Acta. Bot. Neerl. 37(4), December 1988, p. 493–508

Tissue interactions in the developing locule of *Gasteria verrucosa* during microsporogenesis

C. J. KEIJZER and M. T. M. WILLEMSE

Department of Plant Cytology and Morphology, Agricultural University, Wageningen, The Netherlands

SUMMARY

We investigated the development of the locule tissues of *Gasteria verrucosa* (Mill.) H. Duval during microsporogenesis using electron microscopy and summarized it in detailed pen-drawings.

In the early stages of development, the epidermis, endothecium and middle layer show no remarkable processes relating to tapetum and pollen development.

The tapetum regulates the disappearance of the original cell walls and the callose walls from the microspores and itself. These wall materials partly turn into starch in both tissues.

The spherical shape of the meiotic callose walls and the presence of the cytomictic channels enable the pollen mother cells to divide into equally shaped microspores, which is important for random dispersal.

Key-words: anther, callose, meiosis, tapetum, tissue interaction.

INTRODUCTION

During the last decennia many reports have appeared on tapetum and pollen development and the relationship between them (see the reviews of Linskens 1964, 1967; Puri 1972; Vasil 1973; Mascarenhas 1975; Shivanna *et al.* 1979; Bhandari 1984; Knox 1984 and the many works of Heslop-Harrison). Few investigations were carried out on the development of the other locule tissues and these mainly focused on anther dehiscence (many old works, see Keijzer 1987a) or dealt with the systematics of anther tissues or endothecium patterns (Richter 1929; Eames 1961; Davis 1966; Stanley & Kirby 1973). Although the different locule tissues are sometimes described in reports on male sterility, where they often show deviations (see the reviews of Edwardson 1970; Laser & Lersten 1972; Gottschalk & Kaul 1974), investigations on the fertile developing locule as a functional unit are scarce (Reznickova & Willemse 1980).

In the present report the ultrastructural development of the locule of *Gasteria verrucosa* (Mill.) H. Duval is investigated. This work was preceded by some studies on detailed aspects of the stamen in this species (Willemse 1972; Keijzer 1983, 1987a,b; Keijzer *et al.* 1987a,b; Schroder 1985; Van Lammeren *et al.* 1985) and is followed by a second part (Keijzer & Willemse 1988).

MATERIALS AND METHODS

Intact anthers of *Gasteria verrucosa* (Mill.) H. Duval, at different stages of development, were fixed in 3% glutaraldehyde for 2 h and 1% osmium tetroxide for 45 min, both in

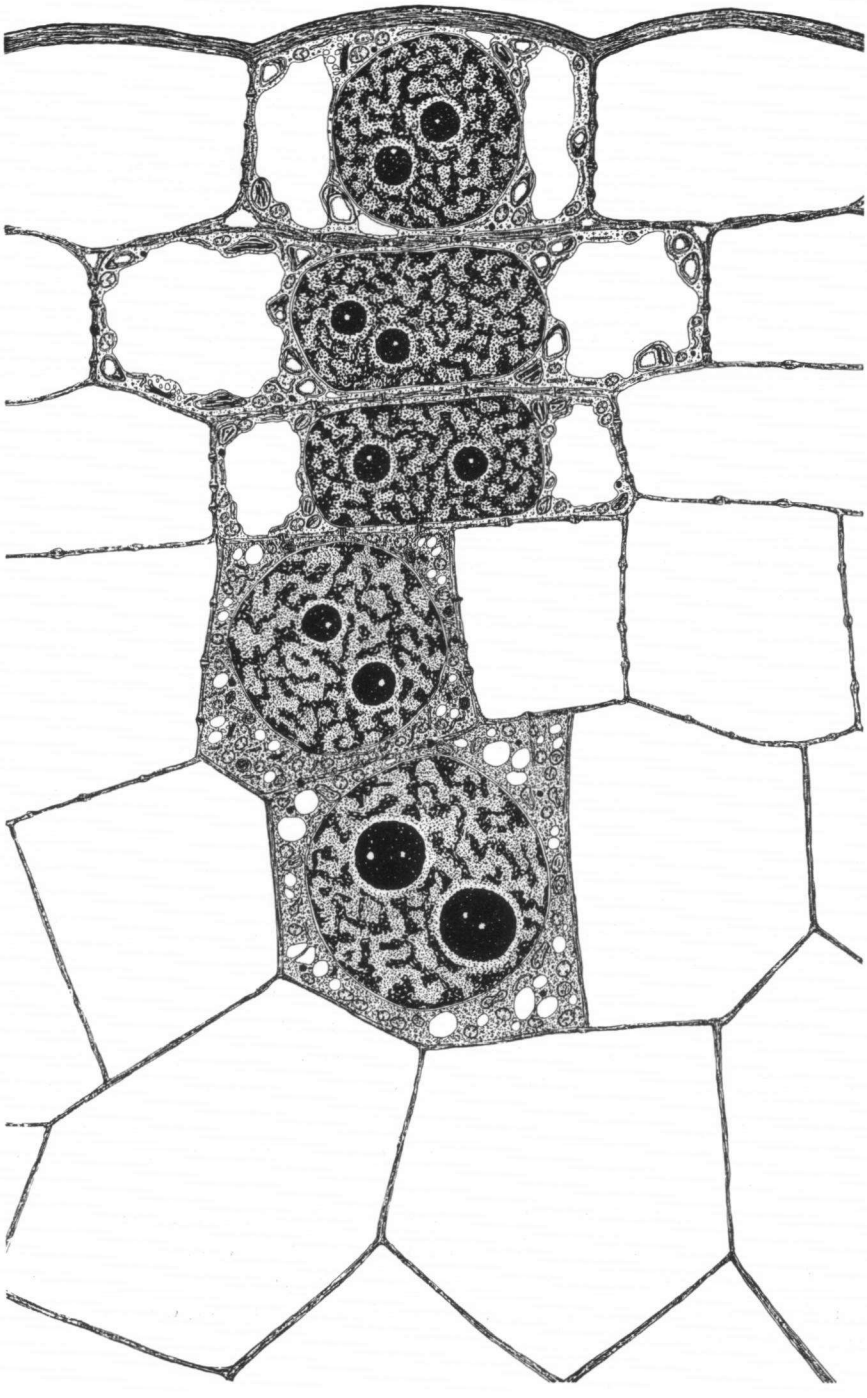
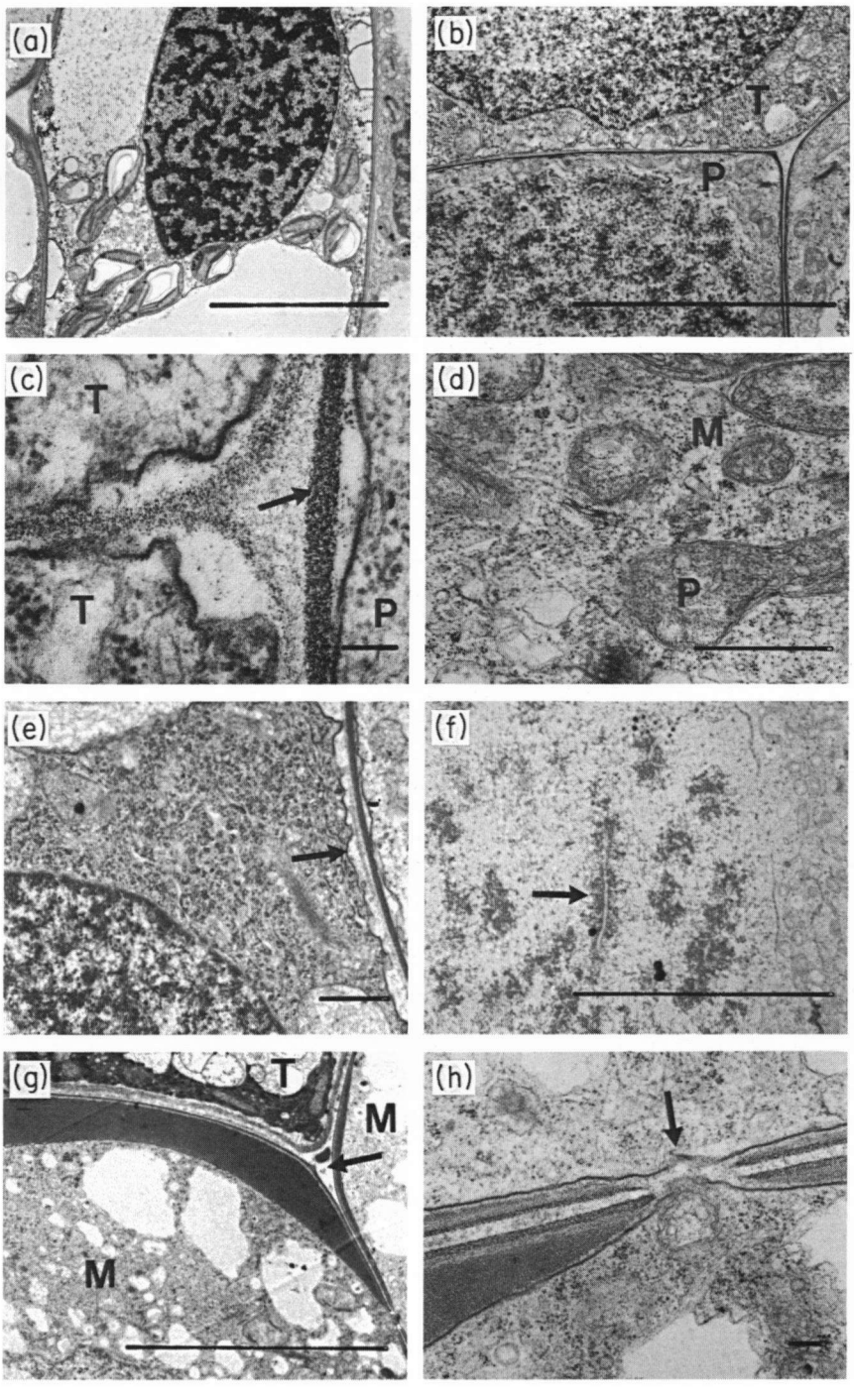


Fig. 1. The pollen mother cell stage. Epidermis, endothecium and middle layer develop in a comparable way by early vacuolation and an almost similar cytoplasmic content. On the other hand, the dense cytoplasm of the tapetum and the pollen mother cells resemble one another.


(a)
(b)
T
P
(c)
T
T
P
(d)
M
P
(e)
(f)
(g)
T
M
M
(h)

0·07 M cacodylate buffer (pH 7·2) at room temperature to prepare them for electron microscopy. After dehydration in ethanol they were embedded in the low viscosity resin of Spurr (1969). Ultra-thin sections were stained with lead citrate and uranylacetate (Reynolds 1963) and observed in a Philips EM 301 at 60 kV.

We investigated twenty cells from each locule tissue and the amount, size shape and position of nuclei, vacuoles, vesicles, plastids, mitochondria, dictyosomes, ER, ribosomes, membranes and walls were determined in the plane of the nucleus. Locule segments were reconstructed by means of pen drawings on the scale 2200:1 to reflect the mean values of these observations.

RESULTS

1. The pollen mother cell stage (Fig. 1)

In the pollen mother cell stage the locule of *G. verrucosa* consists of five tissues: epidermis, endothecium, middle layer and tapetum are single layered, the pollen mother cells fill the centre. The epidermis, endothecium, and middle layer are ultrastructurally similar in many respects (Fig. 2a). All three cell types are vacuolated and have a central nucleus. The plastids contain thylakoids which colour the anther green. Most of them bear a starch grain (Fig. 2a), except in the middle layer where the plastids are smaller. The number of mitochondria is almost equal to the number of plastids; dictyosomes and lipid droplets are scarce.

The tapetum and the pollen mother cells almost resemble one another (Fig. 2b) and can be only distinguished by cytoplasmic details. A clear difference is the more electron-dense cell wall of the pollen mother cells (Fig. 2c). Both cell types are isodiametric, lack any polarity and have a prominent central nucleus. In the pollen mother cells the nucleoles are much larger. The mitochondria are the same size as those in the outer layers, the plastids are much smaller, contain membranous inclusions and ribosomes, and lack starch (Fig. 2d). The ribosome density is larger than in the outer layers. The tapetum cells contain more RER and dictyosomes than the pollen mother cells but lack lipid droplets. The pollen mother cells contain larger vacuoles than the tapetum cells, the amount of lipid droplets is comparable with the outer layers. Plasmodesmata cross all the cell walls in the locule except those between the pollen mother cells.

2. The zygotene stage (Fig. 3)

In the zygotene stage the epidermis and endothecium cells grow in both a tangential and a radial direction, as do their vacuoles. The amount and composition of their cytoplasm

Fig. 2. (a) Cross-section of an endothecium cell in the pollen mother cell stage. In the vacuolated cell the central nucleus is surrounded by most of the mitochondria and the starch- and thylakoid-containing plastids (bar = 10 μm). (b) Cross-section of a tapetum (T) and a pollen mother cell (P). The contents of the cytoplasm that surrounds the large nuclei are almost similar in both layers (bar = 10 μm). (c) The cell walls of the pollen mother cell (P) are very electron dense compared with those of the tapetal cells (T) (bar = 0·1 μm). (d) The poorly differentiated plastids (P) of the pollen mother cell can be hardly distinguished from the mitochondria (M) and lack starch and thyllakoids (bar = 1 μm). (e) In the zygotene stage the density of all organelles, and particularly the ribosome of the tapetal cells, increases compared with the previous stage. The plasma membrane undulates on all sides of the cells (arrow) (bar = 0·1 μm). (f) The zygotene stage can be identified by synaptonemal complexes (arrow) in the huge nucleus of the meiocyte (bar = 10 μm). (g) In the zygotene stage the most distended areas of the meiocytes (M) are filled with callose. Intercellular spaces (arrow) are visible, especially at sites where more than two ceils connected in the previous stage. T = tapetum (bar = 10 μm). (h) Detail of (g). The cytomitic channel (arrow) has the same width as the bordering mitochondrium (bar = 0·1 μm).

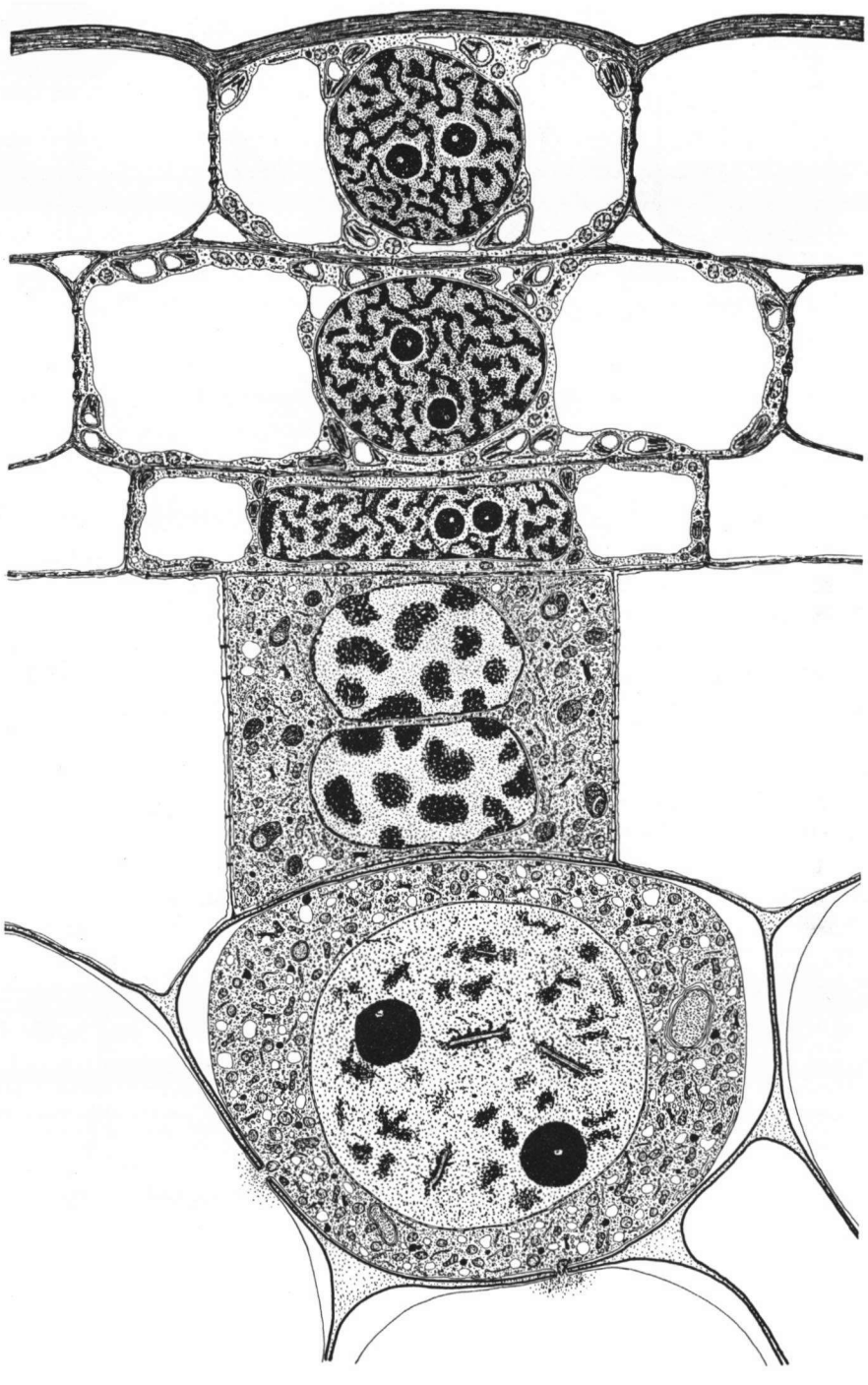


Fig. 3. The zygotene stage. The tapetal cells have just become binucleate and are isolated from the meiocytes by the disappearance of the plasmodesmata. The callose deposition turns the irregularly shaped meiocytes into spherical cells. The cytomictic channels enable the interchange of cytoplasm.

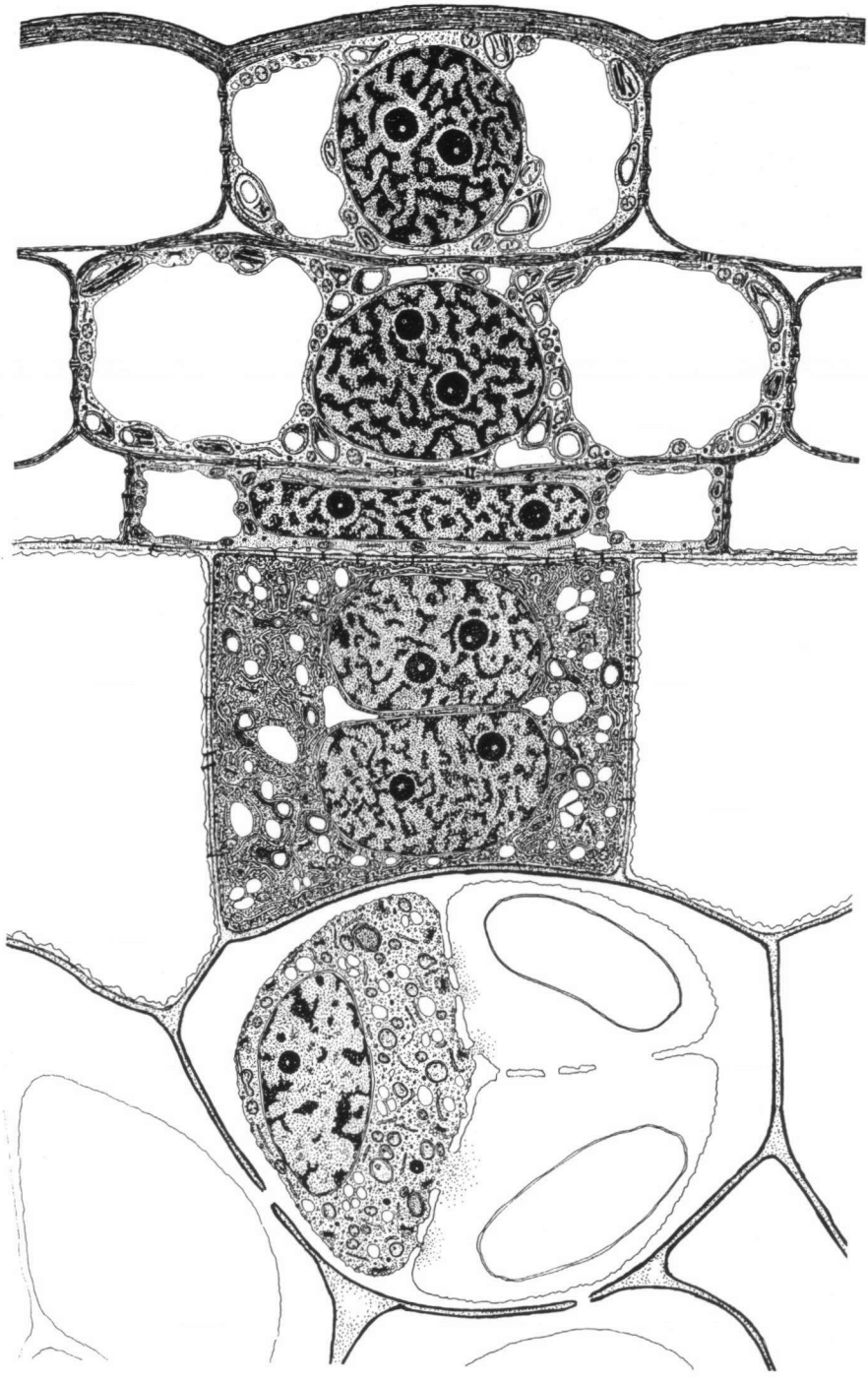
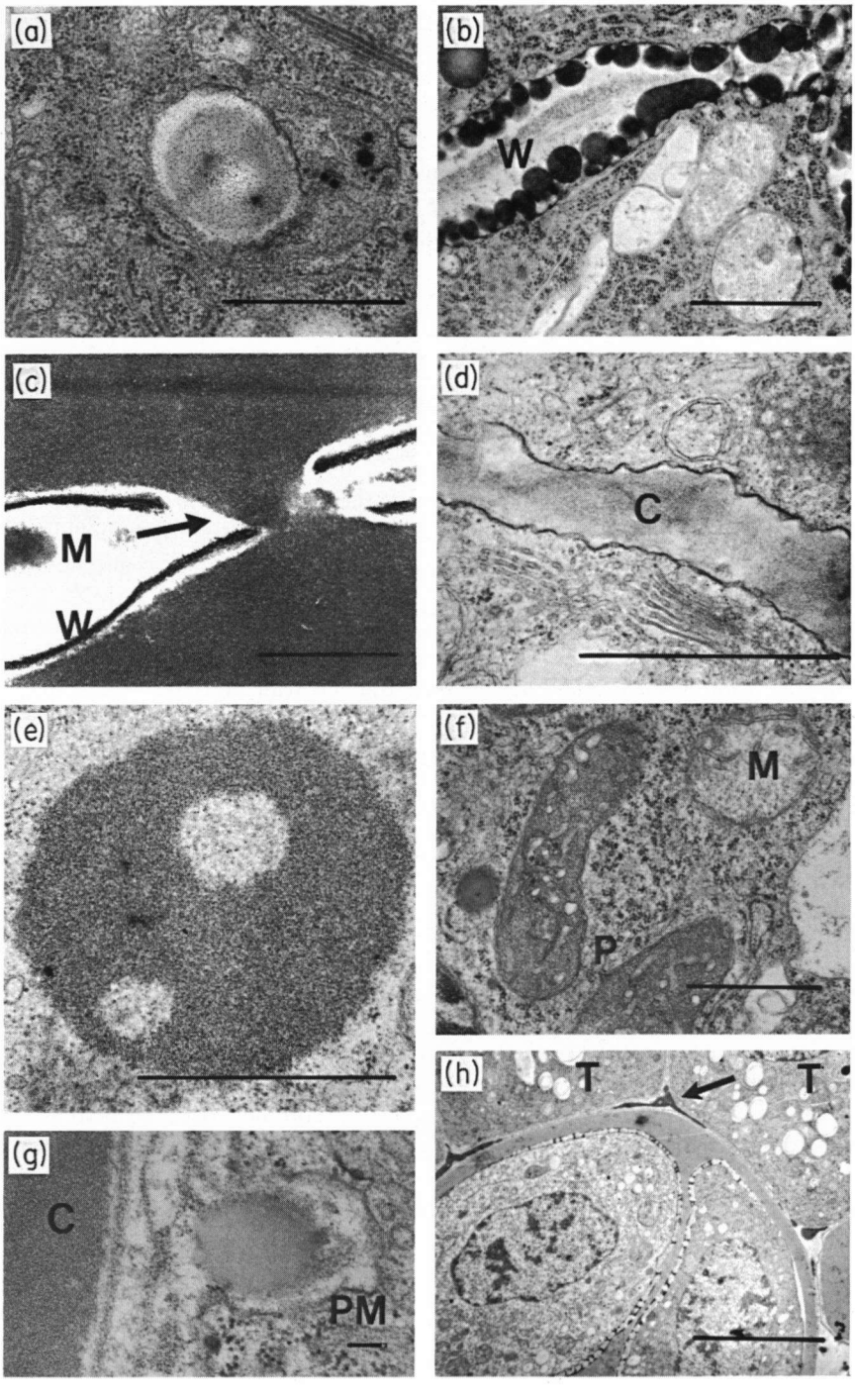


Fig. 4. The early tetrad. The number of organelles increases in the ribosome-dense tapetal cytoplasm. Thin callose walls divide the meiocyte into four equal sized microspores. The latter are also congruent due to the spherical shape of the original cell.


(a)
(b)
W
(c)
M
W
(d)
C
(e)
(f)
M
P
(g)
C
PM
(h)
T
T

remains unchanged, only the size of the starch grains increases. The middle layer cells are tangentially stretched and are flattened in a radial direction.

The tapetum cells grow strongly and become binucleate after endomitosis. The amounts of RER, dictyosomes, lipid droplets, mitochondria and plastids increase (Fig. 2e). The plasma membrane undulates slightly (Fig. 2e).

Both the meiocytes and their nuclei grow strongly, inside the latter, synaptonemal complexes are visiable (Fig. 2f). Their original walls become more electron-dense than those of the tapetum and the site of the middle lamella widens to leave intercellular spaces at the corners (Fig. 2g). Callose deposition starts between the plasma membrane and the original wall and turns the cell shape into a sphere (Fig. 2g). The plasmodesmata towards the tapetum disappear and the meiocytes are mutually interconnected by much wider cytomictic channels in which organelles may be present (Fig. 2h). The amounts of cytoplasm and organelles increase.

3. The early tetrad stage (Fig. 4)

During meiosis no remarkable changes occur in the epidermis, the endothecium and the middle layer cells. The circumference of the locule increases slightly by incidental anticlinal divisions in the epidermis and the endothecium, by which the middle layer cells continue their tangential stretching together with a radial flattening.

The tapetum cells grow slightly in a radial direction. The amount of dictyosomes, RER, ribosomes and vacuoles increases, and the plastids fill with starch (Figs. 5a and b). Electron-dense droplets lie between the cell wall and the plasma membrane (Fig. 5b).

The meiocytes become spherical by further deposition of callose, which also closes the cytomictic channels (Fig. 5c). They divide into four microspores of equal size and shape by the formation of additional thin callose layers, which are bordered by dictyosomes (Fig. 5d). The haploid nuclei are situated near the periphery of the original diploid cell. The plastids contain a few single-membranous structures and sometimes small globules (Fig. 5d). A few nucleoids appear in the cytoplasm (Fig. 5e).

4. The late tetrad stage (Fig. 6)

The ultrastructure of the epidermis, the endothecium and the middle layer remain unchanged, the circumference of the locule increases slightly and the middle layer continues to flatten.

The tapetum cells stretch in a radial direction accompanied by an increase in both cytoplasm and the number of vacuoles. In addition, the amounts of RER, ribosomes,

---

Fig. 5. (a) In the early tetrad stage the tapetal plastids contain starch. The cytoplasm is rather electron dense and contains many ribosomes (bar = 1 μm). (b) In the early tetrad stage the plasma membranes of two bordering tapetum cells are covered with electron-dense droplets. W = cell wall (bar = 1 μm). (c) In the early tetrad stage the growing callose walls close the cytomictic channels that originally crossed the cell walls (W) and the middle lamella (M) in the previous stage (bar = 1 μm). (d) Many dictyosomes border the thin callose walls (C) that divide each meiocyte into four microspores. The plastids are undifferentiated (bar = 1 μm). (e) In the early tetrads free nucleoids can be found in the cytoplasm (bar = 1 μm). (f) In the late tetrad stage the numbers of mitochondria (M) and starch-less plastids (P) in the tapetal cells are larger than in the early tetrad stage (bar = 1 μm). (g) Pro-orbicules are deposited between the plasma membrane (PM) and the cell wall in the tapetal cells. C = callose of the (late) tetrads (bar = 1 μm). (h) Electron-dense material is found inside the intercellular spaces between the tapetal cells (T) and the late tetrads (bar = 10 μm).

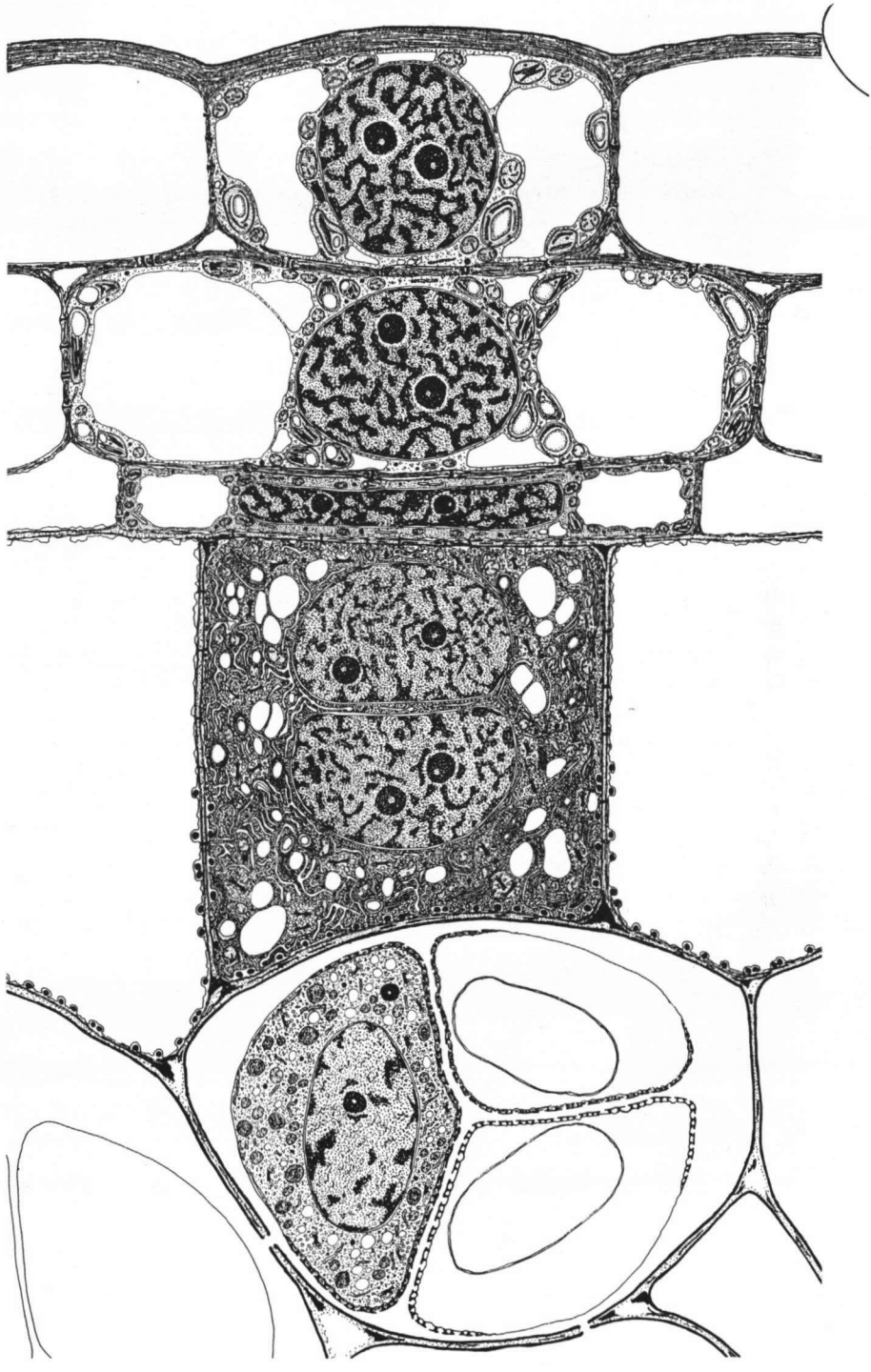
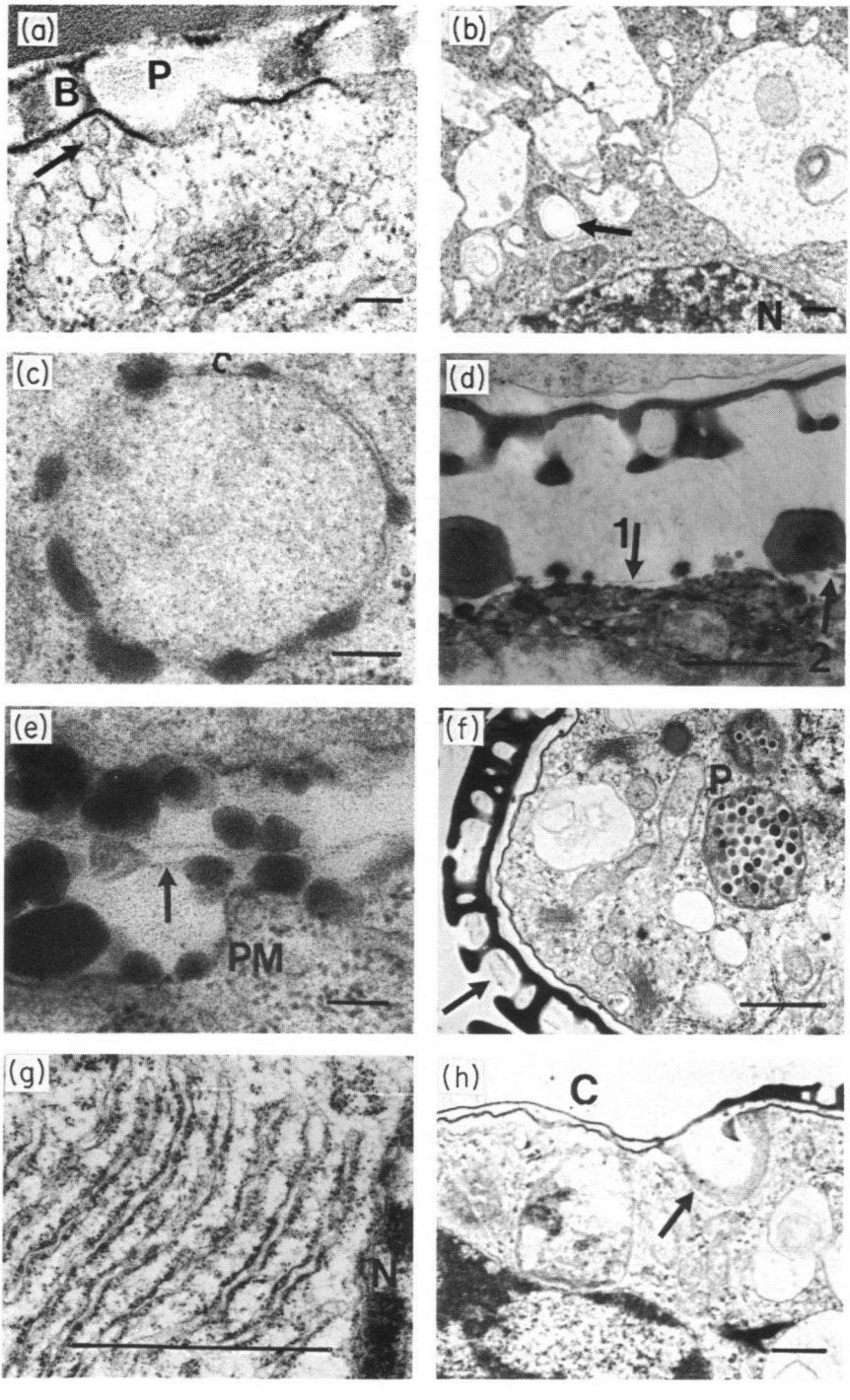


Fig. 6. The late tetrad. Both the tapetal cells and the microspores deposit sporopollenin structures between the plasma membrane and the original cell walls, i.e. the pro-orbicules and the exine. The colpus is situated near the periphery of the original mother cell.


(a)
B
P
(b)
N
(c)
C
(d)
1
2
(e)
PM
(f)
P
(g)
N
(h)
C

mitochondria and plastids increase, the latter sometimes contain starch (Fig. 5f). Along the centripetal part of the cells pro-orbicules appear between the wall and the plasma membrane (Fig. 5g). The middle lamella between the tapetum and the tetrads widens and partly dissolves, in some of these sites electron-dense material appears (Fig. 5h).

The nuclei of the microspores move to the centre of each four-celled complex. The callose wall formation around the cells finishes and a primexine is deposited, invaded by the baculae of the exine and accompanied by the presence of dictyosome vesicles (Fig. 7a). Near the future colpi the exine is very thin and here the plasman membrane is partly lined with RER. The nucleoids are still present. The plastids contain more membranes than in the previous stage.

5. The young free microspore stage (Fig. 8)

The ultrastructure of the epidermis, the endothecium and the middle layer remains unchanged, the circumference of the locule increases slightly and the middle layer continues to flatten radially.

The tapetum cells reach their maximal size, accompanied by a combined vacuolation (Fig. 7b). In their plastids grey material apears and the starch grains are enlarged (Fig. 7b). Dark droplets are often visible between the mitochondrial membranes (Fig. 7c). The amount of lipid droplets increases strongly. The pro-orbicules are covered with fusing drops of sporopollenin and are connected by an electron-dense granular tapetal membrane outside the plasma membrane (Fig. 7d). This surrounds the entire cell and is associated with the extracellular dark droplets which appeared in stage 3 (Fig. 7e). The original cell wall has disappeared (Figs 7d and e).

The microspores lose both their original (pollen mother cell) walls and the callose walls, while the exine thickens and still contains the primexine between the baculae (Fig. 7f). They float in locular fluid. Their plastids grow strongly and store small starch grains (Fig. 7f). A few stacks of RER border the nucleus (Fig. 7g). The nucleoids disappear and near the colpus a few large vesicles are confluent with the plasma membrane (Fig. 7h).

DISCUSSION

1. The development of the epidermis, endothecium and middle layer

In the young anther stages the development of both the epidermis and endothecium shows no remarkable processes which can be related to the development of the inner anther tissues. The circumference of both layers increases due to tangential cell divisions. The

Fig. 7. (a) The primexine (P) of the (late) tetrads is invaded by the baculae (B) of the exine, which is often accompanied by dictyosome vesicles (arrow) (bar = 0·1 μm). (b) In the young, free, microspore stage the tapetal cells contain many vacuoles. The amount of starch (arrow) has increased compared with the previous stage (bar = 1 μm). (c) Dark droplets appear between the membranes of the tapetal mitochondria at this stage (bar = 0·1 μm). (d) A granular membrane (arrow 1) connects the orbicules that fuse with drops of spropollenin (arrow 2) (bar = 1 μm). (e) At this stage two bordering tapetum cells are separated by their plasma membranes (PM) and tapetal membranes (arrow), the latter associated with the electron-dense droplets that appeared in the previous stage. This outer part of the radial cell border lacks orbicules (bar = 0·1 μm). (f) In the exine cavities of the free microspores the primexine (arrow) is still present. Many small starch grains fill the plastids (P) (bar = 1 μm). (g) A RER-stack borders the nucleus of the young free microspore (bar = 1 μm). (h) A large vesicle (arrow) is continuous with the plasma membrane of the colpus (C) of the young free microspore (bar = 1 μm).

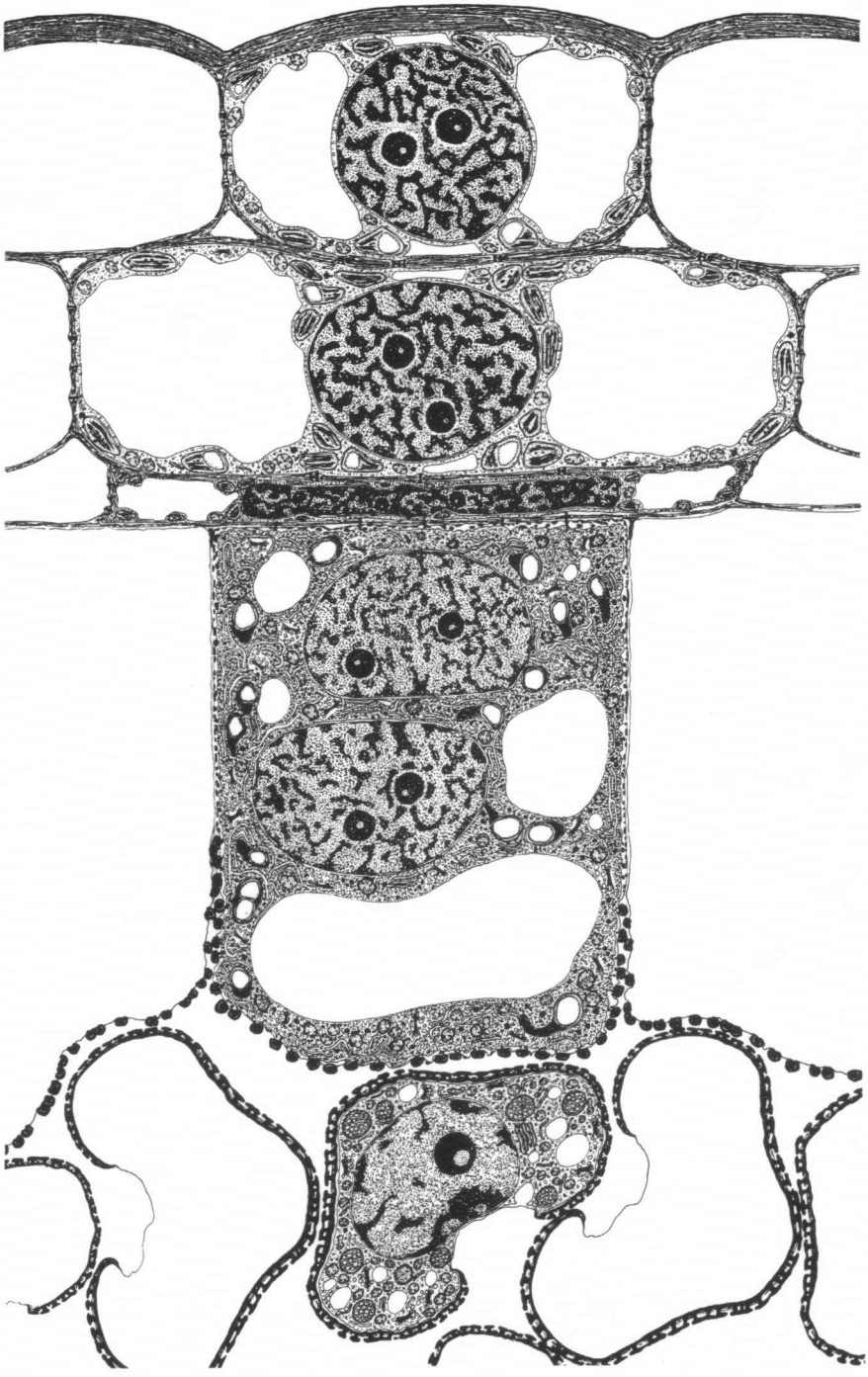


Fig. 8. The young, free microspore stage. The tapetum has reached its largest volume and shows the first signs of degeneration. The original hydrophylic cell walls have been replaced by hydrophobic structures on the border between the sporophytic and gametophytic tissues, i.e. the orbicule-covered tapetal membranes and the exines. The starch in the microspores and the tapetal cells may originate from the breakdown of the callose and cellulose walls.

increase in the amount of starch, already present before the pollen mother cell stage, may be due to photosynthesis in the thylakoid-rich plastids themselves. In contrast with the findings of Reznickova & Willemse (1980) in the *Lilium* hybrid 'Enchantment' (as far as we know the only study on this topic), there are no similarities between the plastidial contents of the middle layer and the tapetum in *G. verrucosa*.

2. The roles of the tapetum

Part of the tapetal functions, reviewed by Pacini *et al.* (1985), can be divided into three main groups, depending upon their final goal. Firstly, the regulation of cell wall changes in the locule; secondly, the supply of energy-rich substances to the developing pollen grains (PAS-positive materials) and, thirdly, the preparation of the pollen grains on recognition and germination (the transfer of sporophytic pollen wall enzymes). Aspects that can be related to our observations will be discussed here.

The organization of the cell wall changes. Our previous histochemical data (Keijzer 1987b) and the present ultrastructural observations indicate that the tapetum has an organizing role in the changes of the cell walls of both the tapetum itself and the developing pollen grains. Shortly after the callose deposition starts, the middle lamellas around both the tapetum and the pollen mother cells desintegrate. The more electron-dense layers of the cell walls in both tissues remain and later disappear in synchony with the callose of the meiocytes. These ultrastructural observations agree with the histochemically tested disappearance of pectins, cellulose and callose, respectively (Keijzer 1987b). The synchrony in both the tapetum cells and the meiocytes, concerning the pectin and the cellulose breakdown, indicates a similar origin for the necessary enzymes. The presence of the callose walls, which may be impermeable to large molecules (Heslop-Harrison & MacKenzie 1967), during the pectin and cellulose digestion makes an enzyme supply from the meiocytes unlikely. Therefore, the tapetum is the source of pectinases and cellulases for both tissues. Mepham & Lane (1969) demonstrated that callase, necessary to digest the callose walls, also orginates from the tapetum. This infers that the tapetum directs the different steps involved in the release of the microspores. The dictyosome vesicles outside its undulating plasma membrane in the meiotic stages may play a role in these excretions (Echlin 1971).

Apart from directing these wall digestions the tapetum is also involved in the synthesis of new walls. The sporopollenin-containing tapetal membranes and orbicules, and part of the sporopollenin precursors for the exines are tapetal excretes, while the early exine and intine originate from the microspore itself (Heslop-Harrison & Dickinson 1969, Heslop-Harrison 1971). This means that the tapetum regulates the shift from hydrophylic (pectins, cellulose, callose) to hydrophobic (sporopollenin) wall substances on the border between the sporophyte and the gametophyte. This change is important for pollen dispersal (Keijzer 1987b).

The tapetal reserves. During the young microspore stage the tapetum cells reach their largest size. The enlarging vacuoles (presumably filled with PAS-positive materials (Pacini & Franchi 1983)) the increase in cytoplasmic lipids and ER, as well as the plastidial increase in starch and grey materials (presumably carotenoids (Reznickova & Willemse 1980)) reflect the accumulation of different substances. In many species there is a close correlation between the tapetal degeneration and the appearance of reserve substances in the developing pollen grains (Echlin 1972, Christensen & Horner 1974). In *G. verrucosa* the

microspores store a large amount of starch before the tapetal degeneration becomes visible, this is due more to the callose breakdown than to tapetal supply (see below).

3. The developing microspores

The cytoplasm during meiosis. During meiosis, dedifferentiation of ribosomes, plastids and mitochondria, as reported by Dickinson & Heslop-Harrison (1977) in *Lilium hybrida*, is not observed. This means that cytoplasmic changes from the diplophase to the haplophase (Dickinson & Andrews 1977) are not observed in *G. verrucosa*. In contrast, the presence of nucleoids related to cytoplasmic redifferentiation (Dickinson & Heslop-Harrison 1970) is evident.

Possible functions of the meiotic callose wall. The meiotic callose wall, also histochemically demonstrated in this species (Keijzer 1987b), presumably originates from materials in the dictyosome vesicles in the proximity of the plasma membrane (Echlin & Godwin 1968; Willemse 1971a,b). Different reports have appeared on the role of these walls and their cytomictic channels. Waterkeyn & Bienfait (1970) demonstrated a negative replica of the exine pattern on the inside of the callose wall of *Ipomoea purpurea* (L) Roth. and supposed a possible role in the determination of this pattern. Such a pattern was also found (M.T.M. Willemse, personal communication) in *G. verrucosa*. Heslop-Harrison & MacKenzie (1967) and Southworth (1971) demonstrated that certain molecules cannot penetrate the callose while others can, which suggests that the wall may act as a selective barrier during meisosis. The observation that premature callose digestion is often accompanied by male sterility indicates that a selective role during development is likely (Izhar & Frankel 1971).

We assume a third function for this wall. In *G. verrucosa*, as in many species, most of the callose is initially deposited in the corners of the original cell walls and turns the differently shaped meiocytes into spherical, equally shaped cells (Van Lammeren *et al.* 1985). This enables them to divide into four microspores of equal size and shape, which excludes any selective influence of size and shape during pollen dispersal. This equal shaping is also effected by the symmetric arrangement of the colpi in the callosic sphere. Furthermore, the amount of callose necessary to reach the spherical shape depends on the size of each individual pollen mother cell and differs between the cells. This causes different voluminal changes in the cells during the callose deposition. As a result the cytomictic channels may play a role in equalizing the pressure between the cells. In this action organelles are also transported. Heslop-Harrison (1968) suggests a different function for these channels, namely the creation of a syncytium which may synchronize the meiotic divisions. This may be true, although synchronous processes also occur in species without cytomictic channels, e.g. *Pinus sylvestris* L. (Willemse 1971a,b).

There are indications for the recycling of a considerable amount of callose breakdown products during the further development. Larson & Lewis (1962) reported that the supply of compounds from the callose wall is in favour of the cellulosic primexine. In *G. verrucosa* we found a sudden increase in the amount of starch in both the tapetum and the microspores shortly after the callose digestion. We presume that these two tissues take up callose-derived sugars from the locular fluid for their starch production, the callose can be regarded as an early reserve substance of the future pollen grains. In addition the pectocellulosic walls of both the tapetum and the pollen mother cells may play such a role (Pacini & Franchi 1983).

ACKNOWLEDGEMENTS

The authors would like to thank Mr A.B. Haasdijk, Mr. P. van Snippenburg and Mr. S. Massalt for preparing the photographs and drawings for publication.

REFERENCES

Bhandari, N.N. (1984): The microsporangium. In: Johri, B.M. (ed): *Embryology of Angiosperms.* 53–121. Springer-Verlag, Berlin.

Christensen, J.E. & Horner, H.T. Jr. (1974): Pollen development and its special orientation during microsporogenesis in the grass Sorghum bicolor. *Am. J. Bot.* **61:** 604–523.

Davis, G.L. (1966): *Systematic Embryology of Angiosperms.* John Wiley, New York.

Dickinson, H.G. & Andrews, L. (1977): The role of membrane-bound cytoplasmic inclusions during gametogenesis in Lilium longiflorum Thunb. *Planta,* **134:** 229–240.

Dickinson, H.G. & Heslop-Harrison, J. (1970): The ribosome cycle, nucleoli, and cytoplasmic nucleoids in the meiocytes of Lilium. *Protoplasma,* **69:** 187–200.

Dickinson, H.G. & Heslop-Harrison, J. (1977): Ribosomes, membranes and organelles during meiosis in angiosperms. *Phil. Trans. Royal Soc. Lond. B.* **277:** 327–342.

Eames, A.J. (1961): *Morphology of the Angiosperms.* McGraw-Hill Book Co. New York.

Echlin, P. (1972): The ultrastructure and ontogeny of pollen in Helleborus foetidus L. 4. Pollen grain maturation. *J. Cell Sci.* **11:** 111–129.

Echlin, P. & Godwin, H. (1968): The ultrastructure and ontogeny of pollen in Helleborus foetidus. 2. Pollen grain development through the callose special wall stage. *J. Cell Sci.* **3:** 175–186.

Edwardson, J.R. (1970): Cytoplasmic male sterility. *Bot. Rev.* **36:** 341–420.

Gottschalk, W. & Kaul, M.L.H. (1974): The genetic control of microsporogenesis in higher plants. *The Nucleus,* **17:** 133–166.

Heslop-Harrison, J. (1968): Pollen wall development. *Science,* **161:** 230–237.

Heslop-Harrison, J. (1971): The pollen wall: structure and development. In: Heslop-Harrison, J. (ed.): *Pollen, Development and Physiology.* 75–98. Butterworths, London.

Heslop-Harrison, J. & Dickinson, H.G. (1969): Time relationships of sporopollenin synthesis associated with tapetum and microspores in Lilium. *Planta* **84:** 199–124.

Heslop-Harrison, J. & MacKenzie, A. (1967): Autoradiography of soluble (2-14C) thymidine derivatives during meiosis and microsporogenesis in Lilium anthers. *J. Cell Sci.* **2:** 387–400.

Izhar, S. & Frankel, R. (1971): Mechanism of male sterility in Petunia: the relationship between pH, callase activity in anthers, and the breakdown of the microsporogensis. *Theoret. Appl. Gen.* **41:** 104–108.

Keijzer, C.J. (1983): Hydration changes during anther development. In: Mulcahy, D.L. & Ottaviano, E. (eds): *Pollen, Biology and Implications for Plant Breeding.* 197–201. Elsevier Biomedical, Amsterdam.

Keijzer, C.J. (1987a): The processes of anther dehiscence and pollen dispersal. 1. The opening mechanism of longitudinally dehiscing anthers. *New Phytol.* **105:** 487–498.

Keijzer, C.J. (1987b): The processes of anther dehiscence and pollen dispersal. 2. The formation and the transfer mechanism of pollenkitt, cell wall development in the loculus tissues and a function of the orbicules in pollen dispersal. *New Phytol.* **105:** 499–507.

Keijzer, C.J., Hoek, I.H.S. & Willemse, M.T.M. (1987a): The processes of anther dehiscence and pollen dispersal. 3. The dehydration of the filament tip and the anther in three monocotylous species. *New Phytol.* **106:** 281–287.

Keijzer, C.J., Hoek, I.H.S. & Willemse M.T.M. (1987b): The development of the staminal filament of *Gasteria verrucosa. Acta Bot. Neerl.* **36:** 271–282.

Keijzer, C.J. & Willemse, M.T.M. (1988). Tissue interactions in the developing locule of *Gasteria verrucosa* during microgametogenesis. *Acta Bot. Neerl.* **37:** 475–492.

Knox, R.B. (1984): The pollen grain. In: Johri, B.M. (ed.): *Embryology of Angiosperms.* 197–271. Springer-Verlag, Berlin.

Larson, D.A. & C.W. Lewis (1962): Pollen wall development in *Parkinsonia aculeata. Grana Palyn.* **3:** 21–27.

Laser, K.D. & N.R. Lersten. (1972): Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. *Bot. Rev.* **38:** 425–454.

Linskens, H.F. (1964): Pollen physiology. *Ann. Rev. Plant Physiol.* **15:** 255–270.

Linskens, H.F. (1967): Pollen. *Handb. Pflanzen Physiol.* **18:** 368–406.

Mascarenhas, J.P. (1975): The biochemistry of angiosperm pollen. *Bot. Rev.* **41:** 259–314.

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

Pacini, E. & Franchi, G.G. (1983): Pollen grain development in Smilax aspera and possible functions of the loculus. In: Mulcahy, D.L. & Ottaviano, E. (eds.): *Pollen Biology and Implications for Plant Breeding*. 183–190. Elsevier, Amsterdam.

Pacini, E., Franchi, G.G. & Hesse, M. (1985): The tapetum: its form, function and possible phylogeny in Embryophyta. *Plant Syst. Evol.* **149:** 155–185.

Puri, V. (1972): Anther sacs and pollen grains: some aspects of their structure and function. *J. Palyn.* **6:** 1–17.

Reynolds, E.S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17:** 208–212.

Reznickova, S.A. & Willemse, M.T.M. (1980): Formation of pollen in the anther of Lilium. 2. The function of the surrounding tissues in the formation of pollen and pollen wall. *Acta Bot. Neerl.* **29:** 141–156.

Richter, S. (1929). Über den Öffnungsmechanismus der Antheren bei einigen Vertretern der Angiospermen. *Planta* **8:** 154–184.

Schröder, M.B. (1985): Ultrastructural studies on plastids of generative and vegetative cells in Liliaceae. 3. Plastid distribution during pollen development in Gasteria verrucosa (Mill.) H. Duval. *Protoplasma,* **124:** 123–129.

Shivanna, K.R., Johri, B.M. & Sastri, D.C. (1979): *Development and Physiology of Angiosperm Pollen.* Today and Tomorrow's Printers and Publishers, New Dehli.

Southworth, D. (1971): Incorporation of radioactive precursors into developing pollen walls. In: Heslop-Harrison, J. (ed.): *Pollen: Development and Physiology*. 115–120. Butterworths, London.

Spurr, A.R.(1969): A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruc. Res.* **71:** 173–184.

Stanley, R.G. & Kirby, E.G. (1973): Shedding of pollen and seeds. In: Kozlowski, T.T. (ed.): *Shedding of Plant Parts*. 295–340. Academic Press, New York.

Van Lammeren, A.A.M., Keijzer, C.J., Willemse, M.T.M. & Kieft, H. (1985): Structure and function of the microtubular cytoskeleton during pollen development in *Gasteria verrucosa* (Mill.) H. Duval. *Planta,* **165:** 1–11.

Vasil, I.K. (1973): The new biology of pollen. *Naturwiss.* **60:** 247–253.

Waterkeyn, L. & Bienfait, A. (1970): On a possible function of the callosic special wall in *Ipomoea purpurea* (L) Roth. *Grana,* **10:** 13–20.

Willemse, M.T.M. (1971a): Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Pinus sylvestris* L. 1. Morphological changes from zygotene until prometaphase-1. *Acta Bot. Neerl.* **20:** 261–274.

Willemse, M.T.M. (1971b): Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Pinus sylvestris* L. 2. Morphological changes from prometaphase-1 until the tetrad stage. *Acta Bot. Neerl.* **20:** 411–427.

Willemse, M.T.M. (1972): Morphological and quantitative changes in the population of cell organelles during microsporogensis of *Gasteria verrucosa. Acta Bot. Neerl.* **21:** 17–31.