MORPHOLOGICAL CHANGES IN THE TAPETAL CELL DURING MICRO-SPOROGENESIS OF PINUS SYLVESTRIS L.

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SUMMARY

After zygotene the tapetal cell becomes osmiophilic. The electron density of the cytoplasm decreases during the early tetrad stage and increases afterwards. During the young microspore stage the tapetal cell degenerates. The electron density of the cytoplasm is caused mainly by the high content of ribosomes.

From interphase II onwards the tapetal cell starts to produce sporopollenin. In the cytoplasm pro-orbicular bodies are found. The formation of orbicules seems connected with the endoplasmic reticulum and ribosomes. Outside the cell the sporopollenin appears around the orbicules and as a pollen sac against the cell wall of the endothecium cells. The electron dense globules on the plasma membrane may be related to the formation of sporopollenin.

1. INTRODUCTION

Some morphological changes in the tapetal cell are related to the development of the microspore.

During the premeiotic stage Golgi vesicles transport cell wall affecting enzymes in the tapetal cell of *Tradescantia bracteata*. During the tetrad stage the Golgi vesicles apparently contain callase for the degradation of the callose wall around the microspore (MEPHAM & LANE 1969). MIKULSKA *et al.* (1969) suppose that the endoplasmic reticulum (ER) and the large quantities of enzymes in *Larix decidua* may be associated with hydrolytic processes in the tapetal cell.

In *Podocarpus macrophyllus* (VASIL & ALDRICH 1970) and in *Paeonia tenuifolia* (MARQUARDT *et al.* 1968) sporopollenin containing droplets appear on the plasma membrane of the tapetal cell. In *Lilium longiflorum* (HESLOP-HARRISON & DICKINSON 1969) and in *Pinus banksiana* (DICKINSON 1971) pro-orbicular bodies are formed in the tapetal cell. ECHLIN & GODWIN (1968) showed in *Helleborus foetidus* a relation of ER and ribosomes with the formation of the pro-orbicular bodies. In *Allium cepa* (RISUENO *et al.* 1969) the formation of the nucleus of the sporopollenin granules starts between the two membranes of the ER. The electron dense material increases in volume to an orbicule and is carried along cytoplasmic channels to the microspore. In different species of *Oxalis* CARNIEL (1967) described a deposition of sporopollenin on lipid droplets which move to the plasma membrane. In all cases the sporopollenin appears around the orbicules outside the cytoplasm of the tapetal cell. The function of the orbicules or Ubisch bodies is not clear (ECHLIN 1971).

During and after the tetrad stage lipid globuli in the tapetal cell of *Lilium*

(HESLOP-HARRISON & DICKINSON 1969) are supposed to be centres of carotenoid accumulation. In the orchid *Eulopedium sandersianum* (CHARDARD 1971) the lipid granules may be associated with the degeneration of the tapetal cell. In the Darwin tulip hybrid 'Apeldoorn'. the production of carotenoids takes place after the tetrad stage; at the lysis of the tapetal cells the presence of anthocyanins has been demonstrated in the loculus fluid (WIERMANN & WEINERT 1969).

During the meiotic divisions of the microspore of *Paeonia* MARQUARDT *et al.* (1968) have shown three subsequent mitoses of the tapetal cell without the formation of a cell wall, followed by an increase of RNA, ER and the number of mitochondria in the tapetal cell. A renewal of the cytoplasm of the tapetal cell in *Pinus banksiana* has been reported by DICKINSON (1971) and in *Tradescantia bracteata* by MEPHAM & LANE (1969).

A tapetal membrane of sporopollenin surrounds the tapetum and pollen in *Ginkgo biloba* and *Taxus baccata* (PETTITT 1966), in grasses (BANERJEE 1967), and in *Pinus banksiana* (DICKINSON 1971).

The volume of the nucleus and the DNA content of the tapetal cell in *Lilium candidum* and *L. henryi* increase just before leptotene. The RNA content increases from leptotene up to the ripe pollen (LINSKENS & SCHRAUWEN 1968; REZNIKOVA 1971). The protein content increases gradually (REZNIKOVA 1971). The tapetal cell shows in every stage a specific protein and enzyme pattern (LINSKENS 1966). Transport of protein to the nucleus of the meiocyte during leptotene and zygotene has been demonstrated in *Rhoeo discolor* by ALBERTINI (1971). In the tapetal cell of *Lilium longiflorum* (TAYLOR 1959) the protein synthesis has been associated with the formation of the pollen wall. In *Petunia hybrida* (LINSKENS 1967) and in *Lilium candidum* (REZNIKOVA 1971) the fat content of the tapetal cell increases after metaphase II.

In the secretory tapetum of *Pinus sylvestris* the morphological changes in the tapetal cell show many similarities with those reported for other plants.

2. MATERIAL AND METHODS

Pieces of the male cone in different stages of development of *Pinus sylvestris* were fixed for one hour in 1 % OsO_4 at 0 °C in phosphate buffer pH 7,2. After washing in water the specimens were stained for 30 minutes in 1 % aqueous uranyl acetate, followed, after washing, by a staining with 1 % aqueous $KMnO_4$ for 15 minutes. After dehydration and embedding in Epon 812 sections were cut using a Porter Blumm ultramicrotome. After 5 minutes staining with Reynolds lead citrate, the sections were examined with the Philips EM 300 electron microscope at 60 KV.

The description of the stages of development of the tapetal cell is based on the different meiotic and postmeiotic stages of microspore development.

3. RESULTS

The secretory tapetum of *Pinus sylvestris* consists of a layer of one or two cells. The tapetal cells are surrounded by two layers of cells with vacuoles: the fibrous layer (endothecium) and the middle layer. The vacuoles of the epidermal cells contain electron dense material (*figs. 1, 2, 3*).

3.1. The tapetal cell during zygotene

The nucleus of the tapetal cell has a heterogeneous nucleolus and contracted chromatine. The karyoplasm contains some granules and membrane-like structures (*fig. 4*).

Plastids in the cytoplasm contain a fine granular content and electron dense material between their membranes, at first as a thin line, thereafter as a globule (*fig. 6*). The cristae in the mitochondria are numerous in comparison to the number in the mitochondria of the developing microspore. Small lipid granules are dispersed in the cytoplasm of the tapetal cell. Golgi bodies are numerous but no vesicles are secreted. Some strands of rough ER (RER) are situated around the nucleus. Ribosomes and polysomes are dispersed in the cytoplasm. Remarkable are the very dilated membranes with an electron transparent content which surrounds a small area of cytoplasm with organelles (*fig. 5*). The tapetal cell wall starts to dissolve. At some places the plasmodesmata are still visible (*fig. 4*).

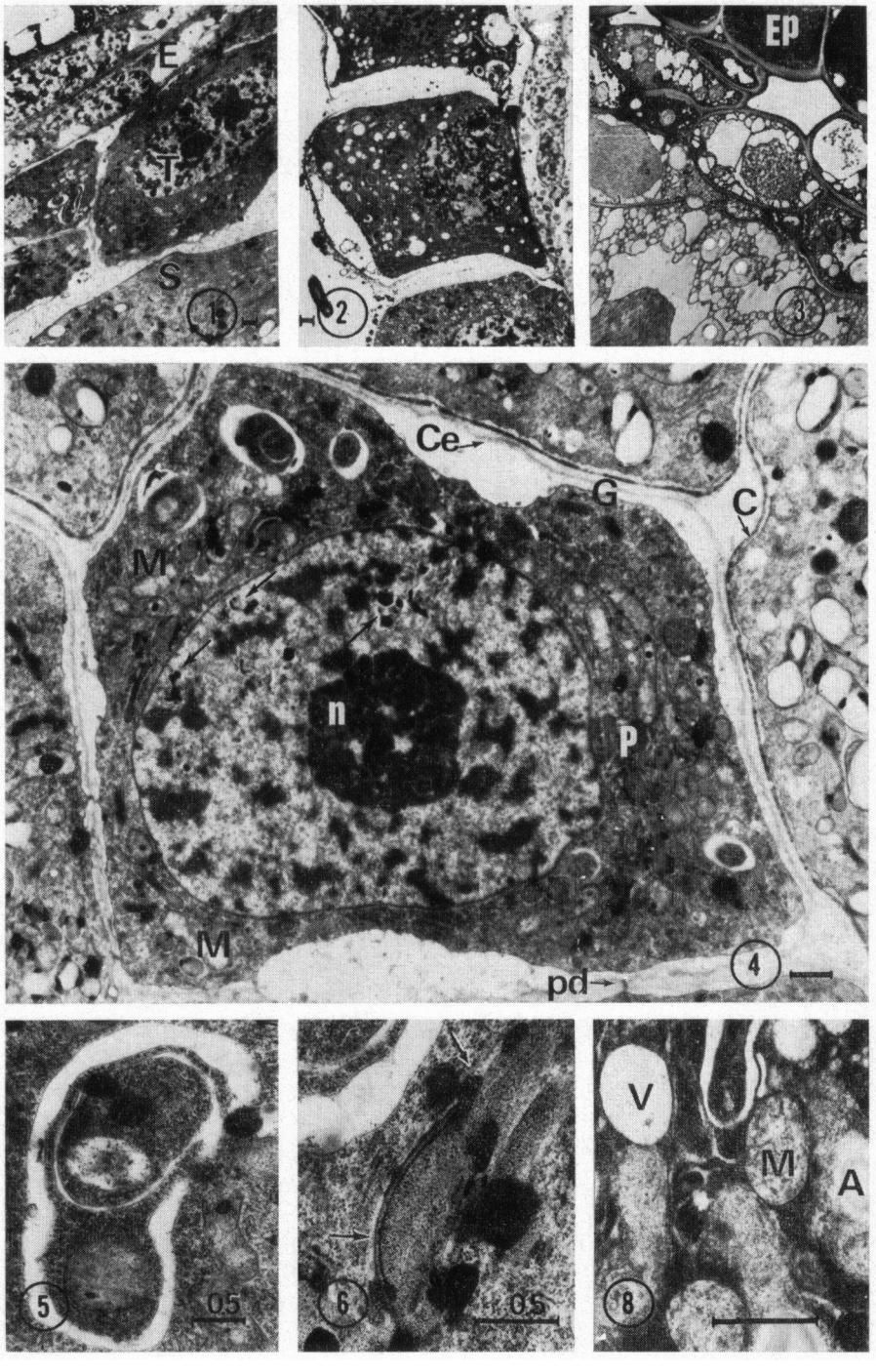
Some differences between the tapetal cell and the developing microspore could be noted. A difference exists between the cell walls. The nucleolus of the tapetal cell nucleus is heterogeneous, the tapetal cytoplasm is more electron dense and contains less lipid granules, less starch in the plastids and areas of cytoplasm surrounded by a dilated membrane. All these features are absent in the developing microspore.

3.2. The tapetal cell from pachytene up to diakinesis

The tapetal cell becomes now more voluminous and osmiophilic and may contain two or three nuclei. The nucleus has a granular karyoplasm and the membrane-like structures have disappeared (*fig. 7*).

The plastids have lost their electron dense globules. Frequently a starch granule is observed in the plastids. The mitochondria are enlarged. Lipid granules are absent. Large vesicles with fine granular fibrillar material become visible (*fig. 8*). Golgi bodies situated in groups produce small vesicles. Long strands of RER are dispersed in the cytoplasm. The high number of ribosomes between the organelles makes the observation of polysomes very difficult (*figs. 10, 11, 12*). The cytoplasmic areas surrounded with dilated membrane remain present.

Outside the plasmamembrane large electron dense droplets are visible (*fig. 7*). On the plasma membrane electron dense globular material is situated (*fig. 12*). Orbicules surrounded by an irregular electron dense thin layer are sometimes found connected with the now increasingly affected tapetal cell wall (*fig. 10*).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3.3. The tapetal cell during interphase II

The Golgi bodies in the cytoplasm, still in groups, produce now more vesicles. In the dilated RER fine fibrillar material is visible (*fig. 11*). Excretion of electron dense globular material continues (*fig. 13*). Outside the cell the orbicules increase in number (*fig. 9*). The cytoplasm contains some pro-orbicular bodies with the same shape and electron density as the centre of the orbicules outside the cell (*fig. 11*). The electron dense material which surrounds the orbicules appears always outside the cell.

Electron dense material accumulates along the inner side of the tapetal cell wall in the direction of the endothecium cells (*fig. 29*). In the locus, fragments of the tapetal cytoplasm are locally observed.

3.4. The tapetal cell during the tetrad stage

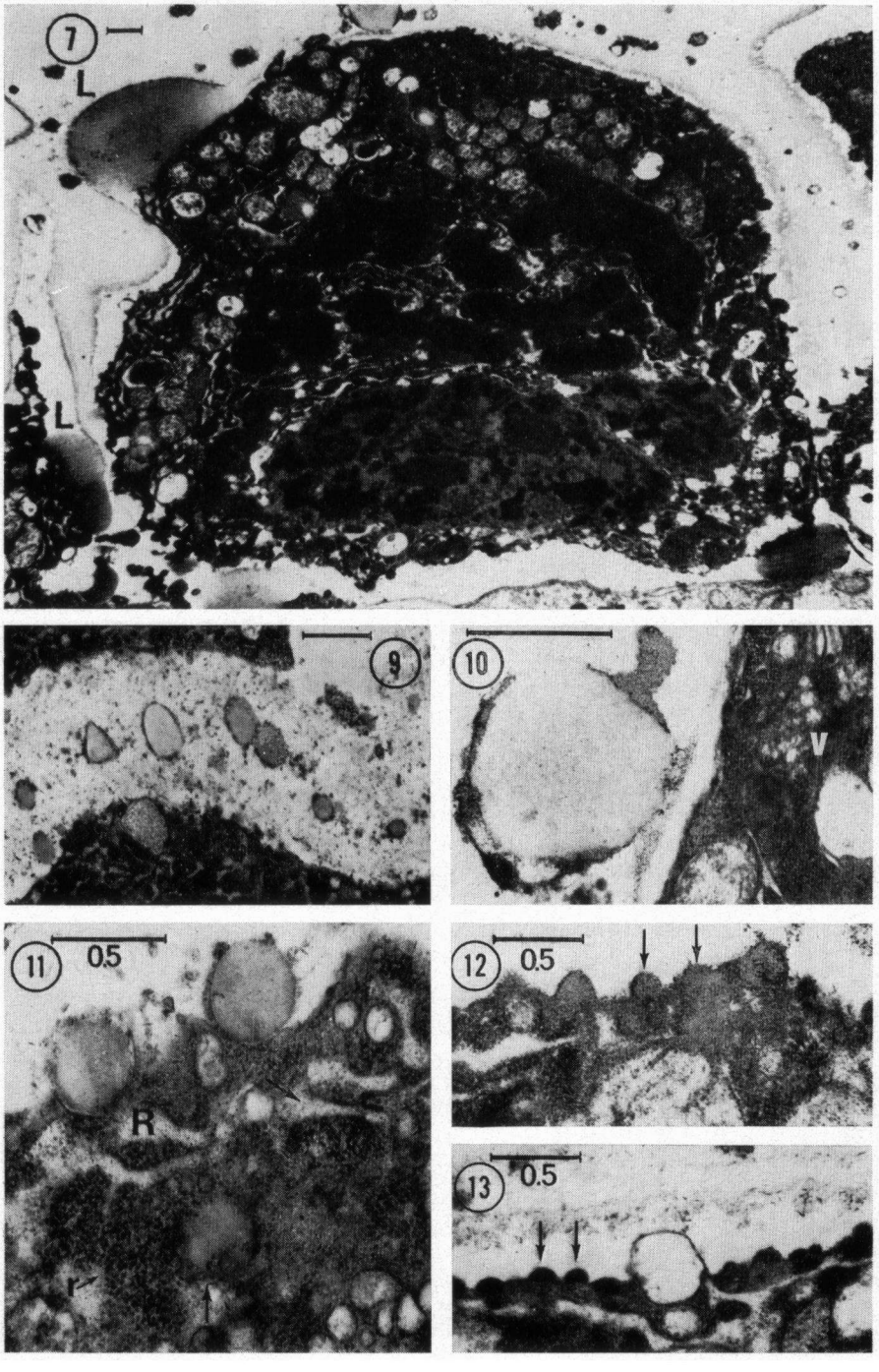
During and after the second meiotic division and at the beginning of the early tetrad stage, three stages of the tapetal cell could be distinguished (*fig. 2*). Firstly an osmiophilic tapetal cell, the same type as exists after zygotene; secondly a less osmiophilic cell, which corresponds to the tapetal cell as described during zygotene; thirdly an intermediate osmiophilic cell (*figs. 16, 14, 15*).

In all three cell types the nucleus has a heterogeneous nucleolus. The osmiophilic cell has granules in the karyoplasm (*fig. 16*). The karyoplasm of the less osmiophilic cell lacks these granules, but contains a dense body (*fig. 19*).

Compared with the tapetal cell during zygotene the less osmiophilic cell shows few differences. Remarkable are the electron dense granules, probably pro-orbicules, sometimes associated with the RER or with ribosomes (*figs. 17, 18*).

Compared with the osmiophilic cell which shows no differences with the cell after zygotene, the less osmiophilic cell has few ribosomes, fewer strands of RER, less voluminous vesicles and the Golgi bodies produce fewer vesicles (*figs. 14, 16*). A transition between the less osmiophilic cell and the osmiophilic cell shows the intermediate osmiophilic cell (*fig. 15*). In this last cell polysomes are present.

Fig. 1. Tapetal cell (T) during zygotene, around the developing microspore (S). Endothecium cells (E) and middle layer contain vacuoles, × 2,200.
Fig. 2. Tapetal cells during early tetrad stage; note the electron density of the cells, × 1,060.
Fig. 3. Degeneration of the tapetal cells. Epidermal cell (Ep) with osmiophilic vacuoles, × 1,060.
Fig. 4. Tapetal cell during zygotene. Nucleus with heterogeneous nucleolus (n) and membrane-like structures (arrows). Cytoplasm with plastids (P), mitochondria (M) and Golgi bodies (G). In the cell wall (Ce) are plasmodesmata (pd). Note the new cell wall (C) of the developing microspore, × 6,500.
Fig. 5. Cytoplasmic area surrounded with dilated ER, × 16,500.
Fig. 6. Plastid with electron dense material (arrows), × 25,800.
Fig. 8. Tapetal cell during diplotene with dividing nucleus. On the plasma membrane electron dense droplets (L), × 4,800.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The cell wall of the three types of tapetal cell has the same structure as the tapetal cell wall during the interphase II.

When the pollen wall formation in the microspore starts, all tapetal cells become osmiophilic again and have the same appearance as during diplotene (*fig. 22*). The Golgi bodies produce many vesicles. A large number of accumulations of electron dense material occur on the plasma membrane (*fig. 20*). The orbicules remain present, in the electron dense surrounding material lamellae of unit membrane dimension become visible (*fig. 27*).

When in the late tetrad stage the pollen wall sexine and nexine I have been formed and the callose wall starts to disappear, the tapetal cell gets more vacuoles (*fig. 23*). In the cytoplasm the Golgi bodies stop their vesicle production. The vacuoles in the cytoplasm originate probably from the large vesicles. Along the plasma membrane many orbicules are observed. Electron dense material increases around the cell (*fig. 21*).

No similarity shows the cytoplasm of a degenerating microspore within a tetrad with the tapetal cell. The degenerating microspore is osmiophilic, although not due to the ribosomes but mainly by the electron dense material around the plastids and mitochondria (*fig. 24*).

3.5. The tapetal cell during the young microspore stage

After the breakdown of the callose wall around the tetrad, the tapetal cell degenerates quickly.

The nucleus remains surrounded by the nuclear membrane, the nucleolus is still recognizable (*fig. 25*). All cell organelles are swollen and have an electron transparent content. Some plastids contain a starch granule; the remnants of the mitochondria have an accentuated membrane (*fig. 25*). Locally ribosomes remain visible (*fig. 26*). The cell wall is absent, the plasmamembrane remains intact.

Outside the cell membrane the orbicules are present (*fig. 26*). The electron dense sporopollenin coat contains lamellae of unit membrane dimension (*fig. 28*). Against the cellulose wall of the endothecium cells borders the electron dense layer of the pollen sac (*fig. 30*).

After the young microspore stage the tapetal cell disappears completely. Sometimes osmiophilic remnants of the tapetal cell remain visible between the microspores.

Fig. 7. Detail Fig. 7. Cytoplasm with plastids with a starch granule (A), mitochondria (M) and vesicle (V), × 14,700.

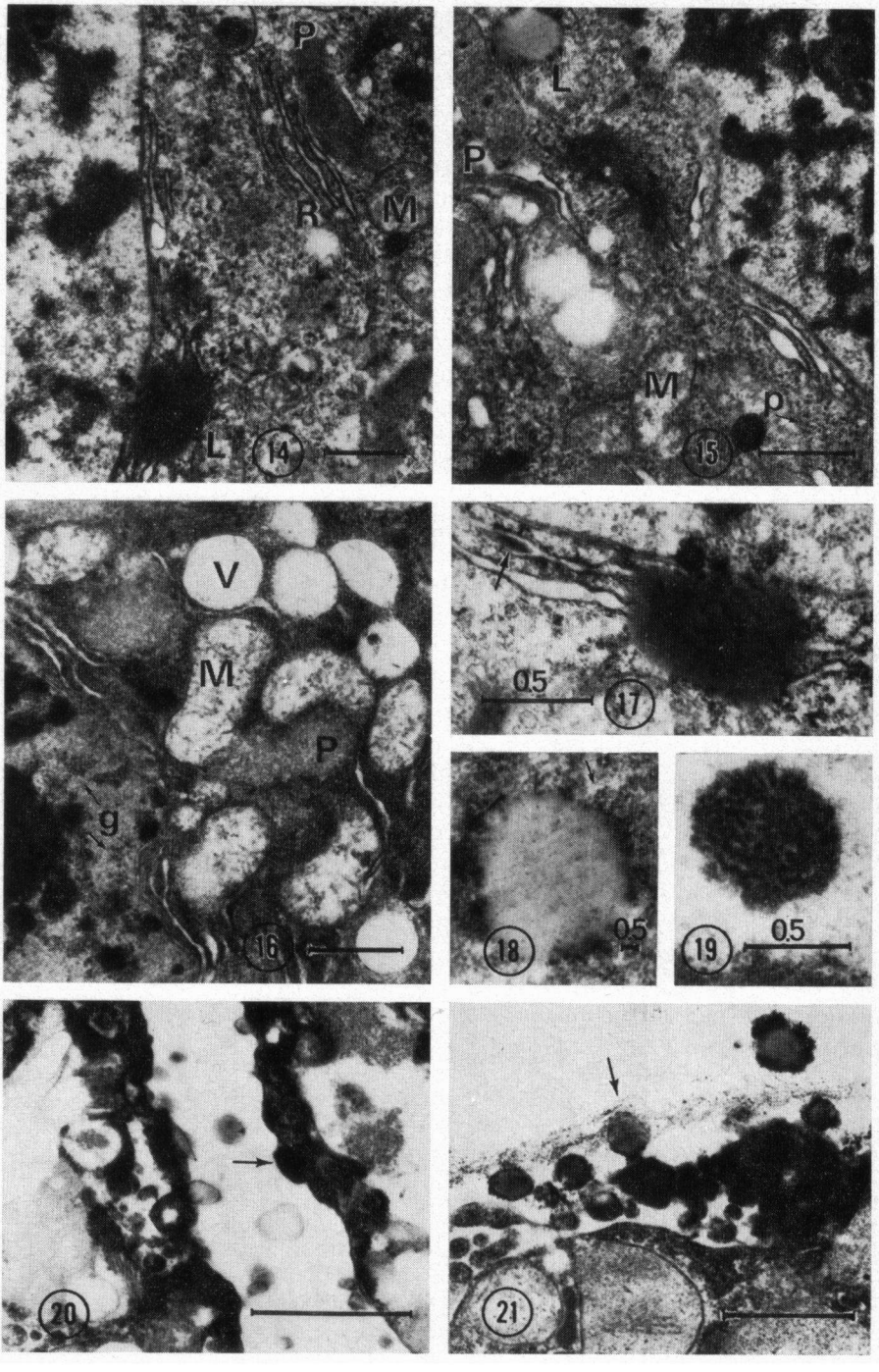
Fig. 9. Orbicules between the tapetal cells, × 10,000.

Fig. 10. Electron dense material around the orbicule. Note Golgi vesicles (v), × 20,000.

Fig. 11. In the cytoplasm a pro-orbicular body (arrow). Note the ribosomes (r) and the dilated RER with fine fibrillar material (arrow), × 32,000.

Fig. 12. Electron dense globular material on the plasma membrane during diplotene, × 25,000.

Fig. 13. Electron dense globular material on the plasma membrane during interphase II, × 25,000.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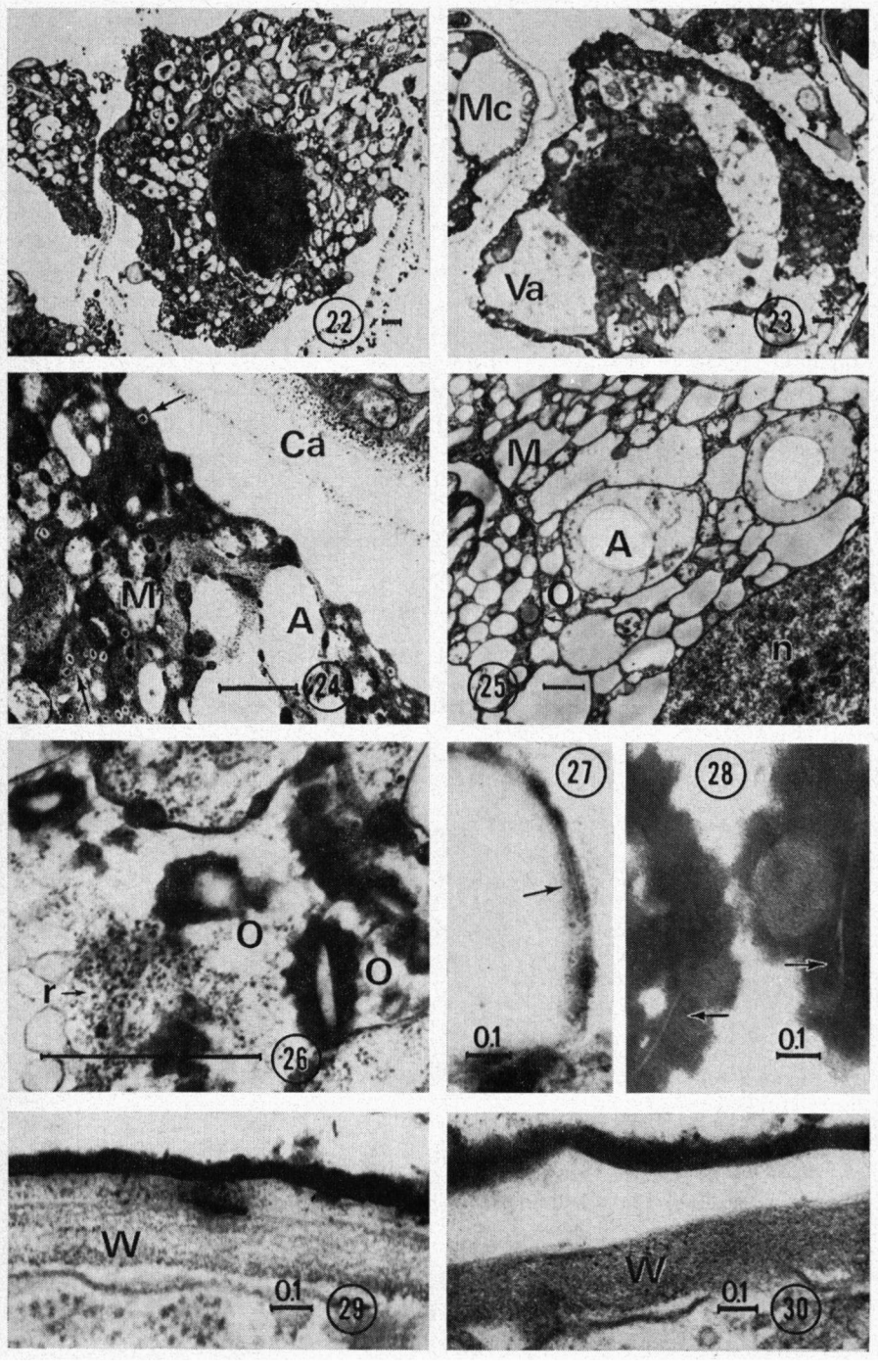
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4. DISCUSSION AND CONCLUSION

Compared with the degenerating microspore in the tetrad, the tapetal cell shows no sign of degeneration before the formation of the young microspore.

After zygotene mainly the number of ribosomes increases, the strands of ER grow and the mitochondria change their number of cristae and enlarge. This increase of ER, mitochondria and ribosomes has also been reported by MIKULSKA *et al.* (1969), MEPHAM & LANE (1969), HOEFERT (1969), and ECHLIN (1971a). During the early tetrad stage mainly the number of ribosomes decreases and the cell becomes similar to the tapetal cell during zygotene. In agreement with the report by MEPHAM & LANE (1969) and DICKINSON (1971) also a renewal of the tapetal cell of *Pinus sylvestris* takes place. A division of the endothecium cell which may be the cause of a new tapetal cell, or division of tapetal cells, has not been observed. Besides, the same structure of the tapetal cell wall persists. For this reason, it seems that in the development of the tapetal cell two identical morphological cycles could be distinguished. The first from zygotene up to early tetrad stage and the second from early tetrad stage up to the degeneration of the tapetal cell.

The granular karyoplasm which appears during zygotene and after the early tetrad stage and the dense bodies in the karyoplasm during the early tetrad stage

Fig. 14. Early tetrad stage: less osmiophilic cell. Cytoplasm with mitochondria (M), plastids (P), RER (R) and pro-orbicule (L), × 11,000.
Fig. 15. Intermediate osmiophilic cell. The RER dilates (R), pro-orbicule (L) with ribosomes. Note the polysomes (p), × 13,000.
Fig. 16. Osmiophilic cell. Vesicles appear (V), karyoplasm with granules (g), × 13,000.
Fig. 17. Detail pro-orbicular body with RER. Between the membranes electron dense material (arrow), × 29,000.
Fig. 18. Pro-orbicular body with ribosomes (arrow), × 32,000.
Fig. 19. Dense body in the karyoplasm of the less osmiophylic cell, × 29,000.
Fig. 20. Electron dense material on the plasma membrane during the tetrad stage (arrow), × 22,000.
Fig. 21. Electron dense material along the cell during break out of the microspores. Orbicule seems to penetrate in the tapetal cell wall (arrow), × 17,000.
Fig. 22. Tapetal cell during the tetrad stage, × 2,200.
Fig. 23. Tapetal cell during the late tetrad stage, vacuoles appear (Va); Mc: microspore, × 2,200.
Fig. 24. Degeneration of a microspore within the tetrad. Plastids with a starch granule (A) and mitochondria (M) surrounded with osmiophilic material; Golgi vesicles remain visible (arrow). Ca: callose wall, × 11,000.
Fig. 25. Degenerating tapetal cell during the young microspore stage. Plastids with a starch granule (A), mitochondria (M) and the nucleolus (n) remain recognizable, × 5,600.
Fig. 26. Orbicules in the tapetal fluid (0), note the ribosomes (r), × 31,000.
Fig. 27. Lamellae of unit membrane dimension in the growing orbicule (arrow), × 57,000.
Fig. 28. Lamellae of unit membrane dimension remain visible in the orbicules (arrows), × 67,000.
Fig. 29. Interphase II: pollen sac appears against the endothecium wall (W), × 56,000.
Fig. 30. Pollen sac during the young microspore stage. W: Cell wall of the endothecium cell, × 56,000.
Unless mentioned otherwise, the line on the figures represents a length of 1 μm.

may be related to the formation of ribosomes, as occurs during diplotene and diakinesis in the developing microspore of pine (WILLEMSE 1971). The high amount of ribosomes, probably polysomes, may be related to the protein synthesis in the tapetal cell (LINSKENS 1966, REZNIKOVA 1971). Polysomes are mainly observed in the intermediate cell.

The production of Golgi vesicles takes place after zygotene and the early tetrad stage. It could not be demonstrated that the production of Golgi vesicles is related to the formation of cell wall affecting enzymes, although during the production of the vesicles the tapetal cell wall and the callose wall around the microspore disappear.

As in *Helleborus* (ECHLIN & GODWIN 1968) the formation of pro-orbicules in *Pinus sylvestris* may have a relation to RER and ribosomes. Outside the cytoplasm the pro-orbicules are surrounded by sporopollenin in which lamellae of unit membrane dimension are present on which the sporopollenin may be formed (ROWLEY & SOUTHWORTH 1967).

The electron dense material on the plasma membrane may contain sporopollenin and/or a carotenoid (WIERMANN 1970), but in another state than that around the orbicules. Around the tapetal cell the same reaction occurs on UV radiation as in the exine of the microspore. The electron dense material on the plasma membrane is probably the source of the fluorescence (WILLEMSE 1971a). The large accumulations of electron dense material on the plasma membrane disappear completely. Finally, it is remarkable that the sporopollenin around the orbicules, of the pollen sac and of the sexine of the pollen wall after the tetrad stage grows while the sporopollenin around the electron dense globular material does not. Whether the production of electron dense globules between the membranes of the plastids is connected with the formation of the electron dense material on the plasma membrane is not clear. Electron dense material on the plastids has been also reported by MARQUARDT *et al.* (1968) and ECHLIN (1971a).

The quick increase of the sporopollenin on the orbicules, pollen sac and pollen wall indicates a very high content of sporopollenin precursors in the tapetal fluid. This non-electron dense material may be produced by the microspore and the tapetal cell. Outside the cell the sporopollenin appears as electron dense material mainly on membranes (WILLEMSE 1971b). The last steps in the formation of sporopollenin apparently occur in the tapetal fluid, as WIERMANN (1970) has shown in the synthesis of flavonol and anthocyanidin. The tapetal fluid plays an important role in the relation between the developing microspore and the tapetal cell (ROWLEY 1963).

A similarity between the tapetal cell and the developing microspore of *Pinus sylvestris* based on the morphology of the cells exists. These are: the presence of membrane-like structures in the karyoplasm during zygotene, the heterogeneous nucleolus in relation to the renewal of the ribosome population and the formation of sporopollenin.

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REFERENCES

ALBERTINI, L. (1971): Action de l'actinomycine D sur les synthèses de RNA et de proteines dans les microsporocytes et le tapis du Rhoeo discolor (Hance) – Etude autoradiographique. *Ann. Univ. et A.R.E.R.S.* **9**: 207–213.

BANERJEE, U. C. (1967): Ultrastructure of the tapetal membranes in grasses. *Grana Palynol.* **7**: 365–377.

CARNIEL, K. (1967): Licht- und electronenmikroskopische Untersuchung der Ubischkörperentwicklung in der Gattung Oxalis. *Österr. Botan. Z.* **114**: 490–501.

CHARDARD, R. (1971): Infrastructure des cellules tapétales des microsporocytes et des grains de pollen de quelques Orchidacées. *Ann. Univ. et A.R.E.R.S.* **9**: 154–178.

DICKINSON, H. G. (1971): The role played by sporopollenin in the development of pollen in Pinus banksiana. In J. BROOKS, P. R. GRANT, M. D. MUIR, P. VAN GIJZEL & G. SHAW (eds), *Sporopollenin*, p. 31–65. Academic Press, London-New York.

ECHLIN, P. (1971): Production of sporopollenin by the tapetum. In J. BROOKS, P. R. GRANT, M. D. MUIR, P. VAN GIJZEL & G. SHAW (eds.), *Sporopollenin*, p. 220–255. Academic Press, London-New York.

— (1971a): The role of the tapetum during microsporogenesis of angiosperms. In J. HESLOP-HARRISON (ed.), *Pollen development and physiology*, p. 41–61. Butterworths, London.

— & H. GODWIN (1968): The ultrastructure and ontogeny of pollen in Helleborus foetidus L. I. The development of the tapetum and Ubisch bodies. *J. Cell Sci.* **3**: 161–174.

HESLOP-HARRISON, J. & H. G. DICKINSON (1969): Time relationships of sporopollenin synthesis associated with tapetum and microspores in Lilium. *Planta (Berl.)* **84**: 199–214.

HOEFERT, L. L. (1969): Ultrastructure of Beta pollen. I. Cytoplasmic constituents. *Amer. J. Bot.* **56**: 363–368.

LINSKENS, H. F. (1966): Die Änderung des Protein- und Enzym-Musters während der Pollenmeiose und Pollenentwicklung. *Planta (Berl.)* **69**: 79–91.

— (1967): Pollen. In W. RUHLAND (ed.) *Encyclopedia of plant physiology* **18**: 368–406. Springer-Verlag, Berlin.

— & J. SCHRAUWEN (1968): Quantitative nucleic acid determinations in the microspore and tapetum fractions of lily anthers. *Proc. Koninkl. Ned. Akad. Wet. Ser. C* **71**, 267–279.

MARQUARDT, H., O. M. BARTH & U. VON RAHDEN (1968): Zytophotometrische und electronenmikroskopische Beobachtungen über die Tapetumzellen in den Antheren von Paeonia tenuifolia. *Protoplasma* **65**: 407–421.

MEPHAM, R. H. & G. R. LANE (1969): Formation and development of the tapetal periplasmodium in Tradescantia bracteata. *Protoplasma* **68**: 175–192.

MIKULSKA, E., H. ZOLNIEROWICZ & A. WALEK-CZERNECKA (1969): Ultrastructure of tapetum cells and periplasmodium in the anthers of Larix decidua Mill. *Acta Soc. Bot. Polon.* **38**: 291–301.

PETTITT, J. M. (1966): A new interpretation of the structure of the megaspore membrane in some gymnospermous ovules. *J. Linn. Soc.(Bot.)* **59**: 253–263.

REZNIKOVA, S. A. (1971): Microsporogenesis and cell differentiation in the anther of Lilium candidum L. in situ and in vitro. *Ann Univ. et A.R.E.R.S.* **9**: 145–150.

RISUENO, M. C., G. GIMÉNEZ-MARTÍN, J. F. LÓPEZ-SÁEZ & M. I. R.-GARCÍA (1969): Origin and development of sporopollenin bodies. *Protoplasma* **67**: 361–374.

ROWLEY, J. R. (1963): Ubisch body development in Poa. *Grana Palynol.* **4**: 25–36.

— & D. SOUTHWORTH (1967): Deposition of sporopollenin on lamellae of unit membrane

dimension. *Nature (Lond.)* **213**: 703–704.

TAYLOR, J. H. (1959): Autoradiographic studies of nucleic acids and proteins during meiosis in Lilium longiflorum. *Amer. J. Bot.* **46**: 477–484.

VASIL, I. K. & H. C. ALDRICH (1970): A histochemical and ultrastructural study of the ontogeny and differentiation of pollen in Podocarpus macrophyllus D.Don. *Protoplasma* **71**: 1–37.

WIERMANN, R. (1970): Die Synthese von Phenylpropanen während der Pollenentwicklung. *Planta (Berl.)* **95**: 133–145.

— & H. WEINERT (1969): Untersuchungen zum Phenylpropanstoffwechsel des Pollens. *Z. Pflanzenphysiol.* **61**: 173–183.

WILLEMSE, M. TH. M. (1971): Morphological and quantitative changes in the population of cell organelles during microsporogenesis of Pinus sylvestris L. I. Morphological changes from zygotene untill prometaphase I. *Acta Bot. Neerl.* **20**: 261–274.

— (1971a): Morphological and fluorescence microscopical investigation on sporopollenin formation at Pinus sylvestris and Gasteria verrucosa. In J. BROOKS, P. R. GRANT, M. D. MUIR, P. VAN GIJZEL & G. SHAW (eds), *Sporopollenin*, p. 68–107. Academic Press, London-New York.

— (1971b): Morphological and quantitative changes in the population of cell organelles during microsporogenesis of Pinus sylvestris L. III. Morphological changes during the tetrad stage and in the young microspore. A quantitative approach to the changes in the population of cell organelles. *Acta Bot. Neerl.* **20**: 498–523.