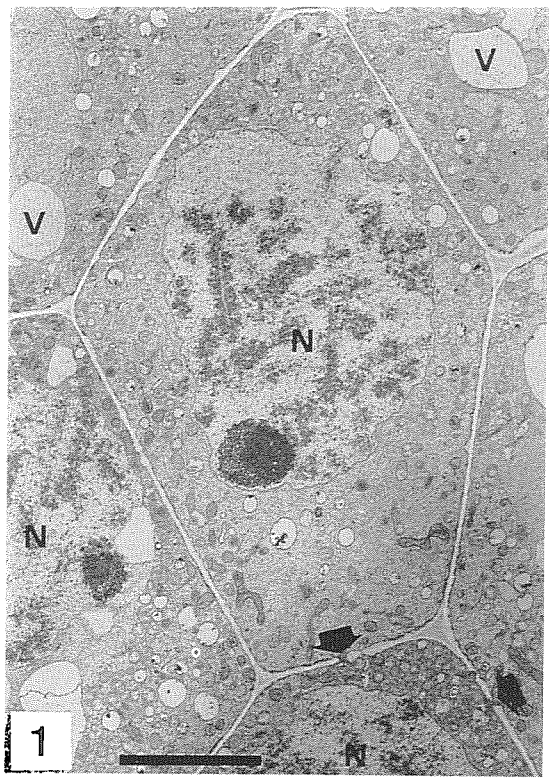
PMC of *Orthilia secunda* (L.) House.

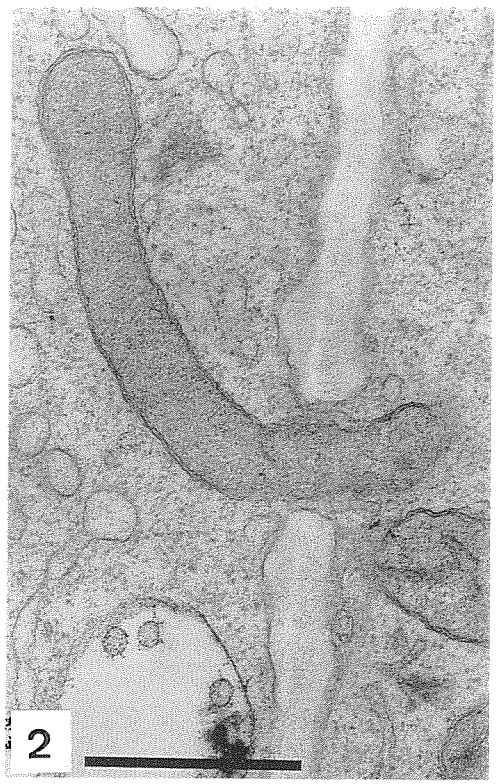
Fig. 1. Each PMC has a large nucleus (N) and many organelles and vesicles (V). Adjoining PMCs are connected by the cytomictic channels (arrows).

Fig. 2. An organelle is found at the cytomictic channel.

Fig. 3. The synaptonemal complex (arrows) is observed in the nucleus. The inner measurement of the synaptonemal complex is 0.13 μm. NM-nuclear membrane.

Bar in Fig. 1 is 5 μm and bars in Figs. 2–3 are 1 μm.


V
V
N
N
N
1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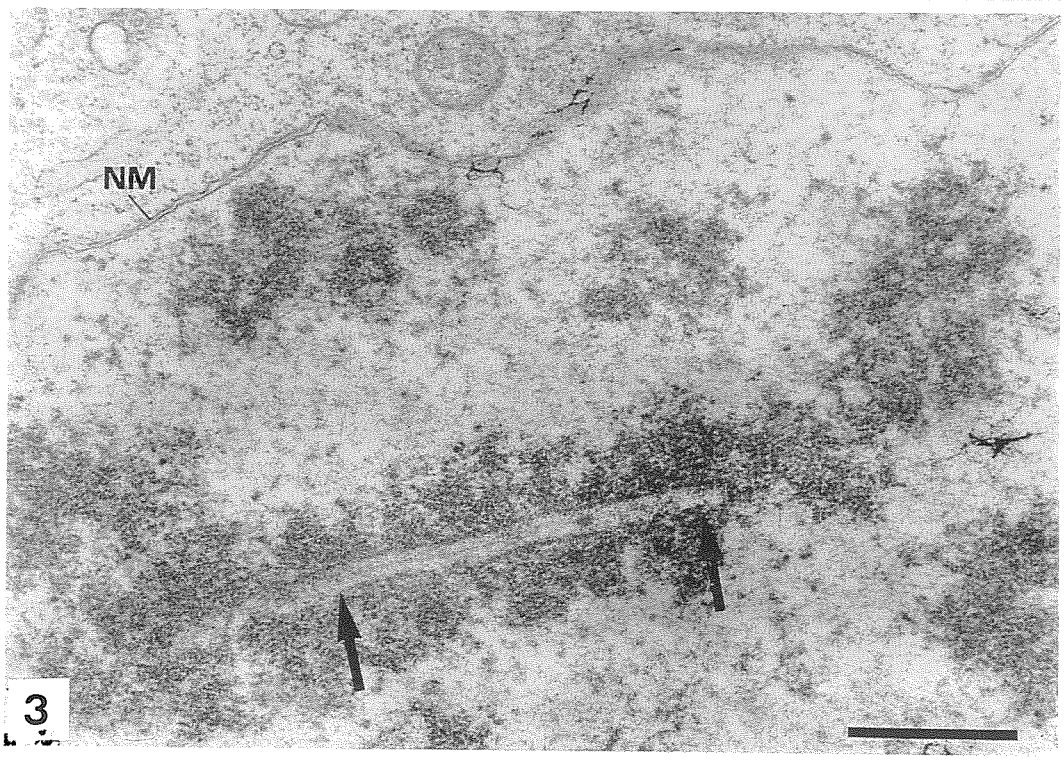

NM
3

Explanation of Plates

Plate 2.

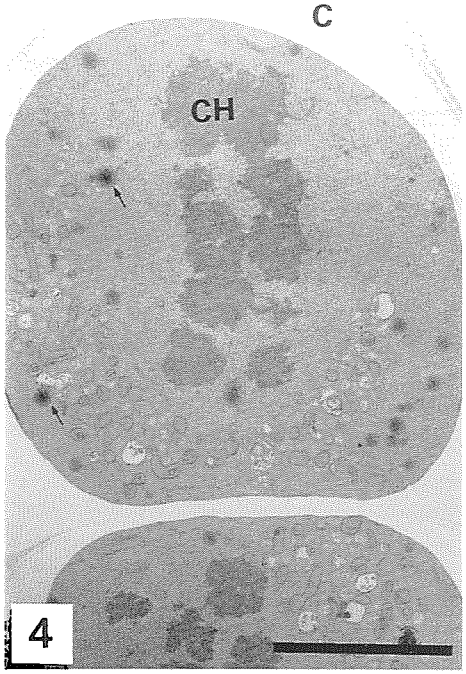
Pollen development of *Orthilia secunda* (L.) House at metaphase I, telophase I and cytokinesis following the meiotic division II.

Fig. 4. Metaphase I. PMC is enclosed in a thick callose wall (C) and chromosomes (CH) are situated at the center of the cell. Osmiophilic droplets (arrows) are found.

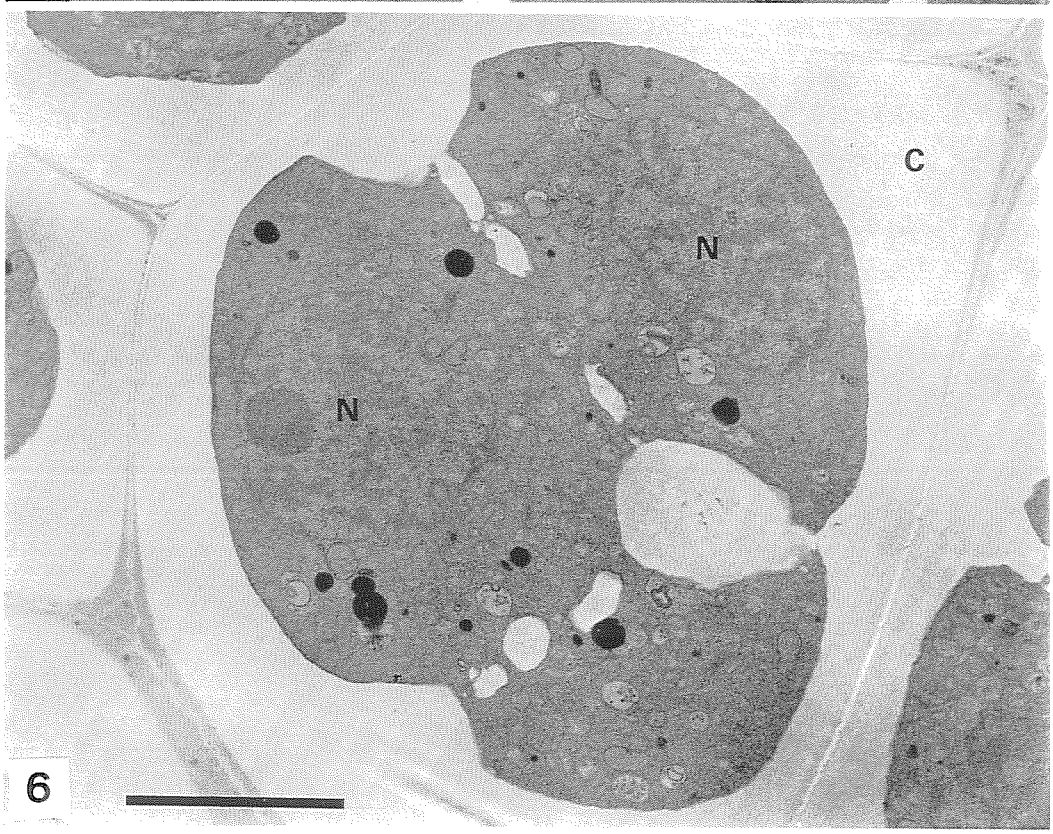
Fig. 5. Telophase I. Adjoining PMCs are sometimes connected by the cytomictic channel (arrow). T-tapetum.

Fig. 6. Cytokinesis following the meiotic division II. The nuclei (N) show a tetrahedral arrangement. C-callose wall.

All bars in Figs. 4-6 are 5 μm.


C
CH
4

T
5


C
N
N
6

Explanation of Plates

Plate 3.

Meiotic tetrad within a callose wall in *Orthilia secunda* (L.) House.

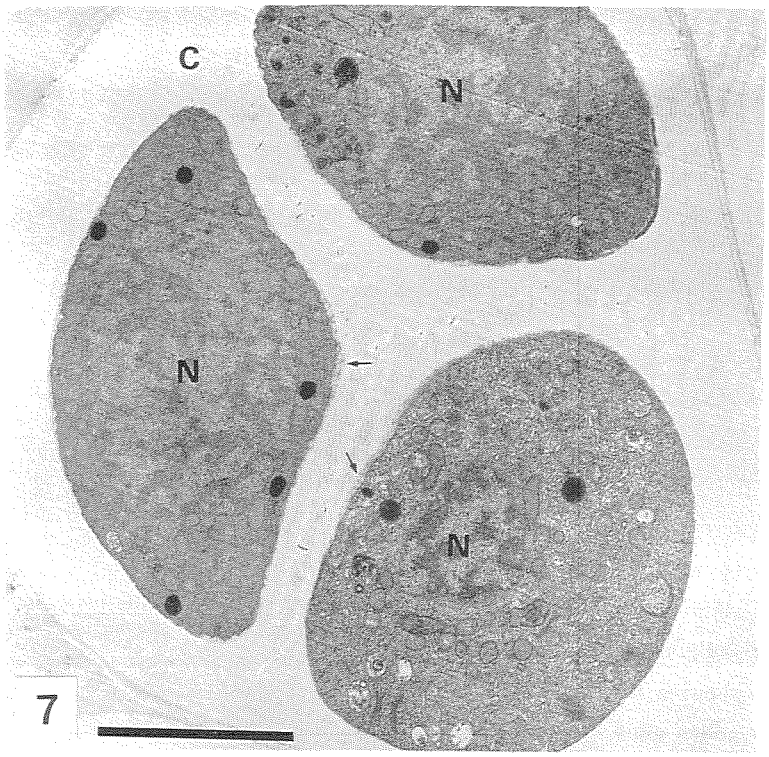
Fig. 7. The early period of the meiotic tetrad within a callose wall. Arrows indicate the first appearance of the primexine. C-callose wall, N-nucleus.

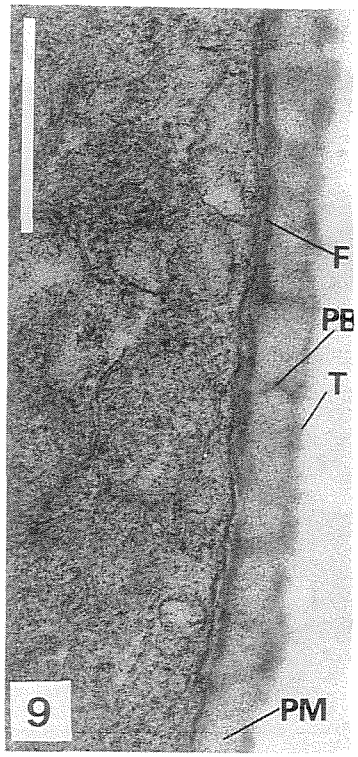
Fig. 8. The primexine becomes distinct on the plasma membrane of each microspore. Arrows indicate the future apertural regions. C-callose wall, N-nucleus.

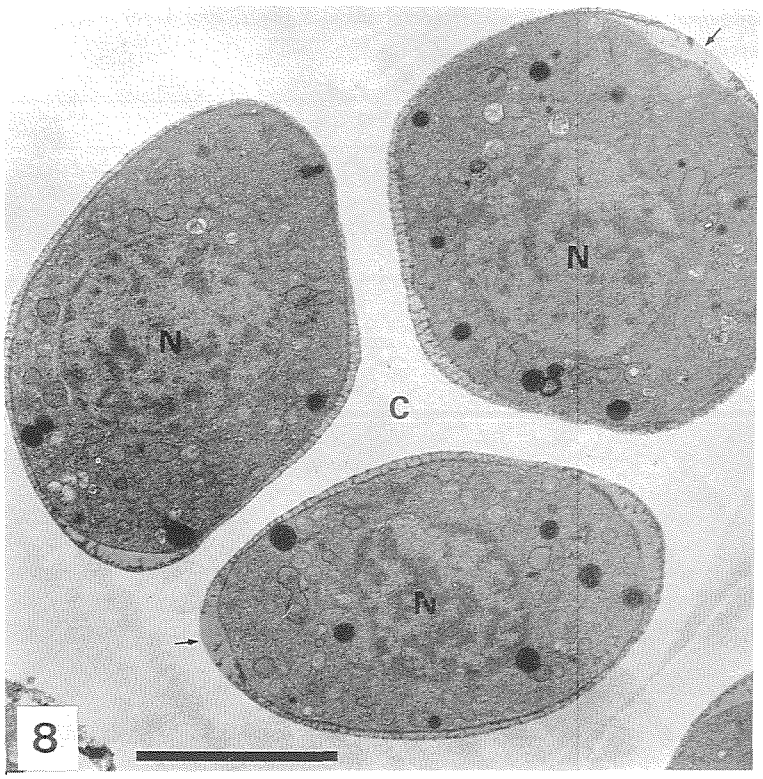
Fig. 9. Enlargement of the primexine. Tectum (T), probacula (PB) and foot layer (F) are formed in the primexine matrix (PM).

Fig. 10. The region indicating the future aperture. Thick primexine matrix (PM) is found and the three exinous sub-layers are not well developed.

Bars in Figs. 7–8 are 5 μm, in Figs. 9–10 are 0.5 μm.


C
N
N
N
7


F
PB
T
PM
9


N
N
C
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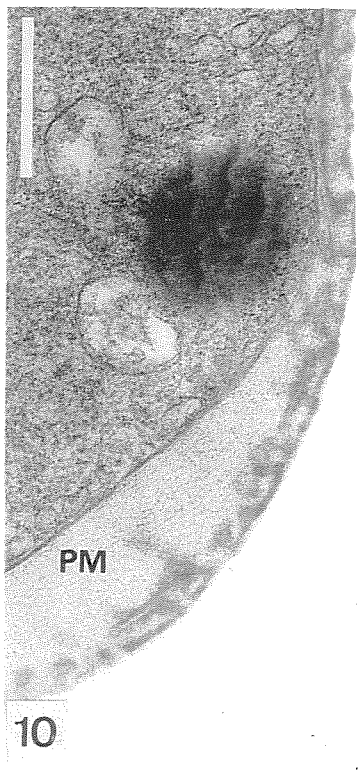
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Explanation of Plates

Plate 4.

Pollen morphology of *Orthilia secunda* (L.) House.

Figs. 11–13. Equatorial view.

Fig. 14. Polar view.

Fig. 15. Abnormal monad with two colpi.

Fig. 16. Equatorial view showing one slit-like os.

Fig. 17. Equatorial view showing two slit-like ora.

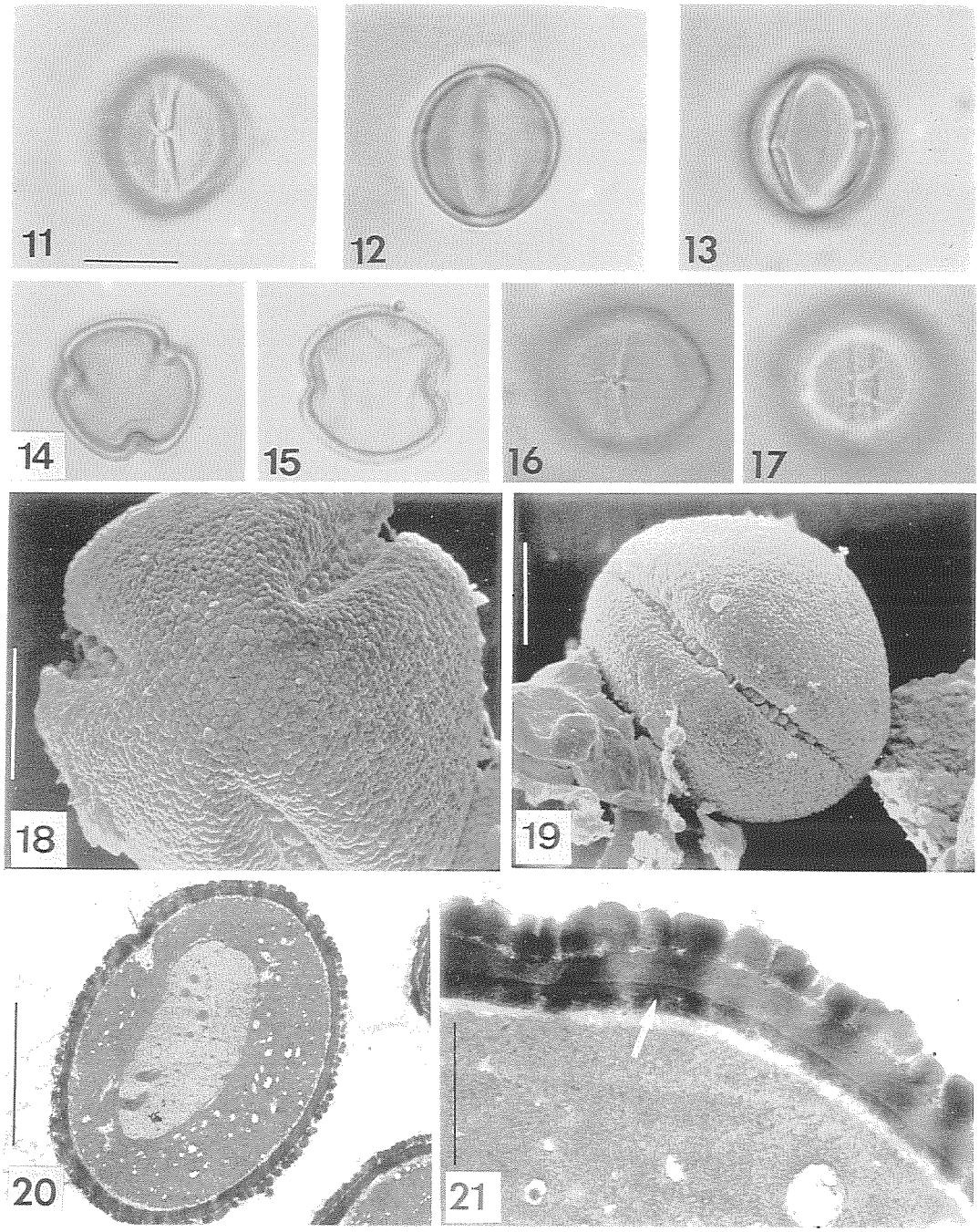
Fig. 18. Verrucate exine sculpture at polar area.

Fig. 19. Equatorial view showing long colpi with verrucae.

Fig. 20. Thin section of pollen grain.

Fig. 21. The exine is composed of tectum, thin infratectal bacular layer, foot layer and endexine (arrow).

Figs. 11–17 are the same magnification (bar is 10 μm), bars in Figs. 18–20 are 5 μm, in Fig. 21 is 1 μm.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